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Pyridobenzodiazepines: A novel class of orally active, vasopressin V_2 receptor selective agonists

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Abstract—Our efforts in seeking low molecular weight agonists of the antidiuretic peptide hormone arginine vasopressin (AVP) have led to the identification of the clinical candidate WAY-151932 (VNA-932). Further exploration of the structural requirements for agonist activity has provided another class of potent, orally active, non-peptidic vasopressin V_2 receptor selective agonists exemplified by the 5,11-dihydro-pyrido[2,3-*b*][1,5]benzodiazepine as a candidate for further development.

Arginine vasopressin (AVP) is a cyclic nonapeptide that exerts its actions by binding to three membrane bound G-protein coupled receptor subtypes, V_{1a}, V_2, and V_3 (also known as V_{1b}).[1] AVP also shows some affinity for the related oxytocin (OT) receptor. The V_{1a} and V_3 receptors are involved mainly in blood pressure control and regulation of ACTH secretion, respectively. AVP is one of several hormones that regulate fluid and salt balance in humans and animals. By stimulating the V_2 receptors found primarily in the principal cells of the renal collecting ducts (aquaporin-2 water channels), AVP increases water reabsorption resulting in a decrease of urine volume and an increase in urine osmolality. As such, AVP plays a vital role in the conservation of water and regulation of plasma osmolality. Vasopressin V_2 receptor selective agonists are a class of antidiuretics with the potential to be useful in treatment of diseases characterized by the production of large volumes of diluted urine or inadequate levels of AVP, such as central and nephrogenic diabetes insipidus, enuresis, and nocturia.[2]

Currently, the only marketed treatment for diabetes insipidus is desmopressin (DDAVP), a peptidic vasopressin V_2 receptor agonist.

For some time we have been interested in non-peptidic, orally active vasopressin V_2 ligands. We have already reported on the genesis of the potent, orally active V_2 selective antagonist VPA-985[3] and the first V_2 selective agonist VNA-932.[4]

In connection with the isosteric replacement of the amide bond of VPA-985 with small heterocyclic rings leading to the discovery of VNA-932 (Fig. 1), we have investigated a number of tricyclic benzodiazepines as potential 'privileged structure' variations.[5] Herein, we report the synthesis and biological activity of a second class of potent vasopressin V_2 receptor selective agonists exemplified by the 5,11-dihydro-pyrido[2,3-*b*][1,5]benzodiazepine **6c**.[6]

The general synthetic route used to prepare the majority of compounds is shown in Scheme 1.

Although **2** has previously been reported,[7,8] experimental protocols were optimized for large-scale preparation (up to 50 g), as shown in Scheme 1. The conditions reported here for the acylation of **2**→**4** were found to be optimal for suppressing competing regioisomer formation (to <5%). Nucleophilic aromatic substitution

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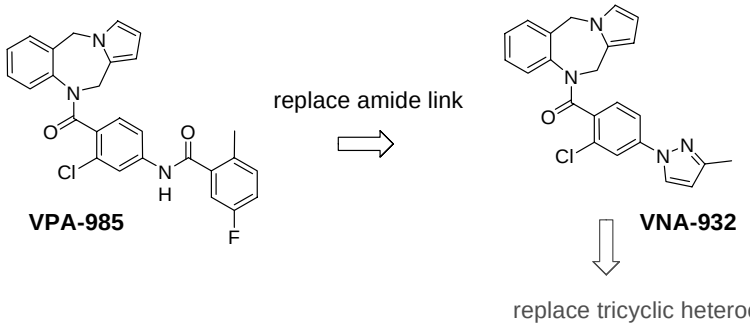
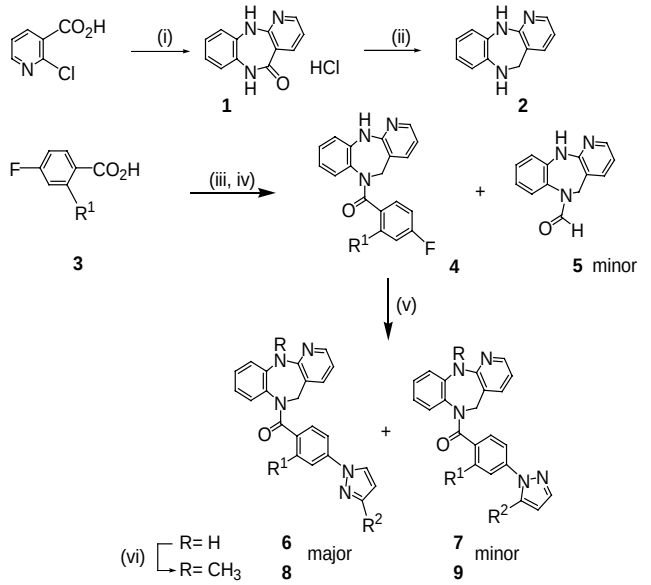
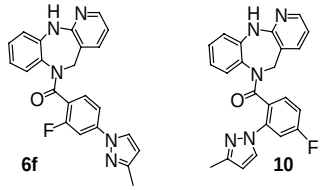


