5-Fluoro-2-methyl-*N*-[4-(5*H*-pyrrolo[2,1-*c*]-[1,4]benzodiazepin-10(11*H*)-ylcarbonyl)-3-chlorophenyl]benzamide (VPA-985): An Orally Active Arginine Vasopressin Antagonist with Selectivity for V_2 Receptors

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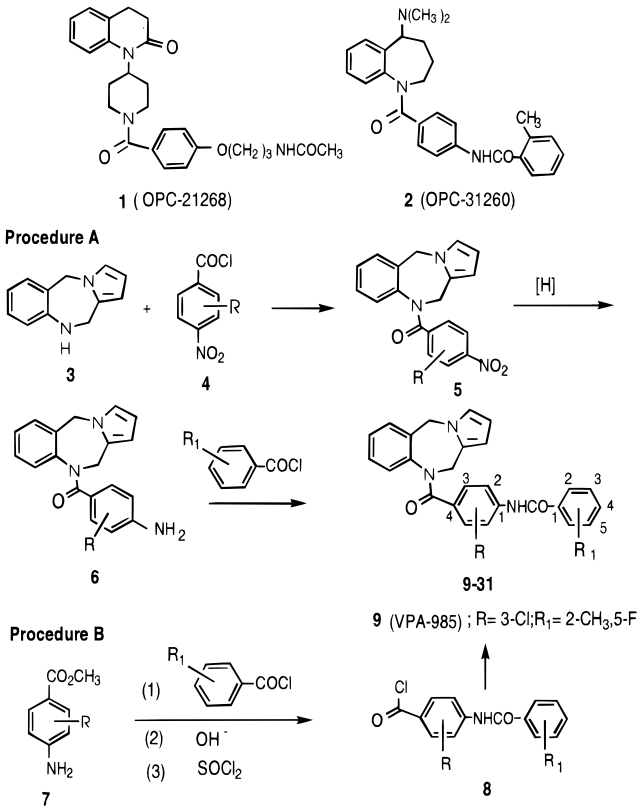
Arginine vasopressin (AVP) is released from the posterior pituitary, in response to either increased plasma osmolality detected by brain osmoreceptors or decreased blood volume and blood pressure sensed by low-pressure volume receptors and arterial baroreceptors. The hormone exerts its actions through three well-defined receptor subtypes: vascular V_{1a}, hormone-releasing V_{1b} (V_3), and renal V_2 receptors.[1−3]

Both V_{1a} and V_2 receptors are found in the kidney. The physiological effects of AVP on different collecting ducts in the kidney as well as the physiology of fluid transport in the kidney are under active investigation.[4−7] The mechanism for the regulation of water and urea transport in the renal collecting duct and the mechanism for AVP regulation of water channels and urea carriers has been reviewed.[8] In brief, AVP regulates a water-selective channel membrane protein aquaporin-2 (also labeled AQP2 or AQP-CD) to control osmotic water permeability of water channels in the kidney.[9−11]

The blockade of V_2 receptors may be useful in treating diseases characterized by excess renal reabsorption of free water. V_2 antagonists may correct the fluid retention in congestive heart failure, liver cirrhosis, nephrotic syndrome, central nervous system injuries, lung disease, and hyponatremia. Thus, antagonizing AVP actions at the receptor level with orally active, non-peptide agents may be the treatment of choice for edematous states.

In 1991 the first non-peptide AVP antagonist **1** (OPC-21268) with selectivity for rat V_{1a} receptor ($V_2/V_{1a} \geq 100$) was reported.[12] Structure−activity relationships among analogues have been published.[13,14] The non-peptide **2** (OPC-31260) with selectivity for rat V_2 receptors ($V_{1a}/V_2 = 86$) and oral activity as an aquaretic was disclosed in 1992.[15] Clinical studies have demonstrated oral efficacy for the hydrochloride as an aquaretic in humans.[16,17] The synthesis and structure−activity relationships of 1-[4-(benzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-benzazepines and 1-[4-(benzoylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1,5-benzodiazepines have been disclosed.[18,19]

As part of a project to develop nonpeptide vasopressin antagonists, we report on 5-fluoro-2-methyl-N-[4-(5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin−10(11*H*)-ylcarbonyl)-3-chlorophenyl]benzamide **9** (VPA-985), and related analogues as a new class of compounds with potent binding affinity for human AVP V_2 receptors and potent aquaretic activity.



