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# Identification of novel selective $V_2$ receptor non-peptide agonists

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ABSTRACT

Peptides with agonist activity at the vasopressin $V_2$ receptor are used clinically to treat fluid homeostasis disorders such as polyuria and central diabetes insipidus. Of these peptides, the most commonly used is desmopressin, which displays poor bioavailability as well as potent activity at the $V_{1b}$ receptor, with possible stress-related adverse effects. Thus, there is a strong need for the development of small molecule chemistries with selective $V_2$ receptor agonist activity. Using the functional cell-based assay Receptor Selection and Amplification Technology (R-SAT®), a screening effort identified three small molecule chemotypes (AC-94544, AC-88324, and AC-110484) with selective agonist activity at the $V_2$ receptor. One of these compounds, AC-94544, displayed over 180-fold selectivity at the $V_2$ receptor compared to related vasopressin and oxytocin receptors and no activity at 28 other G protein-coupled receptors (GPCRs). All three compounds also showed partial agonist activity at the $V_2$ receptor in a cAMP accumulation assay. In addition, in a rat model of central diabetes insipidus, AC-94544 was able to significantly reduce urine output in a dose-dependent manner. Thus, AC-94544, AC-88324, and AC-110484 represent novel opportunities for the treatment of disorders associated with $V_2$ receptor agonist deficiency.



## 1. Introduction

Vasopressin, also known as the anti-diuretic hormone (ADH) or arginine vasopressin (AVP), is a cyclic nonapeptide hormone. AVP has anti-diuretic, hypertensive, and stress modulatory effects, which are mediated by three different receptors, the $V_2$, $V_{1a}$, and the $V_{1b}$ vasopressin receptors, respectively [1]. The vasopressin receptors belong to the G protein-coupled receptor (GPCR) superfamily. The oxytocin (OT) receptor is often grouped with the vasopressin receptors, since these receptors and their endogenous ligands share considerable sequence homology. There is also strong evidence of cross-talk between the vasopressin and oxytocin receptors, as vasopressin can bind to oxytocin receptors and vice versa [2,3].

The $V_2$ receptors, which are predominantly localized in the kidney, are important for the regulation of fluid homeostasis (reviewed in Refs. [1,4–6]). Activation of the $V_2$ receptor results in increased expression of aquaporin-2 water channels in the

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luminal membrane of the kidney, leading to increased water reabsorption by the kidney, and thus resulting in concentration of urine. Further, inactivating mutations in the $V_2$ receptor in humans have been associated with nephrogenic diabetes insipidus, which is characterized by polyuria and excessive thirst [4,6]. As such, agonists at the $V_2$ receptor act as anti-diuretics.

In addition to vasopressin itself, desmopressin, a non-selective $V_2$ peptide agonist has been approved for the treatment of polyuria. Desmopressin (1-desamino-8-D-arginine vasopressin, ddAVP®, Minirin®, Octostim®) is a peptidic analog of vasopressin used in the treatment of central diabetes insipidus, a condition that results from the defective secretion of vasopressin. In addition, desmopressin is also approved as a treatment for other disorders characterized by excessive urine production, such as nocturia and primary nocturnal enuresis. However, desmopressin is not an ideal drug. While desmopressin is active when administered nasally or orally, its bioavailability under these conditions is less than 2% in humans [7]. Furthermore, desmopressin is non-selective, with potent agonist activity at both human $V_2$ and $V_{1b}$ receptors [8]. The $V_{1b}$ receptor mediates depression- and anxiety-like behaviors in rodents [9]. In humans, an association between desmopressin use and depression has not been studied, but evidence suggests a role for desmopressin in anxiety-like behaviors. For instance, depressed individuals that are treated with desmopressin show enhanced cortisol and ACTH secretion compared to normal or non-treated depressed individuals [10,11]. Moreover, intravenous administration of desmopressin in normal individuals has been shown to increase plasma cortisol secretion [12–14].

