Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1915–1920

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS, BIOLOGICAL ACTIVITY, AND ABSOLUTE STEREOCHEMICAL ASSIGNMENT OF NPS 1392: A POTENT AND STEREOSELECTIVE NMDA RECEPTOR ANTAGONIST

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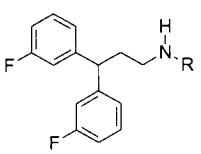
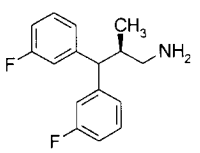
Received 23 March 1999; accepted 19 May 1999

Abstract: The synthesis, biological activity, and single crystal X-ray structure of NPS 1392, (*R*)-(-)-3,3-bis(3-fluorophenyl)-2-methylpropan-1-amine (**3a**), a potent, stereoselective antagonist of the NMDA receptor, are described. The NMDA receptor selectively bound the *levo* isomer (**3a**) over its enantiomer (**3b**), which prompted a rigorous absolute configuration assignment. NPS 1392 has the *R* configuration based on the single-crystal X-ray diffraction analysis of the hydroiodide salt of NPS 1392. This compound is a potential neuroprotective agent for use in the treatment of ischemic stroke. © 1999 Elsevier Science Ltd. All rights reserved.

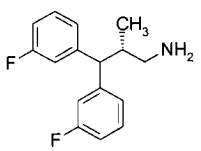
Keywords: Neurologically active compounds; neuroprotective agents; resolution; X-ray crystal structures



1, R = H
2, R = CH_3



3a



3b

Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). Ionotropic glutamate receptors have been classified pharmacologically as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and kainate receptors, according to their preferred agonists. Glutamate receptors have been implicated in the physiology and pathophysiology of various neurological and psychiatric functions and disorders such as ischemic stroke, epilepsy, pain, depression, and various neurodegenerative disorders such as Parkinson's disease. It has been postulated that the NMDA receptor plays a key role in mediating neuronal damage, probably due to its high permeability to calcium, a known mediator of cell damage. In animal models of focal ischemia, NMDA receptor antagonists provide dramatic and consistent cerebroprotection.[1,2]

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PII: S0960-894X(99)00317-0

Our previous efforts to develop NMDA receptor antagonists focused on the Araxin™ compounds, a class of small, highly polar, polycationic arylalkylpolyamine spider toxins, and their ability to selectively block glutamatergic synaptic transmission in the mammalian CNS.[3–5] However, their development as potential therapeutics was hampered due to unresolvable pharmacokinetic and toxicological problems associated with polyamines.

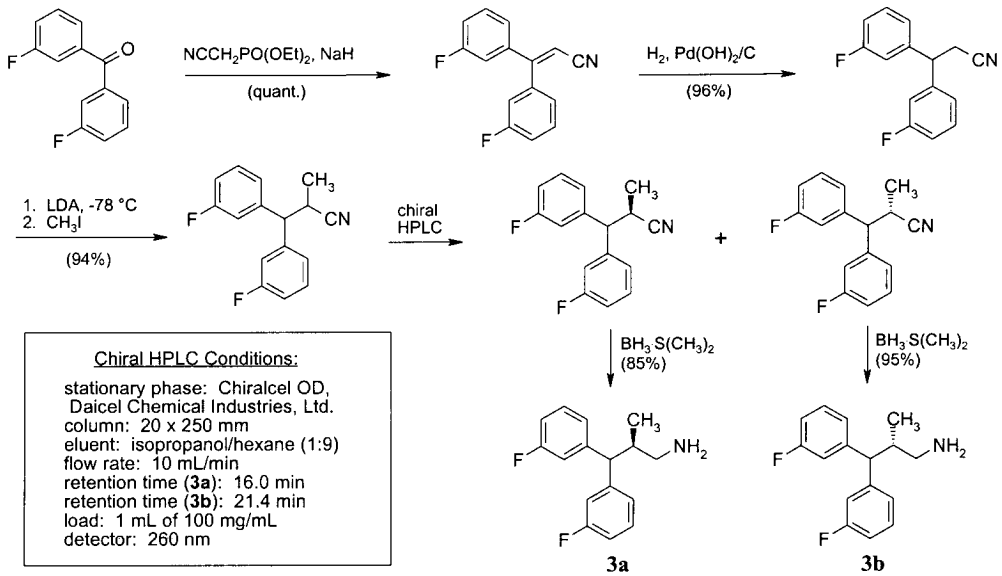
Recently, we reported the discovery of a non-psychotomimetic class of NMDA receptor open–channel blockers; compound **1** (NPS 846) is representative of this class.[3,4,6–9] The *N*-methyl derivative of **1**, NPS 1506 (**2**), is currently in human clinical trials for the treatment of acute ischemic stroke.[10,11] NPS 1392 (**3a**) was prepared during our investigation of the structure–activity relationships of these NMDA receptor antagonists.

