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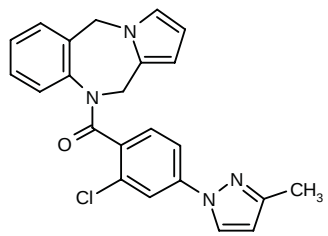
WAY-VNA-932

Treatment of Central Diabetes Insipidus
Treatment of Nocturnal Enuresis
Treatment of Nocturia
Vasopressin V_2 Agonist

VNA-932

10-[2-Chloro-4-(3-methyl-1*H*-pyrazol-1-yl)benzoyl]-10,11-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine
[2-Chloro-4-(3-methyl-1*H*-pyrazol-1-yl)phenyl](10,11-dihydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-10-y-)methanone



$C_{23}H_{19}ClN_4O$

Mol wt: 402.8831

CAS: 220460-92-4

EN: 282109

Abstract

WAY-VNA-932 is a novel, orally active nonpeptide small molecule which exhibits vasopressin V_2 receptor agonism. In CHO cells transfected with human V_{1a}, V_2, V_{1b} and oxytocin receptors, WAY-VNA-932 is a potent agonist at the V_2 receptor with an EC_{50} for second messenger generation (cAMP) of 0.7 nM. In water loaded normotensive rats, Brattleboro rats, dogs and cynomolgus monkeys WAY-VNA-932 lowers urine output in a dose-dependent manner with a concomitant increase in osmolality. It is expected that WAY-VNA-932 will offer several advantages over desmopressin, a peptidic vasopressin V_2 agonist, including greater and more consistent bioavailability and the lack of pressor activity. WAY-VNA-932 is expected to be useful in conditions characterized by excessive production and/or dilution of urine such as central diabetes insipidus and nocturnal enuresis as well as in conditions characterized by inappropriate production of vasopressin. WAY-VNA-932 is the first nonpeptide vasopressin V_2 agonist to enter phase I trials.

Synthesis

WAY-VNA-932 can be prepared by two related pathways starting from 5*H*,11*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine (I). The pyrrolobenzodiazepine (I) is prepared in two steps. The sodium salt of pyrrole-2-carboxaldehyde (II) is prepared in DMF using NaH. Alkylation with 2-nitrobenzyl bromide (III) proceeds in excellent yield. Reduction with hydrogen over Raney nickel effects reduction of the nitro group to the amine which cyclizes in situ to the seven-membered imine that is reduced to the pyrrolobenzodiazepine in excellent yield in one pot (1). Scheme 1.

WAY-VNA-932 is then prepared by two related ways:

1) Acylation of the pyrrolobenzodiazepine (I) with 2-chloro-4-fluorobenzoyl chloride (V) – prepared from the acid (VI) and oxalyl chloride and catalytic DMF – with Hünig's base in dichloromethane gives the acylated pyrrolobenzodiazepine (VII). Treatment of (VII) with a solution of the sodium salt of 3-methylpyrazole (VIII) in DMF yields a 85:15 mixture of WAY-VNA-932 and its 5-methyl isomer (IX) which can be separated by column chromatography using an ethyl acetate/hexane mixture over silica gel. Scheme 2.

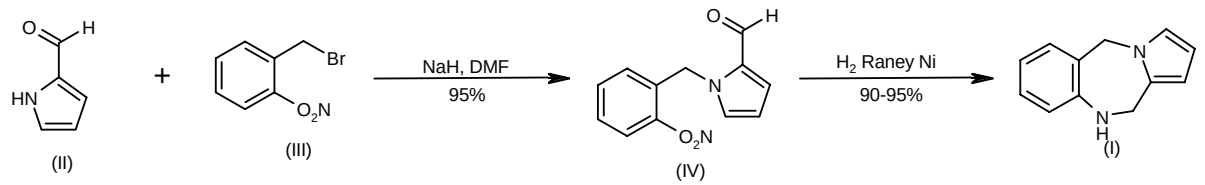
2) Alternatively, treatment of 2-chloro-4-fluorobenzonitrile (X) with the potassium salt of 3-methylpyrazole (VIII) in THF produces an excellent yield of a 9:1 mixture of the 2-chloro-4-(3-methylpyrazol-1-yl)benzonitrile (XI) and 2-chloro-4-(5-methylpyrazol-1-yl)benzonitrile (XII) which can be separated by fractional crystallization. Hydrolysis of the nitrile (XI) to the acid, activation as the acid chloride and acylation of the pyrrolobenzodiazepine using Hünig's base in dichloromethane yields WAY-VNA-932 which is isolated by direct crystallization (2). Scheme 3.

