# New Benzylureas as a Novel Series of Potent, Nonpeptidic Vasopressin V2 Receptor Agonists

Christopher M. Yea,*[,†] Christine E. Allan,[†] Doreen M. Ashworth,[‡] James Barnett,[‡] Andy J. Baxter,[‡] Janice D. Broadbridge,[‡] Richard J. Franklin,[‡] Sally L. Hampton,[†] Peter Hudson,[‡] John A. Horton,[‡] Paul D. Jenkins,[‡] Andy M. Penson,[‡] Gary R. W. Pitt,[‡] Pierre Rivière,[§] Peter A. Robson,[†] David P. Rooker,[†] Graeme Semple,[‡] Andy Sheppard,[†] Robert M. Haigh,[†] and Michael B. Roe[†]

*Vantia Ltd, Southampton Science Park, Southampton SO16 7NP, United Kingdom, Ferring Research Ltd, Southampton Science Park, Southampton SO16 7NP, United Kingdom, and Ferring Research Institute Inc., 3550 General Atomics Court, San Diego, California 92121*

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Vasopressin (AVP) is a hormone that stimulates an increase in water permeability through activation of V2 receptors in the kidney. The analogue of AVP, desmopressin, has proven an effective drug for diseases where a reduction of urine output is desired. However, its peptidic nature limits its bioavailability. We report herein the discovery of potent, nonpeptidic, benzylurea derived agonists of the vasopressin V2 receptor. We describe substitutions on the benzyl group to give improvements in potency and subsequent modifications to the urea end group to provide improvements in solubility and increased oral efficacy in a rat model of diuresis. The lead compound **20e** (VA106483) is reported for the first time and has been selected for clinical development.

## Introduction

The cyclic nonapeptide hormone arginine vasopressin (AVP[a]) plays a crucial role in water balance. It is released from the posterior pituitary in response to decreased blood pressure or volume or by an increase in plasma osmolality. It stimulates an increase in water permeability in the collecting ducts of the kidney by activation of V2 receptors and subsequent regulation of aquaporin-2 water channels, ultimately leading to a decrease in urine volume. Additionally, AVP interacts with receptor subtypes V1a and V1b, resulting in a number of effects including contraction of vascular smooth muscle, platelet aggregation, glycogenolysis, ACTH, and $\beta$-endorphin release.[1−7] The only commercially available V2 agonist, desmopressin (DDAVP), is effective as a treatment for diseases where a reduction of urine output is desired including diabetes insipidus, enuresis, and nocturia.[8,9] While it is successfully administered orally, its peptidic nature limits its bioavailability. Increased utility of this therapeutic class has been enhanced by identification of orally available nonpeptides such as **1** and **2** (Figure 1), but these agonists have yet to progress to market.[10,11] There is therefore a need to develop new orally active agonists that may be used as treatments for indications where decreased urine production would have clinical benefit. We have previously reported the syntheses of libraries targeted to the vasopressin V2 and the related oxytocin receptors.[12,13] Screening of these libraries identified **3a** as a hit compound (Table 1). We report here, for the first time, the functional activity at the human V2 receptor of the resulting series of novel agonists and their SAR, leading to the selection of **20e** (VA106483) as a clinical candidate.

## Chemical Synthesis

The chemistry to prepare the difluorophenyl ureas in Table 1 is shown in Scheme 1, details of which have been disclosed.[14,15]

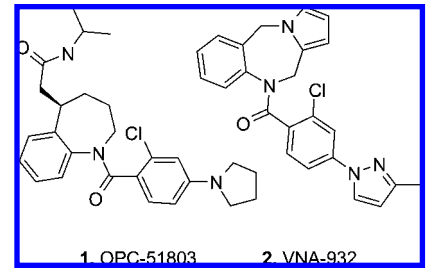
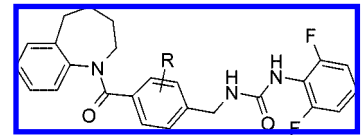



**Figure 1.** Nonpeptide V2 agonists.

**Table 1.** Potencies of Compounds **3a**−**g**



| Compound | R | $EC_{50}$ (nM) | *n* |
|---|---|---|---|
| **3a** | H | 47 ± 15 | 3 |
| **3b** | 2-F | 180 ± 40 | 3 |
| **3c** | 2-Cl | 40 ± 25 | 5 |
| **3d** | 2-OMe | 110 ± 30 | 3 |
| **3e** | 3-Me | 2.1 ± 1.7 | 6 |
| **3f** | 3-Et | 10 ± 4 | 3 |
| **3g** | 3,5-DiMe | 82 ± 73 | 6 |

For example, compound **3a** was prepared starting with 4-cyanobenzoic acid **4a**, which was coupled with 2,3,4,5-tetrahydro-1*H*-1-benzazepine **5** to give **7a**. The nitrile was reduced by hydrogenation to give **8a**. Finally the urea was prepared from 2,6-difluorophenylisocyanate **17** to afford the test compound **3a**.

The aromatic ureas in Table 2 (**18a**−**c**) and the aliphatic ureas in Table 3 (**19a**−**l**) were prepared from the common methyl substituted benzylamine intermediate **8e** (Scheme 2). Reaction with the appropriate isocyanate analogously to the preparation of **3e** provided **18a**, **18b**, and **19c**. Coupling of **8e** to the appropriate amine with CDI gave **19d**, **e**, and **j**. For the preparation of **18c**, diphenylphosphoryl azide was used to prepare the isocyanate in situ via a Curtius rearrangement. Carboxylic acid derivative **19a** was prepared by hydrolysis of glycine ester **19c**. **19a** was subsequently coupled to alcohols or

* To whom correspondence should be addressed. Phone: +44 23 8076 3440. Fax: +44 23 8076 6755. E-mail: chris.yea@vantiatherapeutics.com.
† Vantia Ltd.
‡ Ferring Research Ltd.
§ Ferring Research Institute Inc.

[a] Abbreviations: AVP, arginine vasopressin; CDI, 1,1′-carbonyldiimidazole; DAST, (diethylamino)sulfur trifluoride; DDAVP, 1-desamino-8-D-arginine-vasopressin; WSCDI, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide·HCl.

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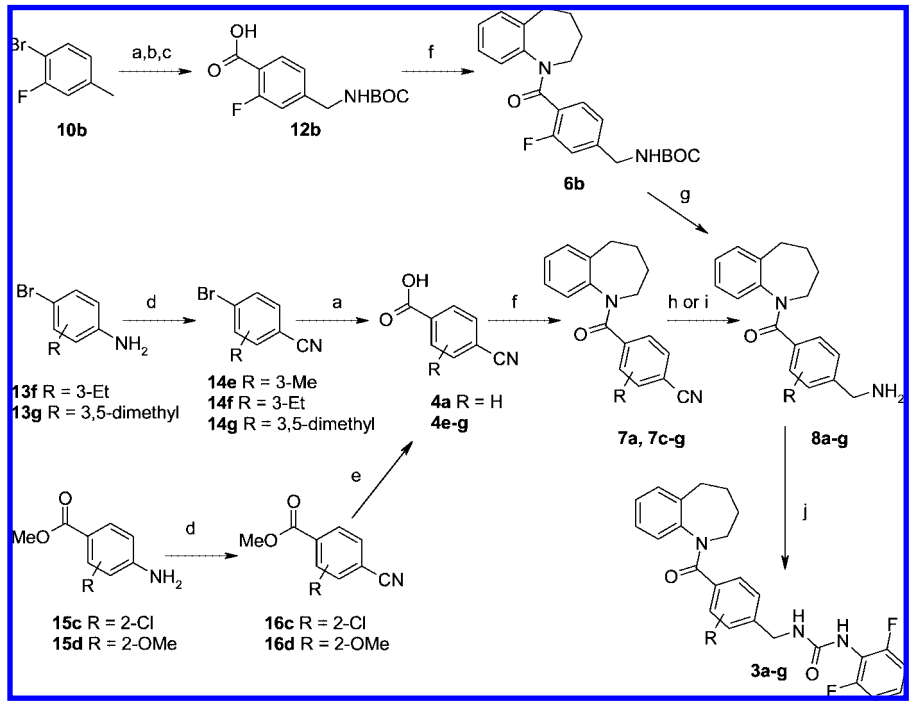
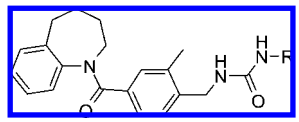
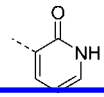
**Scheme 1**[a]




[a] Reagents and conditions: (a) $^{n}$BuLi, THF, $CO_2$, −78°C (56−73%); (b) $SOCl_2$, toluene, reflux, 2.5 h then MeOH, $CH_2Cl_2$ (77%); (c) *N*-bromosuccinimide, $CCl_4$ then $NH_3$, EtOH, $(BOC)_2O$, NaOH, dioxane, $H_2O$ (30%); (d) HCl, $NaNO_2$, $H_2O$, 0−5 °C then $Na_2CO_3$, CuCN, KCN, $H_2O$, 70 °C (50−69%); (e) LiOH, dioxane, $H_2O$ (95−97%); (f) 2,3,4,5-tetrahydro-1*H*-1-benzazepine **5**, DMAP, WSCDI, $Et_3N$, $CH_2Cl_2$, reflux (69−77%); (g) 4N HCl/dioxan (60%); (h) for the preparation of **8a**, **8e**, and **8f**, 10% Pd/C, HCl(aq), MeOH (74−98%); (i) for the preparation of **8c**, **8d**, and **8g**, $NaBH_4$, $CoCl_2 \cdot 6H_2O$ MeOH (57−69%); (j) 2,6-difluorophenylisocyanate **17**, $Et_3N$, $CH_2Cl_2$ (31−88%).