To date, only one non-peptide compound with selective $V_2$ receptor agonist activity, OPC-51803, has been identified [15–18]. However, OPC-51803 shows partial agonist activity in vitro with only 9-fold selectivity for the human $V_2$ receptor over the human $V_{1a}$ receptor. Activation of the $V_{1a}$ receptor might lead to increased blood pressure, adverse circulatory effects, and possibly stress-related disorders [19]. Thus, there is a need for additional small molecule $V_2$ receptor agonists that display an improved safety profile and better pharmacokinetics. Here we report the discovery and biochemical characterization of three small molecule chemotypes with selective agonist activity at the $V_2$ receptor.

## 2. Materials and methods

### 2.1. Drugs

Vasopressin, oxytocin, and desmopressin were obtained from Sigma–Aldrich (St. Louis, MO), and were solubilized as 1 or 10 mM solutions in PBS or $H_2O$. AC-94544 and AC-110484 were synthesized in-house and structures were confirmed by $^1H$ NMR, $^{13}C$ NMR and UV/MS. AC-94544, AC-110484, and AC-88324 were stored as 10 mM stock solutions in DMSO.

### 2.2. Receptor Selection and Amplification Technology (R-SAT®)

The cell-based functional assay, R-SAT®, was performed essentially as previously described [20–22]. R-SAT® is a functional cell-based assay that allows one to monitor receptor-dependent proliferative responses [23]. Briefly, contact-inhibited cells are transiently transfected with the receptor target and a marker gene and maintained in culture in the presence or absence of ligand. Cells expressing the receptor overcome contact inhibition and proliferate in the presence of agonist ligand specific for the target receptor; the extent of proliferation is dependent on the concentration of agonist, and can be quantified using the marker gene. When no receptor is transfected, no agonist response is observed, indicating that all assayed response is from exogenously expressed receptors. The R-SAT® technology has been validated for a number of receptors including GPCRs [20], receptor tyrosine kinases [24], cytokine receptors [25], and nuclear receptors [26,27].

To elaborate, NIH/3T3 cells grown in 840 $cm^2$ roller bottles (Corning Incorporated Life Sciences, Lowell, MA) or 632 $cm^2$ cell factory flasks (Nalge Nunc International, Rochester, NY) to 70% confluency were co-transfected with DNA encoding β-galactosidase or green fluorescent protein, the individual human receptors as described in the text, and ''helper'' DNAs encoding accessory proteins such as chimeric G-proteins [23,28]. Transfection was performed using Polyfect (Qiagen, Valencia, CA) as per manufacturer's instructions. Transfected cells were frozen at −80 °C in DMEM containing 10% calf serum and 10% dimethyl sulfoxide, and subsequently transferred to −135 °C for long-term storage. On day of the assay, cells were thawed and added in DMEM containing 30% Ultraculture (Lonza, Basel, Switzerland) and 0.4% calf serum (Hyclone, Logan, UT) directly to ligands at varying concentrations on 96-well tissue culture plates. Each concentration of a dose–response curve was tested in triplicate. After 5 days in a humidified chamber at 37 °C, 5% ambient $CO_2$, medium was removed from plates and β-galactosidase activity was measured and analysed as previously described using the β-galactosidase substrate o-nitrophenyl-D-galactopyranoside ONPG (Sigma–Aldrich, St. Louis, MO) [25]. Plates were read using a microplate reader at 420 nm. Data from R-SAT™ assays were fit to the equation: $r = A + B(x/(x + c))$, where $A$ = minimum response, $B$ = maximum response minus minimum response, $c$ = EC50, $r$ = response, and $x$ = concentration of ligand. Curves were generated using the curve fitting softwares Excel Fit and GraphPad Prism (San Diego, CA).

### 2.3. Radioligand binding assays

Plasmids expressing human cannabinoid $CB_1$ and $CB_2$ receptors were transfected into HEK293 cells, membranes prepared, and radioligand binding assays performed essentially as described previously using 2 nM [$^3$H]-SR141716 (Amersham Biosciences, Piscataway, NJ) as the radioligand for $CB_1$ and 1.5 nM [$^3$H]-CP-55940 (PerkinElmer Life and Analytical Sciences, Waltham, MA) for $CB_2$ [29].

