Arginine-Vasopressin Analogues with High Antidiuretic/Vasopressor Selectivity. Synthesis, Biological Activity, and Receptor Binding Affinity of Arginine-Vasopressin Analogues with Substitutions in Positions 1, 2, 4, 7, and 8[1]

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In a search for more selective agonists of arginine-vasopressin (AVP), 10 analogues of [Sar[7]]- and [MeLa[7]]AVP with additional substitutions in positions 1 (β-mercaptopropionic acid), 2 (phenylalanine), 4 (valine), or 8 (D-arginine) were synthesized and tested for antidiuretic and vasopressor activities. All analogues are characterized by a relatively high antidiuretic activity and by a sharp decrease in pressor activity. Their antidiuretic/vasopressor (A/P) selectivities were 2–3 orders higher (except for peptides 2 and 3) than that of the parent hormone. The additivity of the effects of changes in positions 1, 2, 4, and 8 combined with the sarcosine or *N*-methylalanine substitutions in position 7 on the biological activity is observed. Binding affinities of AVP analogues to plasma membranes from bovine kidney inner medulla and from rat liver containing specific vasopressin receptors were also determined. Generally, these analogues retained high binding affinities to renal vasopressin receptors, and on the other hand they are characterized by a large decrease in binding affinities to hepatic vasopressin receptors, which share characteristics with vasopressor receptors.

[Sar[7]]- and [MeAla[7]]arginine-vasopressins were found to be highly potent and specific antidiuretic agents.[2] With the exception of D-arginine-vasopressin,[3] these [Sar[7]]- and [MeAla[7]]vasopressins have the highest antidiuretic/pressor (A/P) selectivity observed yet in singly substituted arginine-vasopressin analogues. It was of interest therefore to determine the effects of some other substitutions that are known to bring about enhancement of antidiuretic/pressor selectivity: β-mercaptopropionic acid in position 1,[4] phenylalanine in position 2,[5] valine in position 4,[6,7] and D-arginine in position 8,[3] in combination with Sar[7] or MeAla[7] substitutions. As was shown by us,[8] [Sar[7]]- and [MeAla[7]]vasopressins retained also high binding affinities to the renal vasopressin receptors (V_2), and on the other hand they have drastically reduced binding affinities to hepatic vasopressin receptors (V_1).[9] Thus, the antidiuretic and pressor activities or [Sar[7]]- and [MeAla[7]]arginine-vasopressins correlate quite well with their binding parameters to the corresponding membrane receptors.

The 10 new analogues presented in this paper are as follows: **1**, [2-phenylalanine,7-sarcosine]arginine-vasopressin [Phe[2],Sar[7]]AVP; **2**, [2-phenylalanine,7-*N*-methylalanine]arginine-vasopressin [Phe[2],MeAla[7]]AVP; **3**, [4-valine,7-sarcosine]arginine-vasopressin [Val[4],Sar]AVP; **4**, [1-β-mercaptopropionic acid, 4-valine,7-sarcosine]arginine-vasopressin [Mpa[1],Val[4],Sar[7]]AVP; **5**, [4-valine,7-*N*-methylalanine]arginine-vasopressin [Val[4],MeAla[7]]AVP; **6**, [1-β-mercaptopropionic acid,4-valine,7-*N*-methylalanine]arginine-vasopressin [Mpa[1],Val[4],MeAla[7]]AVP; **7**, [7-sarcosine,8-D-arginine]vasopressin [Sar[7],D-Arg[8]]VP; **8**, [1-β-mercaptopropionic acid,7-sarcosine,8-D-arginine]vasopressin [Mpa[1],Sar[7],D-Arg[8]]VP; **9**, [7-*N*-methylalanine-,8-D-arginine]vasopressin [MeAla[7],D-Arg[8]]VP; **10**, [1-β-mercaptopropionic acid,7-*N*-methylalanine,8-D-arginine]-vasopressin [Mpa[1],MeAla[7],D-Arg[8]]VP.