**Table 2.** Potencies of Compounds **18a−c** Compared to **3e**



| Compd | R | $EC_{50}$ (nM) | n |
|---|---|---|---|
| **3e** | 2,6-Difluorophenyl | 2.1 ± 1.7 | 6 |
| **18a** | 2-Fluorophenyl | 30 ± 42 | 6 |
| **18b** | Phenyl | 130 ± 120 | 6 |
| **18c** |  | 140 ± 40 | 3 |

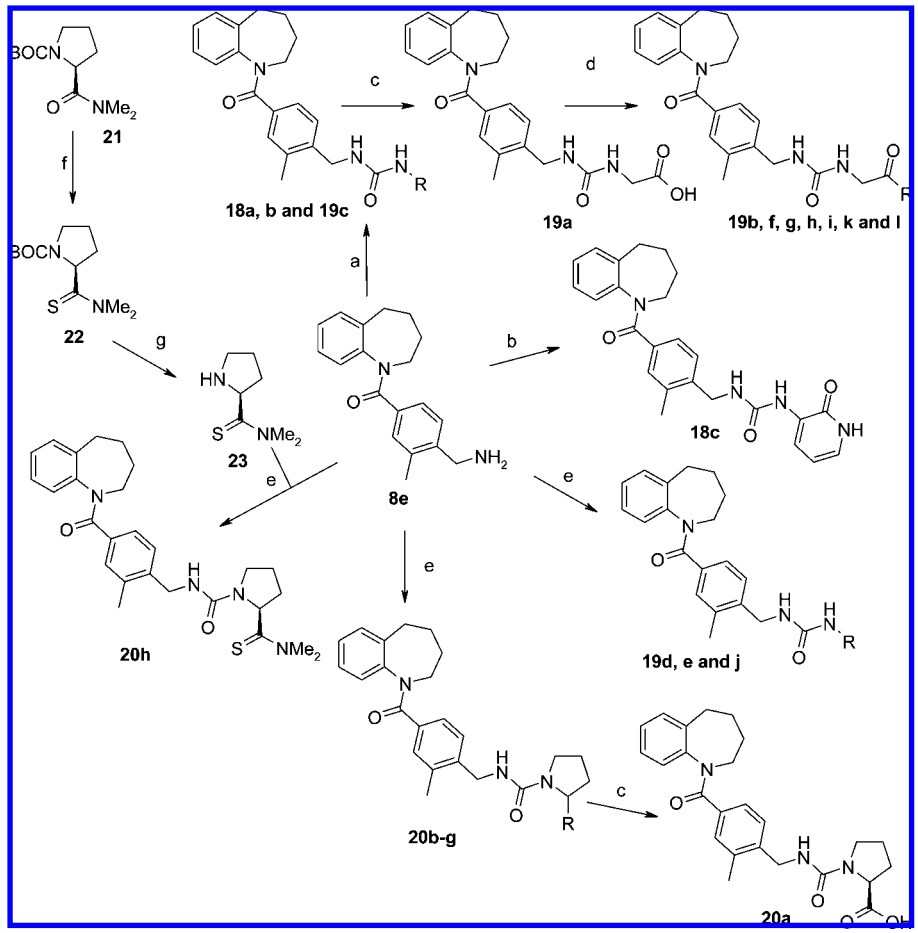
amines using established coupling procedures to yield the remaining compounds in Table 3.

The proline analogues **20a−g** and the thioamide proline analogue **20h** in Table 4 were also prepared from the common intermediate **8e** using CDI (Scheme 2). Compounds **20b−h** were prepared directly from the appropriate proline derivative, whereas the carboxylic acid **20a** was obtained by hydrolysis of the methyl ester **20b**. The starting prolines were available commercially for compounds **20b−e**. The proline derivatives required for the syntheses of compounds **20f** and **20g** were obtained by conventional methods (not shown in scheme). Specifically, BOC-L-proline was coupled with diethylamine and deprotected before coupling with **8e** to provide **20f**. BOC-D-proline was coupled with dimethylamine, deprotected, and coupled with **8e** to provide **20g**. The thioamide **20h** was prepared by treating BOC-proline-*N,N*-dimethylamide **21** with Lawesson's reagent to give **22**, followed by hydrogen chloride solution in 1,4-dioxane to give **23**. The usual coupling with **8e** gave **20h**.

The substituted proline derivatives **21a−h** shown in Table 5 were prepared according to Schemes 3, 4, and 5. Scheme 3 describes the routes that began with (4*R*)-BOC-4-hydroxyproline **24**. This allowed access to the hydroxyl analogue **21a**, by coupling of **24** to dimethylamine, followed by deprotection, then CDI coupling to the intermediate **8e** as described earlier. Subsequent derivatization to the ethyl ether **21c** was achieved with iodoethane. Oxidation of **21a** with Dess−Martin periodinane afforded the carbonyl derivative **21f**. Application of the Dess−Martin periodinane, this time to the hydroxyproline intermediate **25**, provided a substrate **27** suitable for fluorination with DAST. Subsequent deprotection and CDI coupling with **8e** provided the difluoro derivative **21g**. Scheme 4 describes the syntheses of the methyl, *tert*-butyl, and benzyl ether derivatives, **21b**, **21d**, and **21e**. The protected proline methyl ether **30b** is known and the protected benzyl and *tert*-butyl ethers **30e** and **33d** were commercially available.[16] These were coupled with dimethylamine, deprotected, and coupled to intermediate **8e** with CDI. Scheme 5 describes the synthesis of the dimethyl acetal derivative beginning with the known compound 4,4-dimethoxy-L-proline methyl ester, **35h**.[17] In this instance, the order of reactions was modified such that coupling with intermediate **8e** was undertaken first, followed by ester hydrolysis and coupling to dimethylamine.

The syntheses of compounds **22a−g** exploring the SAR about the azepine ring system (Table 6) are shown in Schemes 6, 7, and 8. Scheme 6 begins with the nitrile intermediate **7e** from Scheme 1. Oxidation of the 5-position of the benzazepine end

**Scheme 2**[a]




[a] Reagents and conditions: (a) R-NCO, $Et_3N$, $CH_2Cl_2$ (65−81%); (b) 2-hydroxynicotinic acid, diphenylphosphoryl azide, $Et_3N$, dioxane, reflux then **8e**, reflux, 18 h (43%); (c) LiOH, THF, $H_2O$ (46−99%); (d) coupling to alcohol or amine; (e) CDI, $^iPr_2NEt$, DMF, 90 min then RR′NH, $^iPr_2NEt$, DMF, 18 h (30−65%); (f) Lawesson's reagent, toluene, reflux, 2 h (70%); (g) 4N HCl/dioxane (100%).

group provided the carbonyl derivative **37a**. This was amenable to reduction, concomitantly with the nitrile reduction, to provide the hydroxyl substituted intermediate **38a**. Subsequent CDI coupling to the commercially available L-proline-*N*,*N*-dimethylamide provided the target hydroxylated compound **22a**. However, the diastereoisomeric products of this route were inseparable. The difluoro analogue **22c** was prepared in a similar sequence but with the inclusion of a DAST fluorination of the carbonyl intermediate **37a**. The 5-methylbenzazepine and the benzoxazepine end groups **41b** and **41d** were prepared from the corresponding tetralone **39b** and chromanone **39d**, respectively, as shown in Scheme 7. These were converted to their respective oximes and reduced with regiospecific rearrangement with DIBAL-H.[18] The remaining, tricyclic, top groups shown in Table 6 were prepared according to known procedures.[19−21] Scheme 8 shows the sequence for coupling top end groups **41b** and **41d**−**g** with 4-cyano-3-methylbenzoic acid **4e**, subsequent reduction, and finally the CDI coupling with L-proline-*N*,*N*-dimethylamide to yield the target compounds **22b** and **22d**−**g**. The diastereoisomers of **22b** were inseparable.