The diphenylpropanamine skeleton occurs in a wide variety of pharmaceuticals, including antihistamines, analgesics, antitussives, antidiarrheals, antiarrhythmics, antihypertensives, antidepressants, antispasmodics, and anticholinergics. Examples of pharmacologically (i.e., CNS-) active compounds of this class, substituted with a methyl group beta to the amine nitrogen, include synthetic analgesics such as isomethadone, isomethadol, moramide, and *d*-propoxyphene.[12] Other noteworthy investigations involving the use of β-methyldiphenylpropylamines as NMDA receptor antagonists have been described in the literature.[13] These compounds also contain a γ-hydroxyl moiety, commonly found in many anticholinergic agents, and they evoked significant CNS side effects that precluded their use as pharmaceuticals.

Synthesis

The title compound (**3a**) was synthesized in a five-step reaction sequence. Commercially available 3,3'-difluorobenzophenone was coupled in a Horner-Emmons reaction to the sodium anion of diethyl (cyanomethyl)phosphonate in *N,N*-dimethylformamide (DMF) to provide 3,3-bis(3-fluorophenyl)acrylonitrile. The olefinic double bond was then reduced using catalytic hydrogenation. Deprotonation with lithium diisopropylamide (LDA) followed by treatment with methyl iodide afforded the racemic α-methyl nitrile. The enantiomers of the α-methylated nitrile were separated by chiral stationary-phase HPLC to >99% ee/HPLC (see Reaction Scheme for conditions). Reduction of each nitrile with borane-dimethyl sulfide complex provided, after work-up, enantiomerically pure **3a** and **3b** (NPS 1393), respectively. Full details of the reagents and reaction conditions utilized have been reported.[4] Hydrochloride salts of each isomer, formed in the usual manner, were used in the biological assays.[14] The more pharmacologically active (-)-isomer was then converted to the hydroiodide salt. Subsequent crystallization by slow evaporation from heptane/EtOAc (1/1) provided colorless needles (mp 195–197 °C) of **3a**·HI, which were used in the X-ray crystallographic analysis.

Reaction Scheme



Biological Activity

Biological data for selected compounds are shown in Table 1. Functional NMDA receptor antagonism [i.e., inhibition of NMDA/glycine-induced increases in cytosolic calcium in cultured rat cerebellar granule cells (RCGC's)[15,16] and inhibition of (+)-[3-^{3}H]-MK-801 binding from synaptic plasma membranes obtained from rat cortex][17–19] were used to determine potency at the NMDA receptor. Data for the well-characterized and well-known NMDA receptor antagonist, MK-801 (dizocilpine), are included for comparison.

