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# Identification and optimization of novel 2-(4-oxo-2-aryl-quinazolin-3(4*H*)-yl)acetamide vasopressin V3 (V1b) receptor antagonists

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ABSTRACT

The discovery, synthesis, and preliminary structure–activity relationship (SAR) of a novel class of vasopressin V3 (V1b) receptor antagonists is described. Compound **1**, identified by high throughput screening of a diverse, three million-member compound collection, prepared using ECLiPS™ technology, had good activity in a V3 binding assay ($IC_{50}$ = 0.20 μM), but less than desirable physicochemical properties. Optimization of compound **1** yielded potent analogs **19** ($IC_{50}$ = 0.31 μM) and **24** ($IC_{50}$ = 0.12 μM) with improved drug-like characteristics.



A majority of patients suffering from severe depressive disorder exhibit a profound alteration in their ability to regulate the hypothalamic–pituitary–adrenal (HPA) axis—the major route via which humans (and other mammals) cope with, and adapt to stress.[1] Arginine vasopressin (AVP) is thought to play a pivotal role in HPA axis regulation, particularly in situations of chronic stress.[2] AVP mediates its effects via binding to the V3 (V1b) receptor expressed upon pituitary corticotropes. Through this mechanism, AVP acts synergistically with corticotropin releasing hormone (CRH) to elicit release of adrenocorticotropic hormone (ACTH), thus positively driving the HPA axis. Much evidence indicates that while CRH may be relevant in orchestrating HPA responsivity to acute stress,[3] AVP-induced activity of V3 receptors may be the more important modulator during chronic stress. This in turn may be more clinically relevant to the development of affective disorders.[2,4]

Therefore, a selective antagonist of the V3 receptor represents a therapeutically relevant approach for treatment of the dysfunctional HPA axis in depressive illness. In fact, a previously reported V3 selective antagonist, SSR149415, has been demonstrated to be active in animal models predictive of antidepressant and anxiolytic activity,[5,6] and this compound advanced into phase IIb clinical trials.[7]

In a program aimed at the identification of a novel and structurally distinct class of V3 antagonists, high-throughput screening (HTS) of a diverse three million-member compound collection, prepared using ECLiPS™ technology,[8] was undertaken. The compounds were screened utilizing a 384-well whole cell binding displacement assay using tritium labeled arginine vasopressin ([$^3$H]-AVP).[9] Multiple active structures, or hits, were identified in this HTS campaign. Among these hits was compound **1** (Fig. 1), possessing an $IC_{50}$ value of 0.20 μM. Compound **1** was chosen as a starting point for a hit-to-lead (HtL) program, with the task of evaluating this hit class in terms of both target independent (e.g., physicochemical properties) and target dependent (e.g., potency, selectivity, etc.) criteria.[10]

The main objective of this HtL program was to generate a lead compound with appropriate potency and drug-like physicochemical properties[11] to serve as the basis for further lead optimization efforts. One immediate concern about compound **1**, was that from a perspective of 'drug likeness' it possessed less than optimal physicochemical properties such as high molecular weight ($M_W$), high 'fast polar surface area' (FPSA), a high degree of lipophilicity, and a high degree of conformational flexibility ($M_W$ = 613, FPSA = 148.48 Å$^2$, *A* log *P* = 4.73, # of rotatable bonds = 17).[11,12]

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† In 2008, Pharmacopeia, Inc. was acquired by Ligand Pharmaceuticals, Inc.