Description

M.p. 141-3 °C.

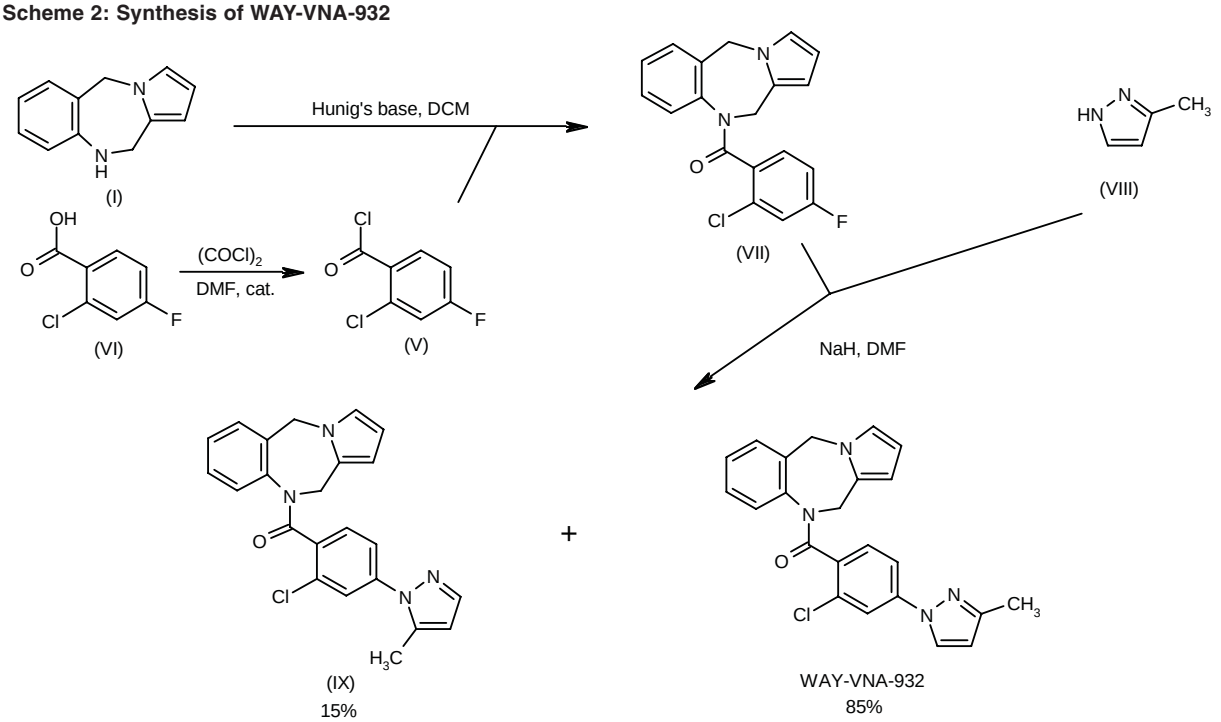
Thomas J. Caggiano. Wyeth Pharmaceuticals, Inc., CN 8000, Princeton, NJ 08540-8000, USA.

Scheme 1: Synthesis of Intermediate (I)



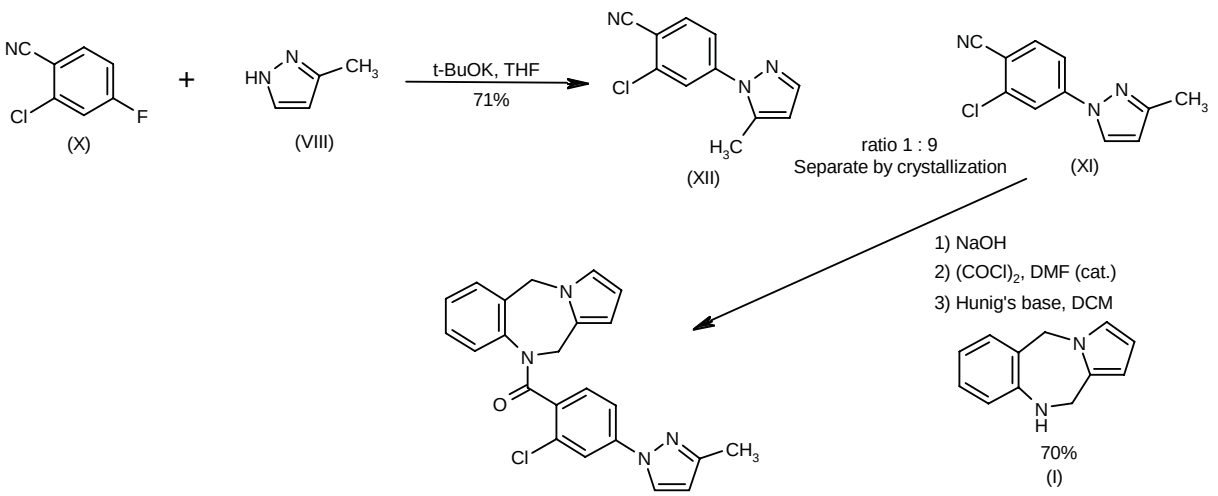
(II) (III) (IV) (I)