Peptide Synthesis. All analogues were obtained by solid-phase peptide synthesis[10–12] as described previously.[2] In general, the *tert*-butyloxycarbonyl group[13–15] was used for the protection of the amino groups and was removed by treatment with 1.3 N HCl/AcOH. Coupling was affected with dicyclohexylcarbodiimide[16] or by *p*-nitrophenyl esters[17] for the asparaginyl and glutaminyl residues. The protected peptides were split from the resin by ammonolysis.[12] They were deblocked with sodium in liquid ammonia,[18,19] and the resulting disulfhydryl derivatives were

Table I. Antidiuretic and Vasopressor Potencies of Arginine-Vasopressin (AVP) Analogues Modified in Positions 1, 2, 4, 7, and 8

no.	peptide[a]	antidiuretic potency (A), IU/mg	vasopressor potency (P), IU/mg	A/P
	AVP	323[b]	369[b]	0.9
	[Sar[7]]AVP	188 ± 19[c]	3.6 ± 0.2	52
	[MeAla[7]]AVP	343 ± 54	10.6 ± 0.4	32
1	[Phe[2],Sar[7]]AVP	39.6 ± 2.3	0.17 ± 0.05	230
2	[Phe[2],MeAla[7]]AVP	14.5 ± 1	0.57 ± 0.04	25
3	[Val[4],Sar[7]]AVP	270 ± 29	34.7 ± 9	8
4	[Mpa[1],Val[4],Sar[7]]AVP	552 ± 35	0.4	1380
5	[Val[4],MeAla[7]]AVP	88.3 ± 11	0.08 ± 0.02	1090
6	[Mpa[1],Val[4],MeAla[7]]AVP	103 ± 28	0.69	150
7	[Sar[7],D-Arg[8]]VP	56 ± 14	0.04	1400
8	[Mpa[1],Sar[7],D-Arg[8]]VP	214.5 ± 18	0.01	2145
9	[MeAla[7],D-Arg[8]]VP	69 ± 3	0.11 ± 0.01	630
10	[Mpa[1],MeAla[7],D-Arg[8]]-VP	368.5 ± 64	0.07 ± 0.01	5260

[a] The symbols Mpa and MeAla are used to indicate the β-mercaptopropionic acid and *N*-methylalanine, respectively. All other symbols follow the recommendations of IUPAC-IUB Joint Commission on Biochemical Nomenclature, 1983: *Eur. J. Biochem.* 1984, *138*, 9. [b] From Manning et al.[21] [c] From Grzonka et al.[2]

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Table II. Parameters of Binding of AVP Analogues of Bovine Kidney Membranes[a]

no.	peptide[b]	b	K_D, M	[^{3}H]AVP
	[^{3}H]AVP	1.09 ± 0.15	(1.1 ± 0.1) × 10^{-9}	
1	[Phe^2,Sar^7]AVP	1.01 ± 0.12	(4.4 ± 0.7) × 10^{-8}	40
2	[Phe^2,MeAla^7]AVP	0.75 ± 0.07	(1.1 ± 0.1) × 10^{-7}	100
3	[Val^4,Sar^7]AVP	1.05 ± 0.17	(9.4 ± 0.2) × 10^{-9}	8.5
4	[Mpa^1,Val^4,Sar^7]AVP	1.05 ± 0.09	(1.4 ± 0.2) × 10^{-9}	1.3
5	[Val^4,MeAla^7]AVP	0.74 ± 0.07	(3.3 ± 0.4) × 10^{-8}	30
6	[Mpa^1,Val^4,MeAla^7]AVP	0.65 ± 0.09	(9.4 ± 1.3) × 10^{-9}	8.5
7	[Sar^7,D-Arg^8]VP	0.67 ± 0.12	(1.0 ± 0.2) × 10^{-8}	9.1
8	[Mpa^1,Sar^7,D-Arg^8]VP	0.83 ± 0.09	(8.7 ± 1.2) × 10^{-9}	7.9
9	[MeAla^7,D-Arg^8]VP	0.76 ± 0.06	(2.1 ± 0.3) × 10^{-8}	19
10	[Mpa^1,MeAla^7,D-Arg^8]VP	0.80 ± 0.19	(5.1 ± 0.9) × 10^{-8}	46

[a] The apparent dissociation constants K_D and the slope factors b of the displacement curves were determined from competition experiments by using a weighted nonlinear least-squares fit to logistic curves as described (Fahrenholz et al.[8]). Parameters for [^{3}H]AVP were obtained from direct binding measurements. Values are the mean ±SE of the two experiments. [b] see corresponding footnote in Table I.

