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New, Potent, Selective, and Short-Acting Peptidic V_{1a} Receptor Agonists

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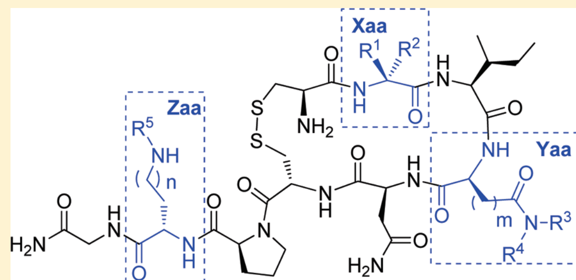
Supporting Information

ABSTRACT: [Arg[8]]vasopressin (AVP) produces vasoconstriction via V_{1a} receptor (V_{1a}R)-mediated vascular smooth muscle cell contraction and is being used to increase blood pressure in septic shock, a form of vasodilatory hypotension. However, AVP also induces V_2 receptor (V_2R)-mediated antidiuresis, vasodilation, and coagulation factor release, all deleterious in septic shock. The V_{1a}R agonist terlipressin (H-Gly$_3$[Lys[8]]VP) also lacks selectivity vs the V_2R and has sizably longer duration of action than AVP, preventing rapid titration of its vasopressor effect in the clinic. We designed and synthesized new short acting V_{1a}R selective analogues of general structure [Xaa[2],Ile[3],Yaa[4],Zaa[8]]VP. The most potent and selective compounds in in vitro functional assays (e.g., [Phe[2],Ile[3],Asn(Me$_2$)[4],Orn[8]]VP (**31**), [Phe[2],Ile[3],Asn((CH$_2$)$_3$OH)[4],Orn[8]]VP (**34**), [Phe[2],Ile[3],Hgn[4],Orn(iPr)[8]]VP (**45**), [Phe[2],Ile[3],Asn(Et)[4],Dab[8]]VP (**49**), [Thi[2],Ile[3],Orn-(iPr)[8]]VP (**59**), [Cha[2],Ile[3],Asn[4],Orn(iPr)[8]]VP (**68**)) were tested by intravenous bolus in rats for duration of vasopressive action. Analogues **31**, **34**, **45**, and **49** were as short-acting as AVP. Compound **45**, FE 202158, is currently undergoing clinical trials in septic shock.



INTRODUCTION

Arginine vasopressin, **1** (AVP), is the endogenous ligand for the three known subtypes of vasopressin receptors, V_{1a}R, V_{1b}R (V_3R), and V_2R, and also acts on the oxytocin receptor (OTR) to regulate a variety of physiological processes both centrally and peripherally (Figure 1).[1−4] **1** is involved in the continuous maintenance of plasma osmolality, and in this role it is also known as the antidiuretic hormone (ADH). In response to elevations of plasma osmolality above a physiological set point, **1** is secreted from the posterior pituitary gland and activates renal V_2R to promote water conservation. **1** also controls the secretion of adrenocorticotropic hormone (ACTH), mediated by both peripherally and centrally located V_{1b}R, and plays an important role in stress/anxiety/depression management by activation of centrally located V_{1b}R.[4] It has been recently suggested that **1** stimulated release of insulin from Islet cells is mediated by the pancreatic V_{1b}R.[5] In sitof severe hypotension, **1** is acutely secreted by the posterior pituitary to produce arterial vasoconstriction by activation of the V_{1a} vasopressin receptor subtype (V_{1a}R) located in vascular smooth muscle cells, with consequent increase in blood pressure.[6,7]

AVP deficiency has been observed in patients with septic shock, a form of vasodilatory hypotension, and low-dose continuous intravenous infusion of **1** effectively increased blood pressure in these patients.[7,8] Numerous small pilot studies using such an "add-back" AVP therapy confirmed these results.[9] Recently, the first large-scale multicenter study, the Vasopressin in Septic Shock Trial (VASST), has suggested that such therapy may reduce mortality in less severe septic shock.[10]

Terlipressin (H-Gly$_3$-LVP), a synthetic analogue of lysine vasopressin (LVP), believed to behave as a prodrug of LVP, is approved in some European and Asian countries for the treatment of bleeding esophageal varices and in France for hepatorenal syndrome.[11] This analogue was shown to be effective in raising mean arterial pressure in septic shock patients after high-dose intravenous bolus administration,[12] which was confirmed by multiple exploratory clinical trials.[13,14]

The lack of V_{1a}R selectivity of **1** could possibly limit its use in vasodilatory hypotension states such as septic shock due to V_2R-mediated unwanted side effects such as vasodilation and coagulation factor release.[15] Likewise, the use of terlipressin in such critical care conditions may be disadvantageous due to the long duration of vasopressive action,[16] which would prevent the desirable rapid titration of effect to optimize safety and clinical efficacy. Furthermore, the lack of selectivity vs the V_2R of its putative active metabolite LVP[16] could also lead to the same V_2R-mediated adverse effects.

There has been substantial progress in discovering selective small-molecule agonists and antagonists for the family of vasopressin/oxytocin receptors with different degrees of receptor selectivity.[4,17,18]

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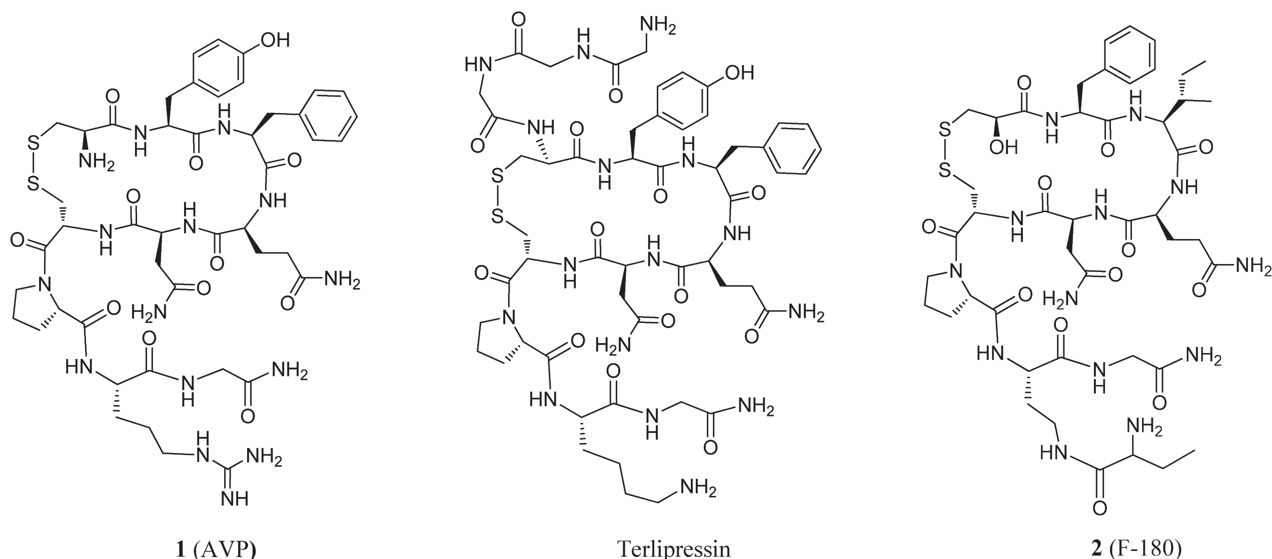


Figure 1. Structures of **1** (AVP), terlipressin, and **2** (F-180).

Table 1. Structure and in Vitro Pharmacological Profile of Reference Compounds 1, 3−7

		in vitro pharmacological profile						
		EC_{50} receptor [nM] (pEC_{50} ± SEM)				selectivity vs receptor[a]		
analogue	structure	hV_{1a}	hV_2	hV_{1b}	hOT	hV_2	hV_{1b}	hOT
1	AVP[19]	0.24 (9.63 ± 0.02)	0.05 (10.3 ± 0.04)	4.3 (8.37 ± 0.02)	15 (7.82 ± 0.07)	0.20	17	62
3	[Orn[8]]VP, OVP[28]	0.69 (9.16 ± 0.17)	0.45 (9.35 ± 0.19)	7.5 (8.12 ± 0.13)	71 (7.15 ± 0.07)	0.65	10	100
4	[Phe[2]]AVP[27]	0.15 (9.81 ± 0.18)	1.4 (8.85 ± 0.15)	16 (7.80 ± 0.04)	>10000[b] (<5.00)	9.3	100	>66000
5	[Phe[2]]OVP[22]	0.14 (9.85 ± 0.06)	9.2 (8.04 ± 0.08)	15 (7.82 ± 0.20)	>10000[b] (<5.00)	65	100	>71000
6	[Ile[3]]OVP[28]	0.23 (9.63 ± 0.08)	4.1 (8.39 ± 0.22)	56 (7.25 ± 0.19)	3.3 (8.48 ± 0.06)	17	240	14
7	[Phe[2],Ile[3]]OVP[22]	0.27 (9.57 ± 0.16)	155 (6.81 ± 0.10)	25 (7.60 ± 0.16)	>10000[b] (<5.00)	570	92	>37000

[a] EC_{50} (receptor)/EC_{50} (V_{1a}R) ratio. If no significant agonism was observed at the highest concentration tested, selectivity is >highest conc tested/EC_{50} (hV_{1a}R). [b] No significant agonism at the highest concentration tested: 10000 nM.

However, for the treatment of patients with vasodilatory hypotension in a hospital setting, a peptide-based drug appears as the most suitable approach to provide very high selectivity for the V_{1a}R coupled with short half-life in vivo. This is expected to allow fast and accurate up and down titration of the infusion rate of the compound to regulate its vasopressor effect in response to the changing demands of critically ill patients, without off target effects due to activation of the V_2, V_{1b}, or OT receptors in the periphery or at these and the V_{1a}R in the CNS.

