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# STRUCTURE–ACTIVITY RELATIONSHIP FOR A SERIES OF 2-SUBSTITUTED 1,2,3,4-TETRAHYDRO-9*H*-PYRIDO[3,4-*b*]INDOLES: POTENT SUBTYPE-SELECTIVE INHIBITORS OF *N*-METHYL-D-ASPARTATE (NMDA) RECEPTORS

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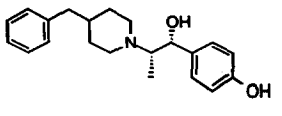
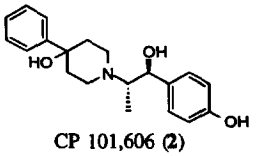
**Abstract**: A series of 2-substituted 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles was synthesized as potential antagonists for the NR1A/2B subtype of *N*-methyl-D-aspartate (NMDA) receptors. Assayed by electrical recording under steady-state conditions, 7-hydroxy-2-(4-phenylbutyl)-1,2,3,4-tetrahydropyrido-[3,4-*b*]indole (**30**) was the most potent compound in the series having an $IC_{50}$ value of 50 nM at the NR1A/2B receptors. 

Glutamate excitotoxicity has been linked to neuronal degeneration in a variety of neurological disease states including cerebral ischemia, epilepsy, Parkinson's disease, and other CNS disorders.[1] Inhibition of NMDA receptors attenuates excitotoxic neuronal death.[2] Animal models suggest that NMDA receptor antagonists hold promise for the treatment of acute ischemic cerebrovascular disorders, and might reduce the progression of chronic neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.[1]

Studies at the molecular level suggest that NMDA receptors are heterooligomeric assemblies of at least two types of polypeptide subunits: NR1 found in eight isoforms, and NR2 found as four distinct subtypes (NR2A-NR2D).[3] Subunit composition and distribution in adult mammalian brain differ significantly from region to region.[4] NMDA receptor subtypes have different pharmacological properties and thus present discrete therapeutic targets. A major advantage in the development of clinically useful subtype selective antagonists of the NMDA receptor complex is their high therapeutic index with respect to sedation and their lack of dose limiting side effects such as neurotoxicity and psychotomimetic behaviors.[5] Thus designing NMDA subtype selective antagonists may provide new treatment strategies for many neurodegenerative disorders. Ifenprodil (**1**), CP 101,606 (**2**),[6] Ro 25-6981 (**3**),[7] Co 101676 (**4**),[8] and Co 101526 (**5**)[9] are examples of NMDA receptor antagonists that have pronounced selectivity for the NR2B receptor subtype.




Ifenprodil (**1**)
(racemate)

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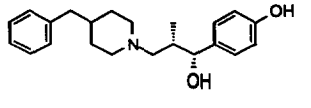
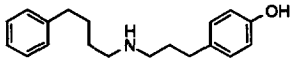
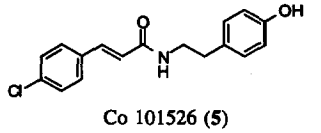



Ro 25-6981 (**3**)




Co 101676 (**4**)



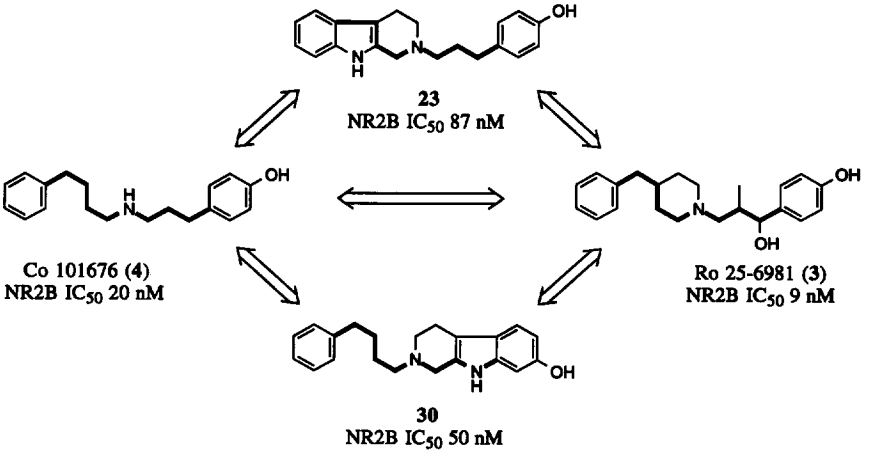

Co 101526 (**5**)

