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STEREOSELECTIVE SYNTHESIS AND EVALUATION OF ALL STEREOISOMERS OF Z4349, A NOVEL AND SELECTIVE μ-OPIOID ANALGESIC

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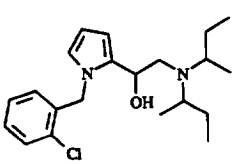
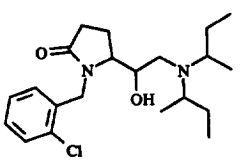
Abstract: A stereoselective synthesis of **1** was devised in order to obtain all 12 possible isomers. The pharmacological data clearly show a highly stereochemical requirement for the affinity and selectivity towards the μ-receptor and the related analgesic activity.

With the realization that the actions of the opioids were mediated through multiple receptors,[1] medicinal chemists and pharmacologists have sought selective agonists as a means to identify therapeutic agents with improved pharmaco-toxicological profile. Recent studies suggest the possible existence of μ-receptor subtypes,[2,3] indicating that various physiological events may not be produced through the same receptor. This gives hope for the development of new μ-potent and selective compounds that would be free of highly undesirable side effects, such as physical dependence and respiratory depression. Our strategy for the obtainement of novel μ-opioid selective agonists was based on modifications of the structure of viminol a central analgesic compound developed in our laboratory in the seventies.[4]



viminol



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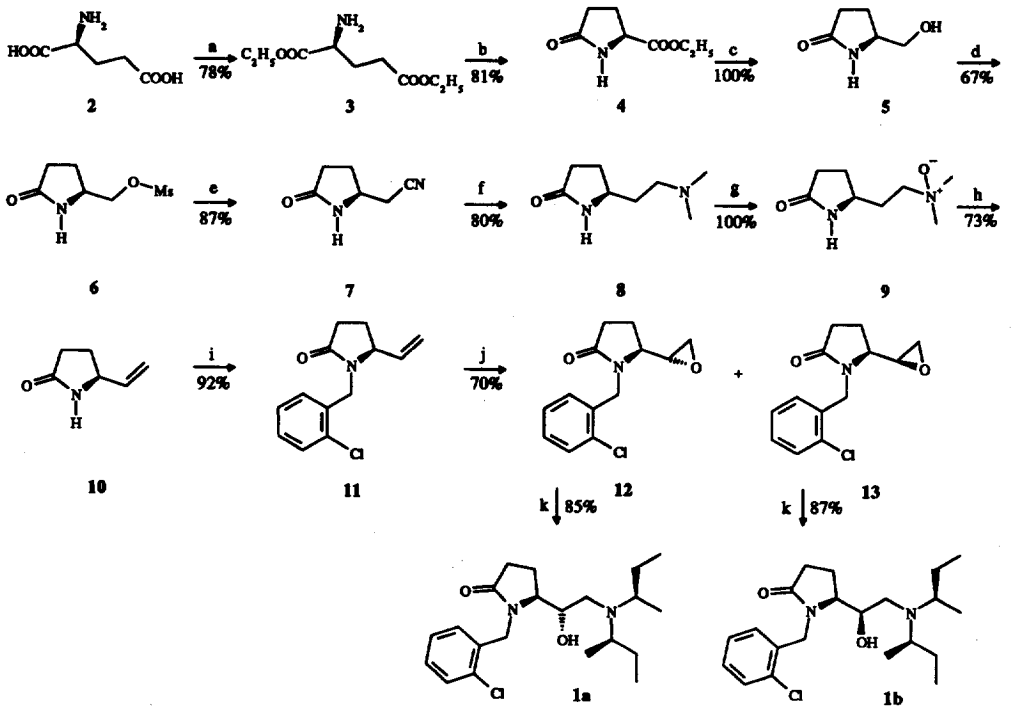
Previous structure-activity studies on viminol clearly pointed out the main structural requirements for the expression of the analgesic activity.[5,6,7,8] In particular, the presence of a tertiary di-sec-butylamine on an ethanolic chain at position 2 of the pyrrole ring and a benzylic substituent at position 1 turned out to be essential in this sense. Moreover, it was shown that the complex pharmacological profile of viminol resulted from contributions of its six stereoisomers.[7] In this communication we describe the synthesis and the preliminary pharmaco-biological data of the stereoisomers of 5-[2-[bis(1-methylpropyl)amino]-1-hydroxyethyl]-1-[(2-chlorophenyl)methyl]-2-pyrrolidinone **1** where the metabolically unstable pyrrole moiety of viminol is replaced by a 2-pyrrolidinone ring.

Chemistry

With the introduction of the pyrrolidinone moiety, compound **1** possesses four stereogenic centers, two of which bear identical substituents, giving rise to twelve possible isomers. In order to obtain all isomers, a stereoselective

synthesis starting from L or D glutamic acid and di-sec-butylamines of defined configuration[7] was devised. In Scheme 1 the representative preparation of isomers **1a** and **1b** is reported.

