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Identification and Characterization of a Potential Ischemia-Selective *N*-Methyl-D-aspartate (NMDA) Receptor Ion-Channel Blocker, CNS 5788†

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Abstract—The identification and characterization of a potentially ischemia-selective and orally-active sulfoxide based NMDA ion-channel blocker showing good neuroprotective activity, (*R*)-(+)-*N*-(2-chloro-5-methylthiophenyl)-*N*′-(3-methylsulfinylphenyl)-*N*′-methylguanidine (CNS 5788), is described.

Many different steps in the cascade of events leading to neuronal cell death have been investigated as possible targets for therapeutic intervention in the treatment of stroke and traumatic brain injury. Amongst the most effective mechanisms studied is by inhibition of the effects of excessive concentrations of the excitatory neurotransmittor glutamic acid, by the blockade of the NMDA receptor directly at its associated ion channel.[1] Many conventional NMDA ion-channel blockers including dizolcipine[2] (MK 801, **1**), aptiganel[3] (CNS 1102, **2**), and *N*-(2-chloro-5-methylthiophenyl)-*N*′-methyl-*N*′-(3-methylthiophenyl)guanidine (CNS 5161, **3**) (Fig. 1) are neuroprotective in animal studies. To date, it has been difficult to demonstrate the efficacy of NMDA antagonists in stroke patients, in part because of behavioral and cardiovascular side effects that appear to limit upper dosage levels in clinical investigations.

We hypothesized that a molecule that is relatively inert under normal physiological conditions but that becomes activated to generate a potent NMDA channel blocker under hypoxic conditions, would be associated with reduced side effects and improved therapeutic utility. Conversion of a sulfoxide derivative to an active aziridinium species within a hypoxic solid tumor provides good analogy for a sulfoxide prodrug for ischemic conditions.[5] There are numerous precedents[5] for sulfoxide-containing drug molecules including the nonsteroidal antiinflammatory drug molecule sulindac.[6] In this report we discuss the synthesis and biological evaluation of compounds containing sulfoxide groups that are derived from the potent NMDA ion-channel blocker **3** (CNS 5161), which is currently under clinical investigation for the treatment of chronic pain.

Synthesis of guanidine derivatives in the present study was achieved through the condensation of the corresponding cyanamides with the aniline hydrogen chloride salts in suitable solvents such as toluene under refluxing conditions[7] (Scheme 1). In our laboratory, 2-chloro-5-methylthioaniline (**5**) was prepared[4] from commercially available 2-chloro-5-methylthiobenzoic acid (**4**) using diphenylphosphoryl azide in the presence of triethyl amine in *N*,*N*-dimethylformamide as solvent in 56% yield. For the preparation of **5** in multigram quantities, this reaction was modified,[8] using *t*-butanol as solvent. Subsequent hydrolysis of the resulting carbamate intermediate (without isolation) using 10% hydrochloric acid led to the isolation of **5** in 79% yield. Reaction of the aniline derivative **5** with cyanogens bromide was carried out in water to yield the desired 2-chloro-5-methylthiophenylcyanamide[4] (**6**) in 61% yield.

Methylation of 3-methylthioaniline was carried out following the reported procedure.[4] Oxidation of *N*-methyl-3-methylthioaniline (**7**) to *N*-methyl-3-methylsulfinylaniline (**8**), was studied under different reaction conditions using different reagents, such as sodium periodate, Oxone®, hydrogen peroxide (alone and in the presence of catalysts)

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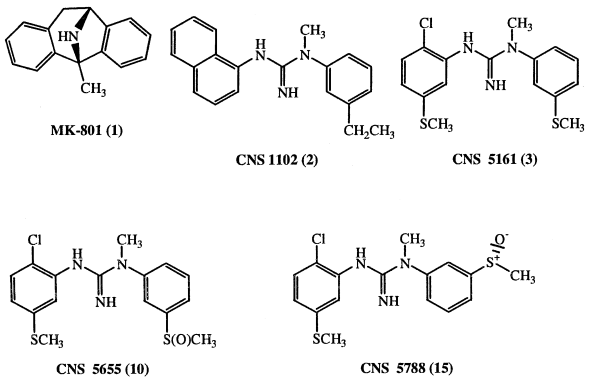
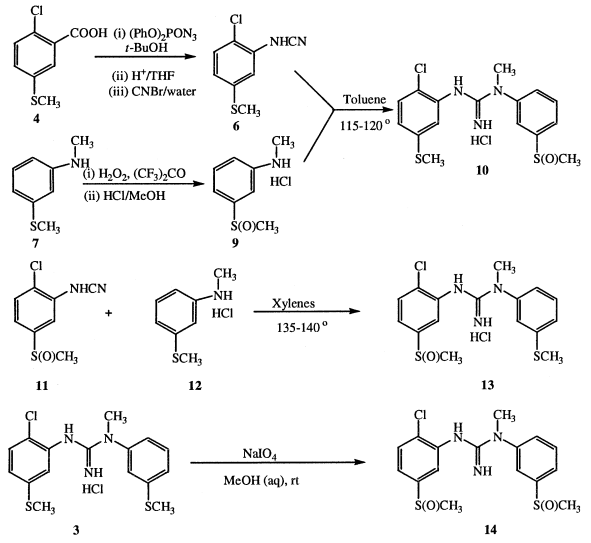


Figure 1.