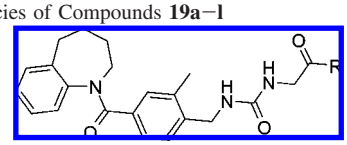
## Biological Assays

In vitro potency and relative efficacy data were obtained by expressing the V2 receptor in human embryonic kidney (HEK293) cells. On agonist stimulation the V2 receptor signals an increase in intracellular cAMP levels. These are amplified through the production of luciferase protein by cotransfected cAMP responsive gene elements ("reporter gene") and subsequently measured as luminescence. $EC_{50}$ values were determined and data are reported as geometric means ± standard deviation. In vivo data were obtained using Brattleboro rats, a strain of Long−Evans hooded rats that have no circulating AVP, thus constituting a model of diabetes insipidus.[22] These animals produce large volumes of dilute urine (approximately 10 mL/h), which were collected and measured over 1 h time periods. Following oral compound administration in carboxymethyl cellulose, voided urine volumes were measured and compared to vehicle-treated animals. Each treatment group contained at least six animals and data are expressed as means.

## Results and Discussion

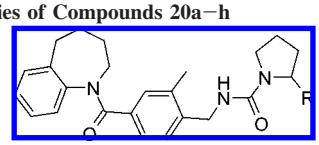
The first SAR to be investigated was around the central linker. The hit compound **3a**, with an unsubstituted aromatic linker, was potent ($EC_{50}$ = 47 nM) and fully efficacious in a human in vitro assay. However, in the equivalent rat assay, the maximum response was only 30% of the maximum AVP response (data not shown), rendering it unsuitable as a pharmacological tool. In an effort to improve the efficacy at the rat receptor and provide a pharmacological tool, a series of compounds with aromatic substituents was prepared. Table 1 shows the potencies of these compounds at the human V2 receptor. Addition of small substituents to either the 2- or the 3-position was generally well tolerated. In particular, small alkyl substituents such as methyl or ethyl improved potency significantly. The 3-methyl substitu-

**Table 3.** Potencies of Compounds **19a−l**



| Compound | R | $EC_{50}$ (nM) | n |
|---|---|---|---|
| **19a** | OH | 180 ± 50 | 3 |
| **19b** | OMe | 37 ± 33 | 6 |
| **19c** | OEt | 140 ± 220 | 6 |
| **19d** | O$^{i}$Pr | 140 ± 25 | 3 |
| **19e** | O$^{t}$Bu | 120 ± 50 | 3 |
| **19f** | OPh | 820 ± 230 | 3 |
| **19g** | $OCH_2Ph$ | 730 ± 80 | 3 |
| **19h** | $NH_2$ | 210 ± 50 | 3 |
| **19i** | $NHCH_2Ph$ | 270 ± 210 | 6 |
| **19j** | $NMe_2$ | 58 ± 35 | 6 |
| **19k** |  | 100 ± 28 | 3 |
| **19l** |  | 150 ± 125 | 6 |

**Table 4. Potencies of Compounds 20a−h**

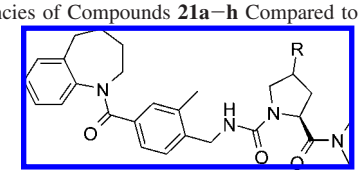


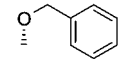
| Compound | R | $EC_{50}$ (nM) | *n* |
|---|---|---|---|
| **20a** | (*S*)-COOH | 680 ± 110 | 3 |
| **20b** | (*S*)-COOMe | 100 ± 75 | 6 |
| **20c** | (*S*)-COO$^{i}$Pr | 950 ± 350 | 3 |
| **20d** | (*S*)-COO$^{t}$Bu | 390 ± 80 | 3 |
| **20e** | (*S*)-$CONMe_2$ | 24 ± 40 | 6 |
| **20f** | (*S*)-$CONEt_2$ | 350 ± 140 | 3 |
| **20g** | (*R*)-$CONMe_2$ | 380 ± 140 | 3 |
| **20h** | (*S*)-$CSNMe_2$ | 3.2 ± 0.5 | 3 |

ent was highly potent (**3e**, $EC_{50}$ = 2.1 nM) and showed 20-fold improvement over **3a**. In addition, the substituted compounds showed full and potent agonism of the rat receptor in the in vitro assay. Using the highly potent **3e** as the lead for the next SAR study, the urea substituent was investigated (Tables 2, 3, and 4).

Removal of one of the fluorine substituents from the difluorophenylurea resulted in a 10-fold reduction in potency (compound **18a**, $EC_{50}$ = 30 nM). Removal of both fluorine substituents reduced potency a further 4-fold (compound **18b**, $EC_{50}$ = 130 nM). It was attempted to mimic the interaction, if any, of the fluorine with the receptor with an oxygen substituent. This was achieved through the pyridine analogue, **18c** ($EC_{50}$ = 140 nM), but it was equipotent with the unsubstituted phenyl group. There was extensive further work on the SAR of aromatic ureas, and while potent and efficacious agonists were designed, the compounds all suffered from limited aqueous solubility.

**Table 5.** Potencies of Compounds **21a−h** Compared to **20e**

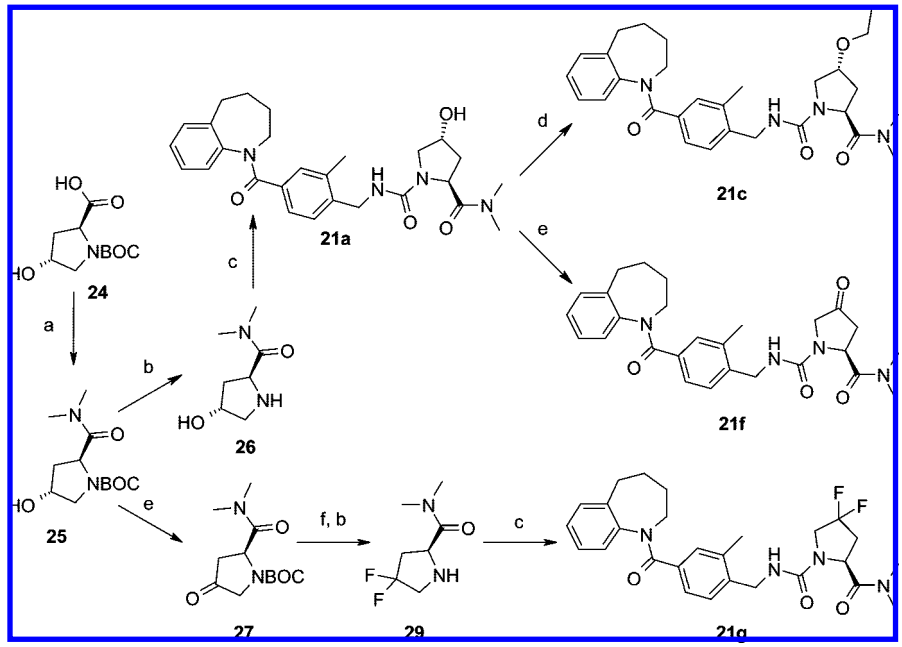


| Compound | R | $EC_{50}$ (nM) | n |
|---|---|---|---|
| **20e** | H | 24 ± 40 | 6 |
| **21a** |  | 2.3 ± 2.1 | 3 |
| **21b** |  | 4.7 ± 1.9 | 3 |
| **21c** |  | 4.3 ± 5.6 | 4 |
| **21d** |  | 46 ± 36 | 3 |
| **21e** |  | 10 ± 5 | 3 |
| **21f** |  | 27 ± 75 | 4 |
| **21g** |  | 50 ± 220 | 4 |
| **21h** |  | 20 ± 43 | 3 |

Alternative derivatizations were therefore designed with the aim of incorporating more hydrophilic groups (Table 3). The glycine derivative, **19a** ($EC_{50}$ = 180 nM), was targeted. Although incorporation of the acidic functionality contributed to a significant loss in potency compared to the difluorophenyl analogue **3e**, the compound retained maximum efficacy and was a useful lead for a new series of amino acid derivatives. Thus a series of esters (**19b−g**) and amides (**19h−l**) were prepared of increasing size. While small ester groups such as methyl, ethyl, *iso*-propyl, and *tert*-butyl (**19b−e**) displayed the highest potency, it was judged that these would likely be hydrolyzed rapidly to the acid in vivo. Incorporation of bulkier esters phenyl and benzyl (**19f−g**) resulted in a reduction in potency. The amides that were prepared retained potency compared to the glycine lead. In particular, the synthesis of **19j** ($EC_{50}$ = 58 nM) resulted in the desired improvement in solubility from <4 $\mu$g/mL to 177 $\mu$g/mL.

