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NMDA-NR2B subtype selectivity of stereoisomeric 2-(1,2,3,4-tetrahydro-1-isoquinolyl)ethanol derivatives

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Dedicated with best wishes to Professor Theodor Severin on the occasion of his 75th birthday

Abstract—Enantiopure 2-(1,2,3,4-tetrahydro-1-isoquinolyl)ethanol derivatives were tested for their affinity to the ifenprodil binding site of the NMDA receptor, their potency to inhibit [^{3}H]MK801 binding and their NMDA-NR2B subtype selectivity. The (1*S*,1′*S*)-configurated series displayed the highest affinity to the ifenprodil binding site. A reasonable potency and NMDA-NR2B subtype selectivity was found for (1*S*,1′*S*)-**4c** (R^1 = Me, R^2 = OMe). A high affinity to HERG K+ channels, however, suggests that (1*S*,1′*S*)-**4c** may involve an increased risk of cardiovascular side effects.

The *N*-methyl-D-aspartate (NMDA) receptor is a glutamate-gated ion channel strongly implicated in neuroprotection, neurodegeneration, long-term potentiation, memory and cognition.[1] Overactivation of NMDA receptors and the resulting calcium overload of neurons is considered to trigger a cascade of intracellular events that alters the cell function and may ultimately lead to the death of neurons.[2] Evidence is accumulating that neurodegenerative and psychiatric disorders including Parkinson's disease, Huntington's chorea, schizophrenia and stroke may be caused, at least in part, by excessive activation of NMDA receptors.[3] NMDA antagonists have been found to show efficacy in animal models of traumatic brain injury[4] and of neuropathic and inflammatory pain.[5] NMDA receptor activity may be attenuated by blockade of the glutamate binding site, the glycine coagonist binding site or the receptor-associated ion channel. At effective dose levels severe side effects have been noted with many, but not all, NMDA receptor antagonists.[3] The NMDA receptors are composed of multiple protein subunits categorized as NR1, NR2 and NR3 with different subunit combinations having diverse functional properties and anatomical distribution.[6] Four distinct NR2 subunits (NR2A-D) have been identified so far.[7] Antagonists selective for NMDA receptors containing a NR2B subunit offer a means of inhibition without the aforementioned side effects.[8] It has been demonstrated that ifenprodil (**1**), the prototype of NMDA antagonists selective for NMDA receptors containing NR2B subunits,[9] has a greater ratio of therapeutic effect to side effect than can be realized with NR2B-unselective antagonists.[10] However, ifenprodil displays a relatively high affinity to non-NMDA receptors, for example, the α1 adrenergic, serotonin and sigma receptors.[11] The tetrahydroisoquinoline derivative *Ro 04-5595* (**2**) is among the newer compounds exhibiting a high selectivity for NMDA receptors containing a NR2B subunit.[12a] In addition to compound **2**, various tetrahydroisoquinolylethanol derivatives **3** have been reported by Hoffmann–La Roche as selective antagonists of NMDA receptors containing NR2B subunits[12b] (Scheme 1). However, no details regarding the stereochemistry of these compounds were given, although it is obvious that they are racemic. Thus, the stereochemistry of the diastereomers evaluated for NMDA antagonistic activity is still unclear. It is even possible that mixtures of diastereomers were used.

Previously, in the context of a study on opioid analgesics, we synthesized a series of 2-(1,2,3,4-tetrahydro-1-

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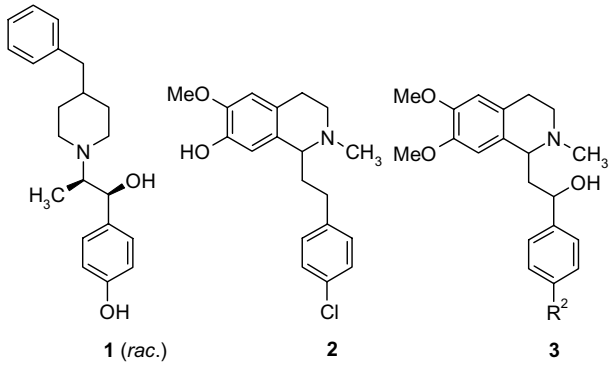
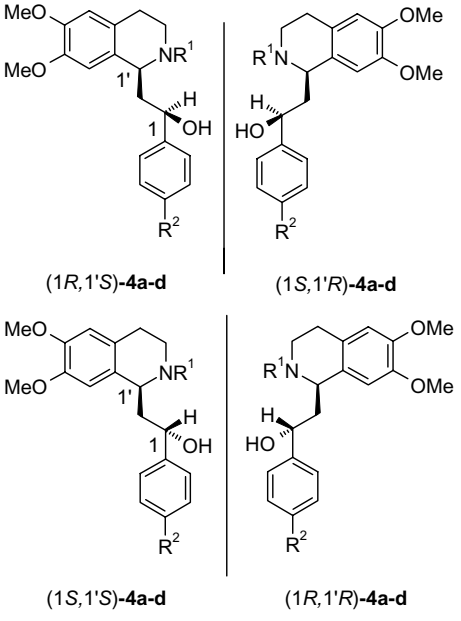