The tricyclic 10,11-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine **3** was synthesized according to the literature procedure[20] by reductive ring closure of 1-(2-nitrobenzyl)-2-pyrrolecarboxaldehyde.[20,21] Two procedures (A and B) were used to synthesize derivatives **9**−**31**. Reaction of **3** with unsubstituted or substituted 4-nitrobenzoyl chlorides **4** afforded the 4-nitrobenzoyl derivatives **5**. Reduction of the nitro group was carried out by one of three procedures: (1) with Pd/C−H_2, (2) with 10% Pd/C−H_2NNH_2 in refluxing ethanol,[22] or (3) with $SnCl_2 \cdot 2H_2O$ in refluxing ethanol to give intermediates **6** in high yields. In derivatives **5** where R is a chloro group, partial dechlorination was observed with Pd/C and hydrazine. In procedure B, methyl esters of unsubstituted or substituted 4-aminobenzoates were reacted with substituted benzoyl chlorides to give intermediate amide esters. The esters were hydrolyzed and converted to the acid chlorides **8**. Reaction of the 10,11-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine **3** with intermediates **8** gave target compounds **9**−**31** (Tables 1 and 2).[24]

Initial binding studies were carried out with receptor membrane preparations isolated from rat liver (V_{1a}) and rat kidney (V_2). Binding affinities were determined by measuring the inhibition of (Phe-3,4,5-^{3}H)-AVP binding to isolated rat hepatic V_{1a} receptors or inhibition of binding of ^{3}H-AVP to isolated kidney medullary V_2 receptors.[24] For binding studies with human receptors,

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Table 1. Affinity for Human Vasopressin Receptors (IC_{50}, nM[a])

compd	R	R_1	V_{1a}	V_2	V_{1a}/V_2	procedure
9	3-Cl	2-CH_3,5-F	230	1.2 ± 0.1	192	B
10	H	2-CH_3,5-F	20 ± 2	1.5 ± 0.2	13	A
11	2-Cl	2-CH_3,5-F	25 ± 5	4	6	B
12	3-OCH_3	2-CH_3,5-F	15 ± 1	2	8	B
13	3-CH_3	2-CH_3,5-F	120	2.1 ± 0.2	80	A
14	3-Cl	2-F,3-CF_3	4400	8.3 ± 0.4	530	A
15	3-Cl	2-CH_3,3-CH_3	430	0.5	860	A
16	3-Cl	2-CH_3,5-CH_3	340	4.2 ± 0.3	81	B
17	H	2-Cl,4-F	6.3 ± 0.3	4.0 ± 0.4	2	A
18	3-Cl	2-Cl,4-F	37 ± 6	0.7	53	A
19	H	2-Cl,4-Cl	15 ± 2	3.2 ± 0.5	5	A
20	3-Cl	2-Cl,4-Cl	640	2	320	B
21	2-OCH_3	2-Cl,4-Cl	32 ± 5	1.9 ± 0.2	17	A
22	3-Cl	2-CH_3,3-F	540 ± 20	1.9 ± 0.3	284	B
23	3-Cl	2-CH_3	59	1	59	B

[a] Displacement of ^{3}H-AVP or ^{3}H-[d$(CH_2)_5$^{1}Tyr(Me)^2-Arg^8)-AVP from specific binding sites in membrane preparations from human platelets (V_{1a} column): Displacement of ^{3}H-AVP from specific binding sites in membrane preparations from a murine fibroblast cell line (LV2) which express human V_2 receptors[25,26] (V_2 column). IC_{50}'s are reported as group means ± SEM for compounds for which there were two or three separate determinations.

Table 2. Affinity for Rat Vasopressin Receptors (IC_{50}, nM)[a]

compd	R	R_1	V_{1a}	V_2	V_{1a}/V_2	procedure
9	3-Cl	2-CH_3,5-F	340	2.3	148	B
19	H	2-Cl,4-Cl	23 ± 2	3.1 ± 1	7	A
20	3-Cl	2-Cl,4-Cl	45 ± 3	8.3 ± 0.3	5	B
23	3-Cl	2-CH_3	54 ± 2	4.5 ± 0.2	12	B
24	H	2-CH_3	38 ± 1.3	4.1 ± 0.2	9	B
25	H	2-Cl	10 ± 0.6	4.6 ± 0.3	2	A
26	H	2-CF_3	26 ± 1	10.2 ± 0.7	3	A
27	H	2-OCH_3	31 ± 3	14 ± 1	2	A
28	H	2-OCH_3,4-Cl	33 ± 5	16 ± 1	2	A
29	H	2-CH_3,3-CH_3	31 ± 2	5.0 ± 0.2	6	A
30	2-CH_3	2-Cl,4-Cl	33 ± 3	8 ± 1	4	A
31	H	2-CH_3,3-F	26 ± 4	4 ± 1	7	A

[a] Displacement of (Phe-3,4,5-^{3}H)vasopressin from specific binding sites in membrane preparations isolated from rat liver (V_{1a} column): Displacement of ^{3}H-AVP from specific binding sites in rat kidney medulla (V_2 column). IC_{50} values are reported as group means ± SEM for compounds for which there were two or three separate determinations.