Scheme 2: Synthesis of WAY-VNA-932



(I) (VI) (V) (VII) (VIII) (IX) 15% WAY-VNA-932 85%

Scheme 3: Synthesis of WAY-VNA-932



(X) (VIII) (XII) (XI) 70% (I)

Introduction

Arginine vasopressin (AVP) is a cyclic nonapeptide which is synthesized in the hypothalamus and stored in the neurohypophysis. AVP is secreted by the posterior pituitary in response to a decrease in blood pressure or blood volume or by an increase in plasma osmolality. Arterial baroreceptors and low pressure volume receptors maintain blood pressure and volume while plasma osmolality is controlled by osmoreceptors in the brain. AVP also regulates other physiologic functions such as stimulation of glycogenolysis in the liver and modulation of ACTH levels (3-5). AVP acts on two classes of G-protein-coupled receptors, V_1 and V_2. The vasopressin V_1 receptor is further classified into two subtypes: vasopressin-1a (V_{1a}) and vasopressin-1b (V_{1b}) (6). Activation of the V_{1a} receptor constricts blood vessels, inhibits platelet aggregation and stimulates glycogenolysis in the liver, while coupling to the V_{1b} receptor potentiates the action of corticotropin releasing hormone and modulates the secretion of adrenocorticotropic hormone. Stimulation of both the V_{1a} and V_{1b} receptors results in the hydrolysis of phosphatidylinositol and mobilization of calcium ion from intracellular stores. The V_2 receptor, which is found primarily in the renal collecting ducts, mediates the diuretic effects of AVP. Activation of the V_2 receptor results in an increase in intracellular cAMP. The resulting signal cascade stimulates the exocytosis of intact water channels (aquaporins) from internal vesicles to the apical membrane in principal cells in the kidney as well as inhibits endocytosis of the aquaporins from the apical membrane and thereby controls water balance (7, 8). AVP also has some affinity for the oxytocin (OT) receptor. OT is a structurally related neurohypophyseal hormone. The OT receptor is expressed in the uterus, mammary gland, ovaries, brain, kidney and lactotrophic cells. Compounds which act as vasopressin V_2 receptor agonists are thought to be of potential use in diseases which are characterized by the production of inappropriate amounts of vasopressin or of large volumes of dilute urine (*e.g.*, SIADH, diabetes insipidus, nocturnal enuresis).

SAR studies led to the identification of WAY-VNA-932 as a clinical candidate, and the compound has entered phase I trials (9-12).

Pharmacological Actions

In vitro *activity*

In studies using CHO cells transfected with human vasopressin (13) or oxytocin (14) receptors, WAY-VNA-932 has K_i values of 39.9 nM (V_2), 465 nM (V_{1a}), >1000 nM (V_{1b}) and 125 nM (OT). ^{3}H-AVP is used as the ligand for the V_2 and V_{1b} receptor. ^{3}H-Manning peptide is used as the ligand for the V_{1a} receptor and ^{3}H-OT as the ligand for the OT receptor. Saturation analysis shows WAY-VNA-932 to be a competitive and reversible binder at the V_2 receptor. In the presence of WAY-VNA-932 (73 nM) the K_d of ^{3}H-AVP at the V_2 receptor increases from 2.14 nM to 4.72 nM, yet the total number of binding sites (B_{max}) is unchanged (1.02 fmole/μg). The Hill coefficient is essentially equal to one. These data are consistent with a single receptor type involved in a simple and reversible bimolecular interaction.