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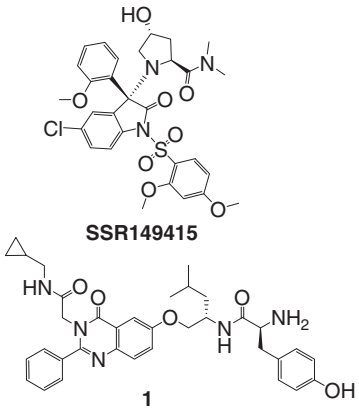


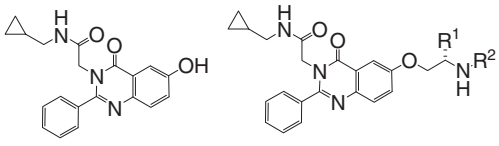

**Figure 1.** V3 antagonists.

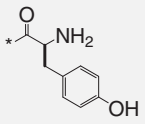
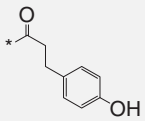
A calculation of the binding energy of compound **1** by Andrew's analysis[13] gave a significantly higher binding energy ($\Delta G_{calc}$ = 18.3 kcal/mol) compared to the experimental value ($\Delta G_{obs}$ = 9.2 kcal/mol). This large difference suggested that not all parts of the molecule were optimally involved in binding to the receptor. This indicated the possibility for deletions and alterations to the structure without a major decrease in binding affinity. We reasoned that the molecule could be greatly simplified, by strategic deletion of unnecessary structural elements, to deliver a potent and tractable lead compound with improved physicochemical properties. In this report, we describe the synthesis and SAR of novel V3 antagonists resulting in the identification of potent lead compounds with improved physicochemical properties versus compound **1**.

The compounds detailed in Tables 1–3 were synthesized via the outlined general syntheses (Schemes 1–3). The synthesis of the penultimate phenol intermediate **2** has been described elsewhere.[14]

As depicted in Scheme 1, a Mitsunobu reaction[15] between phenol **2** and either Boc-L-leucinol or Boc-2-aminoethanol followed by Boc deprotection gave amines **3** and **4**, respectively. Amine **3** was then coupled with Boc-protected tyrosine which, followed by Boc deprotection, yielded compound **1**. In a similar fashion both amines **3** and **4** were coupled with Boc-protected glycine and deprotected to yield compounds **5** and **6**. Amine **4** was also coupled with 3-(4-hydroxyphenyl)propanoic acid giving compound **7**. As

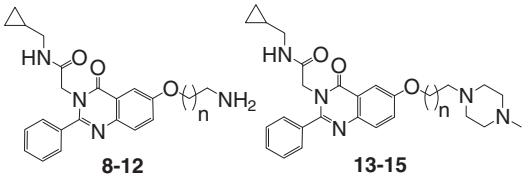
**Table 1**
Simplification/truncation of right-hand side




| Compds | $R^1$ | $R^2$ | hV3 $IC_{50}$[a] (μM) |
|---|---|---|---|
| **1** |  |  | 0.2 (±0.07) |
| **5** |  |  | 5.1 (±3.3) |
| **6** | H |  | 30.5 (±0.7) |
| **7** | H |  | >100 |
| **2** | | | >100 |
| **3** |  | H | 2.1 (±0.1) |

[a] Values are means of at least two experiments, standard deviation is given in parentheses.

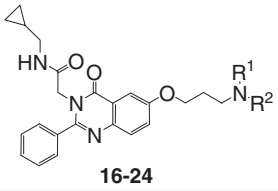
**Table 2**
Exploration of chain length between amino functionality and scaffold

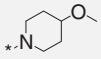
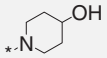



| Compds | *n* | hV3 $IC_{50}$[a] (μM) |
|---|---|---|
| **8** | 1 | 4.0 (±1.6) |
| **9** | 2 | 3.6 (±0.2) |
| **10** | 3 | >30 |
| **11** | 4 | >30 |
| **12** | 5 | >100 |
| **13** | 1 | 22[b] |
| **14** | 2 | 1.7 (±0.6) |
| **15** | 3 | 4.8 (±1.0) |

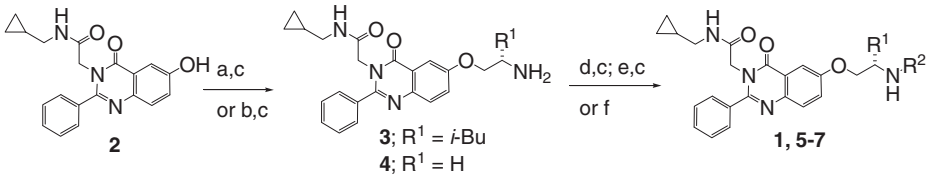
[a] Values are means of at least two experiments unless otherwise indicated, standard deviation is given in parentheses.
[b] Value was obtained from a single experiment.