the compounds were evaluated by displacement of ^{3}H-AVP in membrane preparations from a murine fibroblast cell line (LV2) which express human V_2 receptors[25,26] or the inhibition of ^{3}H-AVP or ^{3}H-[d$(CH_2)_5$-^{1}Tyr(Me)^2,Arg^8)-AVP binding to V_{1a} receptors from human platelet membranes.[27]

In a search for structural parameters which might enhance selectivity for binding to V_2 receptors over V_{1a} receptors, we introduced chloro, fluoro, methoxy, and methyl substituents (R and R_1 in compounds **9**−**31**) in the phenyl rings of the 4-(benzoylamino)benzoyl "tail unit". Introduction of a chloro or a methyl group (R) at the C-3 position enhanced the selectivity for binding to human AVP V_2 receptors while substituents (R = Cl or CH_3O) at the C-2 position (compounds **11** and **21**) had little effect on potency and selectivity compared to their unsubstitued analogues, compounds **10** and **19** (Table 1). This general trend, which showed that a 3-chloro group (R) increased selectivity for human V_2 receptors, was less obvious in binding affinities for rat receptors (Table 2). Derivatives **20** and **23**, which contain a 3-Cl substituent (R), showed no significant selectivity for rat V_2 receptors, while exhibiting selectivity for human V_2 receptors. Analogues **13**−**16**, **20**, **22**, and **23** all exhibit selectivity for human V_2 receptors, due mainly to decreased binding affinities for the human V_{1a} receptors.

Table 3. Vasopressin V_2 Antagonistic Effects in Rats

compd no.	dose,[a] mg/kg	no. of rats	urine vol, mL/4 h	osmolality,[b] mOsm/kg	AVP (ip), 0.4 μg/kg	water (po), 30 mL/kg
9	1*	6	24	393	−	−
	3*	15	38	222	−	−
	10*	13	43	188	−	−
control	−*	22	8	785	−	−
9	1**	8	14	629	+	+
	3**	10	30	366	+	+
	10**	6	37	302	+	+
2	3**	2	10	850	+	+
	10**	2	23	352	+	+
14	10**	2	10	635	+	+
15	10**	2	13	557	+	+
20	10**	4	14	507	+	+
22	10**	3	17	473	+	+
control	−**	10	5	1308	+	+

[a] The vehicle for administration was 20% dimethyl sulfoxide (DMSO) in aqueous 2.5% preboiled starch (test compounds were first dissolved in DMSO). *Conscious rats with free access to water before and during the experiment. **Conscious rats with free access to water before (but not during) the experiment dosed (ip) with 0.4 μg/kg of AVP (in peanut oil): 20 min later given orally (by gavage) 30 mL/kg of deionized water and another 20 min later given vehicle or test compound. [b] Urinary osmolality from urine collected (normotensive Sprague−Dawley rats).

Differences in binding to human versus rat AVP V_{1a} receptors have been reported.[28−30]

On the basis of its selectivity for human V_2 receptors and its in vivo aquaretic activity (Table 3), the derivative **9** (VPA-985) was selected for advanced in vivo studies and clinical trials. Compared to **2** (OPC-31260), the analogue **9** is 3 times more active (in vivo) in rats (Table 3). In conscious dogs, water-loaded with 30 mL/kg (po) and AVP-treated (0.4 μg/kg in oil, sc), **9** (1, 3, and 10 mg/kg po) increased U_{vol} over the AVP-treated vehicle group by 438, 1018, and 1133%, respectively, while U_{osm} decreased from 1222 mOsm/kg (water-loaded and AVP-treated vehicle) to 307, 221, and 175 mOsm/kg, respectively. In homozygous Brattleboro rats lacking AVP, compound **9** at 10 mg/kg po (i.e., 10 times the dose producing V_2 antagonist activity) b.i.d. for 5 days, showed a sustained antagonist action without evidence of agonist effects, while desGlyd$(CH_2)_5$D-Tyr(Et)VAVP (SK&F 101926[31]) (1−30 μg/kg, ip), a peptide AVP V_2 receptor antagonist in normal rats, demonstrated strong agonist effects.

In a randomized double-blind placebo-controlled ascending single dose study, patients (deprived of fluids overnight before dosing) were dosed orally with 30, 75, or 150 mg of **9**. All three doses increased urine flow and serum sodium concentrations and produced significant dose-related decreases in urinary osmolality.[32]

In conclusion, **9** (VPA-985) is a potent, orally active V_2 antagonist in rats and humans and may be useful for the treatment of conditions characterized by water retention and inappropriate secretion of AVP.[33]

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Supporting Information Available: Experimental procedures, analytical data, and melting points for intermediates and derivatives (Tables 1 and 2) (8 pages). Ordering information is given on any current masthead page.

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