Figure 1. Conversion of vasopressin V_2 antagonists to V_2 agonists, exemplified by the clinical candidates VPA-985 (lixivaptan) and VNA-932, respectively.



Scheme 1. Reagents and conditions: (i) 1,2-phenylenediamine, cyclohexanol, reflux, 83%; (ii) $BH_3{\cdot}SMe_2$, dioxane, sonication, rt, overnight, 60%; (iii) oxalyl chloride, DMF (cat), CH_2Cl_2, reflux; (iv) **2**, K_2CO_3 (3 equiv), DMF, 37% (two steps); (v) NaH, pyrazole, DMF, 130 °C, 83%; (vi) NaH, MeI, DMF, 54%. Yields reported for steps (iii)–(vi) are for $R^1 = CF_3$ and $R^2 = CH_3$. For all other substituents, see Ref. 12.

of the fluorine in **4** with the sodium salt of the heterocycle provided the desired targets as a mixture of regioisomers **6** and **7**. The mixtures were separated by chromatography and the structural assignments of the major and minor isomers were confirmed by ^{1}H-NOE NMR experiments. Alkylation of the diazepine NH of **6** and **7** provided the corresponding N-methylated analogs **8** and **9**, respectively. This route was used with a variety of R^1 substituents. However, with R^1=F, substitution occurred at both fluorines in the amide **4**, and pyrazoles **6f** and **10** were obtained by chromatography.



A different synthetic approach (Scheme 2) was used to prepare the isomeric *C*-pyrazoles **19** and **20**.

The key step consisted of the in situ thermal rearrangement of the propargylic amine *N*-oxide **13** to the enone **14**.[9] Alkylation of **15** provided two regioisomeric arylpyrazoles, but only **17** was isolated in a pure form by chromatography. The structural assignment of **19** was confirmed by ^{1}H-NOE NMR experiments.

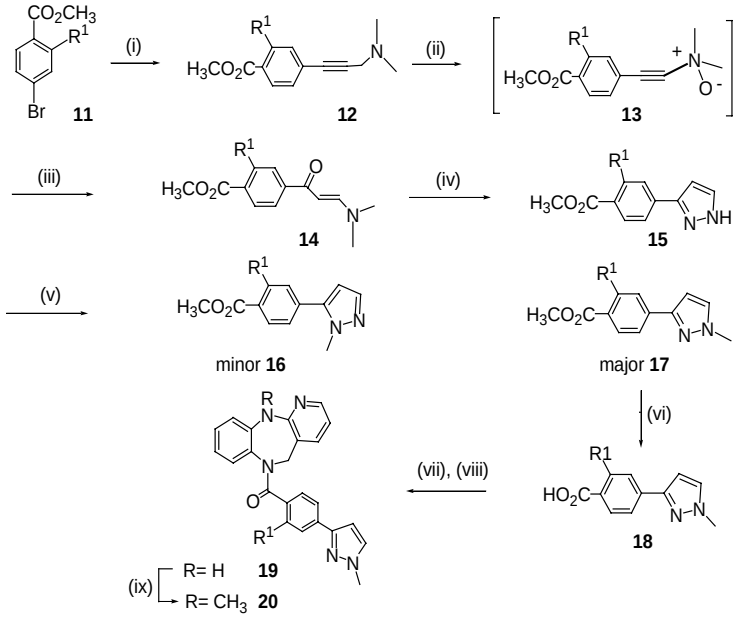
For the synthesis of the triazole analog **28**, a 'tail' to 'headpiece' coupling was utilized as shown in Scheme 3. The triazole NH was protected with a 4-methoxybenzyl group that was later removed by solvolysis in trifluoroacetic acid after the coupling step. It was found that the deprotection proceeded very cleanly but, surprisingly, was much slower (7 days at reflux) than reported in the literature[11] (1.25–6 h at 65 °C).