0960-894X/99/$ - see front matter 
*PII*: S0960-894X(99)00248-6

The family of 1,4-disubstituted piperidines are reported to have neuroprotective effects in animal models of focal cerebral ischemia without themselves inducing neurotoxicity or showing behavioral liability in drug discrimination studies.[6,7] NR2B subtype selective antagonists inhibit NMDA receptor function by a non-competitive allosteric mechanism at a site or sites not located in the membrane-spanning region of the channel pore and that are presumed to bind polyamines.[10]

In an earlier study, we investigated some primary structural determinants required for high potency and selectivity at NR2B subunit containing receptors in a series of bis-alkylphenylamines.[8] In this present study we investigate the effect of structural rigidification through incorporation of a pyridoindole moiety in the backbone of the antagonist. Thus a series of pyridoindole-based molecules was designed and synthesized with the notion that rigidification may result in not only high potency at the NR1A/2B subtype but also high selectivity when compared to phenyl/benzylpiperidine-based molecules previously reported as NR1A/2B selective inhibitors.[6,7] Pyridoindole **30** was identified as a potent and selective inhibitor of the NR1A/2B NMDA receptor subtype showing low nanomolar potency for the NR1A/2B subunit combination and >1000-fold selectivity with respect to NR1A/2A and NR1A/2C. The structural relationship among pyridoindole **23** and transposed analog **30** with respect to the highly potent antagonists **3** and **4** is depicted in Chart 1.

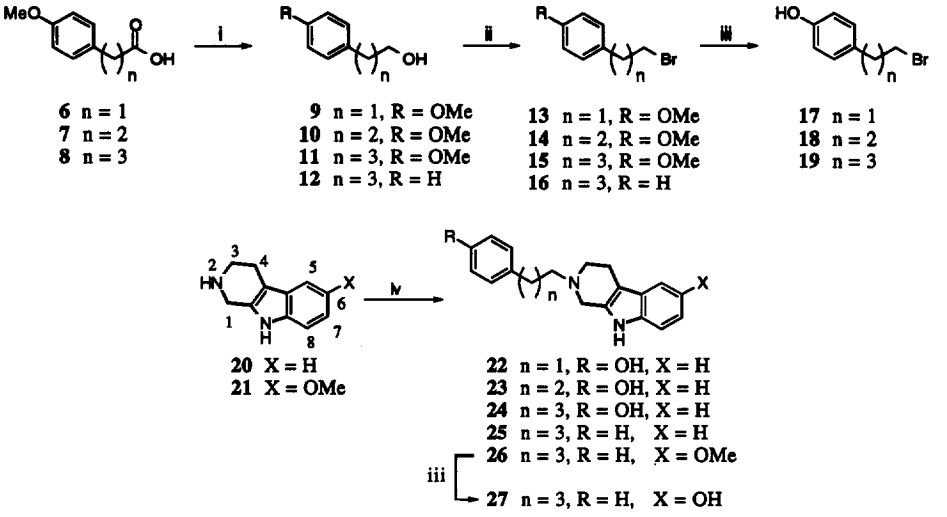
**Chart 1**




## Chemistry

Pyridoindoles **22–27** were synthesized as depicted in Scheme 1. Commercially available carboxylic acids **6–8** were reduced in good yield to the corresponding alcohols **9–11**. Bromides **13–16** were prepared by the reaction of the alcohols **9–12** (alcohol **12** was purchased from Aldrich Co.) with neat $PBr_3$. *O*-Demethylation of ethers **13–15** giving **17–19** was accomplished with $BBr_3$ in $CH_2Cl_2$. Reaction of commercially available pyridoindoles **20** and **21** with the appropriate bromides chosen among **16–19** yielded pyridoindoles **22–26** in good yields. *O*-Demethylation of 6-methoxypyridoindole (**26**) using $BBr_3$ gave **27**. The pyridoindoles **22–27** were crystallized as the free base from an appropriate solvent.[11]

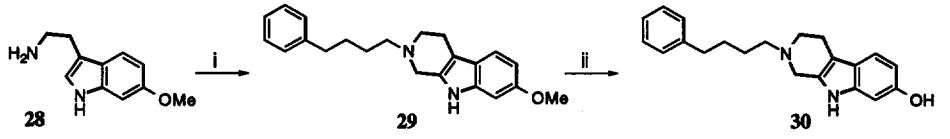
**Scheme 1**




[a] Reagents: (i) $LiAlH_4$, THF; (ii) $PBr_3$ (neat); (iii) $BBr_3$, $CH_2Cl_2$; (iv) corresponding bromide (**17-19**), $NaHCO_3$, $CH_3CN$.