### 2.4. cAMP assay

HEK-293 cells were grown in DMEM containing 10% FBS (Invitrogen, Carlsbad, CA). Cells were plated (20,000 cells/well) in 96-well plates and transfected with DNA encoding human $V_2$ receptor (80 ng/well) using Polyfect (Qiagen) as per manufacturer's instructions. Media was changed 1 day after

**Table 1 – Pharmacological characterization of vasopressin receptor R-SAT® assays**

| | AVP | ddAVP | Oxytocin |
|---|---|---|---|
| $V_2$ | | | |
| %Eff | 100 | 132 ± 7 | 128 ± 5 |
| pEC50 | 9.7 ± 0.2 | 10.0 ± 0.2 | 8.5 ± 0.1 |
| *n* | 15 | 15 | 21 |
| $V_{1a}$ | | | |
| %Eff | 100 | NA | 95 ± 24 |
| pEC50 | 8.3 ± 0.1 | | 6.6 ± 0.05 |
| *n* | 4 | 2 | 2 |
| $V_{1b}$ | | | |
| %Eff | 100 | 132 ± 22 | 105 ± 8 |
| pEC50 | 8.2 ± 0.1 | 8.2 ± 0.1 | 6.8 ± 0.1 |
| *n* | 14 | 2 | 6 |
| OT | | | |
| %Eff | NA | NA | 100 |
| pEC50 | | | 8.7 ± 0.3 |
| *n* | 2 | 3 | 6 |

Potencies (pEC50) and % efficacies (%Eff) for *n* experiments for vasopressin (AVP), desmopressin (ddAVP), and oxytocin at the $V_2$, $V_{1a}$, $V_{1b}$, and oxytocin (OT) receptors (see Fig. 1). Data shown are the average ± standard error for *n* experiments. For each experiment, concentration–response curves were generated with each concentration being tested in triplicate. For $V_2$, $V_{1a}$, or $V_{1b}$ receptors, efficacy is calculated as a percentage of maximum response to that observed for vasopressin in the same experiment. For the OT receptor, efficacy was calculated as a percentage of maximum response to that observed for oxytocin in the same experiment.

transfection. Two days after transfection, media was replaced with 200 μL DMEM + 1% BSA per well. After 30 min incubation, cells were incubated in 100 μL DMEM + 1% BSA + 0.5 mM isobutylmethylxanthine (IBMX, Sigma–Aldrich, St. Louis, MO) for 1 h. Briefly, cells were incubated with vasopressin or test compounds in DMEM + 1% BSA + 0.5 mM IBMX for 30 min at 37 °C. For antagonist experiments, cells were incubated with test compounds in DMEM + 1% BSA + 0.5 mM IBMX for 15 min, followed by the addition of 3 nM vasopressin, and subsequent incubation for 15 min. cAMP accumulation was determined using the cAMP Enzyme Immuno Assay (EIA) Biotrak System as described in manufacturer's protocol (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ).

### 2.5. *Brattleboro rat assay for diabetes insipidus*

Male Brattleboro rats (Harlan, San Diego, CA) were used as subjects and housed two per cage in a room with a 12 h:12 h light:dark cycle and temperature maintained at 22 ± 2 °C. AC-94544 (3, 10, or 30 mg/kg, s.c.), ddAVP (3 μg/kg, s.c.) or vehicle (10% Tween-80 in 90% sterile water, pH 7.5) was administered to Brattleboro rats (*n* = 4/dose). For oral administration, AC-94544 was dispensed by oral gavage in the same vehicle. Immediately after administration, rats were placed individually into a circular metabolic cage with a wire floor and a funnel that led to separate collection of urine and feces. Urine was collected in a graduated tube and the amount of urine present was measured every 15 min over a 2-h session. Water was available ad libitum during the session. Food and water was available ad libitum in the home cages before and after the session. To assess statistical significance, a Student's *t*-test was performed with three degrees of freedom. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health and by the Institutional Animal Care and Use Committee of ACADIA Pharmaceuticals Inc.