The compounds[12] were evaluated for antidiuretic activity in a primary screen using an in vivo rat model (Tables 1 and 2).

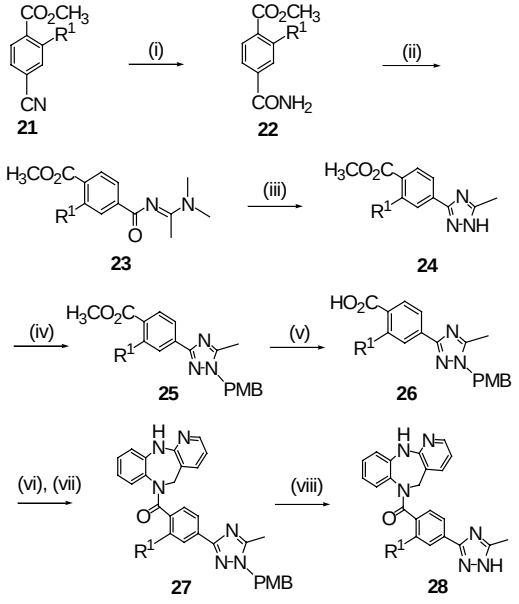
In this protocol normotensive, conscious, water-loaded Sprague–Dawley rats were treated with either a test compound or a reference agent at an oral dose of 10 mg/kg in a volume of 10 mL/kg of a vehicle consisting of 20% DMSO in 2.5% preboiled corn starch. Thirty minutes after dosing the test compound, the rat stomachs were gavaged with 30 mL/kg of deionized water. The urine volume collected over 4 h was measured, and the urine osmolality and electrolyte concentrations (Na^+, K^+, and Cl^-) were determined.[13] The data are reported as percent variation over control at a given dose. The program was driven by this in vivo model that facilitated the rapid identification and ranking of potent orally active agonists.

In general, good activity was observed at the screening dose of 10 mg/kg po. Greater agonist potency in a regioisomeric pyrazole pair appeared to reside in the 3-alkyl pyrazole (major isomer; cf. **6a** and **6c** vs **7a** and **7c**, Table 1), and to some extent, to depend on the nature of the substituent R^2 (cf. **6c** vs **6e**, Table 1).

Agonists with good activity at the screening dose of 10 mg/kg were further evaluated at 3 and 1 mg/kg po. In general, potent agonist activity at the lowest dose of

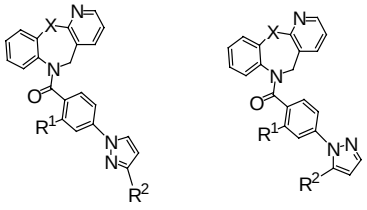


Scheme 2. Reagents and conditions: (i) dichlorobis(triphenylphosphine) Pd(II), CuI (cat), 1-dimethylamino-2-propyne, 95%; (ii) MCPBA; (iii) MeOH, 60 °C, 48% (two steps); (iv) H_2NNH_2, HOAc, 94%; (v) NaH, CH_3I, DMF, 55%; (vi) 2.5 N NaOH, MeOH, then 2 N HCl, 93%; (vii) oxalyl chloride, DMF, CH_2Cl_2; (viii) **2**, K_2CO_3, DMF, 61% (two steps, based on recovered **2**); (ix) NaH, MeI, THF, 47%. Yields reported are for R^1 = Cl.