1 (*rac.*) **2** **3**

Scheme 1.

isoquinolyl)ethanol derivatives **4** (Scheme 2) in enantiomerically pure form by asymmetric electrophilic α-amidoalkylation reactions.[13,14] We discovered that the potency of the amino alcohols **4a**–**d** at the μ and κ opioid receptors is strongly dependent on their configuration. Besides, within a set of isomers, the highest affinity has been always displayed by the same stereoisomer (1*R*,1′*R*)-**4** (Table 1).[14] With this data in mind, we thought it worth to study the effect of the stereochemistry on the biological activity reported for **3** as NMDA antagonists. The enantiopure isoquinoline derivatives **4a**–**d** representing four complete sets of stereoisomers were selected as test compounds. In the first step, the compounds were characterized in a competitive binding assay with respect to their potency at the ifenprodil binding site of the NMDA receptor. The binding experiments were performed using a synaptosomal fraction of porcine hippocampal brain membrane as a source for the NMDA receptor and [^{3}H]ifenprodil as the radioligand.[15]



	R^1	R^2
a	H	OMe
b	H	Cl
c	Me	OMe
d	Me	Cl

Scheme 2.

The results are depicted in Table 1. Two general trends were observed: first, within a set of compounds exhibiting the same stereochemistry [e.g., (1*R*,1′*S*)-**4a**–**d**], the NCH_3 derivatives were more potent than the corresponding N–H compounds. Secondly, compounds with a *p*-chlorophenyl group (**4b**,**d**) displayed lower IC_{50}-values than compounds with a methoxy group in the *para* position (**4a**,**c**). Furthermore, the binding affinity of the amino alcohols **4a**–**d** was found to be strongly dependent on the configuration. Of all the stereoisomers, the (1*S*,1′*S*)-configurated amino alcohols (1*S*,1′*S*)-**4a**–**d** exhibited the highest potencies with IC_{50}-values ranging from 59 nM to 14 μM. Within this series of the (1*S*,1′*S*)-stereoisomers, the ranking of potencies is in accord with the general trends mentioned above: N-methyl/*p*-chloro (1*S*,1′*S*)-**4d** > N-methyl/*p*-OMe (1*S*,1′*S*)-**4c** > N–H/*p*-chloro (1*S*,1′*S*)-**4b** > N–H/*p*-OMe (1*S*,1′*S*)-**4a**. Interestingly, the most potent compound (1*S*,1′*S*)-**4d** (IC_{50} = 59 nM) exhibited an enantioselectivity of binding of ~1/1700. Moreover, the diastereoselectivity of binding of (1*S*,1′*S*)-**4d** was found to be very high [~1/400 as compared to (1*S*,1′*R*)-**4d**, and ~1/900 as compared to (1*R*,1′*S*)-**4d**].

Inhibition of [^{3}H]MK801 binding to brain membranes under non-equilibrium conditions by NR2B subunit selective NMDA receptor antagonists such as ifenprodil or eliprodil is known to proceed in a biphasic manner.[1e,16] The high-affinity phase is thought to reflect the allosteric inhibition at NR2B subunit containing NMDA receptors, whereas the low-affinity phase is assumed to account for inhibition of NMDA receptors lacking a NR2B subunit. Previous studies on antagonists selective for the NMDA receptors containing a NR2B subunit have demonstrated a correlation between the displacement of [^{3}H]ifenprodil and the high-affinity phase of the inhibition of [^{3}H]MK801 binding.[16]

It was therefore of great interest to study the effect of the enantiopure compounds **4a**–**d** on [^{3}H]MK801 binding. The experiments were performed with the aforementioned synaptosomal fraction of porcine hippocampal brain membranes in the presence of 100 μM L-glutamate and 30 μM glycine under non-equilibrium conditions.[15,16b] It was discovered that (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** not only caused inhibition of [^{3}H]MK801 binding in a biphasic fashion, but were also the most potent inhibitors in this assay. The IC_{50}-values for the high-affinity fraction of the inhibition curve were determined to be 477 ± 72 and 103 ± 40 nM for (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d**, respectively. The results were in good accord with the IC_{50}-values found in the [^{3}H]ifenprodil binding assay, whereas the IC_{50}-values of the second binding phase were distinctly higher [(1*S*,1′*S*)-**4c** = 107 ± 13 μM and (1*S*,1′*S*)-**4d** = 23.5 ± 3.4 μM, Table 1, Fig. 1]. According to this data, it seems likely that (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** are characterized by a clear subtype selectivity for NMDA receptors containing a NR2B subunit.