**Table 3**
*N*-Methylpiperazine replacements

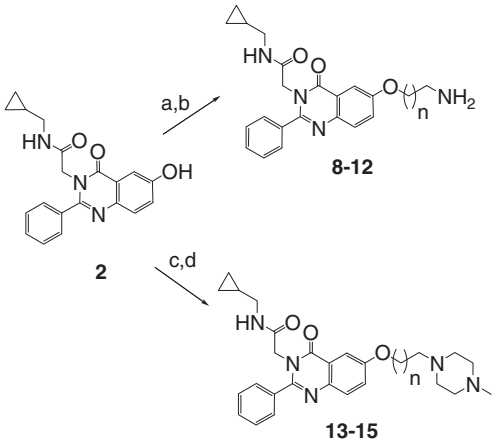



| Compds | $NR^1R^2$ | hV3 $IC_{50}$[a] (μM) |
|---|---|---|
| **16** |  | 25.8 (±2.1) |
| **17** | NHMe | 7.6 (±0.6) |
| **18** | $N(Et)_2$ | 3.8 (±1.9) |
| **19** |  | 0.31 (±0.15) |
| **20** |  | 1.8 (±1.0) |
| **21** |  | 0.34 (±0.11) |
| **22** |  | 0.71 (±0.13) |
| **23** |  | 1.1 (±0.02) |
| **24** |  | 0.12 (±0.046) |

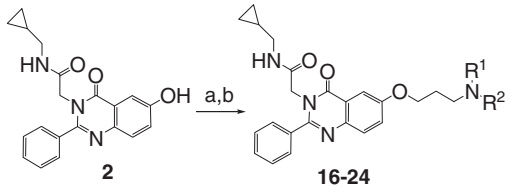
[a] Values are means of at least two experiments, standard deviation is given in parentheses.




**Scheme 1.** Reagents and conditions: (a) *N*-Boc-2-aminoethanol, DIAD, $PPh_3$, THF, 0–23 °C; (b) *N*-Boc-L-leucinol, DIAD, $PPh_3$, THF, 0–23 °C; (c) 50% TFA/DCM; (d) *N*-Boc-L-tyrosine, EDCI, HOBt, DMF; (e) Boc-glycine, EDCI, HOBt, DMF; (f) 3-(4-hydroxyphenyl)propanoic acid, EDCI, HOBt, DMF.




**Scheme 2.** Reagents and conditions: (a) $HO(CH_2)_nNHBoc$, DIAD, $PPh_3$, THF, 0–23 °C; (b) 50% TFA/DCM; (c) $Br(CH_2)_nBr$, $K_2CO_3$, $CH_3CN$, reflux; d) *N*-methylpiperazine, $K_2CO_3$, $CH_3CN$, reflux.




**Scheme 3.** Reagents and conditions: (a) 1,3-dibromopropane, $K_2CO_3$, $CH_3CN$, reflux; (b) $HNR^1R^2$, $K_2CO_3$, $CH_3CN$, reflux.

depicted in Scheme 2, Mitsunobu reaction of the phenol **2** with the appropriate Boc-protected hydroxyalkylamines followed by Boc deprotection yielded compounds **8–12**. Alternatively, alkylation with the appropriate dibromoalkanes followed by reaction with *N*-methylpiperazine yielded compounds **13–15**. Finally, as shown in Scheme 3, phenol **2** was alkylated with 1,3-dibromopropane followed by reaction with the appropriate amines to give compounds **16–24**.