WAY-VNA-932 stimulates formation of cAMP, the V_2 receptor's second messenger, in a dose-dependent fashion with an EC_{50} of 0.73 nM (*vs.* 0.097 nM for desmopressin (DDAVP) and 0.052 nm for AVP). This data shows that WAY-VNA-932, while less potent, is a full agonist with similar efficacy when compared to DDAVP and AVP. Furthermore, the increase in cAMP formation by WAY-VNA-932 and AVP is completely blocked by our V_2 receptor antagonist, WAY-VPA-985 (lixivaptan). This is consistent with the increase in cAMP resulting from V_2 receptor activation. The *in vitro* profiles of WAY-VNA-932, DDAVP, AVP and OT are shown in Table I.

Studies of WAY-VNA-932 (1 μM) in isolated rat tail artery and rat uterine tissue failed to show any agonist activity at either the V_{1a} or OT receptors, respectively. However, WAY-VNA-932 did show weak V_{1a} antagonist activity against AVP-induced vasoconstriction (IC_{50} = 1660 nM) and OT-induced uterine contraction (IC_{50} = 55 nM) (15).

WAY-VNA-932 is very selective for the vasopressin receptor. When the compound was assayed at a high concentration (10 μM) in a panel of 31 different receptors, ion channels and second message systems, no significant activity was observed (<50% inhibition at 10 μM).

Table I: In vitro *profile of WAY-VNA-932 and reference compounds.*

	K_i (nM)			
Ligand	V_2	V_{1a}	V_{1b}	OT
WAY-VNA-932	39.9 ± 1.2 (3)	465 ± 16 (3)	>1000	125 ± (3)
DDAVP	9.6 ± (4)	87.1 ± 4.8 (3)	22.9 (1)	118 (2)
AVP	0.84 ± 0.13 (3)	0.22 ± 0.3 (7)	1.6 (2)	5.0 ± 0.8 (4)
OT	–	–	–	0.48 ± 0.03 (8)

Values are mean ± SEM (n), where n = number of experiments, each performed in triplicate. Ligands used: ^{3}H-AVP for V_2 and V_{1b} receptors, ^{3}H-Manning peptide for the V_{1a} receptor and ^{3}H-OT for the oxytocin receptor.

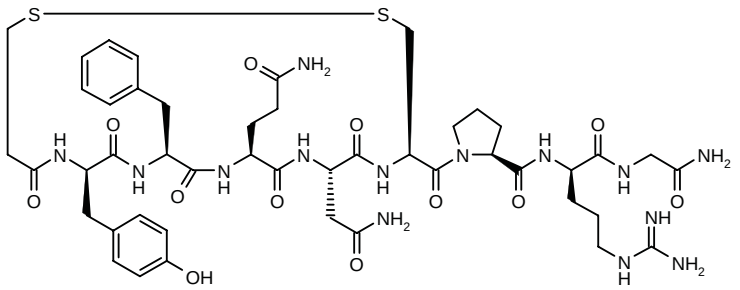


Fig. 1.

In vivo *activity*

The *in vivo* activity of WAY-VNA-932 was assessed initially in conscious normotensive rats under different conditions of water and solute (NaCl) loading and in Brattleboro rats with hereditary central diabetes insipidus (16-18). Oral administration of WAY-VNA-932 (2.5% starch-water suspension; maximum dose of 3 mg/kg p.o.) to water-loaded Sprague Dawley rats produced a dose-dependent decrease in urine volume (82% *vs.* control) along with an increase in urine osmolality (769% over control). No change was observed in the excretion of Na^+, K^+, Cl^- creatinine and urea nitrogen. Similarly, DDAVP (0.25 μg/kg i.p.) produced a 42% drop in urine output accompanied with a 168% increase in urine osmolality with no change in the electrolyte, creatinine and urea nitrogen levels.

Brattleboro rats were used as a model of diabetes insipidus. These rats produce little or no vasopressin yet have normal vasopressin receptors and, as a result, produce large volumes of hypotonic urine and drink frequently to maintain water homeostasis. In this model, orally administered WAY-VNA-932 had an antiduretic ED_{50} of 0.1 ± 0.01 mg/kg. In the same model, when WAY-VNA-932 was administered at a high dose (3 mg/kg/day) in the powdered diet for 5 days a sustained and potent antiduretic effect was observed with no indication of tachyphylaxis.