Table 1.

	μ K_i [μM]	[^{3}H]ifenprodil IC_{50} [μM]	[^{3}H]MK801 EC_{50} [μM][a]
(1*R*,1′*S*)-**4a**	5.8 ± 0.8	243 (*n* = 1)	110 ± 20
(1*R*,1′*S*)-**4b**	2.6 ± 0.1	95 ± 27	34 ± 1
(1*R*,1′*S*)-**4c**	22 (*n* = 1)	100 μM: 34.0%	—[b]
(1*R*,1′*S*)-**4d**	2.7 ± 0.1	52 ± 9.5	45 ± 10
(1*S*,1′*S*)-**4a**	2.2 ± 0.7	14 ± 0.2	29 ± 6
(1*S*,1′*S*)-**4b**	0.92 ± 0.11	**1.1 ± 0.1**	**7.4 ± 2.0**[c]
(1*S*,1′*S*)-**4c**	2.7 ± 0.9	**0.24 ± 0.03**	**4.6 ± 0.9**[d]
(1*S*,1′*S*)-**4d**	0.50 ± 0.03	**0.059 ± 0.002**	**0.96 ± 0.57**[e]
(1*R*,1′*R*)-**4a**	**0.72 ± 0.06**	100 μM: 63%	120 ± 13
(1*R*,1′*R*)-**4b**	**0.26 ± 0.02**	290 (*n* = 1)	37 ± 3
(1*R*,1′*R*)-**4c**	**0.06 ± 0.002**	100 μM: 50%	—[b]
(1*R*,1′*R*)-**4d**	**0.007 ± 0.001**	100 ± 22	56 ± 12
(1*S*,1′*R*)-**4a**	11 ± 2	420 μM (*n* = 1)	140 ± 23
(1*S*,1′*R*)-**4b**	6.7 ± 0.3	43 ± 0.8	40 ± 5
(1*S*,1′*R*)-**4c**	0.38 ± 0.001	100 μM: 87%	120 ± 35
(1*S*,1′*R*)-**4d**	0.11 ± 0.01	24 ± 7	31 ± 6

If not stated otherwise the results are given as means of K_i- and IC_{50}-values ± SEM of three independent experiments each performed in triplicate, *n* = 1 denotes a K_i- or IC_{50}-value from a single experiment, percentages represent specific binding remaining in presence of 100 μM inhibitor.
[a] IC_{50} value for high affinity fraction or monophasic curve, respectively.
[b] Not tested.
[c] 7.37 ± 2.04 (monophasic) 2.60 ± 2.94/42.6 ± 42 μM (biphasic).
[d] 4.55 ± 0.85 (monophasic) 0.477 ± 0.072/107 ± 13 μM (biphasic).
[e] 0.962 ± 0.573 (monophasic) 0.103 ± 0.040/23.5 ± 3.4 μM (biphasic).

For several stereoisomers only monophasic [^{3}H]MK801 inhibition curves were observed. This must be due to a low subtype selectivity for NMDA receptors containing a NR2B subunit.

(1*R*,1′*R*)-**4d** is an example for the type of compounds displaying a monophasic inhibition curve for [^{3}H]MK801 binding only. Consequently, a low affinity or low subtype selectivity for NR2B subunits containing NMDA receptors has to be ascribed to this compound. As a result, (1*R*,1′*R*)-**4d** shows a distinctly less favourable binding profile than its enantiomer. (1*S*,1′*S*)-**4d** turned out to be the most potent inhibitor in the [^{3}H]ifenprodil and [^{3}H]MK801 binding and is presumably associated with a clear subtype selectivity for NMDA receptors containing a NR2B subunit.

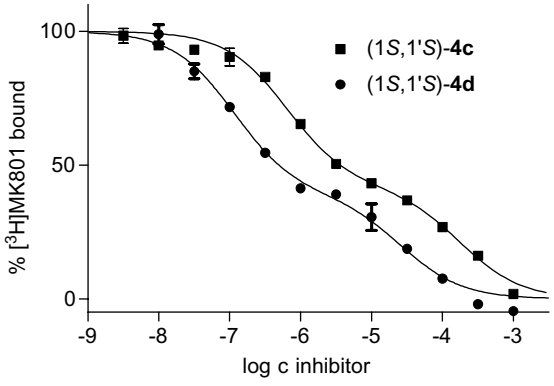


Figure 1. Biphasic inhibition of [^{3}H]MK801 binding to a synaptosomal fraction of porcine hippocampus in presence of 100 μM L-glutamate and 30 μM glycine by (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** from one representative experiment out of three.