Table III. Parameters of Binding to Rat Liver Membranes[a]

no.	peptide	b	K_D, M for [^{3}H]AVP
	[^{3}H]AVP	1.28 ± 0.13	(6.5 ± 0.3) × 10^{-10}
1	[Phe^2,Sar^7]AVP	1.07 ± 0.19	(1.7 ± 0.3) × 10^{-7} 260
2	[Phe^2,MeAla^7]AVP	0.95 ± 0.15	(1.1 ± 0.2) × 10^{-7} 170
3	[Val^4,Sar^7]AVP	1.09 ± 0.07	(1.1 ± 0.1) × 10^{-7} 170
4	[Mpa^1,Val^4,Sar^7]AVP	1.04 ± 0.20	(2.7 ± 0.4) × 10^{-8} 42
5	[Val^4,MeAla^7]AVP	1.03 ± 0.17	(3.1 ± 0.5) × 10^{-7} 480
6	[Mpa^1,Val^4,MeAla^7]AVP	0.93 ± 0.12	(5.3 ± 0.7) × 10^{-8} 82
7	[Sar^7,D-Arg^8]VP	1.01 ± 0.41	(4.2 ± 1.1) × 10^{-7} 650
8	[Mpa^1,Sar^7,D-Arg^8]VP	1.16 ± 0.05	(1.6 ± 0.2) × 10^{-7} 240
9	[MeAla^7,D-Arg^8]VP	1.20 ± 0.26	(2.8 ± 0.5) × 10^{-7} 440
10	[Mpa^1,MeAla^7,D-Arg^8]VP	0.85 ± 0.20	(2.9 ± 0.5) × 10^{-7} 450

[a] See corresponding footnotes in Tables I and II.

Table IV. Physicochemical Properties of the Protected 9-Peptides: A-B-Phe-C-Asn-Cys(Bzl)-D-E-Gly-NH_2

	substitutions								R_f		
no.	A	B	C	D	E	yield, %	mp, °C	$[\alpha]^{20}_D$, deg	BAW	BAWP	formula
I	Z-Cys(Bzl)	Phe	Gln	Sar	Arg(Tos)	80.1	203–207	−20.3[a]	0.53	0.66	$C_{73}H_{87}N_{15}O_{15}S_3$
II	Z-Cys(Bzl)	Phe	Gln	MeAla	Arg(Tos)	74.9	195–196	−34.4[a]	0.54	0.67	$C_{74}H_{89}N_{15}O_{15}S_3$
III	Z-Cys(Bzl)	Tyr(Bzl)	Val	Sar	Arg(Tos)	59.6	214–216	−24.0[b]	0.65	0.74	$C_{80}H_{96}N_{14}O_{15}S_3$
IV	Mpa(Bzl)	Tyr(Bzl)	Val	Sar	Arg(Tos)	55.5	210–213	−21.3[a]	0.68	0.74	$C_{72}H_{89}N_{13}O_{13}S_3$
V	Z-Cys(Bzl)	Tyr(Bzl)	Val	MeAla	Arg(Tos)	67.9	215–218	−23.6[a]	0.73	0.74	$C_{81}H_{98}N_{14}O_{15}S_3$
VI	Mpa(Bzl)	Tyr(Bzl)	Val	MeAla	Arg(Tos)	67.3	199–203	−5.2[a]	0.84	0.84	$C_{73}H_{91}N_{13}O_{13}S_3$
VII	Z-Cys(Bzl)	Tyr(Bzl)	Gln	Sar	D-Arg(Tos)	85.8	203–205	−19.3[a]	0.65	0.76	$C_{80}H_{95}N_{15}O_{16}S_3$
VIII	Mpa(Bzl)	Tyr(Bzl)	Gln	Sar	D-Arg(Tos)	78.0	187–190	−7.7[a]	0.68	0.79	$C_{72}H_{88}N_{14}O_{14}S_3$
IX	Z-Cys(Bzl)	Tyr(Bzl)	Gln	MeAla	D-Arg(Tos)	84.3	192–194	−30.9[a]	0.70	0.79	$C_{81}H_{97}N_{15}O_{16}S_3$
X	Mpa(Bzl)	Tyr(Bzl)	Gln	MeAla	D-Arg(Tos)	81.9	188–190	−21.4[a]	0.72	0.81	$C_{73}H_{90}N_{14}O_{14}S_3$