Addition of a methyl group beta to the amine nitrogen in compound **1** provided a pair of enantiomers (compounds **3a** and **3b**) with significantly different pharmacological activities (Table 1). Both the *R* enantiomer (**3a**) and compound **1** show potent functional *in vitro* NMDA receptor antagonism (IC_{50}, 75 nM and 63 nM, respectively). However, although **3a** is more conformationally restricted than **1**, this did not increase its relative potency. The less active *S* enantiomer, compound **3b** (IC_{50}, 384 nM), has one-fifth the NMDA receptor antagonist potency of **3a**. Similarly, the *R* enantiomer was shown to be approximately fourteen times more potent than the *S* stereoisomer at displacing radioligand from [^{3}H]-MK-801-labelled binding sites.

Compound **3a** was tested in a rat model of temporary focal ischemia by 2-hour occlusion of the middle cerebral artery followed by 48 hours of reperfusion.[20] Two doses (2 mg/kg ip each) of **3a** were administered: the first, 30 minutes prior to ischemia and the second, 3 hours after ischemia (i.e., 1 hour after reperfusion). This double-dose paradigm resulted in a significant (37%) reduction in infarct volume ($p < 0.05$ compared to saline-treated controls, Student's t-test). No behavioral toxicity of compound **3a** was noted in this study.

Table 1. Biological Activities of MK-801, **1**, **3a**, and **3b**.

Compound	Stereochemical configuration	Functional NMDA-Receptor Antagonism IC_{50} (μM)	Displacement of (+)-[3-^{3}H]-MK-801 IC_{50} (μM)
MK-801	-	0.0034 (6)	0.0056 (11)
1	-	0.063 (4)	0.230 (4)
3a	*R*	0.075 (5)	0.141 (5)
3b	*S*	0.384 (3)	2.00 (2)

Numbers in parentheses indicate the number of experimental determinations.

Structural Analysis of NPS 1392·HI (3a)

The enantiomeric pair (compounds **3a** and **3b**) were prepared in such a way that the absolute configurations of their stereocenters were unknown. Pharmacological analysis of the two enantiomers showed that the (-)- isomer was clearly more potent than the (+)-isomer. Single-crystal X-ray diffraction was used to determine the absolute configuration of the hydroiodide salt of **3a** using iodine's anomalous scattering (Figure 1).

The crystal structure was solved to 1 Å resolution.[21] The asymmetric unit contains two independent molecules of **3a**·HI, and their conformations are slightly different. The two conformations are related by rotation of ring A (C5-C10) along the C3-C5 bond of 134° and by rotation of ring B (C11-C16) along the C3-C11 bond of 31°. Iodine and nitrogen atoms of the RNH_3^+ moieties form a stack of distorted I_3N_3 cubes running along the twofold screw axis. The average I-N distance was determined to be 3.55 Å.

Molecular modeling studies using molecular mechanics calculations confirm that the lowest calculated energy and most probable conformation had an identical side-chain (N-C1-C2-C3) orientation as that found in the crystal structure. The crystal structures of similar analogous compounds (such as (5*S*)-(-)isomethadone,[22] (5*S*,6*R*)-*threo*-5-methylmethadone,[23] and (5*S*,6*S*)-(-)-*erythro*-5-methylmethadone[24]) have been reported in the literature, and similar, extended propylamine chain conformations were found for the present compounds. The rotatable bond of highest importance to conformational analysis is the C2-C3 bond. Hydrogen atoms on carbons C2 and C3 are oriented anti-periplanar (*trans*) (Figure 2). This orientation is similar to the minimum energy conformation of 2,3-dimethylbutane. Free rotation of the phenyl groups was anticipated and confirmed by the orientation of the two molecules' phenyl rings in the asymmetric subunit. Our results indicate that there is flexibility of the rotational bonds in compound **3a**. Publication of our studies with more conformationally restricted analogs will follow.

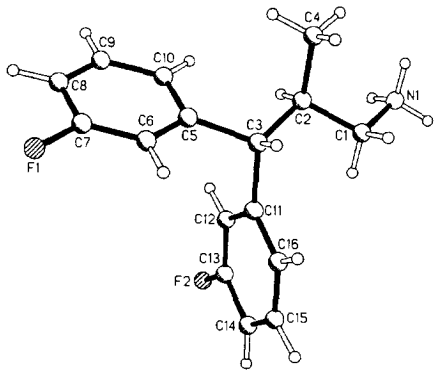


Figure 1. Crystal structure conformation of **3a**.