The three most potent tetrahydroisoquinolylethanol derivatives (1*S*,1′*S*)-**4b–d** were further characterized by electrophysiological recordings on the four binary NMDA receptor subunit combinations NR1a/NR2A, NR1a/NR2B, NR1a/NR2C and NR1a/NR2D expressed in *Xenopus oocytes* (Table 2).[17] For all three compounds (1*S*,1′*S*)-**4b–d**, inhibition of NR1a/NR2B receptors was found to be consistently higher when compared to inhibition of NR1a/NR2A, NR1a/NR2C and NR1a/NR2D receptors. Among these, the amino alcohol (1*S*,1′*S*)-**4d** turned out to be the most potent inhibitor at the NR1a/NR2B subunit displaying an IC_{50}-value of 1.32 μM. This is in line with the results from the [^{3}H]ifenprodil binding studies. However, compound (1*S*,1′*S*)-**4d** was also quite effective at the NR1a/NR2C receptor subtype (IC_{50} = 5.29 μM) resulting in a low subtype selectivity (~1/4). Although the amino alcohol (1*S*,1′*S*)-**4c** was slightly less potent at the NR1/NR2B receptor subtype (IC_{50} = 3.16 μM) compared to (1*S*,1′*S*)-**4d**, it appeared to display a distinctly higher subtype selectivity for the NR1a/NR2B versus the NR1a/NR2C receptor subtype (50/1). Additionally, the subtype selectivity of (1*S*,1′*S*)-**4c** for the NR1a/NR2B versus the NR1a/NR2A (34/1) and the NR1a/NR2D

Table 2. Inhibition of NMDA receptor subtypes (IC_{50} ± SEM in μM at −70 mV)[a]

	NR1a/2A	NR1a/2B	NR1a/2C	NR1a/2D
(1*S*,1′*S*)-**4b**	103.9 ± 23.6	10.54 ± 2.98	24.9 ± 0.6	48.4 ± 8.6
(1*S*,1′*S*)-**4c**	107 ± 23.8	**3.16 ± 0.46**	155.2 ± 38.9	341.0 ± 60.8
(1*S*,1′*S*)-**4d**	136.7 ± 9.2	**1.32 ± 0.42**	5.29 ± 0.34	27.5 ± 1.1

[a] NMDA receptor activation by coapplication of 1 mM L-glutamate and 10 μM glycine for 30–40 s every 2–3 min.

receptor subtypes (108/1) was very promising, too. Finally, compound (1*S*,1′*S*)-**4b** exhibited the lowest potency at the NR1a/NR2B receptor subtype and the lowest subtype selectivity among these three amino alcohols.

HERG (human ether-à-go-go-related gene) K^+ channels are rectifying voltage-activated potassium channels found in the heart. They are responsible for accelerating the repolarizing phase of the cardiac action potential. Unintended blockade of HERG K^+ channels is a side effect of many standard medications and the most common cause of acquired long QT syndrome associated with increased risk of life-threatening arrhythmias.[18] When the amino alcohols (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** were evaluated for their affinity to the HERG protein (HERG; GenBank Acc. No U04270) using patch clamp electrophysiology (tail currents measured at −40 mV following a 1 s depolarization to +20 mV),[19] both compounds [(1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d**] appeared to be inhibitors of the channel. With IC_{50}-values of 0.79 ± 0.04 and 0.40 ± 0.04 μM (means ± SEM, $n \geqslant 3$) the potency of (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** as inhibitors at the HERG K^+ channel was in the same order of magnitude as the potency of these compounds at the NMDA-NR1a/2B receptor subtype. The risk of cardiotoxicity inferred by their activity at the HERG channel certainly makes these compounds [(1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d**] unsuitable for medical use.

In summary, we have evaluated a series of enantiopure 2-(1,2,3,4-tetrahydro-1-isoquinolyl)ethanol derivatives known to be inhibitors of the μ opioid receptor for their affinity to the ifenprodil binding site, their potency to inhibit [^{3}H]MK801 binding, and their NMDA-NR2B subtype selectivity. The results of this evaluation clearly show that, as compared to the μ opioid receptor binding site, the ifenprodil binding site displays a distinctly different stereoselectivity of ligand binding. Whereas the stereoisomeric amino alcohols (1*R*,1′*R*)-**4a**–**d** are most potent for the μ opioid receptor, the highest affinity for the ifenprodil binding was observed for the (1*S*,1′*S*)-configurated series **4a**–**d**. Electrophysiological studies performed with NMDA receptor subtypes in *X. oocytes* revealed the amino alcohol (1*S*,1′*S*)-**4c** as the most promising inhibitor displaying a high potency and reasonable NMDA-NR1a/NR2B subtype selectivity. However, a high affinity to HERG K^+ channels observed for the two most potent amino alcohols (1*S*,1′*S*)-**4c** and (1*S*,1′*S*)-**4d** renders these compounds less suitable for further drug development despite their affinity and selectivity for the NMDA-NR1a/NR2B receptor subtype.

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