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2,5-Disubstituted 3,4-dihydro-2*H*-benzo[*b*][1,4]thiazepines as Potent and Selective V_2 Arginine Vasopressin Receptor Antagonists

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Abstract—A number of 2,5-disubstituted benzothiazepines were synthesized and screened for their ability to inhibit arginine vasopressin binding to the human V_2 and V_{1a} receptor subtypes. The more active compounds were subsequently analyzed for their antagonist activity in in vitro functional assays. The SAR showed a preference for an acidic unit appended from the benzothiazepine scaffold. This substitution pattern afforded the most potent and selective analogues in the series. The carboxymethyl analogue **4**, showed a 140-fold greater selectivity for the V_2 over the V_{1a} receptor in the binding assay. In the cell-based functional assays this analogue was a potent and selective antagonist of the V_2 receptor. The in vitro SAR of the series and a description of the in vivo studies around compound **4** is described.

Vasopressin is a nine amino acid pituitary peptide that exerts multiple actions throughout the body by interacting with three receptor subtypes, V1a, V1b and V2. The V_2 receptors, which are localized in the renal collecting ducts, regulate water resorption.[1] Stimulation of these receptors by vasopressin, reduces the output of urine exerting an antidiuretic or 'water-sparing' affect. A V_2 antagonist acts as an aquaretic, increasing water output without promoting the loss of electrolytes.[2] Diuretics are currently used to treat conditions such as congestive heart failure, inappropriate antidiuretic hormone secretion (SAIDH), brain edema, cirrhosis, and hyponatremia. A V_2 receptor antagonist could serve as a potassium sparing diuretic thereby eliminating the dangerous electrolyte imbalances associated with existing diurectics.[3]

Several non-peptide V_2 receptor antagonists have appeared in the literature over the last decade.[4] A number of the compounds described contain a benzazepine scaffold linked through N-1 to a substituted *p*-amidobenzoyl side chain. This side chain contains key pharmacophore elements and has therefore been preserved in several of the reported compounds. Ancillary substitutions to the benzazepine scaffold ring consist primarily of basic amines or nitrogen containing heterocycles that were appended or fused to the benzazepine ring as in the first example of a non-peptide vasopressin antagonist, OPC 31260[5] (**1**) and the clinical candidate, Lixivaptan[6] (**2**) (Fig. 1). These basic units have been shown to improved compound potency and aqueous solubility.

The biphenyl substitution pattern in the *p*-amidobenzoyl side chain, exemplified in the mixed V_{1a}/V_2 antagonists Conivaptan[7] (**3**), was preserved in several of the benzothiazepines described and the SAR explored. In contrast to many of the previously reported benzazepine compounds, the present studies show that a side chain containing an acidic moiety extended from the 2-position of the benzothiazepine scaffold provided a more potent and selective V_2 receptor antagonist **4** (Fig. 1). In fact most of the derivatives containing an alkyl chain substituted with a carboxylic acid or an acid bioisostere show good potentcy and selectivity for the V_2 receptor.

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The substituted benzothiazepines were synthesized as illustrated in Scheme 1. The intermediate lactam **6** was prepared in two steps by an addition of commercially available 2-aminothiophenol to 5,6-dihydropyran-2-one (**5**) followed by heating in refluxing xylene to promote cyclization in 61% overall yield.[8] Subsequent reduction with borane-tetrahydrofuran complex provided the 2-hydroxyethyl substituted benzothiazepine **7**. The substituted *p*-amidobenzoyl side chain was introduced by an in-situ silyl protection of the hydroxyl group using *N*,*O*-bis(trimethylsilyl)-acetamide, followed by amine acylation to form the 2,5-disubstituted benzothiazepine **8**.