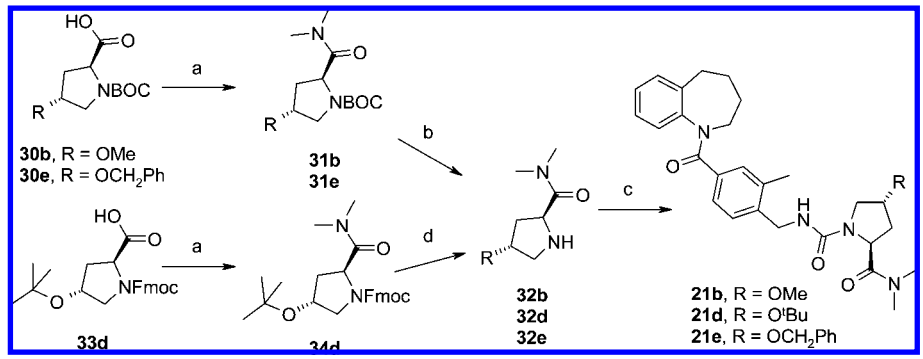
In vivo studies with **3e** and **19j** confirmed the improvement in oral activity hypothesized by increasing solubility. Treatment of Brattleboro rats with **3e** at 1 mg/kg po in methylcellulose had no effect on urine output over a 5 h period. However, treatment with **19j** at the same dose resulted in marked antidiuresis for 1−2 h.[12] Following that period, no reduction in

**Scheme 3**[a]



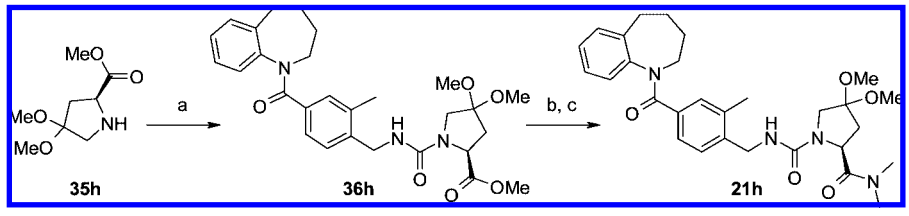

[a] Reagents and conditions: (a) $Me_2NH\cdot HCl$, DMAP, $^iPr_2NEt$, $CH_2Cl_2$ (100%); (b) 4N HCl/dioxane (17−100%); (c) CDI, $^iPr_2NEt$, DMF, 90 min then **8e**, $^iPr_2NEt$, DMF, 18 h (66−80%); (d) NaH, EtI, DMF (39%); (e) Dess−Martin periodinane, $CH_2Cl_2$ (32−50%); (f) DAST, $CH_2Cl_2$ (35%).

**Scheme 4**[a]




[a] Reagents and conditions: (a) $Me_2NH.HCl$, WSCDI, HOBT, $Et_3N$, $CH_2Cl_2$ (57−100%); (b) 4N HCl/dioxane (75−100%); (c) CDI, $^iPr_2NEt$, DMF, 90 min then **8e**, $^iPr_2NEt$, DMF, 18 h (24−75%); (d) piperidine, DMF (100%).

**Scheme 5**[a]

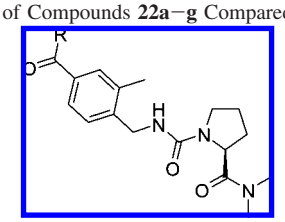


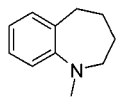
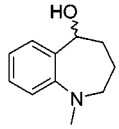
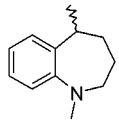
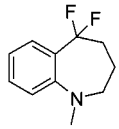
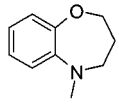
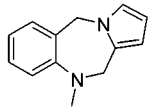
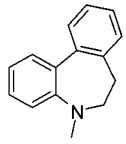
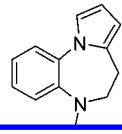

[a] Reagents and conditions: (a) **8e**, CDI, DMF (57%); (b), $LiOH\cdot H_2O$, THF, $H_2O$ (98%); (c) $Me_2NH\cdot HCl$, WSCDI, HOBT, $Et_3N$, $CH_2Cl_2$ (50%).

urine output was detected. It was further hypothesized that the urea functionality may be limiting intestinal permeability. We believed that if the number of hydrogen bond donors could be reduced then absorption may be increased, thus a series of cyclic proline derivatives was prepared (Table 4). The L-proline analogue (**20a**, $EC_{50}$ = 680 nM) showed a reduction in potency compared to the glycine analogue **19a** ($EC_{50}$ = 180 nM). Nonetheless, a series of small ester and amide derivatives was synthesized (**20b**−**20f**). These showed equivalent or improved potency compared to the carboxylic acid **20a**, with the most potent being the dimethylamide derivative **20e** ($EC_{50}$ = 24 nM). Having introduced a chiral center, the opposite, unnatural stereoisomer of this molecule was prepared (**20g**, $EC_{50}$ = 380 nM) but it showed more than 10-fold reduction in potency compared to the natural stereoisomer. Interestingly, the thioamide analogue **20h** ($EC_{50}$ = 3.2 nM) showed a large increase in potency and was the first nonaromatic compound to match the potency of **3e**. However, it was deemed unsuitable for progression because of the well-known toxicity risks associated with thioamides.

Further studies around **20e** were conducted in an effort to explore potency and drug-like properties. The first study explored the effect of substitution on the proline as shown in Table 5. Some of these analogues, substituted in the 4-position,

**Table 6.** Potencies of Compounds **22a**−**g** Compared to **20e**

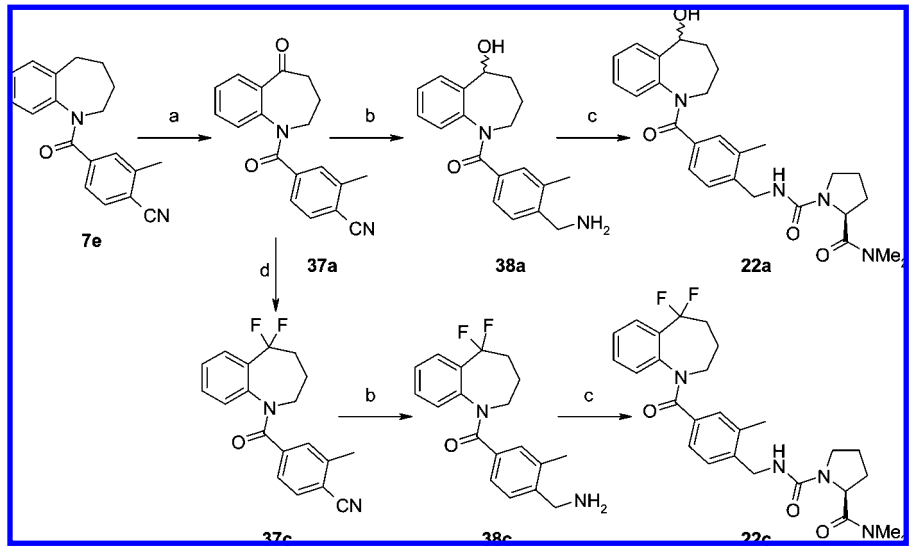


| Compound | R | $EC_{50}$ (nM) | n |
|---|---|---|---|
| **20e** |  | 24 ± 40 | 6 |
| **22a** |  | 230 ± 300 | 4 |
| **22b** |  | 220 ± 420 | 4 |
| **22c** |  | 1700 ± 500 | 4 |
| **22d** |  | 1000 ± 570 | 4 |
| **22e** |  | 120 ± 340 | 4 |
| **22f** |  | 84 ± 50 | 3 |
| **22g** |  | 140 ± 67 | 4 |

were highly potent compounds. For example, incorporation of a hydroxyl group to give **21a** ($EC_{50}$ = 2.3 nM) improved potency 10-fold over the unsubstituted compound **20e**. This provided a compound equipotent with the initial lead of **3e** but which had perceived advantages in terms of drug-likeness. Also, additions of small alkyl groups to provide the methyl ether **21b** ($EC_{50}$ = 4.7 nM) and the ethyl ether **21c** ($EC_{50}$ = 4.3 nM) were well tolerated, giving compounds equipotent with **21a** and **3e**. Larger alkyl ethers such as *tert*-butoxy (**21d**, $EC_{50}$ = 46 nM) and benzyloxy (**21e**, $EC_{50}$ = 10 nM) were slightly less well tolerated but were still equipotent with the unsubstituted proline **20e**. Finally, a number of derivatives were prepared that did not generate an additional chiral center. These included the ketone **21f** ($EC_{50}$ = 27 nM), the difluoro derivative **21g** ($EC_{50}$ = 50 nM), and the dimethylacetal **21h** ($EC_{50}$ = 20 nM). The data in Table 5 suggested that in order to gain potency, the additional chiral center would be required. A selection of compounds were tested in an equivalent in vitro functional reporter gene assay transfected with the rat V2 receptor. These data, shown in Table 7, showed modest improvements in potency in vitro at the rat receptor with compounds **21a**−**c** compared to **20e**. The Brattleboro rat model data shown in Table 7, measuring percentage inhibition of urine output, suggested approximately equivalent responses between these compounds. Thus, given no demonstrable superiority in vivo of any one of substituted proline analogues and given the risk of increased cost of goods of such compounds, it was decided to continue to pursue SAR studies with the unsubstituted dimethylprolinamide analogue, **20e**, as the lead.