The structure of **1** was elucidated by du Vigneaud in the early 1950s,[19] and it was shown to be a nonapeptide consisting of a 20-membered ring, closed by a disulfide bridge between the Cys[1] and Cys[6] residues, and a C-terminal tripeptide amide. The ring is essential for preserving agonistic activity of AVP analogues at the V_{1a}R, but its presence is not required for binding to the V_{1a}R, as potent linear peptidic V_{1a}R and mixed V_{1a}R/V_2R antagonists were discovered by Manning's group.[20] Interestingly, the same group reported linear mixed V_{1a}R antagonists/V_2R agonists with low/moderate antidiuretic potency in a rat in vivo model.[21]

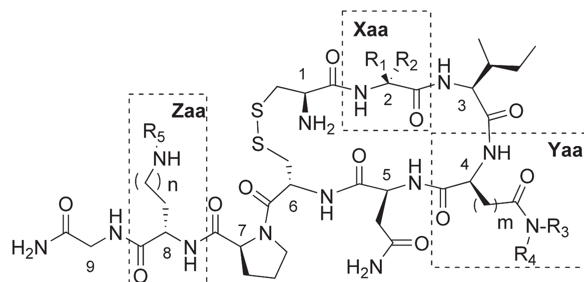
Analogues of **1** showing improved V_{1a}R selectivity were first described by Huguenin: replacements of the Arg[8] residue with Orn and of the Tyr[2] residue with Phe resulted in analogues showing reduced antidiuretic activity in rat.[22] A vasopressin analogue [Hmp[1],Phe[2],Ile[3],Hgn[4],Dab(Abu)[8]]VP, **2** (F-180), was discovered by Ferring scientists and found to be a long-acting vasopressor in vivo[23] and very selective for the human V_{1a}R in vitro.[24]

We report here the synthesis and pharmacological evaluation of a series of analogues of **1** modified in positions 2, 3, 4, and/or 8 designed to provide highly V_{1a}R selective and short-acting peptides for the treatment of vasodilatory hypotension states.

RESULTS AND DISCUSSION

1 and its analogues **3**−**7** were synthesized in our laboratory as reference compounds for this study and tested in vitro in reporter gene assays (RGA) at the hV_{1a} and related receptors (Table 1). In these assays, **1** turned out to be a potent and fully efficacious agonist at all receptors with the lowest potency (EC_{50} = 15 nM, selectivity ratio = 62) at the hOTR. It is noteworthy that **1** has been reported a partial agonist in other in vitro systems (e.g., Wootten et al. demonstrated partial agonism of **1** at the OTR in an inositol triphosphate accumulation assay).[25,26] The improved V_{1a}R selectivity of analogues **3**−**7** as compared to **1** in a rat in vivo model was reported earlier by Huguenin.[22,27,28] This enhanced selectivity was indeed confirmed in our in vitro assays, suggesting

Table 2. Structures and in Vitro Pharmacological Profile of Compounds 8−44



	structure[a]			in vitro pharmacological profile						
				EC50 receptor (nM) (pEC50 ± SEM)				selectivity vs receptor[b]		
analogue	Xaa	Yaa	Zaa	hV_{1a}	hV_2	hV_{1b}	hOT	hV_2	hV_{1b}	hOT
8	Phe	Gln	Dab	0.50 (9.31 ± 0.10)	73 (7.14 ± 0.04)	26 (7.58 ± 0.20)	9.1[c] (8.04 ± 0.04)	140	52	18
9	His	Gln	Dab	510[c] (6.29 ± 0.16)	>10000[d] (<5.00)	>10000[d] (<5.00)	>10000[d] (<5.00)	>19	>19	>19
10	3-Pal	Gln	Dab	30 (7.53 ± 0.12)	>10000[d] (<5.00)	200 (6.71 ± 0.20)	>10000[d] (<5.00)	>330	6.6	>330
11	Ala(BzThi)	Gln	Dab	>1000[e] (<6.00)	>10000[d] (<5.00)	360 (6.44 ± 0.71)	>10000[d] (<5.00)	na[f]	na[f]	na[f]
12	Ala(Thz)	Gln	Dab	19 (7.73 ± 0.21)	2500[c] (5.60 ± 0.07)	430 (6.36 ± 0.27)	1200[c] (5.91 ± 0.30)	130	22	63
13	Thi	Gln	Dab	0.54 (9.27 ± 0.01)	120 (6.94 ± 0.05)	150 (6.82 ± 0.65)	29 [c] (7.53 ± 0.09)	220	270	53
14	3-Thi	Gln	Dab	0.61 (9.21 ± 0.04)	200 (6.69 ± 0.14)	50[c] (7.30 ± 0.56)	30[c] (7.53 ± 0.04)	320	81	49
15	Ala(2-Fur)	Gln	Dab	2.2 (8.65 ± 0.18)	330 (6.48 ± 0.09)	210 (6.68 ± 0.36)	105 (6.98 ± 0.03)	150	95	47
16	Ala	Gln	Dab	>1000[e] (<6.00)	>10000[d] (<5.00)	>10000[d] (<5.00)	>10000[d] (<5.00)	na[f]	na[f]	na[f]
17	Ala(tBu)	Gln	Dab	11 (7.97 ± 0.09)	470 (6.33 ± 0.08)	44 (7.36 ± 0.07)	>10000[d] (<5.00)	42	4.0	>900
18	Leu	Gln	Dab	13 (7.90 ± 0.28)	1200 (5.91 ± 0.03)	250 (6.60 ± 0.47)	>10000[d] (<5.00)	92	19	>760
19	Ala(cPe)	Gln	Dab	0.80 (9.10 ± 0.13)	1200 (5.93 ± 0.07)	20 (7.71 ± 0.21)	>10000[d] (<5.00)	1500	25	>12000
20	Cha	Gln	Dab	0.25 (9.60 ± 0.23)	760 (6.12 ± 0.10)	8.0 (8.10 ± 0.17)	>10000[d] (<5.00)	3000	32	>40000
21	Ac4c[g]	Gln	Dab	0.94 (9.03 ± 0.15)	380 (6.42 ± 0.08)	64 (7.20 ± 0.15)	180[c] (6.76 ± 0.20)	400	68	190
22	Phe	Gln(Me)	Orn	6.0 (8.22 ± 0.20)	210 (6.68 ± 0.09)	59 (7.23 ± 0.21)	>10000[d] (<5.00)	35	9.8	>1600
23	Phe	Gln(Et)	Orn	39 (7.41 ± 0.33)	2600[c] (5.58 ± 0.05)	97 (7.01 ± 0.10)	>10000[d] (<5.00)	66	2.4	>250
24	Phe	Gln(iBu)	Orn	21 (7.68 ± 0.22)	3000[c] (5.52 ± 0.12)	150 (6.83 ± 0.33)	>10000[d] (<5.00)	140	7.1	>470
25	Phe	Gln(OMe)	Orn	1.1 (8.94 ± 0.39)	1700 (5.77 ± 0.03)	28 (7.55 ± 0.04)	>10000[d] (<5.00)	1500	25	>9000
26	Phe	Gln(Me,OMe)	Orn	69 (7.16 ± 0.07)	1200 (5.91 ± 0.05)	540 (6.27 ± 0.17)	>10000[d] (<5.00)	17	7.8	>140
27	Phe	Gln(OH)	Orn	12 (7.93 ± 0.14)	170 (6.78 ± 0.10)	70 (7.15 ± 0.59)	>10000[d] (<5.00)	14	5.8	>830

Table 2. Continued

	structure[a]			in vitro pharmacological profile						
				EC_{50} receptor (nM) (pEC_{50} ± SEM)				selectivity vs receptor[b]		
analogue	Xaa	Yaa	Zaa	hV_{1a}	hV_2	hV_{1b}	hOT	hV_2	hV_{1b}	hOT
28	Phe	Asn	Orn	1.4 (8.84 ± 0.08)	>10000[d] (<5.00)	29 (7.54 ± 0.34)	>10000[d] (<5.00)	>7100	20	>7100
29	Phe	Asn(Me)	Orn	3.6 (8.45 ± 0.16)	>10000[d] (<5.00)	39 (7.41 ± 0.13)	>10000[d] (<5.00)	>2700	10	>2700
30	Phe	Asn(Et)	Orn	5.7 (8.24 ± 0.22)	>10000[d] (<5.00)	30 (7.53 ± 0.05)	>10000[d] (<5.00)	>1700	5.2	>1700
31	Phe	Asn(Me$_2$)	Orn	1.7 (8.78 ± 0.19)	>10000[d] (<5.00)	62 (7.20 ± 0.14)	>10000[d] (<5.00)	>5800	36	>5800
32	Phe	Asn(OH)	Orn	4.1 (8.39 ± 0.09)	2600 (5.58 ± 0.05)	56 (7.25 ± 0.25)	>10000[d] (<5.00)	630	13	>2400
33	Phe	Asn(CH$_2$)$_2$OH)	Orn	8.0 (8.10 ± 0.18)	>10000[d] (<5.00)	26 (7.59 ± 0.15)	>10000[d] (<5.00)	>1200	3.2	>1200
34	Phe	Asn(CH$_2$)$_3$OH)	Orn	2.1 (8.68 ± 0.14)	>10000[d] (<5.00)	19 (7.71 ± 0.15)	>10000[d] (<5.00)	>4700	9.0	>4700
35	Phe	Asn(CH(CH$_2$OH)$_2$)	Orn	16[c] (7.79 ± 0.09)	>10000[d] (<5.00)	62[c] (7.21 ± 0.22)	>10000[d] (<5.00)	>620	3.8	>620
36	Phe	Asn(CH$_2$CH(OH)CH$_3$)	Orn	5.2 (8.29 ± 0.16)	>10000[d] (<5.00)	28 (7.56 ± 0.32)	>10000[d] (<5.00)	>1900	5.3	>1900
37	Phe	Asn((CH$_2$)$_2$O(CH$_2$)$_2$OH)	Orn	4.8 (8.32 ± 0.08)	>10000[d] (<5.00)	41 (7.38 ± 0.24)	>10000[d] (<5.00)	>2000	8.5	>2000
38	Phe	Hgn	Orn	0.90[c] (9.05 ± 0.12)	1600[c] (5.78 ± 0.23)	46 (7.34 ± 0.15)	>10000[d] (<5.00)	1700	51	>11000
39	Phe	Gln	Dap	30 (7.53 ± 0.16)	720 (6.14 ± 0.08)	210 (6.68 ± 0.17)	>10000[d] (<5.00)	24	7.0	>330
40	Phe	Gln	Orn(iPr)	0.38[c] (9.42 ± 0.12)	290[c] (6.55 ± 0.11)	44 (7.35 ± 0.12)	33 (7.48 ± 0.06)	760	110	86
41	Phe	Gln	Orn(Me)	0.26 (9.58 ± 0.11)	130 (6.88 ± 0.21)	74 (7.13 ± 0.44)	16[c] (7.81 ± 0.07)	500	280	61
42	Phe	Gln	Orn(Pr)	0.21 (9.68 ± 0.12)	140 (6.86 ± 0.15)	38 (7.42 ± 0.20)	32 (7.49 ± 0.10)	660	180	150
43	Phe	Gln	Orn(Et)	0.57 (9.24 ± 0.20)	260[c] (6.59 ± 1.02)	32[c] (7.50 ± 0.16)	32 (7.50 ± 0.17)	450	56	56
44	Phe	Gln	Orn(iAm)	0.82 (9.09 ± 0.33)	190 (6.73 ± 1.23)	100 (7.00 ± 0.23)	44 (7.36 ± 0.13)	230	120	53

