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NS 257 (1,2,3,6,7,8-HEXAHYDRO-3(HYDROXYIMINO)-N,N,7-TRIMETHYL-2-OXOBENZO[2,1-b:3,4-c']DIPYRROLE-5-SULFONAMIDE) IS A POTENT, SYSTEMICALLY ACTIVE AMPA RECEPTOR ANTAGONIST

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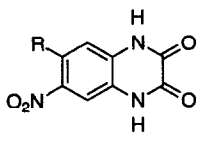
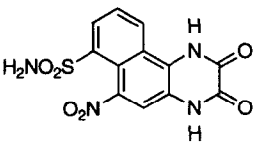
Advances in the molecular biology of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors[1-3] and the discovery of competitive antagonists such as CNQX (**1**) and DNQX (**2**)[4] have helped clarify the role of AMPA receptors in excitatory neurotransmission. However, CNQX and DNQX are essentially inactive when given systemically and therefore lack any clinical application. The discovery of NBQX (**3**) as a potent AMPA receptor antagonist with *in vivo* activity demonstrated the potential clinical utility of this class of compounds.[5] NBQX has shown both anticonvulsant and neuroprotective activity and is effective in animal models of global and focal cerebral ischemia. Other competitive AMPA antagonists with modest affinity at the receptor that have been reported recently that help define receptor binding requirements include the weakly active acidic amino acid, AMOA (**4**)[6] and the systemically active 6-(tetrazolylethyl)decahydroisoquinoline-3-carboxylic acid (**5**).[7] Additional support for the hypothesis that an AMPA receptor antagonist will have significant clinical utility in the treatment of stroke or other neuronal degenerative conditions dependent upon glutamate toxicity was obtained by the neuroprotective activity of the noncompetitive AMPA receptor antagonist, GYKI 52466 (**6**).[8] Unfortunately, both NBQX and GYKI 52466 have very limited aqueous solubility that may limit their use as clinical agents. An objective of our work is to identify novel and potent AMPA receptor antagonists with improved physical characteristics including aqueous solubility.

Our laboratories recently reported the synthesis of a series of 6,7,8,9-tetrahydro-benz[g]-indole-2,3-dione-3-oximes that are selective ligands for the AMPA/kainate glutamate receptor subtype.[9] The most potent AMPA antagonist in this series (**7**, [^{3}H]-AMPA IC_{50} =1.8 μM) is the first compound to show AMPA antagonist activity after oral dosing. Introduction of a fused pyrrolidine ring system in place of the fused cyclohexane ring of **7** provided both increased receptor affinity and the opportunity to prepare water soluble salts. Herein we report the synthesis and preliminary pharmacological characterization of NS 257 (**8**, 1,2,3,6,7,8-hexahydro-3-(hydroxyimino)-N,N,7-trimethyl-2-oxobenzo[2,1-b:3,4-c']dipyrrole-5-sulfonamide) as a competitive AMPA receptor antagonist with *in vivo* anticonvulsant activity. The pharmacological profile of this compound is sufficiently interesting to make it a candidate for clinical consideration for the treatment of stroke.

In our original synthesis of NS 257 (**8**), the key intermediate, 2,3-dihydro-2-methyl-1H-isoindole-4-amine (**12**), was prepared from 3-nitroxylene via the dibromo derivative **10** and subsequent reduction of the nitro group as shown in equation 1. While suitable for preparing small quantities of **12**, the low and variable yields of the alkylation product **11** coupled with the vesicant properties of **10** prevented this from being a useful synthesis for the preparation of large quantities of the title compound **8**.