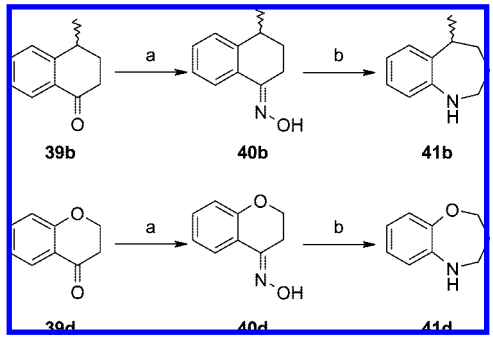
Thus far, all SAR studies had retained a benzazepine end group. Indeed, benzazepines and benzodiazepines have been the predominant scaffolds utilized by many groups for V2 ligands.[23] For example, a 1,4-benzodiazepine based end group features in the V2 agonist **2**.[11] Thus they have been termed privileged structures for vasopressin and oxytocin receptors.[13] To explore the SAR, we sought to utilize similar ring systems in new analogues of **20e**. In particular, we were aware of certain metabolic liabilities in benzazepine. For example, the azepine ring was known to be prone to metabolic hydroxylation although the precise position of hydroxylation had yet to be identified. It was speculated that it was most likely the benzylic 5-position, and we sought to block this with the analogues shown in Table 6. Compound **22a** is the diastereoisomeric mixture of the 5-hydroxylated derivative of the lead compound **20e**. The hydroxyl group was speculated to both reduce the metabolic liability of the end group and to mimic a putative metabolite. However, it demonstrated a 10-fold loss in potency ($EC_{50}$ = 230 nM), so it was decided not to pursue the separation of isomers. A similar conclusion was reached with the methylated derivative **22b** ($EC_{50}$ = 220 nM). The 5,5-difluorobenzazepine derivative **22c** ($EC_{50}$ = 1700 nM) and the 1,5-benzoxazepine derivative **22d** ($EC_{50}$ = 1000 nM) both retained good relative efficacy but were only weakly potent. Inclusion of pyrrole fused to the 3,4-position of the seven-membered ring provided **22e** ($EC_{50}$ = 120 nM), the exact analogue of the known V2 agonist **2**. Interestingly, **2** is reported to be a potent agonist with an $EC_{50}$ value of <1 nM.[11] However, the same end group, when applied to our benzylurea series, gave a reduced potency compared to either **2** or to our own lead compound **20e**. The reasons for this unexpected drop in potency may only be speculated. However, the known agonists **1** and **2** both possess relatively small aniline derived substituents in place of the urea substituents in our series. Also, it is reported that the steric requirements about this end of the molecule are rigorously limited and it may be supposed that urea derivatives of the published compounds would be too large to be tolerated.[10a] Perhaps, therefore, this difference is simply due to there being a different binding mode for our benzylureas compared to other, previously reported, small molecule V2 agonists. Also previously disclosed as having affinity for the vasopressin V2 receptor, albeit as end groups in compounds with antagonist activities, were the tricyclic ring systems exemplified within compounds **22f** ($EC_{50}$ = 84 nM) and **22g** ($EC_{50}$ = 140 nM).[20,21,24] All three tricyclic analogues prepared, **22e**, **22f**, and **22g**, were of similar potency. To summarize Table 6, it was found to be possible to incorporate a number of analogues of the benzazepine end group that retained potency. However, none showed any improvement in potency over benzazepine itself.

**Scheme 6**[a]




[a] Reagents and conditions: (a) $KMnO_4$, $Mg(NO_3)_2 \cdot 6H_2O$, $^tBuOH$, $H_2O$ (25%); (b) $CoCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH (31−60%); (c) CDI, DMF then L-proline-*N*,*N*-dimethylamide (20−86%); (d) DAST, $CH_2Cl_2$, reflux (25%).

**Scheme 7**[a]




[a] Reagents and conditions: (a) $NH_2OH \cdot HCl$, NaOH, EtOH (90%); (b) DIBAL-H, $CH_2Cl_2$ (63−86%).

These structures offer the possibility of alternative routes of metabolism and studies are underway to investigate this.

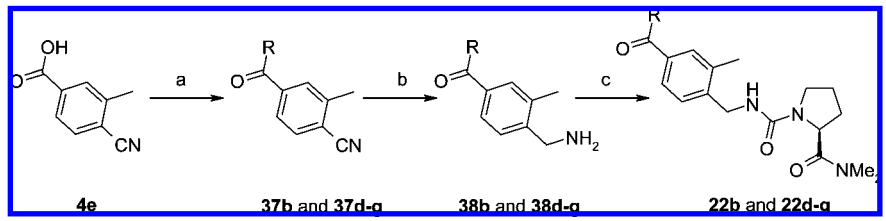
Despite extensive SAR work, there were no analogues prepared that showed any obvious advantages over **20e**. Its dose response curve in the reporter gene assay confirmed its activity at the human V2 receptor, showing 100% efficacy compared to the maximum response of AVP (Figure 2). The specificity of **20e** was confirmed by testing its activity in a sham transfection where the usual assay method was performed except no V2 receptor was transfected into the cells. No biological activity of **20e** in this assay was observed (data not shown). Also, no agonist activities were observed by **20e** or the earlier lead compounds **3a** and **3e** in reporter gene assays utilizing human V1a or V1b receptors at test concentrations up to 10 $\mu$M, thus demonstrating excellent functional selectivity of this series of compounds for the V2 receptor. Selectivity against the closely related oxytocin receptor was obtained through binding studies, which suggested no appreciable binding ($K_i > 10$ $\mu$M) of **3a**, **3e**, or **20e** to this receptor. SAR of V2 antagonists of a similar structural template has been reported to suggest that selectivity could be modified through judicious selection linker substituent. It was suggested a 2-chloro or 2-methyl substituent weakened V1a affinity and enhanced V2 affinity.[23,25] This is consistent with the previously reported V2 agonists **1** and **2**, which both bear a 2-chloro substituent. It is not possible to say whether the source of the V2 selectivity of the series of compounds reported here is driven by the linker substituent and its effect on binding affinity because SAR was driven by a functional assay. In fact, in contrast to the reported binding SAR of V2 antagonists, these compounds actually show enhanced agonist potency when the linker substituent was at the 3-position compared to the 2-position (Table 1). Interestingly, when **3a**, **3e**, or **20e** were tested for binding to the V2 receptor using a radioligand binding assay they showed only weak affinities with $K_i$ values ranging from 0.2 $\mu$M for **3e** ($n = 2$) to 2 $\mu$M for **20e** ($n = 3$). All of these observations are consistent with the lack of correlation between agonist potency and binding affinity previously reported for dopamine receptors.[26] Similarly to this example with dopamine receptors, rather than risk the use of potentially misleading binding data, we found that using SAR generated from a functional assay was the correct approach for optimization of these molecules.[27]

In terms of intestinal absorption, it had been earlier hypothesized that the urea functionality would be limiting. It was observed that in fact **20e** showed no improvement in Caco-2 cell permeability despite the reduced number of hydrogen bond donors compared to **19j** (data not shown). However, studies did show that **20e** was significantly more soluble than other analogues previously studied in animal models (7000 $\mu$g/mL). Thus it was submitted for in vivo study in Brattleboro rats (Figure 3). A reduction in urine volume after oral administration (3 mg/kg) was observed with almost full inhibition of urine output for 2 h. Toward the end of the study, 5 h after dosing, the cumulative volume output was observed to be returning to normal levels.

## Summary and Conclusion

The hit compound **3a** was identified as a novel and potent agonist at the human vasopressin V2 receptor. However, owing to selectivity across species, it was not suitable for study in rat models of antidiuresis. Addition of substituents to the aromatic linker provided compounds such as **3e** with improved efficacy and potency at both human and rat receptors. These suffered from poor aqueous solubility. Replacement of the aromatic urea with aliphatic ureas, such as the glycine amide in **19j**, gave compounds with improved solubility and improved oral efficacy, albeit with reduced potency in vitro. The proline derivative **20e**

**Scheme 8**[a]




[a] Reagents and conditions: (a) $SOCl_2$, toluene, reflux then amine **41b**, **d**, **e**, **f**, or **g**, $Et_3N$, $CH_2Cl_2$ (45−89%); (b) $CoCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH (52−76%); (c) CDI, DMF then L-proline-*N*,*N*-dimethylamide (29−70%).