The compounds in Tables 1–3 were evaluated for their ability to displace the binding of [$^3$H]-AVP to human recombinant V3 receptor expressed in CHO cells.[9] In order to gain insight into the structural requirements for activity in the peptidic, right-hand portion of the molecule, a series of strategic deletions within this domain was performed (Table 1). Removal of the tyrosine sidechain, as in compound **5**, gave an active structure ($IC_{50}$ = 5.1 μM), but with a 26-fold decrease in potency versus compound **1**. Further removal of the isobutyl sidechain led to an additional sixfold loss of potency (compound **6**; $IC_{50}$ = 30.5 μM). Interestingly, incorporation of the tyrosine sidechain back into the molecule with simultaneous deletion of the amine functionality gave compound **7** which was inactive in the assay. Finally, removal of the complete dipeptidic sidechain, resulting in phenol **2**, led to an inactive compound. This preliminary SAR indicated that the basic amine functionality, inherent in the tyrosine, and glycine residues, might be required for activity. This hypothesis was further strengthened by the fact that the synthetic intermediate **3** (see Scheme 1), which also contains a basic primary amine, was found to possess moderate activity ($IC_{50}$ = 2.1 μM).

Next, a series of aminoalkoxy analogs with an amino ($NH_2$) or *N*-methylpiperazine substituent at varying distances from the quinazolinone scaffold were prepared to determine the preference in chain length between the amine functionality and the quinazolinone scaffold. As shown in Table 2, in both the primary amine and *N*-methylpiperazine series, a preference of three carbon atoms was established (compounds **9** and **14**). In this set of analogs, compound **14** had the best activity ($IC_{50}$ = 1.7 μM) and represented a significant advancement in terms of improved physicochemical properties. Compared with compound **1**, compound **14** had a reduced molecular weight (from 612 to 490), a reduced fast polar surface area (from FPSA = 148.48 Å$^2$ to FPSA = 77.72 Å$^2$), a reduced *A* log *P* (from *A* log *P* = 4.73 to *A* log *P* = 2.72), and a reduced number of rotatable bonds (from 17 to 11). Consequently compound **14** was the basis for further modifications to improve potency.

Next, analogs of compound **14** were prepared in which the *N*-methylpiperazine group was replaced with other amines (Table 3). Demethylation of the piperazine, to give **16**, led to a substantial loss of potency. Analog **17** ($IC_{50}$ = 7.6 μM), containing a secondary, *N*-methyl amine, was twofold less active than both the primary amine **9** (i.e., $-NH_2$, see Table 2) and the tertiary, *N*,*N*-diethylamine **18**. The piperidine **19** ($IC_{50}$ = 0.31 μM) resulted in a fivefold increase in potency compared to *N*-methyl piperazine **14** ($IC_{50}$ = 1.7 μM), demonstrating that the presence of the distal, basic piperazine nitrogen was not optimal for activity. The piperidine **19** was also about 10-fold more active than the *N*,*N*-diethylamine **18**, indicating a preference for cyclic amines. Pyrrolidine **20** was sixfold less potent than piperidine **19** whereas homopiperidine **21** was better tolerated, but did not improve potency compared to **19**. Introduction of a methoxy (compound **22**) or hydroxyl (compound **23**) group at the 4-position of the piperidine ring in **19** resulted in a 2- to 4-fold decrease in potency. Finally, replacement of piperidine with morpholine, yielding compound **24**, resulted in a further threefold increase in potency to give the most potent analog ($IC_{50}$ = 120 nM) discussed herein.

The rat V3, and human V1a, V2, and oxytocin affinities were also determined for compound **24** (Table 4). Affinity at the rat V3 receptor was comparable (within twofold) versus the human V3 receptor.