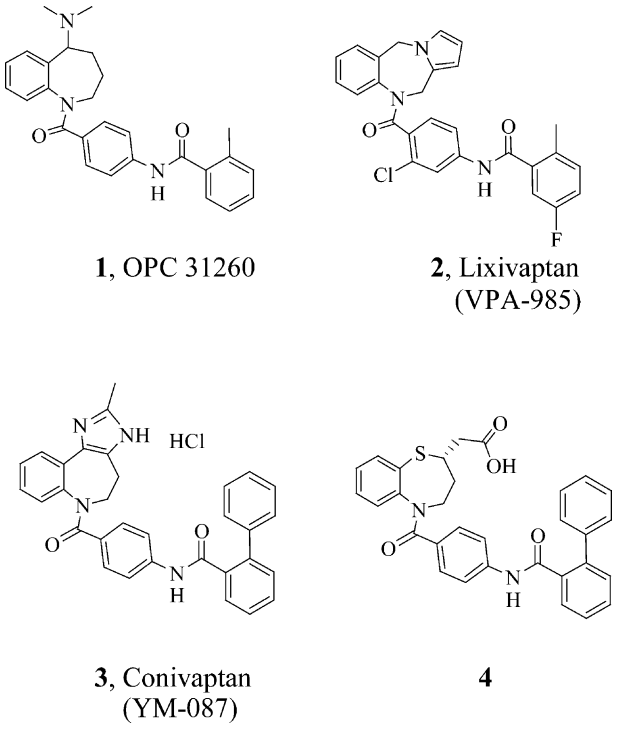
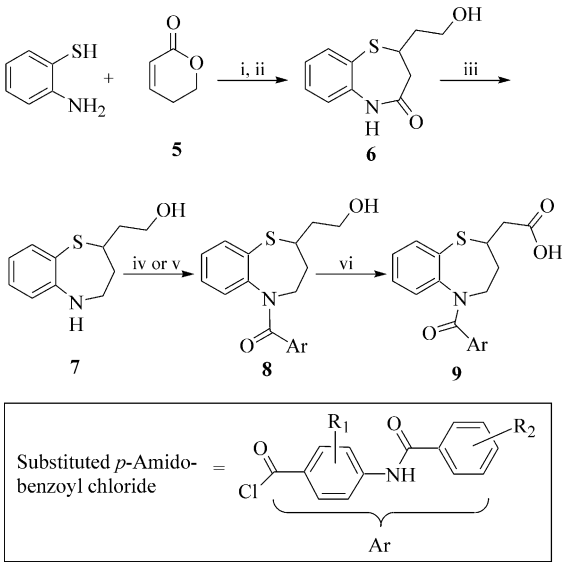


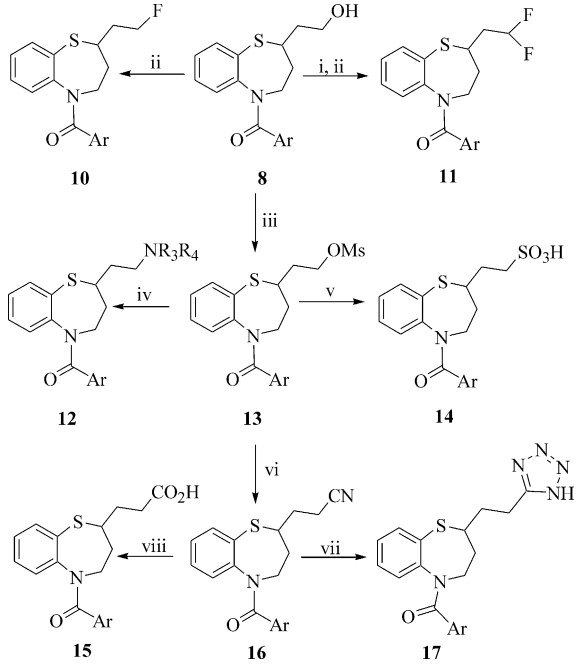
Figure 1. Non-peptide V_2 receptor antagonist.



Scheme 1. (i) CH_2Cl_2, Et_3N (99%); (ii) xylene, reflux (61%); (iii) BH_3 THF; (iv) THF, *N*,*O*-Bis(trimethylsilyl)acetamide; CH_2Cl_2, substituted *p*-amidobenzoyl chloride (85%); (v) (a) THF, *N*,*O*-bis (trimethylsilyl)acetamide; CH_2Cl_2, *p*-nitrobenzoyl chloride; (b) H_2, Pd/C, EtOH; (c) substituted benzoylchloride (vi) PDC, DMF.

Alternatively, the intermediate hydroxyl group could be protected as the TBDMS ether, and the substituted *p*-amidobenzoylchloride introduced in either one step or a stepwise fashion to allow SAR exploration of the aryl rings in this portion of the molecule. The substituted *p*-amidobenzoylchloride was prepared in three steps from commercially available 4-aminomethylbenzoate using standard procedures. The TBDMS protected intermediates were treated with methane sulfonic acid to remove the protecting group. By either route, a final pyridinium dichromate oxidation provided the carboxylic acid product **9**.