**Table 7.** Potencies of Selected Compounds at the Rat V2 Receptor in Vitro and Reduction in Urine Production in Vivo (Brattleboro Rat Model)

| Compound | rat $EC_{50}$ (nM) $n = 1$ | rat $EC_{50}$ (nM) $n = 2$ | % inhibition of urine output; 1 mg/kg po; 1 h post dose |
|---|---|---|---|
| **3e** | 50 | 13 | 0 |
| **19j** | 100 | 50 | 82 |
| **20e** | 150[a] | | 81 |
| **21a** | 41 | | 68 |
| **21b** | 65[b] | | 84 |
| **21c** | 15 | 61 | 60 |

[a] Mean ($n = 24$). [b] Mean ($n = 4$).

showed yet further improvement in aqueous solubility and was used as a lead compound for further SAR studies. In vitro potency was increased further by addition of substituents onto the proline, but there was no parallel increase in in vivo potency. Substituted and tricyclic analogues of benzazepine were incorporated. These did not improve potency but may have potential if they usefully modify the metabolism profile. Thus, compound **20e** remained the lead compound, it is orally active in rat models, and it has been selected for further progression into clinical studies. Compound **20e** essentially mimics the V2 activity of AVP, thus it may be useful in indications where increased V2 activity would be beneficial, for example in diabetes insipidus. Generally, it may have clinical benefit as a treatment for indications where decreased urine output is desired.

## Experimental Section

$^1$H NMR spectra were recorded on a Jeol EX 270 (270 MHz) spectrometer with reference to deuterium solvent and at room temperature. Samples were dissolved in $CDCl_3$ unless stated otherwise. HRMS data were obtained on a Waters LCT Premier time-of-flight mass spectrometer. Samples were dissolved in 0.05% $HCO_2H$ in $H_2O$/MeCN (1:1), which were each combined with a solution of nifedipine and reserpine (reference compounds) in 0.05% $HCO_2H$ in $H_2O$/MeCN (1:1) and infused by MilliGAT pump into the ion source. Data is quoted for $MH^+$ unless otherwise stated. Elemental analyses were carried out by Medac Ltd. (Egham, UK). Compounds were routinely checked by TLC and/or by LCMS. TLC was carried out on Merck silica gel 60 $F_{254}$ precoated plates. LCMS was carried out using a Chromolith Speedrod RP-18e column, 50 mm × 4.6 mm, with a linear gradient 10% to 90% 0.1% $HCO_2H$/MeCN into 0.1% $HCO_2H/H_2O$ over 11 min, flow rate 1.5 mL/min. Data was collected using a Thermofinnigan Surveyor MSQ mass spectrometer with electospray ionization in conjunction with a Thermofinnigan Surveyor LC system. All target compounds were analyzed by HPLC and were found to be >95% pure. HPLC was carried out with a Varian Pursuit XRS-Diphenyl column, 50 mm × 4.6 mm, 5 μm particle size, with a linear gradient 10% to 90% 0.1% $CF_3CO_2H$/MeOH into 0.1% $CF_3CO_2H/H_2O$ over 25 min, flow rate 1.5 mL/min. UV detection was carried out at wavelength 254 nm. Full HPLC data and chromatograms are provided in Supporting Information. Chromatography refers to flash chromatography, which was carried out on Merck silica gel 60 (0.040−0.063 mm). All materials were commercially available unless otherwise noted. **3a**, **3b**, **3e**, **3g**, **4e**, **4g**, **6b**, **7a**, **7e**, **7 g**, **8a**, **8b**, **8e**, **8g**, **9b**, **11b**, **12b**, **14g**, **18c**, **19a**, **19b**, **19c**, and **19h** were prepared as described previously and **3c**, **3d**, **3f**, **7c**, **7d**, **7f**, **8c**, **8d**, **8f**, **18a**, and **18b** were prepared using analogous methods.[14] **4c**, **4d**, **4f**, **14f**, **16c**, and **16d** were prepared as described previously.[15]

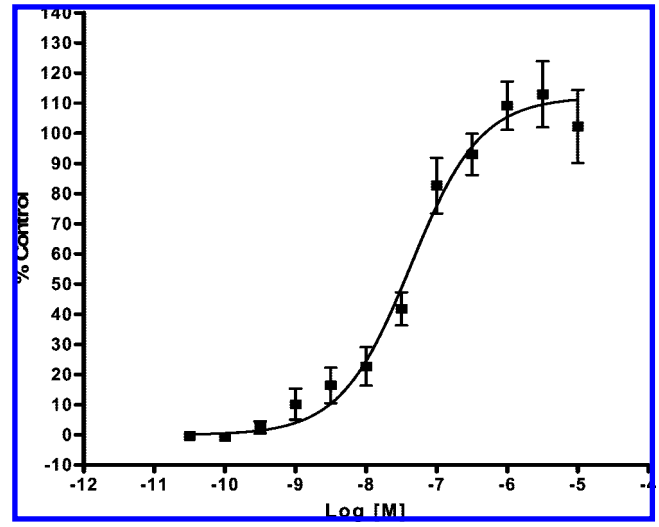



**Figure 2.** Dose response curve of **20e** at the human V2 receptor in the reporter gene assay. The control used is AVP and data are reported as the mean of data from six independent experiments, ± standard deviation. $EC_{50}$ = 24 nM.

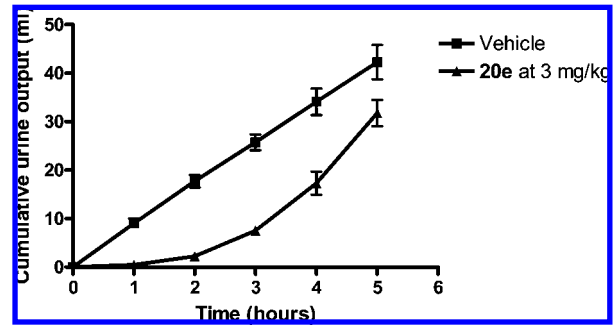



**Figure 3.** Antidiuretic action of compound **20e** dosed orally to Brattleboro rats.

**1-(4-[*N*-(2-Phenoxy-2-oxoethylcarbamoyl]aminomethyl]-3-methylbenzoyl)-2,3,4,5-tetrahydro-1*H*-1-benzazepine (19f).** To a solution of **19a** (0.134 g, 0.33 mmol) in $CH_2Cl_2$ (25 mL) were added phenol (0.155 g, 1.6 mmol), 4-(dimethylamino)pyridine (0.04 g, 0.33 mmol), and WSCDI (0.135 mg, 0.66 mmol). The mixture was stirred at reflux for 48 h and cooled. The mixture was washed with 1 M $KHSO_4$, saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude material was purified by chromatography (eluant EtOAc:pet. ether 80:20) to give a white solid; yield 0.038 g (33%). $^1$H NMR $\delta$ 1.40−1.60 (1H, m), 1.62−2.08 (6H, m), 2.66−3.08 (3H, m), 4.12 (2H, d, $J$ = 5.0 Hz), 4.19 (2H, d, $J$ = 5.6 Hz), 4.95 (1H, d, $J$ = 13.2 Hz), 5.62 (1H, br

s), 5.82 (1H, t, $J = 5.0$ Hz), 6.57 (1H, d, $J = 7.6$ Hz), 6.64−6.96 (4H, m), 6.98−7.14 (3H, m), 7.16−7.26 (2H, m), 7.28−7.42 (2H, m). HRMS: 472.2229 (calculated) and 472.2119 (observed).

**19g**, **19i**, **19k**, and **19l** were prepared using the same procedure as **19f**.

**1-(2-Methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzylcarbamoyl)-L-proline (20a).** To a solution of **20b** (0.176 g, 0.39 mmol) in THF (20 mL) and water (5 mL) was added $LiOH \cdot H_2O$ (0.038 g, 0.91 mmol). The mixture was stirred at room temperature for 4 h, concentrated in vacuo, and the residue diluted with water and washed with $Et_2O$. The aqueous layer was acidified to pH 1 by addition of 1 M HCl and extracted with EtOAc (3 times). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo to give a white solid; yield 0.119 g (70%). $^1$H NMR $\delta$ 1.35−1.52 (1H, m), 1.75−2.10 (6H, m), 2.05 (3H, s), 2.30−2.40 (1H, m), 2.60−3.00 (3H, m), 3.10−3.35 (2H, m), 4.20 (2H, d, $J = 5.2$ Hz), 4.30−4.36 (1H, m), 4.82−4.90 (1H, m), 5.24−5.30 (1H, m), 6.54 (1H, d, $J = 7.6$ Hz), 6.70−7.02 (6H, m), 7.13 (1H, d, $J = 6.9$ Hz). HRMS: 436.2235 (calculated) and 436.1905 (observed).