In normotensive rats, the oral ED_{50} was 0.4 ± 0.05 mg/kg (male) and 0.2 ± 0.23 mg/kg (female). Onset of action occurred within 30 min of the oral administration and was dose-dependent. In conscious male dogs and conscious cynomolgus monkeys (male and female), the respective oral ED_{50} values were 0.1 ± 0.04 mg/kg and 0.28 ± 0.05 mg/kg. In all cases, the drop in urine output was accompanied by a comparable increase in osmolality with no effect on excretion of electrolytes, creatinine or urea nitrogen.

When normotensive rats were dosed with the compound at levels 25-1000 times higher than the ED_{50} no statisticallly significant effect was observed in either the blood pressure or heart rate, which suggests that WAY-VNA-932 lacks V_1a agonist activity *in vivo*. A weak V1a antagonist effect was evident in the conscious rats at 200 mg/kg po. At a dose of 5 mg/kg po, WAY-VNA-932 showed no statistically significant adverse effects in the CNS (based on behavioral assessment and scoring).

Other Vasopressin V_2 Agonists

Desamino-D-arginine vasopressin (DDAVP Fig. 1, Ferring; Aventis) (19-22), a synthetic cyclic peptide analog of AVP, is currently the only vasopressin agonist approved for use in man. DDAVP is available in injectable, intranasal and oral formulations. It is indicated in the treatment of central diabetes insipidus, primary nocturnal enuresis and in certain conditions such as hemophilia A (23, 24) and von Willebrand's disease (25, 26).

When DDAVP is administered by injection the antiduretic effect is approximately 10 times that of an equivalent dose of DDAVP given via the intranasal route. The bioavailability of DDAVP oral tablets is about 5% compared to intranasal DDAVP, and about 0.16% compared to intravenous DDAVP. The dosage of DDAVP tablets must be determined for each individual patient and adjusted according to response. Onset of the antiduretic effect is rapid (~ 30 min) and reaches a maximum at about 90 min. The half-life is biphasic with a $t_{1/2}$ of 7.8 min and 75 min for the fast and slow phases, respectively (27).

Recently, workers at Otsuka have reported two series of substituted benzazepine vasopressin V_2 receptor agonists. One series is exemplified by OPC-51803 (Fig. 2) (28-31). In HeLa cells transfected with human V2 receptors, OPC-51803 showed a 9-fold selectivity for the V_2 receptor (over V_{1a}). Data from studies which examined the inhibitory effect of OPC-51803 on the cAMP production by AVP suggest that OPC-51803 is a partial agonist

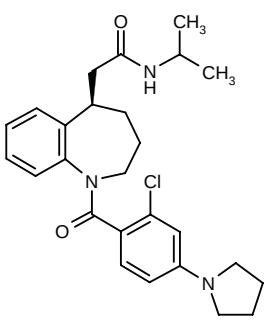


Fig. 2.

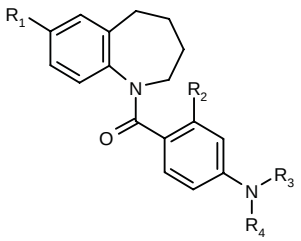


Fig. 3.

at the V_2 receptor. The second series, discovered as a result of metabolism studies in Otsuka's V_2 receptor antagonists program, is described in Fig. 3 (32). Based on limited SAR studies, the authors proposed structural requirements for a substituted benzazepine vasopressin V_2 receptor agonist.

Summary

WAY-VNA-932 is a novel, orally active nonpeptide small molecule which exhibits activity consistent with vasopressin V_2 receptor agonism. In CHO cells transfected with human V_{1a}, V_2, V_{1b} and OT receptors, WAY-VNA-932 was a potent agonist at the V_2 receptor, a weak antagonist at the V_{1a} and OT receptors, and had virtually no affinity for the V_{1b} receptor. In several animal models, WAY-VNA-932 lowered urine output in a dose-dependent manner with a concomitant increase in osmolality. In rats, suprapharmacologic oral doses of WAY-VNA-932 elicited no effects on blood pressure, heart rate or on the CNS. Based on this data it is expected that WAY-VNA-932 will offer several advantages over DDAVP, including greater and more consistent bioavailabilty and the lack of pressor activity. WAY-VNA-932 is expected to be useful in conditions characterized by excessive production and/or dilution of urine such as central diabetes insipidus and nocturnal enuresis. It may also have utility in certain clinical settings for the management of patients with hemophilia A or von Willebrand's disease.

Manufacturer

Wyeth Pharmaceuticals, Inc. (US).

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