1 R = CN
2 R = NO_2



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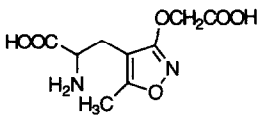

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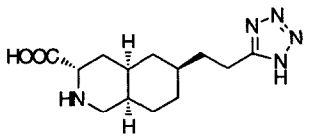

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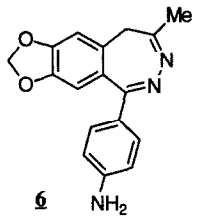

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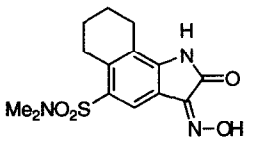

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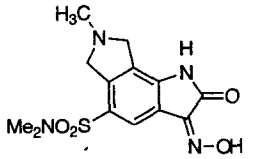
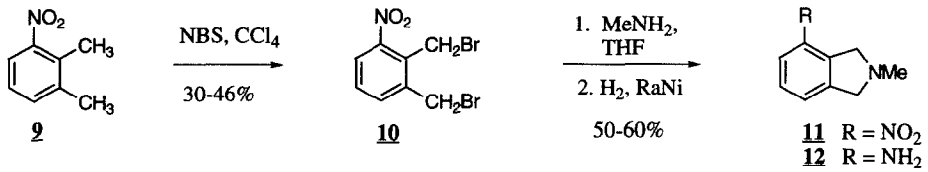


8 (NS 257)

Equation 1.