[a] See the list of abbreviations for unnatural amino acid residues definitions. [b] EC_{50} (receptor)/EC_{50} ($hV_{1a}R$) ratio. If no significant agonism was observed at the highest concentration tested selectivity is >highest conc. tested/EC_{50} ($hV_{1a}R$). [c] Partial agonist, efficacy <70%. [d] No significant agonism at the highest concentration tested: 10000 nM. [e] No significant agonism at the highest concentration tested; 1000 nM. [f] Nonapplicable, EC_{50} at the $hV_{1a}R$ not calculated due to low efficacy. [g] In the case of Ac4c $R^1 = R^2 = -(CH_2)_3-$, in all other cases R^2 is H.

positions 2, 3, and 8 of the AVP molecule are important to attain $V_{1a}R$ specificity. The presence of the N-terminal amino group appears to be beneficial for $V_{1a}R$ selectivity, as the desamino analogue dDAVP is a V_2R selective compound[29] with some residual V_{1b} agonistic activity.[30] In addition, the replacement of the Gln[4] residue with homoglutamine in F-180 confers enhanced $V_{1a}R$ selectivity.[23] Therefore, we decided to explore positions 2, 3, 4, and/or 8 for designing new, selective and short-acting $V_{1a}R$ agonists. A total of 63 new compounds (**8**−**70**) were prepared by either Fmoc or Boc strategy. The peptides with alkylated side chains in position 8 were assembled by Fmoc chemistry. The diamino acid residue in position 8 was introduced with the *N*-δ-Mmt (methoxytrityl) protecting group.[31] The Mmt group was removed and the *N*-ω-isopropyl analogues were synthesized by reductive alkylation of the resin bound peptides with acetone/NaBH(OAc)$_3$. To prepare peptides with straight *N*-alkyl groups, the side chain amino function was derivatized with *o*-NBS-Cl (2-nitrobenzenesulfonyl chloride)[32] and the resulting resin-bound sulfonamide was alkylated with an appropriate alcohol under Mitsunobu reaction conditions.[33] The peptides *N*-alkylated in position 4 were assembled by Boc chemistry, and the position 4 residue was introduced in the sequence as Boc-Asp(OFm)-OH or Boc-Glu(OFm)-OH.[34,35] After the complete assembly of the peptide, the side chain protection was removed

Table 3. Structures and in Vitro Pharmacological Profile of Compounds 45−70

	structure[a]			in vitro pharmacological profile						
				EC_{50} receptor (nM) (pEC_{50} ± SEM)				selectivity vs receptor[b]		
analogue	Xaa	Yaa	Zaa	hV_{1a}	hV_2	hV_{1b}	hOT	hV_2	hV_{1b}	hOT
45	Phe	Hgn	Orn(iPr)	2.4 (8.62 ± 0.07)	2700[c] (5.58 ± 0.05)	340 (6.47 ± 0.16)	1100[c] (8.62 ± 0.07)	1100	140	450
46	Phe	Hgn	Dab(iPr)	18 (7.74 ± 0.24)	1100 (5.95 ± 0.09)	960 (6.02 ± 0.25)	>10000[d] (<5.00)	61	53	>550
47	Phe	Asn	Dab	2.9 (8.54 ± 0.23)	>10000[d] (<5.00)	37 (7.43 ± 0.29)	>10000[d] (<5.00)	>3400	12	>3400
48	Phe	Asn(Me)	Dab	3.9 (8.41 ± 0.13)	5300[c] (5.28 ± 0.10)	8.7 (8.06 ± 0.43)	>10000[d] (<5.00)	1300	2.2	>2500
49	Phe	Asn(Et)	Dab	2.7 (8.57 ± 0.10)	1600[c] (5.79 ± 0.05)	21 (7.68 ± 0.17)	>10000[d] (<5.00)	590	7.7	>3700
50	Phe	Asn	Orn(iPr)	1.4 (8.87 ± 0.10)	3700[c] (5.43 ± 0.11)	250 (6.60 ± 0.16)	110[c] (6.98 ± 0.12)	2600	170	78
51	Phe	Asn	Orn(Me)	2.4 (8.62 ± 0.30)	>10000[d] (<5.00)	65 (7.19 ± 0.01)	>10000[d] (<5.00)	>4100	27	>4100
52	Phe	Asn	Orn(Et)	0.99 (9.00 ± 0.13)	>10000[d] (<5.00)	120 (6.93 ± 0.04)	>10000[d] (<5.00)	>10000	120	>10000
53	Phe	Asn	Orn(Pr)	1.5 (8.82 ± 0.40)	>10000[d] (<5.00)	31[c] (7.51 ± 0.19)	>10000[d] (<5.00)	>6600	20	>6600
54	Ala(2-Fur)	Gln	Orn(iPr)	2.6 (8.58 ± 0.22)	950 (6.02 ± 0.10)	570 (6.24 ± 0.06)	260 (6.59 ± 0.07)	360	210	100
55	Ala(2-Fur)	Asn	Orn	10 (8.00 ± 0.24)	>10000[d] (<5.00)	530 (6.28 ± 0.28)	>10000[d] (<5.00)	>1000	53	>1000
56	Ala(2-Fur)	Asn	Orn(iPr)	15 (7.81 ± 0.42)	>10000[d] (<5.00)	1800 (5.75 ± 0.19)	970 (6.01 ± 0.09)	>660	120	64
57	Ala(2-Fur)	Asn	Dab	14 (7.84 ± 0.24)	>10000[d] (<5.00)	460 (6.34 ± 0.16)	780[c] (6.11 ± 0.12)	>710	32	55
58	Thi	Asn	Orn	15 (7.84 ± 0.28)	>10000[d] (<5.00)	220 (6.65 ± 0.26)	>10000[d] (<5.00)	>660	14	>660
59	Thi	Gln	Orn(iPr)	0.69 (9.16 ± 0.15)	390[c] (6.41 ± 0.20)	370 (6.44 ± 0.24)	110 (6.96 ± 0.06)	560	530	150
60	Thi	Asn	Dab	12 (7.94 ± 0.13)	>10000[d] (<5.00)	510 (6.29 ± 0.52)	>10000[d] (<5.00)	>830	42	>830
61	Thi	Asn	Orn(iPr)	14 (7.86 ± 0.18)	>10000[d] (<5.00)	260 (6.58 ± 0.09)	860 (6.06 ± 0.04)	>710	18	61
62	3-Thi	Asn	Orn	7.3 (8.14 ± 0.11)	>10000[d] (<5.00)	350 (6.46 ± 0.26)	>10000[d] (<5.00)	>1300	47	>1300
63	3-Thi	Asn	Dab	7.4 (8.13 ± 0.12)	>10000[d] (<5.00)	380 (6.42 ± 0.44)	>10000[d] (<5.00)	>1300	51	>1300
64	Ac4c[e]	Asn	Orn	7.9 (8.10 ± 0.11)	>10000[d] (<5.00)	110 (6.98 ± 0.11)	>10000[d] (<5.00)	>1200	13	>1200
65	Ac4c[e]	Asn	Dab	12 (7.91 ± 0.11)	>10000[d] (<5.00)	120 (6.93 ± 0.21)	>10000[d] (<5.00)	>830	10	>830
66	Ala(cPe)	Asn	Orn	7.7 (8.11 ± 0.23)	>10000[d] (<5.00)	120 (6.92 ± 0.17)	>10000[d] (<5.00)	>1200	15	>1200
67	Ala(cPe)	Asn	Dab	11 (7.98 ± 0.25)	>10000[d] (<5.00)	76 (7.12 ± 0.17)	>10000[d] (<5.00)	>900	6.9	>900
68	Cha	Asn	Orn(iPr)	1.4 (8.85 ± 0.14)	970 (6.01 ± 0.04)	110 (6.96 ± 0.11)	>10000[d] (<5.00)	690	78	>7100
69	Cha	Asn	Orn	3.2 (8.49 ± 0.57)	>10000[d] (<5.00)	140 (6.84 ± 0.30)	>10000[d] (<5.00)	>3100	43	>3100
70	Cha	Asn	Dab	1.6 (8.80 ± 0.16)	>10000[d] (<5.00)	18 (7.75 ± 0.05)	>10000[d] (<5.00)	>6200	11	>6200

[a] See Table 2 for general structure and the list of abbreviations for unnatural amino acid residues definitions. [b] EC_{50} (receptor)/EC_{50} ($hV_{1a}R$) ratio. If no significant agonism was observed at the highest concentration tested selectivity is >highest conc tested/EC_{50} ($hV_{1a}R$). [c] Partial agonist, efficacy <70%. [d] No significant agonism at the highest concentration tested: 10000 nM. [e] In the case of Ac4c $R^1 = R^2 = -(CH_2)_3-$, in all other cases R^2 is H.