## 3. Results

### 3.1. *Development of proliferation assays for vasopressin and oxytocin receptors*

Using the functional mammalian cell-proliferation assay, R-SAT®, pharmacologically predictive assays were developed for the $V_2$, $V_{1a}$, $V_{1b}$, and OT receptors [23]. As shown in Table 1 and Fig. 1A, the known peptide agonists, vasopressin, desmopressin and oxytocin show expected responses at the vasopressin and oxytocin receptors compared to published functional and radioligand binding data [3,8]. The rank order of potency of the peptides at the $V_2$ and $V_{1b}$ receptor was AVP = ddAVP > OT, whereas at the $V_{1a}$ receptor the rank order

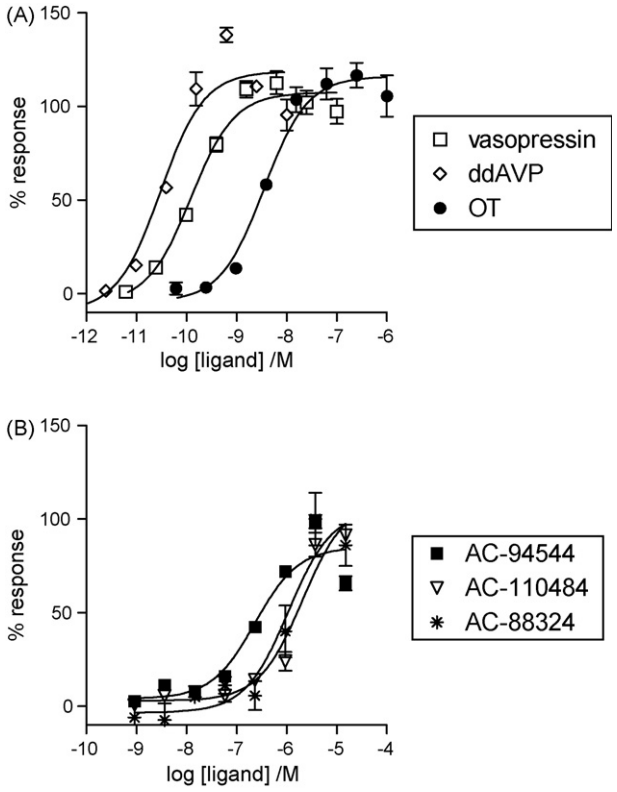
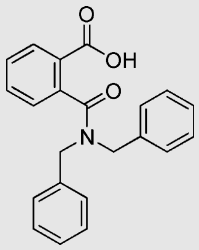
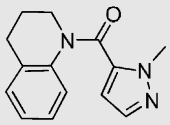



**Fig. 1 – $V_2$ agonist hits from ultra high-throughput screening using the R-SAT® assay. Concentration–response curves shown for R-SAT® activity of (A) vasopressin, oxytocin, and desmopressin (ddAVP) or (B) AC-94544, AC-110484, or AC-88324 at the vasopressin $V_2$ receptor. Efficacy is relative to vasopressin defined as 100%. A representative experiment is shown where each point represents the average of three determinations.**

**Table 2 – Novel selective small molecule $V_2$ agonists**

| | AC-94544 | AC-88324 | AC-110484 |
|---|---|---|---|
| |  |  |  |
| $V_2$ | | | |
| %Eff | 117 ± 14 | 100 ± 8 | 143 ± 11 |
| pEC50 | 7.1 ± 0.2 | 5.9 ± 0.1 | 5.7 ± 0.3 |
| *n* | 9 | 2 | 4 |
| $V_{1a}$ | | | |
| %Eff | NA | NA | NA |
| pEC50 | | | |
| *n* | 5 | 2 | 3 |
| $V_{1b}$ | | | |
| %Eff | NA | NA | NA |
| pEC50 | | | |
| *n* | 6 | 3 | 5 |
| OT | | | |
| %Eff | NA | NA | NA |
| pEC50 | | | |
| *n* | 6 | 2 | 4 |

Average potencies (pEC50) and % efficacies (%Eff) as obtained using R-SAT®. For each experiment, concentration–response curves were generated with each concentration being tested in triplicate. For $V_2$, $V_{1a}$, or $V_{1b}$ receptors, efficacy is calculated as a percentage of maximum response to that observed for vasopressin in the same experiment. For the OT receptor, efficacy is calculated as a percentage of maximum response to that observed for oxytocin in the same experiment.

was AVP > OT ≫ ddAVP. As expected, ddAVP had no detectable activity at the $V_{1a}$ receptor at 15 μM. In addition, the peptide antagonist ($d(CH_2)_5^1$, $Tyr(Et)^2$, $Val^4$, $Arg^8$, des-Gly-$NH_2^9$)-Vasopressin (Manning compound) [30] was able to inhibit the vasopressin response at the $V_2$ receptor in R-SAT® with a $pK_i$ of 6.8, similar to its reported binding affinity [3] (data not shown).