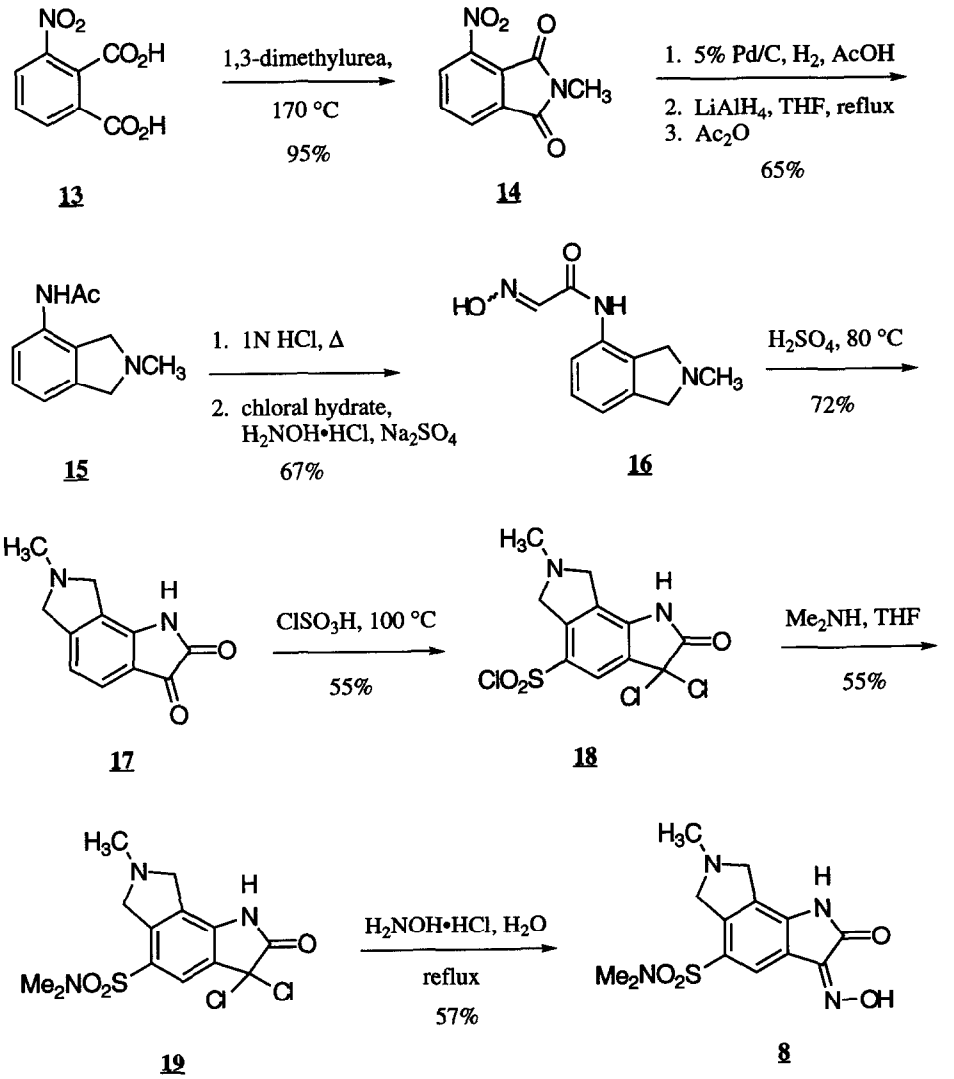


11 R = NO_2
12 R = NH_2

An improved synthesis of **8** is outlined in Scheme I. An alternative procedure for the preparation of **12** was developed that allows hundred gram quantities of the isatin **17** to be prepared readily. 2-Methyl-4-nitro-1H-isoindole-1,3(2H)-dione **14** was available on large scale by the reaction of 1,3-dimethylurea with 3-nitrophthalic acid at 170° C. Hydrogenation of the nitro group was followed by lithium aluminum hydride reduction and treatment with acetic anhydride to provide the acetanilide **15** in 60% overall yield. Hydrolysis gave 2,3-dihydro-2-methyl-1H-isoindole-4-amine (**12**) which was converted to the isatin **17** using the classical Sandmeyer method.[10] The second key step in the synthesis is the chlorosulfonylation which was done in chlorosulfonic acid (neat, 80° C, 5 min). The dimethylsulfonamide **19** was formed after treatment with dimethylamine in THF, and the 3,3-dichloro moiety converted to the oxime **8** by treatment with hydroxylamine.

The hydrochloric acid salt has an aqueous solubility of ≈ 7 mg/mL. Conversion into the methanesulfonic acid salt dramatically increased the aqueous solubility of **8** to ≈ 70 mg/mL. This solubility is sufficient to allow concentrated parenteral solutions to be used for *in vivo* studies.

Scheme 1.



NS 257 (**8**) was evaluated for affinity at excitatory amino acid receptor subclasses using selective radioligand binding to rat cortical membrane tissue - [^{3}H]-AMPA IC_{50} = 0.70 ± 0.08 μM;[11] [^{3}H]-kainate IC_{50} = 13 ± 2 μM;[12] NMDA-sensitive [^{3}H]-glutamate IC_{50} = 44 ± 6.4 μM;[13] [^{3}H]-glycine IC_{50} > 100 μM[14]. These results confirm that **8** binds selectively to the AMPA receptor with limited affinity for the NMDA receptor. To functionally measure AMPA antagonist activity, we examined the effects of **8** on AMPA-induced neuronal damage in primary cortical neuronal cultures using techniques similar to those outlined in Koh et al (1990)[15]. The neuronal damage produced by long-term exposure to 100 μM AMPA, measured by the release of the cytosolic enzyme lactate dehydrogenase, was reduced by treatment with **8** with an IC_{50}-value of 9 ± 2.5 μM (n = 4) (Fig 1). The neuronal damage produced by application of 500 μM kainate was blocked by **8** with an IC_{50}-value of 10 μM (n = 2). These results are similar to those seen with NBQX (Fig 1).