As shown in Scheme 2, the hydroxyl intermediate **8** provided several opportunities to expand the SAR in this series. Treatment of this intermediate **8** with diethylaminosulfurtrifluoride (DAST) provided the alkyl fluoride analogue **10**. Oxidation of **8** with PCC to the corresponding aldehyde followed by treatment with DAST afforded the difluorinated analogue **11**. PCC oxidation also oxidized the sulfur to the sulfoxide; however, subsequent treatment with DAST converted the sulfoxide back to the sulfide.[9] The hydroxyl group of **8** was converted to the mesylate to provide the intermediate **13**. This versatile intermediate was readily displaced with aliphatic amines to introduce basic groups as in example **12**, converted to the bromide then heated in the presence of sodium sulfite to provide the sulfonic acid analogue **14** or treated with sodium cyanide to yield the nitrile **16**. The nitrile was then further hydrolyzed to yield the homologated carboxylic acid analogue

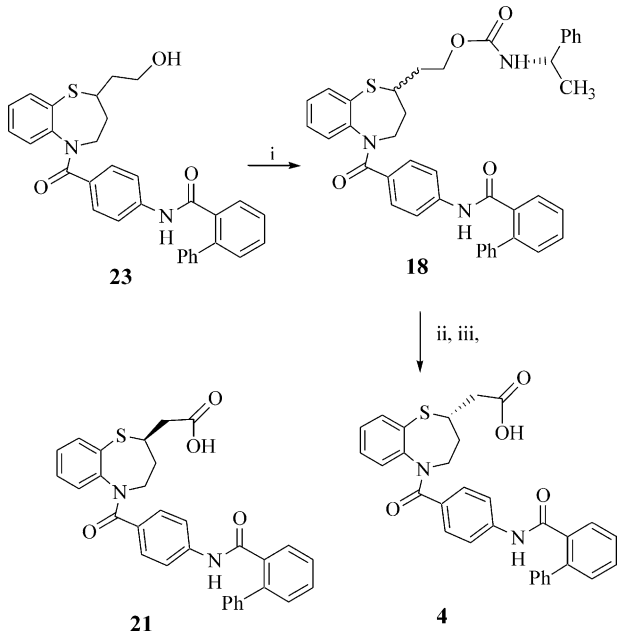


Scheme 2. (i) PCC, Celite, CH_2Cl_2 (55%); (ii) DAST, CH_2Cl_2 (61%); (iii) MsCl, Et_3N, CH_2Cl_2; (iv) NR_2R_4, 80 °C; (v) (a) LiBr, THF; (b) Na_2SO_3, EtOH/H_2O; (vi) NaCN, DMF; (vii) NaN_3, NH_4Cl, DMF; (viii) NaOH/ MeOH.

15 or treated with sodium azide to provided the tetrazole **17**, a carboxylic acid bioisostere.[10]

The enantiomers of the intermediate **23** were resolved by the addition of a chiral isocyanate, to form diastereomeric carbamates **18** as illustrated in Scheme 3. The diastereomers were easily separated by column chromatography.[11] Once isolated, each pure diastereomer was hydrolyzed back to the corresponding alcohols using sodium methoxide. The final PDC oxidation step provided the enantiomerically pure acids **4** and **21** in greater that 98% ee.[12]

In the in vitro binding assay, substituted benzothiazepines analogues were evaluated for their ability to displace ^{3}H-arginine vasopressin from cloned human V_2 and V_{1a} receptors. The functional activity was then subsequently determined by measuring the activation or inhibition of vasopressin induced cAMP accumulation and calcium immobilization, respectively, in V_2 and V_{1a} receptor expressing cells. Table 1 summarizes the results of these studies. A biphenyl substitution on the *p*-amidobenzoyl side chain was preferred to achieve the highest level of potency. However, selectivity for the V_2 receptor was clearly enhanced with the addition of the appended carboxylic acid side chain to the 2-position of the benzothiazepine scaffold as in example **4**, **20** and **21**. Diminished activities were observed in both V_{1a} and V_2 binding assays with the addition of amine appendages as in example **22**. The analogues containing alkyl chains terminating with either a hydroxyl group (**23**) or with fluorides (**24**, **25**) maintained good binding affinity yet displayed a mixed selectivity profile for the two receptor subtypes. Good potency and selectivity were preserved with bioisosteric replacements (**28**, **29**) and with carboxy side chain homologation (**26, 27**). In the functional assay, both enantiomers **4** and **21** of the most potent and selective benzothiazepine analogue displayed a 120- and 148-fold greater selectivity for the V_2 receptor. Compound **4** was selected for further studies.