SCHEME 1[a]



[a] ***Key:*** *(a) $SOCl_2$, EtOH; (b) neat 160°C, 15 mbar; (c) $NaBH_4$, EtOH; (d) MsCl, Py; (e) NaCN, NaI, DMF; (f) H_2, $Pd/BaSO_4$, Me_2NH, EtOH; (g) H_2O_2, H_2O; (h) K_2CO_3, xylene, Δ ; (i) NaH, DMF,*  *; (j) MCPBA, $CHCl_3$, MPLC separation of two diastereomers; (k) (R,R)-di-sec-butylamine, n-BuOH, Δ .*

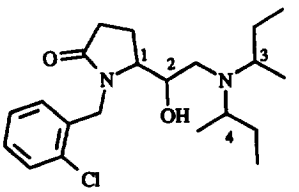
(5S)-5-Ethenyl-2-pyrrolidinone **10** was prepared in eight steps starting from L-glutamic acid modifying a reported procedure.[9] Alkylation with o-chlorobenzyl chloride gave compound **11** in high yield. Treatment of **11** with m-chloroperbenzoic acid resulted in a 30:70 mixture of epoxides **12** and **13** which were separated by medium pressure liquid chromatography. The attribution of the relative configuration of stereogenic centers of compounds **12** and **13** was obtained by means of ^1H NMR studies and subsequently confirmed by X-ray analysis of epoxide **12**. Finally desired compounds **1a** and **1b** were prepared refluxing respective epoxides **12** or **13** with optically pure (R,R)-di-sec-butylamine in n-butanol. Adopting the same procedure utilizing proper isomer of glutamic acid and di-sec-butylamine as starting materials, all stereoisomers **1a-l** were obtained. The

stereoisomeric purity of compounds **1a-l** was evaluated by HPLC method and resulted in greater than 99.5% except for compound **1d** which contained about 1% of isomer **1a**.

Pharmacology

Binding. The affinity of all compounds towards opioid receptors was determined as previously described by their ability to displace specific radioactive ligands in Sprague-Dawley male rats brain homogenates (minus cerebellum).[10] Incubations were performed at 25°C and lasted 30 minutes for μ-and κ-receptors and 45 minutes for δ-receptors. Specific radioligand were [^{3}H]-dihydromorphine 1 nM for μ-receptors, [^{3}H]-[D-Pen^2, D-Pen^5]enkephalin 2 nM for δ-receptors and [^{3}H]-U69593 2 nM for κ-receptors. The inhibition constant values (Ki) for compounds **1a-l** are reported in Table 1.

TABLE 1



1

Compound	Absolute configuration				Binding Affinity; Ki, nM			Hot Plate Test
	1	*2*	*3*	*4*	μ	κ	δ	ED_{50} *mg/kg, s.c.*
1a (Z4349)	S	S	R	R	0.25	62.5	265	0.01
1b	S	R	R	R	390	>5000	550	>100
1c*	R	S	R	R	100	>5000	1850	10.4
1d**	R	R	R	R	35	4850	>5000	0.47
1e	S	R	S	S	145	>5000	>5000	>100
1f	S	S	S	S	2250	>5000	>5000	>100
1g	S	R	R	S	340	3650	>5000	>100
1h	S	S	R	S	32	n.d.	>5000	1.5
1i	R	S	S	S	>5000	270	>5000	>100
1j	R	S	R	S	>5000	198	n.d.	>100
1k	R	R	S	S	>5000	n.d.	n.d.	>100
1l	R	R	R	S	87	260	1778	14.3
Morphine					3.0	27	15	4.6

* Data obtained as sulphate

** These data were obtained with a sample containing about 1% of compound 1a (Z4349)

n.d. = not detectable

In vivo antinociceptive studies. Analgesia was determined by the hot plate test.[11] Before the treatment, the basal reaction time (0-13 sec) was determined for each animal and those animals whose latency on the plate, fixed at a temperature of 51.5°C, was 14 sec or over were rejected. The selected mice were subcutaneously (sc) treated and placed at different times after treatment (10,20,30,45 and 60 min) on the heated plate until they reacted by licking their forepaws or shaking their hind legs. If they failed to react, we considered cut-off time 30 sec. The analgesic activity was expressed as ED_{50} (mg/kg) calculated on number of animals in analgesia. Reaction-time areas among 0 and 30 min after treatment were evaluated to identify animals under analgesic effect. We considered animals under analgesia when their area values were 45% higher than the mean area value of 100 vehicle treated animals (311 ± 3.59). The obtained results for compounds **1a-l** are reported in Table 1.

Results

The preliminary pharmaco-biological results and the absolute configurations of isomers **1a-l** are summarized in Table 1. These data clearly show how the stereochemistry of compounds **1** deeply influences their affinity towards the opioid receptors and the related analgesic activity. In particular the S,S,R,R isomer **1a** (Z4349) is endowed with very high affinity and good selectivity (κ/μ=250 and δ/μ=1060) for μ-receptor subtypes. These binding data well correlate with the in vivo antinociceptive potency of Z4349 in the hot plate test. The novelty of the structure of Z4349 and its configuration requirements for binding to μ-opioid receptors adds further data for the modeling of the opioid receptors.[12]

In particular questions arise about the role of the absolute configuration of the stereogenic center of the di-sec-butyl chains. In order to elucidate the influence of these stereocenters on the conformation of the whole molecule, as a possible explanation for the receptor binding stereochemical requirement, X-ray and computer modeling studies of isomers **1a**, **1f** and **1h** are in progress.[13]

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