Attempts to synthesize 7-methoxypyridoindole according to the procedure of Callaway et al.[12] failed in our hands. Thus a one-pot synthesis was devised to synthesize pyridoindole **30** in good yield (Scheme 2). The HCl salt of commercially available 6-methoxytryptamine (**28**) was allowed to react with formaldehyde in a Pictet-Spengler reaction. Addition of a mixture of bromide **16** and $NaHCO_3$ in DMF gave **29**. *O*-Demethylation to give **30** was achieved using the $BBr_3$ protocol.

**Scheme 2**



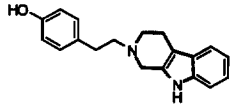
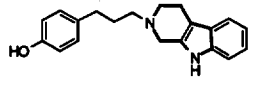
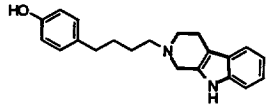
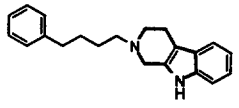
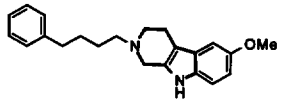
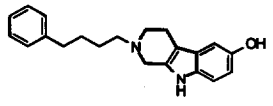
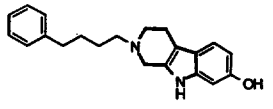

[a] Reagents: (i) a) HCl/ether; b) $CH_2O$ (37% in $H_2O$); c) **16**, $K_2CO_3$, DMF; (ii) $BBr_3$, $CH_2Cl_2$

**Structural–Activity Relationships**

Potency and selectivity of ligands were assessed by functional assays in *Xenopus* oocytes expressing recombinant NMDA receptor subunit combinations. The $IC_{50}$ values (Table 1) were determined by curve fitting to concentration–inhibition data pooled from 2–7 separate experiments.[8] All compounds with the exception of **26** showed high or moderate selectivity for NR1A/2B subunit combinations ($IC_{50}$ < 5 μM). The most potent compound at the NR1A/2B subtype in this series is **30**, which possesses a 7-hydroxy group in the pyridoindole

moiety and a 4-phenylbutyl substituent on the basic nitrogen atom. The presence of the phenolic hydroxy group is essential for activity throughout this series. Moving the hydroxy group from the 7 to the 6 position on the pyridoindole moiety as seen in **27** results in a fourfold drop in potency. Transfer of the hydroxy group to the A-ring (see Table 2 for the A, B numbering convention) results in **24** and a fourfold drop in potency. Removal of the hydroxy group gives **25** and renders the molecule essentially inactive. Shortening the chain length towards the A-ring to three methylene groups results in **23** with a potency comparable to **30**. Reduction of this N–O distance in **23** by one methylene unit results in **22** which is now 44-fold less active than **23**. Extending the methylene count in **23** gives **24** which is 6-fold less potent than **23**, yet more active as compared to **22**. Antagonists **23**, **27** and **30** all have similar distances between the hydroxy group and the basic piperidine nitrogen atom (Table 2).

**Table 1.** Functional Antagonism of Substituted Amines at NMDA Receptor Subtypes

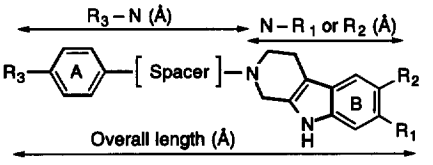
| Compd No. | Structure | $IC_{50}$ (μM)[a] 1A/2A | 1A/2B | 1A/2C | No. of Oocytes n (A, B, C) |
|---|---|---|---|---|---|
| **22** |  | 74 ± 11 | 3.8 ± 0.3 | 140 ± 11 | 2, 3, 2 |
| **23** |  | 42 ± 21 | 0.087 | 170 ± 13 | 2, 4, 2 |
| **24** |  | 58 ± 16 | 0.56 ± 0.05 | 150 ± 93 | 2, 3, 2 |
| **25** |  | 68 ± 15 | 3.3 ± 0.4 | 65 ± 11 | 2, 2, 2 |
| **26** |  | 110 ± 36 | 14 ± 4.2 | 132 ± 31 | 2, 3, 2 |
| **27** |  | 97 ± 30 | 0.14 ± 0.03 | 180 ± 21 | 2, 3, 2 |
| **30** |  | 57 ± 42 | 0.05 ± 0.02 | 67 ± 9.0 | 2, 3, 2 |