Scheme 3. Reagents and conditions: (i) 30% H_2O_2, K_2CO_3, DMSO,[10] 74%; (ii) *N*,*N*-dimethylacetamide dimethyl acetal, 90 °C; (iii) NH_2NH_2, HOAc, 67% (two steps); (iv) NaH, 4-methoxybenzyl chloride, 66%; (v) 2.5 N NaOH, MeOH, then 1 N HCl, 81%; (vi) oxalyl chloride, DMF (cat), CH_2Cl_2, reflux; (vii) **2**, K_2CO_3, DMF, 38% (two steps); (viii) TFA, reflux, 7 days, 55%. Yields reported are for R^1 = Cl.

Table 1. In vivo antidiuretic activity of *N*-pyrazoles in water-loaded Sprague–Dawley rats



6a-h, 8a, 8c **7a, 7c**

Compound	X	R^1	R^2	Urine volume[a]	Osmolality[b]
6a	NH	Cl	CH_3	80	172
6b	NH	Cl	CF_3	72	162
6c	NH	CF_3	CH_3	87	239
6d	NH	CF_3	CF_3	69	183
6e	NH	CF_3	Bu^t	6	−7
6f	NH	F	CH_3	59	272
6g	NH	Br	CH_3	85	247
6h	NH	Br	4-Pyr	24	11
7a	NH	Cl	CH_3	61	189
7c	NH	CF_3	CH_3	25	4
8a	NCH_3	Cl	CH_3	71	275
8c	NCH_3	CF_3	CH_3	72	198
10				63	177
VNA-932				74	286

[a] % decrease over control (10 mg/kg po).
[b] % increase over control (10 mg/kg po).

1 mg/kg was maintained when R^1 was Br, Cl or CF_3. Based upon the in vivo screening and physicochemical profiling (solubility, permeability, and crystallinity), **6c** was selected for further evaluation.

Oral administration of **6c** to water-loaded conscious rats produced a dose-dependent decrease in urine volume (ED_{50} = 0.1 mg/kg, 2.5% starch in water vehicle) and a corresponding increase in osmolality without altering the urine electrolyte excretion profile. In the same model, VNA-932 had a similar profile with an ED_{50} = 0.14 mg/kg. However, compound **6c** showed weaker binding affinity for the cloned human vasopressin

Table 2. In vivo antidiuretic activity of *C*-pyrazoles and [1,2,4]triazole in water-loaded Sprague–Dawley rats

Compound	R[1]	Urine volume[a]	Osmolality[b]
19	Cl	76	134
20	Cl	10	−11
28	Cl	86	516

[a] % decrease over control (10 mg/kg po).
[b] % increase over control (10 mg/kg po).

V_2 receptors than VNA-932 (IC_{50} = 493.1 ± 21.1 nM *vs* 80.3 ± 2.4 nM),[4] with somewhat greater selectivity for the hV_2 over hV_{1a} receptors (IC_{50} = 5954 ± 812 nM vs 778 ± 27 nM, Table 3) and no measurable affinity at the hV_3 receptors.

To clarify the apparent discrepancy observed with **6c** between binding affinity and in vivo efficacy, we looked into second messenger responses. Binding of AVP at the V_2 receptors in the renal collecting ducts activates the adenyl cyclase system and stimulates cAMP production. Compound **6c** stimulated cAMP formation in LV2 cells expressing the hV_2 receptors in a dose-dependent manner with an EC_{50} = 1.67 ± 0.17 nM (vs 0.74 ± 0.07 nM for VNA-932 and 0.052 ± 0.003 nM for AVP).[15] The cAMP data were more consistent with the in vivo than with the binding data, and confirmed that like VNA-932, **6c** also acts as a vasopressin V_2 mimetic. The concept of 'spare receptors' may be invoked[16] to help explain differences between binding affinity and the cAMP functional assay. However, the discrepancy between binding affinity and in vivo efficacy may be due to several factors, such as species differences or the PK/PD profile of **6c**, although we have no data to put forward at this time to clarify this aspect.