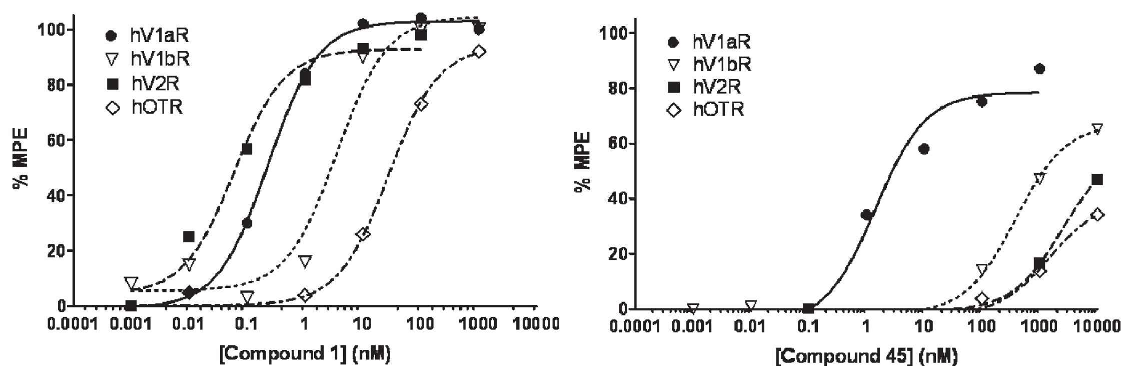


Figure 2. Agonist activity of **1** (AVP) and compound **45** in reporter gene assays at the $hV_{1a}R$ and related receptors.

with 30% piperidine/DMF. The resulting free carboxylic group was converted to the desired amide by coupling with an appropriate amine mediated by PyBOP or BOP/DIPEA. The structures and the results of in vitro evaluation of the new analogues **8**−**70** are reported in Tables 2 and 3. Compounds for which a full dose response curve could be completed were found to have efficacy greater than 70% at the $hV_{1a}R$ and $rV_{1a}R$ by comparison with the maximum efficacy of **1**, unless noted otherwise in the tables.

Replacement of the Tyr^2 residue with Phe in reference compounds **1** and **6** results in equipotent V_{1a} agonists **4** and **7**, respectively. Analogues **4** and **7** show better overall selectivity profile than their parent compounds. Therefore, we prepared a series of analogues **8**−**15**, where position 2 Xaa was a residue

Table 4. Duration of Action of Selected Analogues in Vivo

	structure[a]			in vitro potency at $rV_{1a}R$	relative duration of action		
analogue	Xaa	Yaa	Zaa	EC_{50} (nM) ($pEC_{50} \pm SEM$)	equieffective dose (nmol/kg)	mean ± SEM	no. of rats tested
1[a]	Tyr	Gln	Arg	0.07 (10.2 ± 0.02)	0.1	1.0 ± 0.1[b]	9
2[a]	Phe	Hgn	Dab(Abu)	0.54 (9.27 ± 0.39)	1.0	5.0 ± 0.6[c]	7
3[a]	Tyr	Gln	Orn	0.35 (9.46 ± 0.21)	0.1	1.1 ± 0.1[b]	3
4[a]	Phe	Gln	Arg	0.07 (10.1 ± 0.12)	0.3	1.4 ± 0.2[b]	3
7	Phe	Gln	Orn	0.12 (9.91 ± 0.34)	0.3	2.3 ± 0.2[c]	3
19	Ala(cPe)	Gln	Dab	4.2 (8.38 ± 0.24)	3.0	2.3 ± 0.4[b,c]	5
20	Cha	Gln	Dab	2.1 (8.68 ± 0.22)	2.0	3.1 ± 0.5[c]	5
28	Phe	Asn	Orn	8.2 (8.09 ± 0.15)	3.0	1.4 ± 0.1[b,c]	6
31	Phe	Asn(Me_2)	Orn	1.0 (9.00 ± 0.15)	1.0	1.6 ± 0.1[b,c]	3
34	Phe	Asn($(CH_2)_3OH$)	Orn	2.7 (8.56 ± 0.16)	2.0	1.7 ± 0.3[b,c]	3
38	Phe	Hgn	Orn	0.34 (9.47 ± 0.14)	2.0	3.4 ± 0.3[c]	6
40	Phe	Gln	Orn(iPr)	0.13 (9.90 ± 0.14)	0.2	2.3 ± 0.2[c]	3
41	Phe	Gln	Orn(Me)	0.29 (9.54 ± 0.15)	0.3	2.6 ± 0.5[c]	4
42	Phe	Gln	Orn(Pr)	0.10 (9.98 ± 0.06)	0.3	1.9 ± 0.1[c]	3
43	Phe	Gln	Orn(Et)	0.16 (9.79 ± 0.19)	0.2	2.1 ± 0.2[c]	3
45	Phe	Hgn	Orn(iPr)	0.55 (9.26 ± 0.05)	0.3	1.4 ± 0.1[b]	3
47	Phe	Asn	Dab	2.0 (8.71 ± 0.19)	6.0	2.0 ± 0.2[b,c]	4
49	Phe	Asn(Et)	Dab	4.8 (8.32 ± 0.09)	3.0	1.3 ± 0.1[b]	3
52	Phe	Asn	Orn(Et)	8.0 (8.10 ± 0.40)	6.0	2.4 ± 0.3[c]	3
54	Ala(2-Fur)	Gln	Orn(iPr)	0.62 (9.21 ± 0.15)	1.0	1.5 ± 0.1[b,c]	3
59	Thi	Gln	Orn(iPr)	0.32 (9.49 ± 0.10)	0.6	2.7 ± 0.5[c]	3
68	Cha	Asn	Orn(iPr)	57 (7.24 ± 0.18)	60	4.1 ± 0.8[c]	6

[a] Compounds **1**, **3**, and **4** contain Phe and all other analogues contain Ile in position 3. Compound **2** is an Hmp[1] analogue. See abbreviations list for definitions of unnatural amino acid residues. [b] Significantly different from **2**. [c] Significantly different from **1**.

containing an unsubstituted aromatic or heteroaromatic ring and the diaminobutyric acid (Dab) residue in position 8 as the starting point for a systematic exploration of the effect of the side chain length in this position. The compounds containing nitrogen heterocycles were generally less potent at the $V_{1a}R$ than the Phe[2] analogue **8**, with the loss of potency apparently correlated to the basicity of the aromatic ring. The compound containing an imidazole ring (**9**, Xaa = His) was the least potent in this series, and the compounds with pyridine (**10**) and thiazol (**12**) rings were markedly less active as $V_{1a}R$ agonists than **8**. The introduction of the bulky heterocyclic *β*-(3-benzothiazolyl)alanine in position 2 resulted in an inactive analogue **11**. The Thi[2] (**13**) and the 3-Thi[2] (**14**) compounds were equipotent with **8** as $V_{1a}R$ agonists but showed a better selectivity profile. The replacement of the phenyl ring with the oxygen-containing furan ring resulted in slightly less potent but more selective compound **15**.

Next, we explored the possibility of replacing the aromatic residue in position 2 with an aliphatic one. As in the case of previously reported compound [Ala[2]]AVP,[36] the Ala[2] analogue **16** turned out to be completely inactive (no significant $V_{1a}R$ agonist activity up to 1000 nM). Surprisingly, the compounds with bulkier acyclic side chains **17** (R^1 = neopentyl, EC_{50} = 11 nM) and **18** (R^1 = isobutyl, EC_{50} = 13 nM) were found to be moderately potent as V_{1a} agonists. Analogues **19**−**21** with cyclic aliphatic residues in position 2 were equipotent with the Phe[2] analogue **8**, but their selectivity versus other related receptors (i.e., OTR) was substantially improved. Although AVP analogues with cyclic aliphatic residues in position 2 have been previously reported,[37−39] and a few fully nonaromatic naturally occurring vasopressin/conopressin analogues have been described,[40] compounds **19**−**21** appear to be the first examples of fully nonaromatic $V_{1a}R$ agonists.