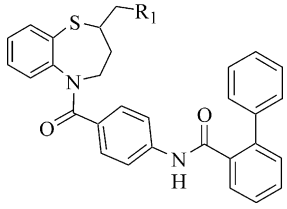


Scheme 3. (i) *S*-(−)-α-Methylbenzylisocyanate, $(CH_3)_2NCH_2CH_2OH$, toluene (97%); (ii) $NaOCH_3/CH_3OH$ (97%); (iii) PDC, DMF, (60%).

Strong species differences have been reported for vasopressin antagonists.[13] To explore that possibility, compound **4** was tested in functional assays using cells expressing rat V_{1a} or V_2 receptors. Compound **4** had functional IC_{50} values of 0.017 and 0.133 μM against the rat V_2 and V1a, respectively. This compound was therefore similar in potency at both the human and rat V_2 receptor but showed a greater selectivity with the human receptors.

The acute diuretic effect of **4** was measured in vivo in Sprague–Dawley rats (Table 2). The animals were dosed orally with either the vehicle (0.5% hydroxypropyl–methylcellulose), or the test compound. The urine was then collected over a 4-h period. As shown in Table 2, compound **4** demonstrated good aquaretic activity, producing a dose-related increase in urine volume and an associated decrease in urine osmolality. A significant increase in urine output was observed at doses of 10 mg/kg

Table 1. In-vitro binding and functional data



Compd	R_1	Config.	Binding (IC_{50}, μM[a])		Functional (IC_{50},μM[a])	
			V1a	V_2	V1a	V_2
4	CO_2H	*S*	1.14	0.008	1.76	0.03
20	CO_2H	*R*/*S*	0.729	0.007	0.74	0.04
21	CO_2H	*R*	0.44	0.007	2.13	0.036
22	$N(CH_3)_2$	*R*/*S*	1.2	0.43	ND[c]	ND[c]
23	CH_2OH	*R*/*S*	0.097	0.008	0.15	0.1
24	CH_2F	*R*/*S*	0.18	0.085	ND[c]	ND[c]
25	CHF_2	*R*/*S*	0.16	0.1	ND[c]	ND[c]
26	CH_2CO_2H	*R*	0.14	0.015	2.01	0.03
27	CH_2CO_2H	*S*	0.314	0.016	4.63	0.04
28	CH_2SO_3H	*R*/*S*	1.15	0.012	ND[c]	ND[c]
29	CH_2tetrazole	*R*/*S*	65%[b]	0.02	ND[c]	ND[c]

[a]IC_{50} values unless otherwise specified.
[b]Percent inhibition at 0.2 μM.
[c]ND, not determined.

Table 2. Effect of Compound **4** on urine volume and osmolality in conscious hydrated male rats

Treatment dose	Number of animals[b]	Urine volume (mL)[a]	Urine osmolality (mOsmol/Kg)[a]
Vehicle	10 (9)	1.3 (±0.2)	843 (±123)
3 mg/kg	10	2.2 (±0.3)	529 (±63)*
10 mg/kg	10	7.3 (±1.0)*	299 (±31)*
30 mg/kg	10 (9)	15.7 (±2.1)*	161 (±15)*
100 mg/kg	10 (9)	13.8 (±0.9)*	177 (±11)*

*$p < 0.05$ versus vehicle values.
[a]Each value represents the mean ± SE.
[b]Values in parentheses indicate the number of samples available for osmolality determination.

and greater while osmolality decreased at all doses tested (3 mg/kg and above). Maximal aquaretic efficacy was seen at 10 mg/kg with no greater effects apparent at 100 mg/kg.

A new series of substituted benzothiazepine analogues were synthesized and shown to inhibit binding of vasopressin to the V_{1a} and V_2 receptors. Several of these compounds also demonstrated good antagonist activity in an in vitro functional assay. Analogues containing carboxylic acid or acid isosteres appended from the benzothiazepine ring, showed higher selectivity for the V_2 over the V_{1a} receptor subtype in vitro. One example of this, compound **4**, was orally active in vivo, showing aquaretic activity at doses as low as 3 mg/kg. The production of hypo-osmotic urine differentiates this and other V_2 antagonists from the diuretics, such as furosemide and hydrochlorothiazide, now used in the clinic.

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