**1-(2-Methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzylcarbamoyl)-L-proline-*N*,*N*-dimethylamide (20e).** [14] To a solution of **8e** (0.10 g, 0.302 mmol) in DMF (10 mL), under a nitrogen atmosphere, were added *N*,*N*-diisopropylethylamine (0.043 g, 0.332 mmol) and CDI (0.074 g, 0.453 mmol). The mixture was stirred at room temperature for 40 min. A solution of proline-*N*,*N*-dimethylamide (0.107 g, 0.76 mmol) in DMF (1 mL) was added. The mixture was stirred at room temperature for a further 16 h. The solvent was removed in vacuo, and the crude material was purified by chromatography (eluant MeOH:$CH_2Cl_2$ 5:95) to give a white solid; yield 0.115 g (82%). $^1$H NMR $\delta$ 1.34−1.55 (1H, m), 1.70−2.20 (7H, m), 2.10 (3H, s), 2.60−3.10 (3H, m), 2.89 (3H, s), 3.04 (3H, s), 3.20−3.34 (1H, m), 3.40−3.58 (1H, m), 4.00−4.18 (1H, m), 4.28−4.45 (1H, m), 4.58−4.84 (2H, m), 4.95 (1H, d, $J =$ 13.1 Hz), 6.56 (1H, d, $J = 7.4$ Hz), 6.73−6.89 (2H, m), 6.90−7.08 (3H, m), 7.15 (1H, d, $J = 7.4$ Hz). HRMS: 463.2708 (calculated) and 463.2450 (observed). Anal. ($C_{27}H_{34}N_4O_3 + 0.25\ H_2O$) C, H, N.

**19d**, **19e**, **19j**, **20b**, **20c**, **20d**, **20f**, **20g**, **20h**, **21a**, **21b**, **21d**, **21e**, and **21g** were prepared using the same procedure as **20e**.

**(4*R*)-4-Ethoxy-1-(2-methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzyl-carbamoyl)-L-proline-*N*,*N*-dimethylamide (21c).** To a solution of **21a** (0.080 g, 0.17 mmol) in DMF (10 mL) at −5 °C was added NaH (60%, 8 mg, 2.0 mmol). The mixture was stirred for 20 min, iodoethane (0.155 g, 10 mmol) was added and was allowed to warm to room temperature and stirred for 3 days. The mixture was reduced in vacuo. The residue was purified by chromatography (eluant $CHCl_3$:MeOH:acetic acid 98:1:1) and freeze-dried to give a white solid; yield 0.034 g (39%). $^1$H NMR $\delta$ 1.16 (3H, t, $J = 7.2$ Hz), 1.35−1.55 (1H, m), 1.85−2.13 (5H, m), 2.14 (3H, s), 2.66−3.16 (3H, m), 2.91 (3H, s), 3.11 (3H, s), 3.20−3.30 (1H, m), 3.44 (2H, q, $J = 7.4$ Hz), 3.65−3.68 (1H, t, $J = 6.7$ Hz), 4.05−4.20 (1H, m), 4.25−4.39 (3H, m), 4.81−5.10 (2H, m), 6.61 (1H, d, $J = 7.7$ Hz), 6.80−7.00 (3H, m), 7.07−7.17 (2H, m), 7.20−7.24 (1H, m). HRMS: 507.2973 (calculated) and 507.2576 (observed).

**1-(2-Methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzyl-carbamoyl)-4-oxo-L-proline-*N*,*N*-dimethylamide (21f).** To a solution of **21a** (0.080 g, 0.20 mmol) in $CH_2Cl_2$ (20 mL) at 0 °C was added Dess−Martin periodinane (0.176 g, 0.50 mmol). The mixture was allowed to warm to room temperature and stirred for 18 h. The mixture was adsorbed onto silica gel. Chromatography (eluant $CHCl_3$:MeOH:acetic acid 97:2:1) afforded a white solid; yield 0.040 g (50%). $^1$H NMR $\delta$ 1.38−1.70 (1H, m), 1.86−2.18 (3H, m), 2.15 (3H, s), 2.45 (1H, d, $J = 17.6$ Hz), 2.66−3.10 (4H, m), 2.93 (3H, s), 3.20 (3H, s), 3.65 (1H, d, $J = 15.6$ Hz), 3.95 (1H, d, $J = 15.6$ Hz), 4.14−4.30 (1H, m), 4.32−4.48 (2H, m), 4.98 (1H, d, $J = 13.2$ Hz), 5.33 (1H, d, $J = 7.7$ Hz), 6.61 (1H, d, $J = 7.9$ Hz), 6.78−6.96 (3H, m), 7.00−7.28 (3H, m). HRMS: 477.2499 (calculated) and 477.2122 (observed).

**BOC-proline-*N*,*N*-dimethylthioamide (22).** A solution of BOC-proline-*N*,*N*-dimethylamide (**21**) (0.85 g, 3.51 mmol) and Lawesson's reagent (0.78 g, 1.93 mmol) in toluene (30 mL) was stirred at reflux for 2 h. The mixture was cooled, diluted with EtOAc, washed with 0.5 M $KHSO_4$, saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and reduced in vacuo. The crude material was purified by chromatography (eluant EtOAc:pet. ether 50:50) to give a colorless gum; yield 0.63 g (70%).

**Proline-*N*,*N*-dimethylthioamide (23).** A solution of **22** (0.63 g, 2.44 mmol) in 4N HCl/dioxan was stirred for 90 min. The mixture was reduced in vacuo to give a white solid that was used without further purification; yield 0.52 g.

**27** was prepared using the same procedure as **21f**.

**BOC-4,4-difluoroproline-*N*,*N*-dimethylamide (28).** To a solution of **27** (0.30 g, 1.17 mmol) in $CH_2Cl_2$ at 0 °C was added DAST (155 $\mu$L, 0.189 g, 1.17 mmol). The mixture was stirred for 4 h. A further amount of DAST (78 $\mu$L, 0.095 g, 0.59 mmol) was added and allowed to warm to room temperature. The mixture was stirred for 18 h, and a trace of water was added. The mixture was reduced in vacuo and adsorbed onto silica gel. Chromatography (eluant EtOAc:pet. ether 50:50) afforded a yellow gum; yield 0.11 g (35%).

**4-Difluoroproline-*N*,*N*-dimethylamide hydrochloride (29). 28** (0.11 g, 0.40 mmol) was reacted with 4N HCl/dioxan (20 mL) following the method of **26** and used immediately in the next step without purification.

**4,4-Dimethoxy-1-(2-methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzyl-carbamoyl)-L-proline methyl ester (36h).** To a solution of **8e** (0.281 g, 0.90 mmol) in DMF (15 mL) at 0 °C was added CDI (0.176 g, 0.90 mmol). The mixture was allowed to warm to room temperature and stirred for 50 min. 4,4-Dimethoxy-L-proline methyl ester (**35h**)[17] (0.180 g, 0.90 mmol) was added, and the mixture was stirred at room temperature for a further 16 h. The solvent was removed in vacuo, and the crude material was purified by chromatography (eluant MeOH:$CH_2Cl_2$ 2:98) to give a white solid; yield 0.260 g (57%).

**4,4-Dimethoxy-1-(2-methyl-4-(2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)benzyl-carbamoyl)-L-proline-*N*,*N*-dimethylamide (21h).** To a solution of the methyl ester **36h** (0.230 g, 0.45 mmol) in water (5 mL) and THF (15 mL) was added $LiOH \cdot H_2O$ (56 mg, 1.4 mmol) and stirred for 18 h. Water (20 mL) was added and the mixture washed with $Et_2O$. The aqueous layer was acidified with 2 M citric acid solution and extracted with EtOAc. The organic phase was washed with water, brine, dried over $MgSO_4$, and reduced to give the carboxylic acid as a white solid; yield 219 mg (98%). To a solution of the carboxylic acid (0.209 g, 0.42 mmol) in $CH_2Cl_2$ (10 mL) were added WSCDI (0.101 g, 0.50 mmol) and hydroxybenzotriazole (0.057 g, 0.46 mmol). The mixture was stirred and to it was added $Et_3N$ (150 $\mu$L, 0.105mmol). After 10 min, $Me_2N \cdot HCl$ (0.086 g, 0.105 mmol) was added. The mixture was stirred for 18 h and adsorbed onto silica gel. Chromatography (eluant MeOH:$CH_2Cl_2$ 3:97) gave a glassy solid; yield 0.11 g (50%). $^1$H NMR $\delta$ 1.38−1.58 (1H, m), 1.82−2.18 (3H, m), 2.21 (3H, s), 2.36 (1H, dd, $J = 7.9$, 12.1 Hz), 2.60−3.26 (4H, m), 2.88 (3H, s), 3.07 (3H, s), 3.17 (3H, s), 3.21 (3H, s), 3.45 (1H, d, $J = 9.6$ Hz), 3.54 (1H, d, $J = 9.6$ Hz), 4.02−4.18 (1H, m), 4.28−4.44 (1H, m), 4.46−4.64 (1H, m), 4.76 (1H, t, $J = 8.2$ Hz), 4.97 (1H, d, $J =$ 13.6 Hz), 6.60 (1H, d, $J = 7.7$ Hz), 6.76−6.98 (3H, m), 7.00−7.10 (2H, m), 7.18 (1H, d, $J = 6.7$ Hz). HRMS: 523.2923 (calculated) and 523.2510 (observed).