Position 4 (Yaa) seems to be essential for tuning the receptor specificity in the family of vasopressin/oxytocin receptors. Compounds with improved V_2 selectivity have been obtained by replacing the Gln[4] residue with hydrophobic amino acids such as Val.[41] $V_{1b}R$ selective compounds have been discovered by replacing the Gln[4] residue in the dAVP molecule with a variety of basic (Lys, Orn) or hydrophobic (Cha, Leu) amino acids.[42−45] Consequently, position 4 of compound **7** was modified with a variety of polar amino acids (analogues **22**−**38**). Shortening the position 4 side chain length resulted in analogue **28** showing similar in vitro profile as **7**, whereas analogue **38** with the longer side chain of homoglutamine, Hgn, exhibited high selectivity versus both hV_2R and hOTR. The N-substitutions on the glutamine primary amide in most cases led to compounds with decreased potency (**23**, **24**, **26**, and **27**). Only the *N*-methyl (**22**) and the *N*-methoxy (**25**) substituents were well tolerated but selectivity versus related receptors was not improved. Interestingly, N-substitutions on the primary amide of an asparagine residue with alkyl, hydroxyalkyl, or hydroxyl groups resulted in a series of relatively potent compounds **29**−**34**, **36**, **37**, with the *N*,*N*-dimethyl analogue **31** and the *N*-3-hydroxypropyl analogue **34** showing remarkable selectivity versus both V_2R and OTR.

The length of the side chain in position 8 has a significant impact on potency and selectivity of $V_{1a}R$ agonists. The diaminopropionic acid Dap[8] compound (**39**) is a substantially less potent $V_{1a}R$ agonist than the Dab (**8**) and Orn (**7**) analogues. Compound **8** is equipotent with but slightly less selective than compound **7**. *N*-Alkylation of the Orn[8] side chain with small alkyl

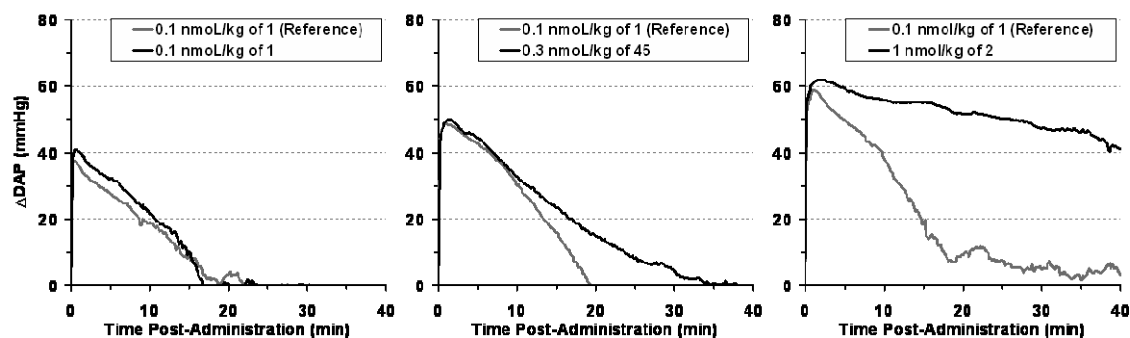


Figure 3. Typical traces of diastolic (DAP) arterial pressure time course (mmHg over baseline DAP) showing the decay of vasopressor effect of analogues **1**, **45**, and **2** after an intravenous bolus injection of equieffective doses to the reference dose of **1**. Traces in a given graph were obtained in the same animal.

groups (analogues **40**−**44**) was well tolerated and resulted in potent $V_{1a}R$ agonists with improved selectivity versus both V_2R and $V_{1b}R$. It should be pointed out that the enhanced V_{1b} selectivity could not be achieved by any other modifications described in this paper.

Analogues **45**−**70** combine two or more of the single modifications in positions 2, 4, and/or 8 employed in compounds **8**−**44**. Although a number of potent/selective $V_{1a}R$ agonists were found in this set (e.g., compounds **45**, **47**, **49**−**52**, **54**, **59**, **68**, **70**), the in vitro data suggests that the effects of the structural changes introduced in compounds **8**−**44** are not fully additive. For example, the combination of aromatic residues containing five-membered rings (Thi, Ala(2-Fur)) in position 2 with the Asn^4 residue (**55**−**58**, **60**−**63**) yielded analogues significantly less potent at the $V_{1a}R$. Nonaromatic residues in position 2 in combination with other modifications (**64**−**67**) led to markedly less potent $V_{1a}R$ agonists with the notable exception of the Cha^2 compounds **68**−**70**.

The analogues with the best in vitro pharmacological profile (potent and $V_{1a}R$-selective) contain the Phe or the Thi residues in position 2, the *N*-alkylated $Asn(R^3)$, the Gln or the Hgn residues in position 4, and/or the Dab, the Orn or the *N*-alkylated $Orn(R^5)$ residues in position 8 (e.g., **31**, **34**, **38**, **45**, **49**, **59**, and **68**). A graphical illustration of in vitro receptor selectivity of compound **45** as compared to **1** is shown in Figure 2. Although maximum efficacy of **45** in the RGA in vitro assay at the $hV_{1a}R$ appears to be slightly lower than that of **1**, we believe the difference is insignificant as the analogue has elicited full agonistic response in various in vitro, ex vivo and in vivo assays.[46]

The analogues showing a favorable in vitro profile at the human receptors were tested in vivo in a rat vasopressor model for their duration of action. Differences in intrinsic potency at the rat $V_{1a}R$ (see Table 4 for in vitro EC_{50} values) and interanimal variation in V1aR agonist responsiveness were compensated for by experimentally selecting the dose of each compound that produced an elevation of diastolic blood pressure equivalent to that of a 0.1 nmol/kg dose (ED_{80}) of **1**. All compounds were effective in raising arterial blood pressure at the equieffective doses but showed considerable differences in the rates of disappearance of the vasopressor effect (Table 4).

The analogues were considered short-acting when the rate of the decay of the vasopressor effect was less than 2 times of that of **1**. For comparison, the relative duration of action of the previously described long-acting[23] analogue **2** used as a reference compound in this assay was 5 times longer than that of **1**. While no obvious SAR rules for the duration of action in vivo could be identified, compounds **20** and **68**, which contain a cyclic aliphatic Cha residue in position 2, had considerably long duration of action. Compounds **31**, **34**, **45**, and **49** displayed the most favorable overall pharmacological profile, and analogue **45** was selected for further evaluation as a potential treatment in critical care medicine. The differences in vasopressive duration of action in vivo for compounds **1**, **2**, and **45** are presented in Figure 3.

■ CONCLUSION

We have designed, synthesized, and pharmacologically characterized a series of vasopressin analogues modified in position 2, 3, 4, and/or 8. We identified compounds with high potency as $V_{1a}R$ agonists, with high selectivity versus related receptors, in particular V_2R, and short duration of action in vivo. Several peptides, such as $[Phe^2,Ile^3,Asn(Me_2)^4,Orn^8]VP$ (**31**), $[Phe^2,Ile^3,Asn((CH_2)_3OH)^4,Orn^8]VP$ (**34**), $[Phe^2,Ile^3,Hgn^4,Orn(iPr)^8]VP$ (**45**), and $[Phe^2,Ile^3,Asn(Et)^4,Dab^8]VP$ (**49**), showed the desired pharmacological profile. Compound **45** (FE 202158)[47] is currently undergoing clinical trials for the treatment of vasodilatory hypotension conditions such as septic shock. The full pharmacological characterization of peptide **45** has just been published.[46]

■ EXPERIMENTAL SECTION

Synthesis. *General.* Amino acid derivatives and resins were purchased from Novabiochem, Bachem Peptide International, and PepTech Corporation. Fmoc-Hgn-OH was synthesized according to the published procedure.[48] Other chemicals and solvents were purchased from Sigma-Aldrich, Fisher Scientific, and VWR.

Mass spectra were recorded on a Finnigan MAT spectrometer. Preparative HPLC was performed on a Waters 2000 liquid chromatograph using a 15 μm PrePak 47 mm × 300 mm cartridge at a flow rate of 100 mL/min. Final purity of analogues was assessed on a 1100 Agilent liquid chromatograph using the following analytical method: column, Vydac C18, 5 μm, 2.1 mm × 250 mm; column temperature −40 °C; flow rate −0.3 mL/min; solvent A, 0.01% TFA; solvent B, 70% CH_3CN, 0.01% TFA; gradient, 0−20% B in 1 min, then 20−40% B in 20 min, then held at 100% B for 5 min; when necessary, the first two segments of the gradient were adjusted for compound lipophilicity; UV detection at 214 nm. The purity of analogues exceeded 95% unless stated otherwise in the Supporting Information.

HPLC retention times (t_R) were determined on a 1200rr Agilent liquid chromatograph using the following analytical method: column,

Zorbax SB-C18, 1.8 μm, 50 mm × 4.6 mm; column temperature −40 °C; flow rate −1.5 mL/min; solvent A, 0.05% TFA; solvent B, 90% CH_3CN, 0.05% TFA; gradient, 10% B in 1 min then 10−50% B in 9 min; UV detection at 214 nm. HPLC capacity factors (k') were calculated using the equation $k' = (t_R - t_0)/t_0$ where t_0 is retention time of the unretained species (actual value was $t_0 = 0.425 \pm 0.002$ min). The purities, t_R, and k' values are reported in the online Supporting Information.

*Synthesis of Peptides with Alkylated Side Chain in Position 8 (**40**−**46**, **50**−**54**, **56**, **59**, **61**, **68**).* The peptides were assembled by Fmoc chemistry. The diamino acid residue in position 8 was introduced with the *N*-δ-Mmt (methoxytrityl) protecting group[31] and the N-terminal Cys residue was coupled as Boc-Cys(Trt)-OH. The resin bound peptide was treated with the DCM/TIS/TFA 93/5/2 (v/v/v) cocktail (2 × 1.5 h) to remove the Mmt group. The *N*-ω-isopropyl analogues (**40**, **45**, **46**, **50**, **54**, **56**, **59**, **61**, and **68**) were synthesized by reductive alkylation of the resin bound peptides with acetone/$NaBH(OAc)_3$ in DCE/TMOF.