[a] c = 1, DMF. [b] c = 0.5, DMF.

subjected to oxidative cyclization with $K_3Fe(CN)_6$.[20] The vasopressin analogues were purified by gel filtration on Sephadex G-15.[21]

Bioassays. Antidiuretic activity was assessed in hydrated anaesthetized rats by intravenous injection as described by Larsson et al.[22] The change in urine conductivity was taken as the measure of the effect. Arginine-vasopressin was used as the standard. Vasopressor assays were performed on rats treated with phenoxybenzamine as described by Dekanski.[23]

Receptor Binding Assays. Binding parameters of arginine-vasopressin analogues to plasma membranes from bovine kidney inner medulla[24] and from rat liver[8] were determined by using a weighted nonlinear least-squares fit to logistic curves as described.[8] The incubation time was 30 min. For the assays of binding to liver membranes, bacitracin (1 mg/mL) was included in the standard medium.

Results and Discussion

The antidiuretic (A) and vasopressor (P) potencies of analogues 1–10 together with their A/P ratios are shown

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in Table I. All the new analogues are characterized by a sharp decrease in pressor activity, and on the other hand they retain high antidiuretic activity. Thus, they possess selective A/P properties, and they could become useful pharmacological tools.[25] The binding parameters of analogues 1–10 to bovine kidney and rat liver membranes are presented in Tables II and III, respectively. Comparison of the binding affinities shows clearly that these analogues are bound much more strongly to the antidiuretic receptors in bovine kidney membranes than to the vasopressin receptors in rat liver membranes.

Effects of Phe^2 on Pharmacological Potencies of [Sar^7]AVP and [MeAla^7]AVP. The replacement of the Tyr residue in position 2 by a Phe residue in combination with Sar or MeAla substitutions in position 7 results in decreases of both antidiuretic and vasopressor activities, relative to [Sar^7]AVP and [MeAla^7]AVP, respectively. However, due to the higher diminution of pressor activity, the A/P selectivity of [Phe^2,Sar^7]AVP is 4 times that or [Sar^7]AVP. The A/P selectivity of [Phe^2,MeAla^7]AVP is roughly similar to that of [MeAla^7]AVP. The difference in selectivities of analogues 1 and 2 is confirmed by the difference in binding affinities of both compounds to the antidiuretic and hepatic vasopressin receptors.

Effects of Val^4 on Pharmacological Potencies of [Sar^7]AVP and [MeAla^7]AVP. Replacement of the Gln residue in position 4 by a Val residue in [Sar^7]AVP enhances the antidiuretic activity. The combination of deamination in position 1 (Cys replaced by Mpa), Val in position 4, and Sar in position 7 led to the compound having the highest antidiuretic activity among analogues presented in Table I. The analogue [Mpa^1,Val^4,Sar^7]AVP also has a very high A/P selectivity ratio. On the contrary, when deamination in position 1 is combined with Val and MeAla substitutions in positions 4 and 7, respectively, to give [Mpa^1,Val^4,MeAla^7]AVP, no such additivity in antidiuretic response is observed. It can be pointed out from the data presented in Tables II and III that the Sar-containing analogues 3 and 4 retain higher binding affinities to the antidiuretic and vasopressor receptors than the MeAla-containing analogues 5 and 6. The binding affinity is increased when the amino group in position 1 is replaced by hydrogen. [Mpa^1,Val^4,Sar^7]AVP (4) has a very high affinity for antidiuretic receptor, close to that of AVP.

Effects of D-Arg^8 on Pharmacological Potencies of [Sar^7]AVP and [MeAla^7]AVP. The replacement of the L-Arg residue in position 8 by its enantiomer gave an expected high increase in A/P selectivity, mainly because of a dramatic drop in vasopressor potency (Table I, analogues 7 and 9). The combination of D-Arg^8 substitution with deamination in position 1 was very effective in further enhancing A/P selectivity. Thus, both analogues 8 and 10 are very selective antidiuretic/vasopressor compounds with A/P selectivity comparable to that of [Mpa^1,D-Arg^8]AVP[26,27] (dDAVP or desmopressin), which is used as the drug of choice for treatment of diabetes insipidus. Only some of Manning's multisubstituted analogues have higher A/P selectivities.[25] The binding affinities of the D-Arg^8-substituted analogues to the bovine kidney and rat liver membranes follow also to some extent their pharmacological activities. Their affinities to vasopressor re-