### 3.2. *Identification of three small molecule agonists at the vasopressin $V_2$ receptor*

Using the $V_2$ R-SAT® assay, a high-throughput screen of an internal compound library of 176,277 small molecules was performed to identify compounds with agonist activity at the human $V_2$ receptor. This compound library is heavily biased in favor of compounds displaying drug likeness characteristics [31,32], and has chemical properties which are in broad agreement with those properties from the MDL Drug Data Report (MDDR) and the Comprehensive Medicinal Chemistry (CMC) library. Moreover, the library exhibits unique chemical space properties.

The screening effort identified three non-peptidic entities with $V_2$ agonist activity: *N*,*N*-dibenzyl-phthalmic acid (AC-94544), (3,4-dihydro-2*H*-quinolin-1-yl)-(2-methyl-2*H*-pyrazol-3-yl)-methanone (AC-88324) and 3-[1-(2-ethyl-phenyl)-1*H*-tetrazol-5-yl]-4-trifluoromethyl-pyridine (AC-110484). AC-94544 had potency of (pEC50) 7.1 at the $V_2$ receptor, while AC-88324 and AC-110484 were approximately 15-fold lower in potency (pEC50, 5.9, 5.7, respectively). AC-94544 lacked any detectable activity at up to 15 μM at the related $V_{1a}$, $V_{1b}$, and OT receptors and showed toxic effects at 50 μM (Fig. 1B and Table 2). AC-88324 and AC-110484 lacked any detectable activity up to 50 μM at $V_{1a}$, $V_{1b}$, and OT receptors. Also, AC-94544 and AC-110484 did not show antagonist activity up to 10 μM at the OT receptor. Thus, AC-94544 was extremely selective, showing over 180-fold selectivity for $V_2$ over related vasopressin receptors. AC-88324 and AC-110484 displayed moderate selectivity of at least 20-fold.

Of the three classes of non-peptide agonists found, the tetrahydroquinoline class (AC-88234) was structurally similar to the benzoazepine class (OPC-51803) previously reported and therefore not pursued further. However, the two remaining classes had novel characteristics compared with known vasopressin compounds. Unlike vasopressin and desmopressin which both incorporate a basic motif (arginine), the AC-94544 class incorporates an acidic functionality, a carboxylic acid pharmacophore. Although speculative at this point, this could be the major contribution to the high selectivity seen with AC-94544; in support of this idea, a modeling study indicates that the arginine in vasopressin binds to negatively charged amino acids in the $V_1$ receptors [33]. The third class, AC-110484, has a rigid pyridine structure which may be useful for determining the spatial arrangement for the pharmacophores for selective $V_2$ agonist activity. Both AC-94544 and AC-110484 have drug-like chemical character-