To prepare other *N*-alkyl peptides (**41**−**44**, **51**−**53**), the side chain amino group was derivatized with *o*-NBS-Cl (2-nitrobenzenesulfonyl chloride)[32] and the resulting resin bound sulfonamide was alkylated with an appropriate alcohol under Mitsunobu reaction conditions (TPP/DIAD in 1,2-dimethoxyethane).[33] The *o*-NBS group was then removed with 5% potassium thiophenolate in DMF,[32] and finally the peptides were cleaved from the resin.

*Synthesis of Peptides with N-Hydroxy (**27**, **32**) or N-Alkylated Side Chain in Position 4 (**22**−**26**, **29**−**37**, **48**, **49**).* The peptides were assembled by Boc chemistry. The residue in position 4 was introduced in the sequence as Boc-Asp(OFm)-OH or Boc-Glu(OFm)-OH.[34,35] After complete assembly, the side chain protection was removed with 30% piperidine/DMF. The resulting free carboxylic group was converted to the desired amide by coupling with an appropriate amine mediated by PyBOP or BOP/DIPEA. The N-terminal Boc group was removed prior to the HF cleavage.

Cleavage and Purification. The peptides synthesized by Fmoc chemistry were cleaved with the TFA/TIS/H_2O 96/2/2 cocktail and the ones synthesized by Boc chemistry were cleaved with 90% HF/10% anisole. The ring formation was achieved by oxidation of linear peptides dissolved in 10% aqueous TFA with iodine. The peptides were purified by preparative HPLC in triethylammonium phosphate buffers. Finally, the compounds were converted to their acetates by a modification of a published HPLC method.[49] The fractions with purity exceeding 97% were pooled and lyophilized.

Biological Methods. *In Vitro Receptor Assays.* Agonist activity of compounds at the human or rat vasopressin $V_{1a}R$ were determined in a reporter gene assays (RGA) in HEK293 cells by transiently cotransfecting recombinant vasopressin $V_{1a}R$ expression vectors and the reporter plasmid containing a luciferase gene under the control of NFAT response elements (NFAT-luciferase).[50,51] Assays for human vasopressin V_2R were performed in HEK293 cells cotransfected with recombinant human vasopressin V_2R expression vector and the reporter plasmid containing a luciferase gene under the control of cAMP responsive elements.

Assays for V_{1b} receptor activity were performed in a Flp-In-293 cell line (Invitrogen) stably expressing recombinant human vasopressin V_{1b} receptor and transiently transfected with the NFAT-luciferase reporter construct.[50,51] Assays for OT receptor activity were performed in CHO-K1 cells stably expressing human OT receptor and transiently transfected with the NFAT-luciferase reporter construct.[50,51] Two days following transfection, cells were treated with appropriate doses of peptides, incubated at 37 °C for 5 h, lysed in the presence of luciferin, and total luminescence measured. **1** was used as an internal control for the vasopressin V_{1a} and V_{1b} receptors, dDAVP was used as an internal control for the vasopressin V_2 receptor, and carbetocin, a selective OT analogue, was used as an internal control for the OTR assay.

Compounds were tested in at least three independent experiments. Dose−response curves were generated using a one-site four-parameter model from Xlfit (IDBS) and used to estimate EC_{50} and efficacy values. Agonist potency is presented as geometric means in nanomol/L (nM). Selectivity values are given as EC_{50} ratios of the test compound at a receptor vs $V_{1a}R$.

In Vivo Pharmacological Methods. The duration of the vasopressive effect (i.e., the increase in arterial pressure) induced by intravenous bolus administration of test compound was determined and compared to the duration of action of equieffective doses of the short-acting **1** and the long-acting and selective $V_{1a}R$ agonist **2**.[23,24]

Specifically, 91 male Wistar rats (206−340 g) were obtained from Harlan (Indianapolis, IN) for this assay. Upon delivery, rats were housed two per microisolator cage at Ferring Research Institute Animal Care Facility under controlled environmental conditions (12 h/12 h light −dark cycle, lights on at 7 a.m.; 22−25 °C) in a small animal housing cabinet (Scantainer Classic, Scanbur A/S, Karlslunde, Denmark) or in a positive-pressure clean room (BioBubble, BioBubble, Fort Collins, CO) with free access to standard rodent chow (5001 Rodent Diet, PMI Nutrition International, Richmond, IN, or 18% Protein Rodent Diet, product no. 2018, Harlan Teklad, Madison, WI) and acidified tap water (pH 2.5−3.0). Housing conditions were in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. The rats were allowed to acclimate for at least 2 days prior to study use. The in vivo portion of all study protocols was approved by the Institutional Animal Care and Use Committee.

After overnight fasting, rats were anesthetized with isoflurane (2.0−2.5% in oxygen), and anesthesia was maintained with thiobutabarbital sodium (150 mg/kg intraperitoneally, redosed at 50 mg/kg if needed), mechanically ventilated with 36% oxygen compressed air (HSE-HA Multiple-Channel Ventilator, Harvard Apparatus, Holliston, MA), and their body temperature maintained at 37−38 °C using a water circulating heating pad (MUL-T-PAD, Gaymar Industries, Orchard Park, NY). A carotid artery was catheterized to monitor arterial blood pressure (disposable pressure transducer sets "DTX Plus TNF-R, Becton Dickinson Critical Care Systems, Franklin Lakes, NJ), and the opposite jugular vein was catheterized for vehicle and drug administration. Arterial pressure data was continuously acquired with Notocord-hem (Notocord Systems SAS, Croissy sur Seine, France). Prior to agonist administration, rats were pretreated intravenously with the α-adrenergic receptor antagonist dibenamine (1.8 mg/rat administered divided into 7 boluses spread over 30 min) to stabilize arterial blood pressure and enhance vasopressor responsiveness to $V_{1a}R$ agonists.[52−54]

After stable hemodynamic recordings were established (~45 min), animals were administered intravenous boluses at a volume of 0.5 mL/kg of: (i) heparinized vehicle solution (20 unit/mL heparin in 2.5% w/v dextrose and 0.45% w/v NaCl) to assess any nonspecific hemodynamic effects, followed by (ii) **1** (0.1 nmol/kg; ~ED_{80}) to assess vasopressive integrity of the animal preparation, (iii) a second administration of **1**, also at 0.1 nmol/kg, to induce an internal (control) reference response, and (iv) two doses of test compound to identify the equieffective dose to the second administration of the 0.1 nmol/kg dose of **1** in terms of diastolic arterial pressure (DAP) rise. For each compound, these two doses were estimated based on the in vitro potency of the compound at the $rV_{1a}R$, relative to **1** (Table 4). The first potentially equieffective dose was calculated as 0.1 nmol/kg·EC_{50}(Compound)/EC_{50}(**1**). The second dose was set to three times this value. The actual equieffective dose was selected as the one that produced the elevation of diastolic arterial pressure closer to that produced by the 0.1 nmol/kg dose of **1**. In time control rats, the test compound administration was replaced by repeated administration of the 0.1 nmol/kg dose of **1**. An additional control group received an equieffective dose (1 nmol/kg) of the long-acting selective $V_{1a}R$ agonist **2** instead of test compound. Dosing intervals were set as the time required for the DAP to decrease to a stable baseline (~20 min for **1** to ~120 min for **2**). The vehicle solution did not produce any measurable hemodynamic effect in any of the animals.

For each rat, the absolute duration of action of the vasopressor effect of either test compound, **1**, or **2** was calculated as the average of the DAP decay rate, measured over 10 s intervals, from the time of peak DAP increase to the time where 80% of this increase had dissipated. To normalize for interindividual variation in $V_{1a}R$ agonist responsiveness, the relative duration of action of the vasopressor effect for the dose of test compound equieffective to the 0.1 nmol/kg dose of **1** inducing the internal (control) reference response was calculated for each rat as the ratio of the average decay rate for **1** divided by the average decay rate for test compound or **2** such that a ratio greater than 1 is indicating a longer duration of action and a ratio smaller than 1 a shorter duration of action. Results are expressed as mean ± SEM and are presented in Table 4. Statistical comparison was done using a one-way ANOVA after transforming the relative duration of action into its reciprocal to ensure that the assumptions of normal distribution and equal variance were not violated. The significant ANOVA ($P < 0.05$) was followed by all pairwise multiple comparison procedures using the Holm Sidak method (SigmaStat v. 3.11, Aspire Software International, Ashburn, VA).