To further assess the agonist or antagonist activity of **6c** at the V_{1a} and OT receptors, we studied its effects in isolated tissues. In rat tail arteries and uterine strips, AVP (10 nM) and OT (10 nM) produced strong contractions by stimulating the V_{1a} and OT receptors, respectively. Compound **6c** (up to 1 μM) elicited no contractions in either preparation, indicating lack of V_{1a} or OT agonist activity. Compound **6c** antagonized AVP and OT induced responses with IC_{50} values of >30,000 nM (rat tail artery) and 1259 nM (rat uterine strip), respectively. For comparison, atosiban (Tractocile®), a non-selective peptidic OT antagonist, had an IC_{50} = 12 nM (rat uterine strip). Thus, in functional studies, compound **6c** showed a very weak V_{1a} and OT antagonist activity.

Table 3. In vitro binding data for the pyridobenzodiazepines

Compound	hV_{1a} binding,[14] IC_{50}[a] (nM)	hV_2 binding,[14] IC_{50}[b] (nM)
6a	(38 at 1 μM)	91.5
6b	nd	(13)
6c	5954	493
6d	nd	(−7)
6e	nd	(26)
6f	nd	933
6g	(30 at 1 μM)	296
6h	nd	(26)
7a	nd	390
7c	(11 at 0.1 μM)	413
8a	(20 at 1 μM)	475
8c	(32 at 10 μM)	(10)
10	nd	(5)
19	1783	183
VNA-932	778	80

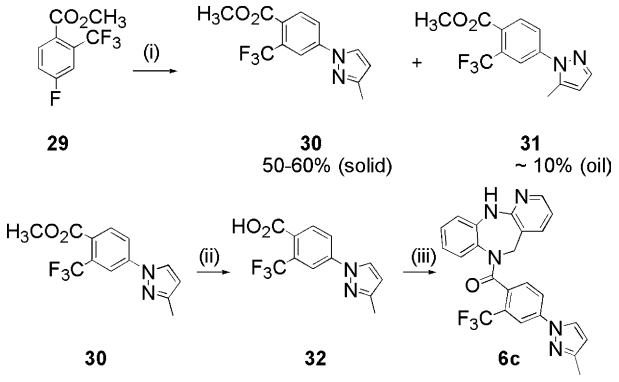
[a] % inhibition at the stated concentration is given in parentheses (nd, not determined). IC_{50} values are means of three experiments.
[b] % inhibition at 300 nM is given in parentheses. IC_{50} values are means of two to five experiments.

Conscious Brattleboro rats provide a convenient animal model of hypophyseal diabetes insipidus.[17] These rats produce negligible amounts of AVP, excrete large volumes of hypotonic urine, and need to drink frequently to maintain water homeostasis. In this animal model, orally administered **6c** had potency and efficacy comparable to that shown in normal rats (ED_{50} = <0.1 mg/kg). Preliminary data also showed that **6c** was effective in conscious dogs and cynomolgus monkeys (ED_{50} = 3 mg/kg po, in both species; vehicle: 1% Tween 80–0.5% methylcellulose–water).

To scale up the supply of **6c**, a streamlined synthetic route was desirable. The general route shown in Scheme 1 had a number of shortcomings associated with it: (i) the formyl by-product **5** was isolated in 10–30% yield from the coupling reaction (**2**→**4**, Scheme 1), (ii) efficient chromatographic separation of the regioisomers proved to be very tedious, and (iii) the ratio of regioisomers **6**:**7** was 5–6:1.

A number of different solutions were implemented to address the synthetic limitations. Reducing the amount of K_2CO_3 to 1.1 equiv in the acylation step caused **5** to remain in the aqueous phase during work-up. Since the formation of **5** was likely due to the use of DMF as a solvent, a convenient solution was found using the Yamaguchi coupling protocol in dichloromethane.[18] An alternative, successful approach to address problems (ii) and (iii) was to assemble the tailpiece first and then couple it to **2** using Yamaguchi conditions (Scheme 4).