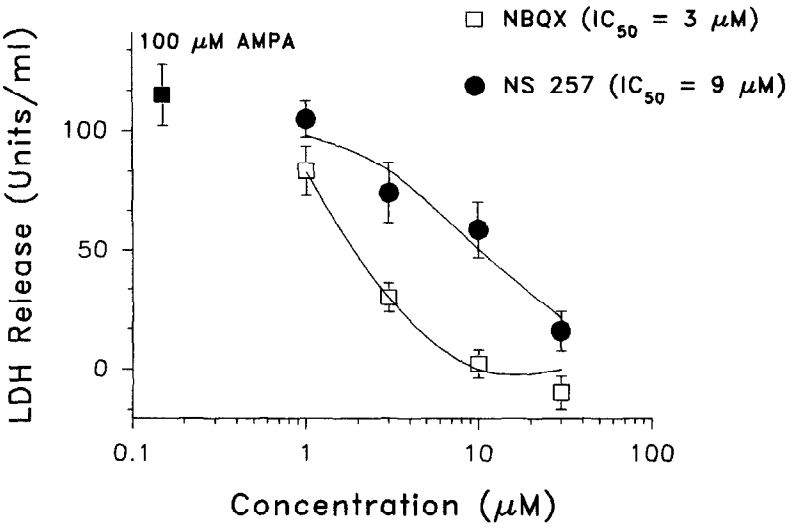


Fig 1. NS 257 (**8**, closed circles) and NBQX (**3**, open squares) blocked AMPA-induced neuronal damage in rat neocortical cell cultures. Neuronal damage was quantified by measuring the activity of the cytosolic enzyme lactate dehydrogenase (LDH) that was released into the culture medium during a 20 hr exposure to 100 μM AMPA. Points represent the mean of 9 to 12 observations with standard errors.

Intracerebroventricular (icv) injection of AMPA produces tonic-clonic seizures in NMRI mice and the ability of an agent to block the AMPA-induced convulsant effect is a measure of its *in vivo* AMPA receptor antagonist activity.[16] A 5 min pretreatment with **8** (iv) protected animals from these seizures (ED_{50} = 10 mg/kg). The reference AMPA antagonist NBQX and **8** were nearly equipotent in this assay, but neither was capable of fully blocking AMPA induced seizures (Fig 2). **8** was ineffective when administered either orally or intraperitoneally.

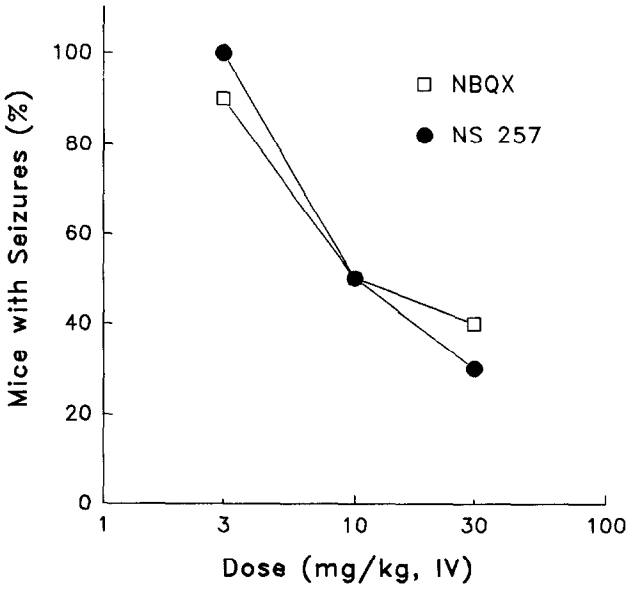


Fig 2. Intracerebroventricular (ICV) AMPA (0.3 g in 10 μL) produces tonic-clonic seizures in ≥ 90% of NMRI mice. NS 257 (**8**, closed circles) and NBQX (**3**, open squares) (5 min pretreatment, IV) were equipotent in preventing AMPA-induced seizures (ED_{50}'s = 10 mg/kg).

Pharmacological activity of the current reference AMPA antagonists, NBQX and GYKI 52466, has generated significant interest in the development of this class of compounds. In this report we introduce a novel, selective AMPA antagonist, NS 257 (**8**), that is active *in vivo* following systemic administration. Systemic administration of **8** can prevent the seizures of AMPA administered icv, showing that the compound is a functional antagonist of AMPA receptor activity *in vivo*. At anticonvulsant doses, there is little noticeable impairment of motor function which suggests that **8** may be a development candidate for either its anticonvulsant or neuroprotective (focal or global ischemia) properties.

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16. Male or female NMRI mice (20 - 25 g) were used. AMPA was dissolved in distilled water. NS 257 (**8**) was administered as a solution (iv) to 10 mice per dose 5 minutes before a challenging dose of 0.3 μg of AMPA administered icv in a volume of 10 μL. The number of mice having clonic seizures within the next minute were recorded. An ED_{50}-value was determined by graphical interpolation from at least three doses of NS 257 as the dose protecting 50% of the mice from having clonic seizures.

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