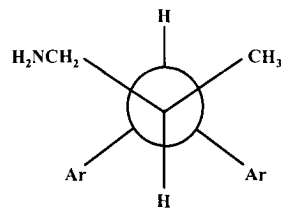


Figure 2. Newman Projection of **3a**.

Summary

In this communication we have described the synthesis, biological activity, and absolute stereochemical assignment of NPS 1392·HCl, a potent and stereoselective NMDA receptor antagonist. The title compound (**3a**) was synthesized from its corresponding enantiomerically pure intermediate nitrile, which was obtained by means of chiral stationary-phase chromatography. In vitro pharmacological assays showed that the *levo* isomer (**3a**) was the more potent enantiomer. The absolute stereochemistry of the more active isomer (**3a**) was determined to be *R* by an X-ray crystallographic analysis. Full details of the structure–activity relationships, chemical syntheses, and pharmacological studies will be the subject of future publications.

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14. Compound **3a**: mp 260–270 °C dec; $[\alpha]_{365}^{26}$ +6.6 ± 0.4° (*c* 1.0, EtOH); $[\alpha]_D^{20}$ -1.7 ± 0.2° (*c* 2.9, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$/methanol-d_4 [3/1]) δ 0.94 (d, 3H, CH_3), 2.52 (dd, J_1 = 13 Hz, J_2 = 10 Hz, 1H, pro-*S* CH_2), 2.64–2.78 (m, 1H, C*H*CH_3), 2.83 (dd, J_1 = 13 Hz, J_2 = 3 Hz, 1H, pro-*R* CH_2), 3.57 (d, *J* = 11 Hz, 1H, Ar_2CH), 6.78–7.23 (3m, 8H, ArH). Compound **3b**: mp 270–272 °C dec; $[\alpha]_{365}^{26}$ -6.1 ± 0.3° (*c* 1.0, EtOH); ^{1}H NMR data were consistent with that of the *R* enantiomer. Enantiomeric purities of **3a** and **3b** were each estimated to be >99% ee by ^{1}H-NMR ($CDCl_3$) studies of their corresponding diastereomeric urea derivatives, formed with (*R*)- and (*S*)-1-(1-naphthyl)ethyl isocyanate.
15. Following a reported method,[16] primary cultures of RCGC's obtained from 8-day-old rats were incubated with fura 2-AM to measure intracellular calcium concentrations. A combination of NMDA and glycine-induced response was used as the stimulus. Multiple cumulative concentration-response curves for blocking the stimulus were performed for each antagonist tested. IC_{50} values were determined by logit analysis. Five different concentrations of each test substance were used in the determination of each IC_{50}.
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21. Crystal data for NPS 1392 (**3a**): Crystal dimensions 0.50 x 0.05 x 0.03 mm, $C_{16}H_{17}F_2N{\cdot}HI$, M = 389.21, monoclinic, *a* = 33.175(4) Å, *b* = 7.134(10) Å, *c* = 14.747(10) Å, α = 90°, β = 103.830(10)°, γ = 90°, *V* = 3389.0(7) Å^3, space group P1 (C2), *Z* = 8, *Dc* = 1.522 Mg m^{-3}, μ(Cu-Kα) = 21.56 cm^{-1}, *F*(000) = 1536. Data were obtained at 20 °C on a Siemens R3m/V diffractometer using graphite monochromated Cu-Kα radiation. A total of 4794 reflections (4272 unique) were collected using the ω/2θ scan technique within a 2θ range of 110.12°. The structure was solved by direct methods and refined by a full-matrix least-squares method using 4272 reflections [F > 4σ(F)]. The final refinement converged to R_1 = 0.0570 and wR_2 = 0.1463.
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