Table V. Physicochemical Properties of Arginine-Vasopressin Analogues Modified in Positions 1, 2, 4, 7, and 8

no.	peptide	yield,[a] %	$[\alpha]^{20}_D$, deg (AcOH)	R_f BAW	R_f BAWP	mol wt[b]	amino acid anal. ratios: $^1/_2$ Cys	Tyr	Phe	Glu	Asp	Arg	Gly	Val
1	[Phe^2,Sar^7]AVP	37.6	+2.1	0.22	0.20	1042	2.00		2.06	1.00	0.95	0.93	1.00	
2	[Phe^2,MeAla^7]AVP	28.9	−7.0	0.17	0.22	1056	2.09		2.11	1.06	1.00	0.99	1.00	
3	[Val^4,Sar^7]AVP	33.7	+4.5	0.14	0.32	1029	1.90	0.94	1.00		0.98	0.95	1.00	0.96
4	[Mpa^1,Val^4,Sar^7]AVP	21.6	−61.3	0.22	0.47	1014	0.96	1.00	1.01		1.05	1.01	1.00	0.99
5	[Val^4,MeAla^7]AVP	34.8	+33.3	0.16	0.40	1043	1.92	0.97	1.00		0.99	0.98	1.00	0.98
6	[Mpa^1,Val^4,MeAla^7]AVP	13.4	−35.3	0.26	0.55	1028	0.97	1.02	1.00		1.02	0.96	1.00	0.99
7	[Sar^7,D-Arg^8]VP	48.0	+17.9	0.21	0.22	1058	2.10	1.01	1.03	1.08	1.11	1.03	1.00	
8	[Mpa^1,Sar^7,D-Arg^8]VP	32.7	−38.5	0.33	0.34	1043	1.03	0.94	0.95	1.01	0.97	1.04	1.00	
9	[MeAla^7,D-Arg^8]VP	42.2	+2.0	0.29	0.30	1072	2.02	1.05	0.98	1.04	1.05	1.03	1.00	
10	[Mpa^1,MeAla^7,D-Arg^8]VP	22.6	−58.7	0.36	0.41	1057	0.95	0.94	0.94	1.03	1.01	1.01	1.00	

[a] Overall yield of chromatographically pure peptide after reduction, oxidation, and purification steps. [b] By field desorption technique.

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ceptors in rat liver are the lowest among all analogues studied, in support of the findings in the literature that these receptors are highly intolerant of conformational changes arising from substitutions in the C-terminal tripeptide, -Pro-Arg-Gly-NH_2.[2,28]

Conclusion

Replacement of the Pro residue in position 7 of AVP by Sar or MeAla combined with substitutions in position 1 (Mpa), position 4 (Val), or position 8 (D-Arg) leads to analogues that are characterized by a relatively high antidiuretic activity and by a sharp decrease in pressor activity. Thus, these new analogues exhibit a selective increase in antidiuretic/vasopressor potencies. In general, the change in pharmacological profile of these analogues is consistent with the tendency in the change of their binding affinities to the appropriate vasopressin receptors from bovine kidney inner medulla (V_2) and from rat liver (V_1). However, it appears from our results that there are different structural requirements for the receptor recognition (binding) and for the transduction (activation of the biological response) at both V_2 and V_1 vasopressin receptors. The data presented here have obvious potential for the design of even more specific vasopressin agonists.