This indicated that further optimization of this hit class was likely to deliver tool compounds for evaluation in rat in vivo mod-

**Table 4**
Evaluation of compound **24** for affinity at rat (r) V3 and human (h) V1a, V2 and oxytocin receptors versus affinity at human V3 receptor

| Compd | IC50[a] (μM) | | | | |
|---|---|---|---|---|---|
| | hV3 (V1b) | rV3 | hV1a | hV2 | hOxt |
| **24** | 0.12 (±0.046) | 0.21 (±0.016) | 6% inh. at 5 μM | >100 | >100 |

[a] Values are means of at least two experiments, standard deviation is given in parentheses.

els. In addition, compound **24** demonstrated excellent selectivity (>1000-fold) for the human V3 receptor versus the related human V1a, V2, and oxytocin receptors.[16,17]

In conclusion, hit-to-lead efforts starting from the initial library hit, compound **1**, led to the identification of highly active lead compounds **19** and **24**. Importantly, a significant improvement in physicochemical properties versus hit compound **1** was achieved (e.g., compound **24**: $M_W = 477$, $A\log P = 2.45$, FPSA = 83.30 Å$^2$ versus compound **1**: $M_W = 612$, $A\log P = 4.73$, FPSA = 148.48 Å$^2$). In addition, compound **24** was demonstrated to have comparable affinity for the rat V3 receptor versus the human V3 receptor, and an excellent selectivity profile versus the related human V1a, V2, and oxytocin receptors. Further optimization within this series to provide highly selective V3 antagonists with improvements in affinity and with in vivo activity will be reported in due course.

## Acknowledgments

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9. *V3 (V1b) whole cell binding assay:* The initial HTS is performed in 384-well format whereas the compound evaluation is done in 96-well format. The CHO cells stably expressing human V3 (V1b) receptor are cultured at 37 °C with 5% CO2 in DMEM/F12 (Invitrogen) with 10% FBS (HyClone Labs, FetalClone II, #206007), 0.4 mg/mL G418, and 1% penicillin and streptomycin. Cells are seeded at 30,000 cells into either 384-well (50 μL/well) or 96-well (100 μL/well) clear bottom plate and cultured at 37 °C overnight. Cells are washed twice with 100 μL/well (for 384-well plate) or 200 μL/well (for 96-well plate) of PBS. For initial HTS, 384-well plates containing dried down compounds are resuspended with 30 μL/well of 5 nM [$^3$H]-AVP in assay buffer (PBS with 10 mM $MgCl_2$ and 0.1% BSA) with 1% DMSO. After solubilization, 20 μL/well is transferred from compound plate to cell plate. For compound evaluation, test compounds are initially serially diluted in DMSO and then intermediately diluted at 1:50 with assay buffer (PBS with 10 mM $MgCl_2$ and 0.1% BSA). To each well, 25 μL of serially diluted compounds are added followed by 25 μL of 10 nM [$^3$H]-AVP in assay buffer. The final reaction contains 5 nM [$^3$H]-AVP and 1% DMSO. The mixtures are incubated at room temperature for 30 min. The plates are washed twice with 100 μL/well (for 384-well plate) or 200 μL/well (for 96-well plate) of PBS and air dried. Detection is performed by adding 50 μL of scintillation fluid and shaking for 30 min followed by counting at 1 min/well in the microbeta.
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17. Human V1a membrane binding assay performed in 96-well microtitre plates containing (final concentrations): 50 μg/well membrane protein prepared from CHO cells stably expressing human V1a receptor, 5 nM [$^3$H]-AVP and test compounds in a total volume of 100 μL of assay buffer (50 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 1 mg/mL BSA, and 0.5% DMSO). The assay mixture was incubated at room temperature for 60 min. The reaction was terminated by rapid filtration of the mixture over 96-well GF/B filters (pre-soaked in 0.3% polyethylenimine) using a Tomtec Harvester. The filters were washed four times with approximately 200 μL of ice-cold 50 mM Tris–HCl (pH 7.4), then dried at 55 °C for 30 min. 50 μL scintillation fluids were then added and the filters counted using a Packard Topcount.