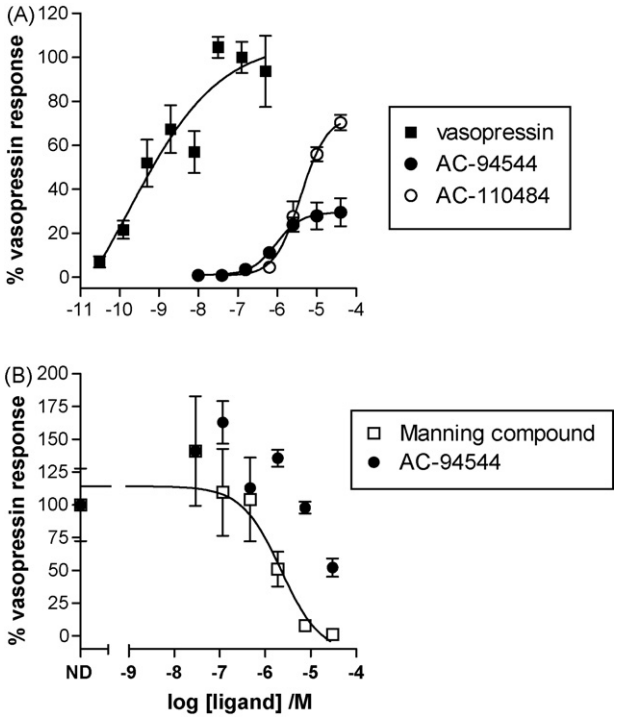



**Fig. 2 – Effect of AVP, AC-94544, and AC-110484 on production of cAMP. (A) Concentration–response curves shown for effect of AVP, AC-94544, and AC-110484 on production of cAMP in HEK-293 cells expressing human $V_2$ receptor. (B) Concentration–response curves for effect of Manning compound and AC-94544 on cells treated with compound and 3 nM vasopressin. For each curve, a representative experiment is shown where each point represents the average of three determinations.**

istics in-line with the rule of 5 with molecular weights less than 500 g/mol (AC-94544, MW = 345; AC-110484, MW = 319) and *c* log *P* values below 5 (AC-94544, 3.1; AC-110484, 4.5) [31]. In addition, both AC-94544 and AC-110484 classes are amenable to further optimization by high-throughput medicinal chemistry.

### 3.3. *Broad profiling of compounds*

AC-94544, AC-110484, and AC-88324 did not show activity when tested at other GPCRs. No R-SAT® agonist activity at 10 μM was observed at the adrenergic $\alpha_{2A}$, $\alpha_{1D}$, muscarinic $M_5$, serotonergic 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{1D}$, 5-$HT_{2A}$, 5-$HT_{2B}$, 5-$HT_{2C}$, histamine $H_1$, $H_2$, dopamine $D_2$, corticotropin releasing factor $CRF_1$, $CRF_2^{\beta}$, and calcium-sensing CaSR receptors for all three compounds. No agonist activity at 10 μM was observed at the prostanoid TP, formyl peptide receptor (FPR), formyl peptide receptor like-1 (FPRL-1), histamine $H_3$, dopamine $D_1$, $D_3$, cholecystokinin $CCK_A$, $CCK_B$, or neurotensin $NTS_1$, $NTS_2$ receptors for AC-94544 or AC-110484. No inverse agonist activity was observed for AC-94544 or AC-110484 at the chemokine $CCR_{2A}$ or $CCR_{2B}$ receptors. No competitive antagonist activity was observed for AC-94544 or AC-110484 at the adrenergic $\alpha_{1B}$, $\alpha_{2A}$, or histamine $H_1$/$H_3$ receptors. Finally, no binding affinity was observed for AC-94544 and AC-110484 at the cannabinoid $CB_1$ and $CB_2$ receptors.

### 3.4. *Effect of AVP, AC-94544, and AC-110484 on cAMP accumulation*

To further characterize these compounds, we evaluated their ability to modulate second messenger release, a classical function of GPCRs. In HEK-293 cells transiently transfected with the $V_2$ receptor, vasopressin stimulated significant cAMP accumulation through coupling to $G_s$ protein (pEC50, 9.4 ± 0.5, $n$ = 3) (Fig. 2A). Similar to the R-SAT® assay, AC-94544 (pEC50, 5.9 ± 0.3, $n$ = 3) was more potent in the cAMP assay compared to AC-110484 (pEC50, 5.1 ± 0.5, $n$ = 2). However, unlike the full response observed in R-SAT®, AC-94544 and AC-110484 showed partial agonism compared to vasopressin with 27 ± 4% and 68 ± 7% efficacy in stimulating cAMP production, respectively. AC-88324 was tested at a single 10 μM dose, and showed 21 ± 9% efficacy ($n$ = 2). By contrast, oxytocin showed 182 ± 69% efficacy ($n$ = 3) in stimulating cAMP production at the $V_2$ receptor, as expected [34]. When tested as an antagonist, AC-94544 showed extremely weak activity ($pK_i < 5$) in the ability to inhibit vasopressin response, while the Manning compound showed weak sub-micromolar potency as expected [3] (Fig. 2B). Thus, all three compounds were revealed to be partial agonists at the $V_2$ receptor in the cAMP assay, compared to the full agonists vasopressin and oxytocin.