ASSOCIATED CONTENT

Supporting Information. Detailed synthetic procedures and physicochemical properties of compounds **1**−**7** and **8**−**70**. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

Ac4c, 1-aminocyclobutane-1-carboxylic acid; ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; Ala(BzThi), β-3-benzothienylalanine; Ala(cPe), β-cyclopentylalanine; Ala(2-Fur), β-(2-furyl)alanine; Ala(*t*-Bu), β-(*tert*-butyl)alanine; Ala(Thz), β-4-(1,3-thiazolyl)alanine; Asn($CH(CH_2OH)_2$), N^{γ}-((1,1-dihydroxymethyl)methyl)asparagine; Asn($CH_2CH(OH)CH_3$), N^{γ}-(2-hydroxypropyl)asparagine; Asn($(CH_2)_2O(CH_2)_2OH$), N^{γ}-(5-hydroxy-3-oxapentyl)asparagine; Asn($(CH_2)_2OH$), N^{γ}-(2-hydroxyethyl)asparagine; Asn($CH_2)_3OH$), N^{γ}-(3-hydroxypropyl) asparagine; Asn(Et), N^{γ}-ethylasparagine; Asn(Me), N^{γ}-methylasparagine; Asn(Me_2), N^{γ},N^{γ}-dimethylasparagine; Asn(OH), N^{γ}-hydroxyasparagine; AVP, 8-arginine vasopressin; BOP, benzotriazol-1-yloxy trisdimethylaminophosphonium hexafluorophosphate; Cha, β-cyclohexylalanine; Dab, 2,4-diaminobutyric acid; Dap, 2,3-diaminopropionic acid; DAP, diastolic arterial pressure; dAVP, desamino-8-arginine vasopressin; DCE, 1,2-dichloroethane; dDAVP, desamino-8-D-arginine vasopressin; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DIPEA, *N*,*N*-diisopropyl-*N*-ethylamine; Gln(Et), N^{δ}-ethylglutamine; Gln(iBu), N^{δ}-isobutylglutamine (N^{δ}-(2-methylpropyl)glutamine); Gln(Me), N^{δ}-methylglutamine; Gln(Me,OMe), N^{δ}-methoxy-N^{δ}-methylglutamine; Gln(OMe), N^{δ}-methoxyglutamine; Gln(OH), N^{δ}-hydroxyglutamine; HF, hydrogen fluoride; Hgn, homoglutamine; LVP, 8-lysine vasopressin; Mmt, methoxytrityl; MPE, maximum possible effect; Orn(iPr), N^{δ}-isopropylornithine; Orn(Me), N^{δ}-methylornithine; Orn(Pr), N^{δ}-propylornithine; Orn(Et), N^{δ}-ethylonithine; Orn(iAm), N^{δ}-isoamylornithine (N^{δ}-(3-methylbutyl)-ornithine); OT, oxytocin; OTR, oxytocin receptor; OVP, 8-ornithine vasopressin; 2-Pal, β-(2-pyridyl)alanine; 3-Pal, β-(3-pyridyl)alanine; 4-Pal, β-(4-pyridyl)alanine; PyBOP, benzotriazol-1-yloxy trispyrrolidinephosphonium hexafluorophosphate; RGA, reporter gene assay; TFA, trifluoroacetic acid; Thi, β-(2-thienyl)alanine; 3-Thi, β-(3-thienyl)alanine; TIS, triisopropylsilane; TMOF, trimethyl orthoformate; TPP, triphenylphosphine; $V_{1a}R$, vasopressin V_{1a} receptor; VP, vasopressin; $V_{1b}R$ (V_3R), vasopressin V_{1b} receptor; V_2R, vasopressin V_2 receptor; Xaa, any amino acid residue in position 2; Yaa, any amino acid residue in position 4; Zaa, any amino acid residue in position 8

REFERENCES

(1) Barberis, C.; Mouillac, B.; Durroux, T. Structural Bases of Vasopressin/Oxytocin Receptor Function. *J. Endocrinol.* **1998**, *156*, 223–229.
(2) Caldwell, H. K.; Young, W. S., III. Oxytocin and Vasopressin: Genetics and Behavioral Implications. In *Handbook of Neurochemistry and Molecular Neurobiology: Neuroactive Proteins and Peptides* (3rd ed.; Lim, R., Lajtha, A., Eds.; Springer: New York, 2006; pp 573−607.
(3) Jard, S. Vasopressin Receptors: A Historical Survey. *Adv. Exp. Med. Biol.* **1998**, *449*, 1–13.
(4) Ring, R. H. The Central Vasopressinergic System: Examining the Opportunities for Psychiatric Drug Development. *Curr. Pharm. Des.* **2005**, *11*, 205–225.
(5) Oshikawa, S.; Tanoue, A.; Koshimizu, T.; Kitagawa, Y.; Tsujimoto, G. Vasopressin Stimulates Insulin Release from Islet Cells through V_{1b} Receptors: A Combined Pharmacological/Knockout Approach. *Mol. Pharmacol.* **2004**, *65*, 623–629.
(6) Howl, J.; Wheatley, M. Molecular Pharmacology of V_{1a} Vasopressin Receptors. *Gen. Pharmacol.* **1995**, *26*, 1143–1152.
(7) Oliver, J. A.; Landry, D. W. Endogenous and Exogenous Vasopressin in Shock. *Curr. Opin. Crit. Care* **2007**, *13*, 376–382.
(8) Landry, D. W.; Levin, H. R.; Gallant, E. M.; Ashton, R. C., Jr.; Seo, S.; D'Alessandro, D.; Oz, M. C.; Oliver, J. A. Vasopressin Deficiency Contributes to the Vasodilation of Septic Shock. *Circulation* **1997**, *95*, 1122–1125.
(9) Holmes, C. L.; Walley, K. R. Arginine Vasopressin in the Treatment of Vasodilatory Septic Shock. *Best Pract. Res. Clin. Anaesthesiol.* **2008**, *22*, 275–286.
(10) Russell, J. A.; Walley, K. R.; Singer, J.; Gordon, A. C.; Hebert, P. C.; Cooper, D. J.; Holmes, C. L.; Mehta, S.; Granton, J. T.; Storms, M. M.; Cook, D. J.; Presneill, J. J.; Ayers, D. Vasopressin Versus Norepinephrine Infusion in Patients with Septic Shock. *N. Engl. J. Med.* **2008**, *358*, 877–887.
(11) Halimi, C.; Bonnard, P.; Bernard, B.; Mathurin, P.; Mofredj, A.; di Martino, V.; Demontis, R.; Henry-Biabaud, E.; Fievet, P.; Opolon, P.; Poynard, T.; Cadranel, J. F. Effect of Terlipressin (Glypressin) on Hepatorenal Syndrome in Cirrhotic Patients: Results of a Multicentre Pilot Study. *Eur. J. Gastroenterol. Hepatol.* **2002**, *14*, 153–158.
(12) O'Brien, A.; Clapp, L.; Singer, M. Terlipressin for Norepinephrine-Resistant Septic Shock. *Lancet* **2002**, *359*, 1209–1210.
(13) Delmas, A.; Leone, M.; Rousseau, S.; Albanese, J.; Martin, C. Clinical Review: Vasopressin and Terlipressin in Septic Shock Patients. *Crit. Care* **2005**, *9*, 212–222.
(14) Pesaturo, A. B.; Jennings, H. R.; Voils, S. A. Terlipressin: Vasopressin Analog and Novel Drug for Septic Shock. *Ann. Pharmacother.* **2006**, *40*, 2170–2177.
(15) Kaufmann, J. E.; Vischer, U. M. Cellular Mechanisms of the Hemostatic Effects of Desmopressin (DDAVP). *J. Thromb. Haemost.* **2003**, *1*, 682–689.
(16) Wiśniewski, K.; Alagarsamy, S.; Taki, H.; Miampamba, M.; Laporte, R.; Gaylean, R.; Croston, G.; Schteingart, C. D.; Riviere, P. J.-M.;

Trojnar, J. Synthesis and Biological Activity of Terlipressin and Its Putative Metabolites. In *Understanding Biology Using Peptides, Proceedings of the19th American Peptide Symposium*; Blondelle, S. E., Ed.; Springer: New York, 2005; pp 489−490.

(17) Freidinger, R. M.; Pettibone, D. J. Small Molecule Ligands for Oxytocin and Vasopressin Receptors. *Med. Res. Rev.* **1997**, *17*, 1–16.

(18) Serradeil-Le Gal, C.; Wagnon, J.; Valette, G.; Garcia, G.; Pascal, M.; Maffrand, J. P.; Le Fur, G. Nonpeptide Vasopressin Receptor Antagonists: Development of Selective and Orally Active V_{1a}, V_2 and V_{1b} Receptor Ligands. *Prog. Brain Res.* **2002**, *139*, 197–210.

(19) du Vigneaud, V.; Gish, D. T.; Katsoyannis, P. G. Synthetic Preparation Possessing Biological Properties Associated with Arginine Vasopressin. *J. Am. Chem. Soc.* **1954**, *76*, 4751–4752.

(20) Manning, M.; Przybylski, J. P.; Olma, A.; Klis, W. A.; Kruszynski, M.; Wo, N. C.; Pelton, G. H.; Sawyer, W. H. No Requirement of Cyclic Conformation of Antagonists in Binding to Vasopressin Receptors. *Nature (London)* **1987**, *329*, 839–840.

(21) Manning, M.; Kołodziejczyk, A. S.; Kołodziejczyk, A. M.; Stoev, S.; Klis, W. A.; Wo, N. C; Sawyer, W. H. Highly Potent and Selective Tyr-NH_2^9-Containing Linear V_1 Antagonists and D-Tyr^2-Containing Linear V_2 Agonists: Potential Radioiodinated Ligands for Vasopressin Receptors. In *Peptides 1990, Proceedings of the 21st European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM, Leiden, The Netherlands, 1991; pp 665−667.

(22) Huguenin, R. L. Synthesis of Phe^2-Orn^8-Vasopressin and of Phe^2-Orn^8-Oxytocin, Two Analogs of Vasopressin with Selective Pressor Activity. *Helv. Chim. Acta* **1964**, *47*, 1934–1941.

(23) Aurell, C. J.; Bengtsson, B.; Ekholm, K.; Kasprzykowska, R.; Nilsson, A.; Persson, R.; Trojnar, J.; Abbe, M.; Melin, P. Development of Vasopressor Specific Vasotocin Analogs with Prolonged Effects. In *Peptides 1990, Proceedings of the 21st European Peptide Symposium*; Giralt, E., Andreu, D., Eds.; ESCOM, Leiden, The Netherlands, 1991; pp 671−673.

(24) Andres, M.; Trueba, M.; Guillon, G. Pharmacological Characterization of F-180: A Selective Human V_{1a} Vasopressin Receptor Agonist of High Affinity. *Br. J. Pharmacol.* **2002**, *135*, 1828–1836.

(25) Favre, N.; Fanelli, F.; Missotten, M.; Nichols, A.; Wilson, J.; di Tiani, M.; Rommel, C.; Scheer, A. The Dry Motif as a Molecular Switch of the Human Oxytocin Receptor. *Biochemistry* **2005**, *44*, 9990–10008.