[a] $IC_{50}$ values (±S.E.M) were determined by electrical assays in *Xenopus* oocytes expressing the NMDA receptor combinations.

Also, the molecules are apparently able to interact with the receptor pocket in either orientation given a similar spatial position of the phenolic hydroxyl group and the basic nitrogen atom on the piperidine ring (Table 2). We made a similar observation in another series of NR2B subtype selective antagonists.[9]

Intramolecular distances (Table 2) were measured (Å) corresponding to the fully extended minimized conformer using AM1 semiempirical calculations. After geometry optimization, the fully elongated conformer of amine **30** has an overall length of 17.8 Å. While amines **30** and **24** have similar overall lengths, **30** is 11-fold more potent at the NR1A/2B subtype. On the other hand, compounds **23**, **27**, and **30** have a similar distance between the basic piperidine nitrogen atom and the corresponding oxygen atom of the hydroxy group and are all potent at the NR1A/2B subtype. This observation confirms that the potency of an antagonist at the NR1A/2B subtype in this series depends on the position of the basic nitrogen atom relative to the phenolic hydroxy group. A variation of ±2 Å in the overall length of the molecule and/or the distance between the two aromatic rings (A–B) is tolerated without much change in potency (**23** vs **30**).

**Table 2.** Intramolecular Distances (Å) Between Atoms Measured on the Fully Extended Conformer Calculated at the Semiempirical (AM1) Level.




| Compd No. | $R_1$ | $R_2$ | $R_3$ | N–O (Å) | Overall Length (Å)[a] | A–B (Å)[b] | $IC_{50}$ 1A/2B (μM) |
|---|---|---|---|---|---|---|---|
| **23** | H | H | OH | 8.8 | 15.0 | 11.1 | 0.087 |
| **24** | H | H | OH | 10.1 | 17.7 | 12.9 | 0.56 |
| **27** | H | OH | H | 7.8 | 17.5 | 12.9 | 0.14 |
| **30** | OH | H | H | 8.3 | 17.8 | 12.9 | 0.05 |

[a]Distance measured from the para hydrogen of the A/B-ring to the hydroxy oxygen of the B/A-ring respectively. [b]The distance measured from the center point of the A-ring to the center point of the B-ring.

Inhibition of NR1A/2A and NR1A/2C receptors by this series of compounds was consistently weaker when compared to inhibition of NR1A/2B receptors. In general, compounds with high potency at the NR1A/2B receptors also show a trend towards moderate potency at the NR1A/2A receptors. There are no a priori reasons why there should be parallels between SARs for the different subunit combinations since the low potency inhibition at 1A/2A and 1A/2C receptors is voltage dependent hence mechanistically distinct from the 2B receptors.[13] However, to the extent that such relationships exist, it would suggest that the sites mediating the high potency and low potency inhibition may share common structural features.

## Conclusion

The 2-substituted 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indoles studied are selective antagonists of NR1A/2B receptors, pyridoindole **30** being the most potent ($IC_{50}$ = 50 nM). In this series potency at 1A/2B receptor subtype is strongly dependent on the presence of a phenolic hydroxyl group and its spatial relationship with the nitrogen atom of the piperidine ring.

**Acknowledgment:** The cDNAs encoding the rat NR1A, NR2A, NR2B and NR2C subunits were a generous gift from Dr. P. H. Seeburg (University of Heidelberg, Heidelberg, Germany). We thank Drs. V. I. Ilyin (CoCensys, Inc.) and J. E. Huettner (Washington University Medical School) for help with statistical methodology, and Dr. L. Zhang for the preparation of cRNA. Financial support to the University of Oregon was provided by CoCensys, Inc.

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