### 3.5. *Effect of AC-94544 in rat model for diabetes insipidus*

$V_2$ agonists are known to display anti-diuretic effects in vivo. The Brattleboro rats harbor an autosomal recessive trait that results in their inability to synthesize detectable amounts of vasopressin. These rats are thus commonly used as a model to assess anti-diuretic properties of test compounds. AC-94544 and ddAVP were administered to vasopressin-deficient Brattleboro rats ($n$ = 4) and effects on urine output were recorded over a 2-h session. As expected, ddAVP (3 μg/kg, s.c.) decreased urine output significantly, from 10.8 ± 2.3 mL (vehicle) to 1.68 ± 0.95 mL (Fig. 3A) ($p < 0.01$). AC-94544 was evaluated at 3, 10 and 30 mg/kg (s.c.). AC-94544 significantly reduced urine output in a dose-dependent fashion at 10 and 30 mg/kg over the 2-h session. At 10 mg/kg, AC-94544 decreased urine output to 3.9 ± 1.5 mL ($p < 0.05$), and at 30 mg/kg AC-94544 decreased urine output to 3.9 ± 2.0 mL ($p = 0.06$) comparable in magnitude to the effect seen with ddAVP. In a different experiment, the decrease in urine output compared to vehicle was steady throughout the 2 h following AC-94544 administration (10 mg/kg, s.c., $p < 0.001$) (Fig. 3B). Finally, when administered orally, AC-94544 decreased urinary output compared to vehicle at 2 h following administration (Fig. 3C). However, the effect did not reach statistical significance, possibly due to the use of a vehicle which did not exhibit consistent absorption (Fig. 3C). These data demonstrate that AC-94544 can act as a $V_2$ agonist in vivo.

## 4. Discussion

Using the functional cell-based assay screening platform R-SAT®, three small molecule entities with potent and selective

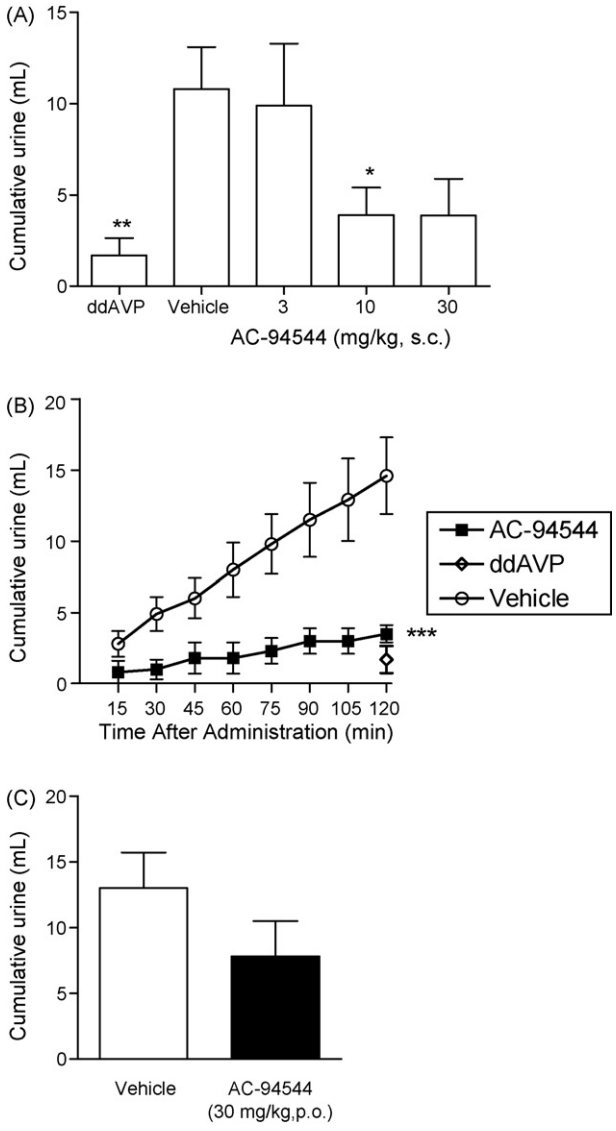



**Fig. 3 – Effect of AC-94544 in the Brattleboro rat model for diabetes insipidus. (A) Brattleboro rats ($n = 4$) were dosed with ddAVP (3 μg/kg, s.c.), AC-94544 (s.c.), or 10% Tween-80 pH 7.5 (vehicle). Cumulative urine output was measured for 2 h following compound administration. (B) In a separate experiment, time course of urine output shown at 15 min intervals following administration of ddAVP, AC-94544 (10 mg/kg, s.c.) or vehicle. (C) Cumulative urine output shown at 2 h following administration of AC-94544 (30 mg/kg, p.o.) or vehicle. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, when compared to the vehicle.**