**1-(4-Cyano-3-methylbenzoyl)-5-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepine (37a).** To a stirred suspension of **7e** (4.00 g, 13.78 mmol) in *tert*-butanol (120 mL) and water (300 mL) were added $KMnO_4$ (15.25 g, 96.50 mmol) and $Mg(NO_3)_2 \cdot 6H_2O$ (24.68 g, 96.25 mmol). The mixture was stirred for 72 h and diluted with 3 N HCl(aq) (100 mL). Sodium bisulfite was added portionwise until a clear colorless solution was obtained. The mixture was extracted with $CH_2Cl_2$. The organic extract was washed with saturated brine, dried over $MgSO_4$ and reduced. The residue was purified by chromatography (eluant EtOAc:pet. ether 40:60) to give a pale-orange gum; yield 1.06 g (25%).

**(±)-1-(4-Aminomethyl-3-methylbenzoyl)-5-hydroxy-2,3,4,5-**

**tetrahydro-1*H*-1-benzazepine (38a).** To a solution of **37a** (0.450 g, 1.48 mmol) and $CoCl_2 \cdot 6H_2O$ (706 mg, 2.97 mmol) in MeOH (40 mL) was added $NaBH_4$ (0.573 g, 15.15 mmol) portionwise. The mixture was stirred for 90 min, acidified with 1 N HCl(aq), and reduced in vacuo. The residue was taken up in water, washed with $Et_2O$, basified with saturated $NaHCO_3$ solution, and extracted with $CHCl_3$. The organic extract was washed with saturated brine, dried over $MgSO_4$, and reduced to give a white solid; yield 0.142 g (31%).

**38b**, **38c**, **38d**, **38e**, **38f**, **38g** were prepared using the same procedure as **38a**.

**1-(4-(5-Hydroxy-2,3,4,5-tetrahydro-1-benzazepin-1-ylcarbonyl)-2-methylbenzylcarbamoyl)-L-proline-*N*,*N*-dimethylamide (22a).** To a solution of **38a** (0.142 g, 0.46 mmol) in DMF (10 mL), under a nitrogen atmosphere, was added CDI (0.083 g, 0.52 mmol). The mixture was stirred at room temperature for 1 h. A solution of L-proline-*N*,*N*-dimethylamide (0.107 g, 0.76 mmol) in DMF (1 mL) was added. The mixture was stirred at room temperature for a further 4 h, and the solvent was removed in vacuo. The residue was taken up in EtOAc, washed with 0.3 M $KHSO_4$, saturated brine, dried over $MgSO_4$, and reduced in vacuo. Chromatography (eluant $CHCl_3$:MeOH 92:8) gave a white solid; yield 0.045 g (20%). HPLC data showed evidence of diastereoisomers, isomer A 15.86 min (35.2%) and isomer B (61.3%). NMR data showed evidence of diastereoisomers. $^1$H NMR $\delta$ 1.50−2.25 (11H, m), 2.64−2.90 (1H, m), 2.83 and 2.85 (total 3H, each s), 3.01 (3H, s), 3.27−3.50 (1H, m), 3.54−3.66 (1H, m), 3.98−4.30 (3H, m), 4.60−5.05 (3H, m), 6.48−6.60 and 6.70−7.20 (total 7H, each m), 7.60 (1H, d, $J = 7.4$ Hz). HRMS: 479.2658 (calculated) and 479.2288 (observed).

**22b**, **22c**, **22d**, **22e**, **22f**, and **22g** were prepared using the same procedure as **22a**.

**1-(4-Cyano-3-methylbenzoyl)-5,5-difluoro-2,3,4,5-tetrahydro-1*H*-1-benzazepine (37c).** To a solution of **37a** (0.60 g, 1.97 mmol) in $CH_2Cl_2$ (20 mL) at 0 °C was added DAST (522 $\mu$L, 3.95 mmol). The mixture was allowed to warm to room temperature and stirred for 18 h. Additional amounts of DAST were successively added to the mixture and heated at reflux until TLC indicated a significant amount of product had formed. The mixture was cooled to room temperature and poured into water (5 mL). Saturated $NaHCO_3$(aq) was added slowly until effervescence ceased. The mixture was extracted with $CH_2Cl_2$. The organic phase was washed with water, dried over $MgSO_4$, and reduced in vacuo. Chromatography (eluant EtOAc:pet. ether 30:70) afforded a white solid; yield 164 mg, (25%).

**(±)-4-Methyl-1-tetralone oxime (40b).** To a solution of (±)-1-methyl-4-tetralone (**39b**) (2.0 g, 0.012 mol) and $NH_2OH \cdot HCl$ (1.388 g, 0.020 mol) in EtOH was added a solution of NaOH (2.4 g, 0.060 mol) in water (2.0 mL). The mixture was stirred at reflux for 2 h, cooled, poured into hydrochloric acid, and stirred for 30 min. The precipitate was filtered and dried to give a yellow solid; yield 1.90 g (90%).

**40d** was prepared using the same procedure as **40b**.

**(±)-5-Methyl-2,3,4,5-tetrahydro-1*H*-1-benzazepine (41b).** To a solution of DIBAL-H (4.7 mL, 0.026 mol) in $CH_2Cl_2$ (20 mL) under $N_2$ was added slowly a solution of **40b** (1.9 g, 0.0108 mol) in $CH_2Cl_2$ (10 mL) so as to achieve a gentle reflux. The mixture was stirred at room temperature for 18 h. KF (11.2 g, 0.176 mol) was added portionwise (CAUTION) and the mixture stirred for 10 min. Water was added dropwise (CAUTION), and the mixture stirred for 20 min. The mixture was poured into $CH_2Cl_2$ (200 mL), stirred for 30 min, filtered, and reduced in vacuo. The residue was purified by chromatography (eluant EtOAc:pet. ether. 10:90) to afford a pale-yellow oil; yield 1.20 g (86%).

**41d** was prepared using the same procedure as **41b**.

**5-(4-Cyano-3-methylbenzoyl)-2,3,4,5-tetrahydro-1,5-benzoxazepine (37d).** To a solution of **4e** (2.16 g, 13.4mmol) in toluene (20 mL) was added $SOCl_2$ (2.93 mL, 40.2 mmol). The mixture was heated at reflux for 90 min and reduced in vacuo. The residue was taken up in $CH_2Cl_2$ (5.0 mL) and added to a solution of **41d** (2.0 g, 13.4 mmol) and $Et_3N$ (3.74 mL, 26.8 mmol) in $CH_2Cl_2$ (2.0 mL) at 0 °C. The mixture was allowed to warm to room temperature, stirred for 18 h, and reduced in vacuo. The residue was taken up in $CHCl_3$ and washed with 0.3 M $KHSO_4$(aq), $NaHCO_3$(aq), and brine. The organic phase was dried over $MgSO_4$ and reduced to afford a brown solid; yield 3.50 g (89%).

**37b**, **37e**, **37f**, and **37g** were prepared using the same procedure as **37d**.

**CRE-Luciferase Reporter Gene Assay. Cell Transfection.** The human V2 receptor was cloned from kidney total RNA using the RT-PCR. The human receptor was subcloned into pcDNA5/FRT (InVitrogen). The cAMP response element and the synthetic Firefly Luciferase gene *Luc2* were provided in the pGL4.29-vector (Promega). Plasmids were amplified in Novablue *Escherichia coli* (Merck Biosciences Ltd.) and plasmid DNA was purified using UltraMobius Endotoxin free plasmid purification kits (Merck Biosciences Ltd.).

**Cell Culture.** Human embryonic kidney (HEK293) cells (ECCAC, UK) were maintained in Dulbecco's modified Eagles medium without phenol red, supplemented with 50000 U penicillin, 50 mg streptomycin, and 10% fetal calf serum (InVitrogen). Cells were grown to 70−80% confluency and transiently transfected with the V2 receptor and pGL4 CRE-Luc reporter constructs using Genejuice (Merck Biosciences Ltd.). These transfected cells were incubated at 37 °C with 5% $CO_2$ for 24 h before plating out at 10000 cells/well into 96 well white Optiplates (Perkin-Elmer). The plates were incubated for a further 24 h at 37 °C with 5% $CO_2$ before testing for functional agonism.

**Functional Assays.** Compounds were prepared in DMSO to a concentration of 10 mM and 0.5 log serially diluted in duplicate to give the appropriate concentrations when added to the cells. Final DMSO concentrations were 0.1%. Levels of luciferase synthesis were measured following 5 h incubation at 37 °C using SteadyGlo luciferase reagent (Promega). The data were expressed as percentage of the 100 nM Arginine-Vasopressin (AVP) (Sigma no. V-9879) controls and then plotted against the log of the concentration. The data were fitted to a 4-parameter logistic equation and $EC_{50}$ values determined. Data are reported as geometric means ± standard deviation.

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**Supporting Information Available:** Routine experimental and spectroscopic data, elemental analyses on selected compounds, and HPLC traces on target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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