Synthesis of the tailpiece starting with the benzoate ester **29** gave a mixture of regioisomers **30** and **31**. However,



Scheme 4. Reagents and conditions: (i) 3-Me pyrazole, NaH, DMF, 130 °C, 60%; (ii) NaOH, MeOH, reflux, then 1 N HCl, 98%; (iii) 2,4,6-trichlorobenzoyl chloride, TEA, **2**, DMAP, CH_2Cl_2, 58%.

two recrystallizations from 10% ethyl acetate in hexanes provided the major, desired isomer **30** in >99% purity. Yamaguchi coupling of the carboxylic acid **32** to the headpiece proceeded in yield higher than that of the acid chloride coupling used previously. This route also avoided formation of the formyl by-product **5**. Purification of **6c** was accomplished by direct crystallization from ethanol, thus eliminating the need for chromatography.

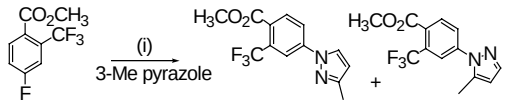
Finally, an additional improvement in the ratio of regioisomers **30** and **31** was sought by varying the conditions used in the nucleophilic displacement of the aryl fluoride **29** (Table 4). No improvement in selectivity was achieved when NaH was replaced by KH or KO^{t}Bu as the base.

However, by switching to the nitrile **33**, the ratio of regioisomers **34**:**35** increased to 9:1 using KO^{t}Bu in THF at room temperature (Table 5). Direct recrystallization from ethanol provided the pure, desired isomer **34** that, in turn, was hydrolyzed to the acid **32** by heating with 1 N NaOH in ethanol.

A combination of the above improvements was then utilized to scale up preparation of **6c** (15 g).

In summary, a novel 5,11-dihydro-pyrido[2,3-*b*][1,5]-benzodiazepine class of non-peptidic, orally active vasopressin V_2 receptor selective agonists, was identified. Based on the in vitro and in vivo profiles, **6c** was selected as a candidate for further development. Oral administration of **6c** potently decreased urine volume and increased urine osmolality in conscious laboratory animals. Its effects on Brattleboro rats suggest that **6c** should be effective in treating hypophyseal diabetes insipidus, thus providing a therapeutic alternative to desmopressin.

Table 4. Improving regioisomer ratio (ester route)

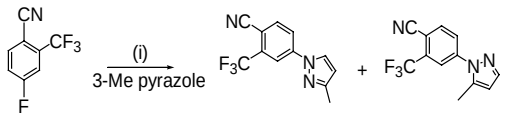


29 **30** **31**

(i) Conditions	Ratio (**30**:**31**)	Comments
KH, DMF, 130 °C, 15 min	53:15	
KO^{t}Bu, THF, 0 °C ⇒ rt, 24 h	20:3	[a]
KO^{t}Bu, DMF, 0 °C ⇒ rt, 24 h	45:16	[a]

[a] Acid and O^{t}Bu ester present.

Table 5. Improving regioisomer ratio (nitrile route)



33 **34** **35**

(i) Conditions	Ratio (**34**:**35**)	Comments
NaH or KO^{t}Bu, DMF, rt, 1 h	3:1	80% yield
NaH or KO^{t}BU, THF, 0 °C ⇒ rt, 18 h	3:1	50% yield
KO^{t}Bu, THF, rt, 30 min	9:1	80% yield[a]

[a] Solution of pyrazole salt added to aryl fluoride.

Acknowledgments

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13. Urine osmolality was measured using an Advance Cryomatic Osmometer Model 3C2 (Advanced Instruments, Norwood, MA, USA). Determination of urinary electrolytes was performed using ion selective electrodes on a Synchron EL-ISE analyzer (Beckman, Fullerton, California, USA).
14. Binding affinities were determined by measuring the inhibition of ^{3}[H]AVP binding to CHO cell membranes expressing human V_{1a} receptors or membranes of LV2 cells transfected with human V_2 receptors.
15. Accumulation of cyclic AMP was measured in transfected LV2 cells expressing human V_2 receptors. The cells were incubated with the test compound for 15 min and the cAMP assay was run in triplicate wells according to manufacturer's protocol (cAMP Biotrak Scintillation Proximity Assay, Code RPA 556 Amersham Biosciences Corp., Piscataway, N.J., USA).
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