agonism at the $V_2$ receptor were identified. Two compounds, AC-94544 and AC-110484, showed partial agonist activity in a cAMP accumulation assay. AC-94544 was also able to decrease urine output in a rat model of central diabetes insipidus.

AC-94544, AC-110484, and AC-88324 appear more selective than previously described $V_2$ agonists. AC-94544 displayed over 180-fold selectivity at the $V_2$ receptor compared to the $V_{1a}$, $V_{1b}$, and OT receptors, while AC-88324 and AC-110484 showed moderate >20-fold selectivities. In contrast, the vasopressin analog, ddAVP, displays nanomolar potency at both $V_2$ and $V_{1b}$ receptors [8]. Moreover, OPC-51803, the first reported small molecule agonist at the $V_2$ receptor, exhibits less than 10-fold selectivity at the $V_2$ receptor compared to $V_{1a}$ based on binding data, and a 50-fold selectivity at the $V_2$ receptor based on calcium signaling data [18]. Notably, OPC-51803 does not show $V_{1a}$-mediated blood pressure effects in vivo in rats and dogs, but binding data indicate it is more $V_2$ receptor selective (20-fold and 40-fold, respectively) in these species compared to the human [16,17].

The three identified compounds behaved as full agonists in R-SAT® but displayed partial agonism in the cAMP accumulation assay. The cAMP accumulation assay, like most second messenger assays, measures the amount of cAMP amassed after a short 2 h incubation of ligand on cells expressing the $V_2$ receptor. In contrast, the R-SAT® assay measures cellular transformation observed occurring over a 5-day time course, integrating multiple signaling events such as cAMP accumulation. This results in a highly amplified, highly coupled functional assay with a large receptor reserve [23]. Thus it is not surprising these compounds display much greater efficacy in the R-SAT® assay compared to the cAMP assay.

In vivo, AC-94544 behaved as a full agonist in the Brattleboro rat model for diabetes insipidus. The full agonism of AC-94544 in this rat model is not inconsistent with the partial agonism observed in vitro in the cAMP assay. The Brattleboro rat strain is a vasopressin-deficient strain, which has been shown to have a higher sensitivity to agonists than normal rats, and has been used to detect weak agonist activity of reported $V_2$ antagonists. For instance, the peptide antagonist, SKF-101926, which has antagonist properties in several animal species and models, acts as an agonist in the Brattleboro rat as well as in man [35]. It is also notable that, like AC-94544, OPC-51803 is also a partial $V_2$ agonist in vitro with full agonist activity in vivo in the Brattleboro rat model [17]. Taken together, these results indicate that there is likely to be substantial receptor reserve for $V_2$ receptors in vivo, and that partial agonists of the $V_2$ receptor could be efficacious drugs.

The chemical class defined by AC-94544 shows promise as therapeutic agents. In addition to selective $V_2$ agonist activity both in vitro and in vivo, AC-94544 did show oral activity, although additional studies will be required to quantify oral bioavailability. Moreover, AC-94544 was found to be stable in the presence of human liver microsomes (data not shown). In addition, further chemical optimization of AC-94544 has revealed structurally related compounds with selective agonist and competitive antagonist activities at the $V_2$ receptor (data not shown). AC-94544 and related compounds might thus be used to modulate disorders of fluid homeostasis. Current $V_2$ receptor agonists are anti-diuretics, and as such are used to treat central diabetes insipidus, polyuria, nocturia and primary nocturnal enuresis. However, administration of the full $V_2$ agonist desmopressin requires careful medical monitoring to avoid the risk of water intoxication when co-administered with other water-retaining drugs. Compounds with partial and selective agonism at the $V_2$ receptor might modulate vasopressin tone in a more subtle manner than a

potent full agonist or antagonist, and thus might be used to treat fluid homeostasis disorders with less adverse effects.

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