(26) Wootten, D. L.; Simms, J.; Massoura, A. J.; Trim, J. E.; Wheatley, M. Agonist-Specific Requirement for a Glutamate in Transmembrane Helix 1 of the Oxytocin Receptor. *Mol. Cell. Endocrinol.* **2011**, *333*, 20–27.

(27) Huguenin, R. L.; Boissonnas, R. A. Syntheses of Phe^2-Arginine-Vasopressin and of Phe^2-Arginine-Vasotocin and New Syntheses of Arginine-Vasopressin and Arginine-Vasotocin. *Helv. Chim. Acta* **1962**, *45*, 1629–1643.

(28) Huguenin, R. L.; Boissonnas, R. A. Synthesis of Orn^8-Vasopressin and Orn^8-Oxytocin. *Helv. Chim. Acta* **1963**, *46*, 1669–1676.

(29) Zaoral, M.; Kolc, J.; Sorm, F. Amino Acids and Peptides. LXXI. Synthesis of 1-Deamino-8-D-G-Aminobutyrine Vasopressin, 1-Deamino-8-D-Lysine Vasopressin, and 1-Deamino-8-D-Arginine Vasopressin. *Collect. Czech. Chem. Commun.* **1967**, *32*, 1250–1257.

(30) Saito, M.; Tahara, A.; Sugimoto, T. 1-Desamino-8-D-Arginine Vasopressin (DDAVP) as an Agonist on V_{1b} Vasopressin Receptor. *Biochem. Pharmacol.* **1997**, *53*, 1711–1717.

(31) Barlos, K.; Gatos, D.; Chatzi, O.; Koutsogianni, S.; Schaefer, W. Solid Phase Synthesis Using Trityl Type Side Chain Protecting Groups. In *Peptides 1992, Proceedings of the 22nd European Peptide Symposium*; Schneider, C. H., Eberle, A. N., Eds.; ESCOM, Leiden, The Netherlands, 1993; pp 283−284.

(32) Fukuyama, T.; Jow, C.-K.; Cheung, M. 2- and 4-Nitrobenzenesulfonamides: Exceptionally Versatile Means for Preparation of Secondary Amines and Protection of Amines. *Tetrahedron Lett.* **1995**, *36*, 6373–6374.

(33) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1–28.

(34) Al-Obeidi, F.; Sanderson, D. G.; Hruby, V. J. Synthesis of β- or γ-Fluorenylmethyl Esters of Respectively N^{α}-Boc-L-Aspartic Acid and N^{α}-Boc-L-Glutamic Acid. *Int. J. Pept. Protein Res.* **1990**, *35*, 215–218.

(35) Bolin, D. R.; Wang, C. T.; Felix, A. M. Preparation of *N-tert*-Butyloxycarbonyl-*O*-ω-9-Fluorenylmethyl Esters of Aspartic and Glutamic Acids. *Org. Prep. Proced. Int.* **1989**, *21*, 67–74.

(36) Konieczna, E.; Czaja, M.; Kasprzykowska, R.; Kozlowski, A.; Dzieciol, K.; Trzeciak, H. I.; Lammek, B. Synthesis and Biological Activity of Arginine-Vasopressin Analogs Modified with L-Alanine or L-Proline. *Pol. J. Chem.* **1994**, *68*, 1535–1543.

(37) Jastrzebska, B.; Derdowska, I.; Kowalczyk, W.; Machova, A.; Slaninova, J.; Lammek, B. Influence of 1-Aminocyclohexane-1-Carboxylic Acid in Position 2 or 3 of AVP and Its Analogues on Their Pharmacological Properties. *J. Pept. Res.* **2003**, *62*, 70–77.

(38) Kowalczyk, W.; Prahl, A.; Dawidowska, O.; Derdowska, I.; Sobolewski, D.; Hartrodt, B.; Neubert, K.; Slaninova, J.; Lammek, B. The Influence of 1-Aminocyclopentane-1-Carboxylic Acid at Position 2 or 3 of AVP and Its Analogues on Their Pharmacological Properties. *J. Pept. Sci.* **2005**, *11*, 584–588.

(39) Kowalczyk, W.; Prahl, A.; Derdowska, I.; Dawidowska, O.; Slaninova, J.; Lammek, B. Highly Potent 1-Aminocyclohexane-1-Carboxylic Acid Substituted V_2 Agonists of Arginine Vasopressin. *J. Med. Chem.* **2004**, *47*, 6020–6024.

(40) Kruszynski, M.; Manning, M.; Wo, N. C.; Sawyer, W. H. Invertebrate Neuropeptides Resembling Vasotocin and Some Analogs: Synthesis and Pharmacological Properties. *Experientia* **1990**, *46*, 771–773.

(41) Sawyer, W. H.; Acosta, M.; Balaspiri, L.; Judd, J.; Manning, M. Structural Changes in Arginine Vasopressin Molecule That Enhance Antidiuretic Activity and Specificity. *Endocrinology* **1974**, *9*, 1106–1115.

(42) Cheng, L. L.; Stoev, S.; Manning, M.; Derick, S.; Pena, A.; Ben Mimoun, M.; Guillon, G. Design of Potent and Selective Agonists for the Human Vasopressin V1b Receptor Based on Modifications of [Deamino-Cys^1]Arginine Vasopressin at Position 4. *J. Med. Chem.* **2004**, *47*, 2375–2388.

(43) Guillon, G.; Pena, A.; Murat, B.; Derick, S.; Trueba, M.; Ventura, M. A.; Szeto, H. H.; Wo, N.; Stoev, S.; Cheng, L. L.; Manning, M. Position 4 Analogues of [Deamino-Cys^1] Arginine Vasopressin Exhibit Striking Species Differences for Human and Rat V_2/V_{1b} Receptor Selectivity. *J. Pept. Sci.* **2006**, *12*, 190–198.

(44) Pena, A.; Murat, B.; Trueba, M.; Ventura, M. A.; Bertrand, G.; Cheng, L. L.; Stoev, S.; Szeto, H. H.; Wo, N.; Brossard, G.; Serradeil-Le Gal, C.; Manning, M.; Guillon, G. Pharmacological and Physiological Characterization of D[Leu^4, Lys^8]Vasopressin, the First V_{1b}-Selective Agonist for Rat Vasopressin/Oxytocin Receptors. *Endocrinology* **2007**, *148*, 4136–4146.

(45) Pena, A.; Murat, B.; Trueba, M.; Ventura, M. A.; Wo, N. C.; Szeto, H. H.; Cheng, L. L.; Stoev, S.; Guillon, G.; Manning, M. Design and Synthesis of the First Selective Agonists for the Rat Vasopressin V_{1b} Receptor: Based on Modifications of Deamino-[Cys^1]Arginine Vasopressin at Positions 4 and 8. *J. Med. Chem.* **2007**, *50*, 835–847.

(46) Laporte, R.; Kohan, A.; Heitzmann, J.; Wiśniewska, H.; Toy, J.; La, E.; Tariga, H.; Alagarsamy, S.; Ly, B.; Dykert, J.; Qi, S.; Wiśniewski, K.; Galyean, R.; Croston, G.; Schteingart, C. D.; Riviere, P. J-M. Pharmacological Characterization of FE 202158, a Novel, Potent, Selective, and Short-Acting Peptidic Vasopressin V_{1a} Receptor Full Agonist for the Treatment of Vasodilatory Hypotension. *J. Pharmacol. Exp. Ther.* **2011**, *337*, 786–796.

(47) Lovgren, U.; Johansson, S.; Jensen, L. S.; Ekstrom, C.; Carlshaf, A. Quantitative Determination of Peptide Drug in Human Plasma Samples at Low pg/mL Levels Using Coupled Column Liquid Chromatography-Tandem Mass Spectrometry. *J. Pharm. Biomed. Anal.* **2010**, *53*, 537–545.

(48) Wiśniewski, K.; Kołodziejczyk, A. S. The Efficient Synthesis of Fmoc-L-Homoglutamine. *Org. Prep. Proced. Int.* **1997**, *29*, 338–340.

(49) Hoeger, C.; Galyean, R.; Boublik, J.; McClintock, R.; Rivier, J. Preparative Reversed Phase High Performance Liquid Chromatography: Effects of Buffer pH on the Purification of Synthetic Peptides. *BioChromatography* **1987**, *2*, 134–142.

(50) Boss, V.; Talpade, D. J.; Murphy, T. J. Induction of NFAT-Mediated Transcription by Gq-Coupled Receptors in Lymphoid and Non-Lymphoid Cells. *J. Biol. Chem.* **1996**, *271*, 10429–10432.

(51) Fitzgerald, L. R.; Mannan, I. J.; Dytko, G. M.; Wu, H. L.; Nambi, P. Measurement of Responses from Gi-, Gs-, or Gq-Coupled Receptors by a Multiple Response Element/Camp Response Element-Directed Reporter Assay. *Anal. Biochem.* **1999**, *275*, 54–61.

(52) Dekanski, J. The Quantitative Assay of Vasopressin. *Br. J. Pharmacol. Chemother.* **1952**, *7*, 507–572.

(53) Erker, E. F.; Chan, W. Y. The Site and the Mechanism of Phenoxy-Benzamine Potentiation of the Pressor Response to Oxytocin and Vasopressin: In Vivo and Isolated Aortic Strips Studies. *J. Pharmacol. Exp. Ther.* **1977**, *202*, 287–293.

(54) Hoffman, W. E. Regional Vascular Effects of Antidiuretic Hormone in Normal and Sympathetic Blocked Rats. *Endocrinology* **1980**, *107*, 334–341.