Experimental Section

The procedure of solid-phase peptide synthesis conformed to that published.[2] Chloromethylated resin (Bio-Rad, Bio-Beads SX-1) was esterified[29] to Boc-Gly to an incorporation of 0.51 mmol/g. Triethylamine was distilled from ninhydrin; dimethylformamide was distilled under reduced pressure. Other solvents and reagents were of analytical grade. Thin-layer chromatography was carried out on silica gel plates (Merck), and the products were detected by ninhydrin or iodine vapor. The following solvent systems were used: BAW, 1-butanol–acetic acid–water (4:1:5, v/v, upper phase); BAWP, 1-butanol–acetic acid–water–pyridine (15:3:3:10, v/v). Loads of 10–80 μg were applied, and chromatograms were of minimum length (10 cm). Melting points are uncorrected. Optical rotations were determined with a Hilger-Watts polarimeter with an accuracy of 0.01°. Samples for analytical purposes were dried over P_2O_5 in vacuo for 24 h. Analytical results determined on a Carlo-Erba Model 1106 analyzer indicated by the elemental symbols were ±0.4% of theoretical values. For amino acid analysis,[30] peptides (ca. 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (400 μL) containing phenol (20 μL) in evacuated and sealed ampules for 18 h at 110 °C. The analyses were performed on a AAA 881 Mikrotechma analyzer. Sarcosine and *N*-methylalanine ratios were not calculated, considering the difficulties in detection of *N*-methyl amino acids.[31] The presence of Sar and MeAla in the structure of the synthesized peptides was determined by TLC of hydrolysates on silica gel. Two-dimensional solvent systems: (1) 1-butanol–acetic acid–water (4:1:5, v/v, upper phase); (2) phenol–water (3:1, v/v). Molecular ions of pure peptides were determined by mass spectrometry field desorption technique with a Varian MAT 711 instrument.

Z-Cys(Bzl)-Phe-Phe-Gln-Asn-Cys(Bzl)-Sar-Arg(Tos)-Gly-NH_2 (I). Boc-Gly resin (0.784 g, 0.4 mmol) was subjected to eight cycles of deprotection, neutralization, and coupling.[2] The nonapeptidyl resin (1.388 g) was ammonolyzed[12] in methanol. Following the evaporation of the solvent, the product was extracted into hot DMF, precipitated with boiling water, and left overnight at room temperature. The peptide was collected by filtration, washed with water, and dried over P_2O_5. The product was further purified by dissolution in DMF and reprecipitation with boiling EtOH; yield 489 mg (80.1% based on substituted Gly). The physicochemical properties of this peptide (I), as well as all of the protected peptides (II–X) synthesized by the same procedure, are given in Table IV.

[Phe^2,Sar^7]Arginine-vasopressin (1). The protected 9-peptide I (177 mg, 0.117 mmol) was dissolved in 400 mL of ammonia freshly distilled from sodium and treated at the boiling point, with stirring, with sodium from a stick of the metal contained in a small-bore glass tube, until a light blue persisted in the solution for 20 s. The color was discharged by the addition of a few drops of glacial acetic acid, and the clear solution was evaporated. The residue was dissolved in N_2-flushed 0.2% aqueous AcOH, and aqueous ammonia (2 N) was added gradually to give a solution of pH 7. The solution was treated with 0.02 M $K_3Fe(CN)_6$ until a permanent yellow color was observed and stirred for next 20 min. Anion-exchange resin (Amberlite IR 45, acetate form) was added to remove the ferri- and ferrocyanide ions. The mixture was filtered through a bed of the resin, and the filtrate was lyophilized. The residue was desalted on Sephadex G-15 (column 100 × 2.5 cm), eluting with 50% AcOH with a flow rate of 4 mL/h. The eluate was monitored for absorbance at 280 nm and fractionated. Fractions consisting of the major peak were pooled and lyophilized, and the residue (54 mg) was further subjected to gel filtration on Sephadex G-15 (column 120 × 1.2 cm), eluting with 0.2 M AcOH with a flow rate of 2.5 mL/h. The arginine-vasopressin analogue (45.8 mg, 37.6%) was isolated from the fractions comprising the single symmetrical peak by lyophilization (Table V).

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Registry No. 1, 98525-39-4; 2, 98525-40-7; 3, 98509-74-1; 4, 97868-95-6; 5, 98509-75-2; 6, 97884-18-9; 7, 97906-81-5; 8, 97906-82-6; 9, 97906-83-7; 10, 97906-84-8; I, 98509-78-5; II, 98525-41-8; III, 98509-76-3; IV, 98525-42-9; V, 98539-79-8; VI, 98509-77-4; VII, 98632-66-7; VIII, 98575-33-8; IX, 98575-34-9; X, 98575-35-0; [MeAla⁷]AVP, 84558-81-6; [^{3}H]AVP, 63493-11-8.

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