Scheme 1.

to reduce the formation of sulfone derivative and to avoid the coloration of final product. After considerable experimentation, sulfoxide (**8**) was prepared in ~90% yield through oxidation[9] using hydrogen peroxide (5%) in chloroform in the presence of trifluoroacetone.[10] Reaction of *N*-methyl-3-methylsulfinylaniline (**8**) with hydrogen chloride in methanol gave the corresponding hydrochloride (**9**). Condensation of the cyanamide **6** and anilinehydrochloride **9** in refluxing toluene afforded **10** in ~20% yield initially. Following reinvestigation[11] of the synthesis of **10**, the yield was improved from 17% to 50% using a mixture of solvents (dichloromethane/toluene 1:1) for the condensation step.

Oxidation of 2-chloro-5-methylthiophenylcyanamide (**6**) was carried out using sodium periodate in aqueous methanol at room temperature to the corresponding sulfoxide derivative **11** in 65% yield. Condensation of **11** with *N*-methyl-3-methylthioaniline hydrochloride (**12**) at 135–140 °C led to the isolation of **13** in 32% yield.

Synthesis of the disulfoxide **14** was achieved by sodium periodate oxidation of **3** (CNS 5161) in aqueous methanol in 73% yield.

All the three sulfoxide derivatives (**10**, **13**, **14**) exhibited reduced in vitro affinity {[^{3}H]-MK-801 binding assay}[12] for the NMDA ion-channel binding site and markedly reduced sodium channel blocking activity (^{14}C-guanidinium flux assay)[13] compared with parent sulfide, **3**. The sulfoxide **10** (CNS 5655), retained the highest level of NMDA binding affinity (~80-fold reduction) compared to the other sulfoxides **13** and **14** (Table 1).

Sulfoxides **10**, **13** and **14** were studied in various animal models[14] and **10** showed the preferred pharmacological profile, including activity in the rat middle cerebral artery occlusion model[15] (MCAO model) for stroke (32% protection at 4.5 mg/kg) and reduced side effects, based on the Irwin general behavioral screen[16] and the rotarod assay for motor effects[17] (Table 2).

Both the enantiomers of **10** were obtained through preparative chiral HPLC separation (Fig. 2) of the racemic compound as the free base.[18] Further evaluation of the enantiomers revealed that the in vitro potencies of the enantiomers do not appear to be significantly different from the racemate **10** (Table 1). However, based on the rat MCAO data, the (+)-enantiomer, **15** (CNS 5788) appears to be at least as potent as **10** (32% protection at 4.5 mg/kg) whereas the (−)-enantiomer, did not show significant neuroprotection at this dose.

The absolute configuration of the active enantiomer **15** was determined as *R* by X-ray crystallography[19] and this enantiomer retains the favorable pharmacological profile of the racemate in exhibiting good neuroprotection at doses not associated with the unwanted CNS side effects as revealed by the Irwin test and rotarod assays.

In pharmacokinetics experiments for monitoring the conversion of racemic sulfoxide **10** (CNS 5655) to the parent sulfide **3** (CNS 5161) in vivo, a plasma HPLC

Table 1. In vitro properties of parent sulfide **3** and derived sulfoxides

Compound	NMDA ^{3}H-MK-801 Binding K_i (nM)	Neuronal Guan. Flux IC_{50} (μM)	Cardiac Guan. Flux IC_{50} (μM)
3 (CNS 5161)	1.94±0.37	1.75±0.41	2.25±0.25
10 (CNS 5655)	179±23	83.1±0.50	25.98±3.55
13	834±114	>25	29.5±2.1
14	6587±2150	>25	81.0±37.4
15 (CNS5788)	233±29	>25	23.7±2
(−)-Enantiomer	321±5	>25	26.0±2.05

Table 2. In vivo properties and comparison of CNS 5161 (**3**) and CNS 5655 (**10**)

Assay system	Measure	**3** (CNS 5161)	**10** (CNS 5655)	Comparison of potency CNS 5655 versus CNS 5161
Rat pup hypoxia-ischemia neuroprotecion model	% Reduction in lesion volume@the dose (mg/Kg) tested (ip)	74±14%@4 (11)[a]	100±0%@20 (13)[a] 60±22%@10 (15)[a]	3- to 4-fold less potent
Rat MCAO Neuroprotection model	% Reduction in total lesion volume at the dose tested (combination iv and ip)	33±13%@1.5 (6)[a]	32±8%@4.5 (12)[a]	~3-fold less potent
Rat rotarod motor coordination test	ED_{50} To cause rats to fall from the rod (iv)	ED_{50}=0.14 mg/kg	ED_{50}=8.8 mg/kg	63-fold less potent
Irwin general behavior screen	Lowest dose at which definite behavioral effects seen (esp. excitation) (iv)	0.25 mg/kg	4 mg/kg	16-fold less potent
Audiogenic seizure mouse model (DBA/2)	ED_{50} To block the audiogenic seizures	ED_{50}=0.9 mg/kg	ED_{50}=12 mg/kg	13-fold less potent
Oral activity	DBA/2 test—CNS 5655 Irwin test—CNS 5161			~25% for CNS 5655 <5% for CNS 5161

[a]Numbers in parentheses refer to the number of animals tested.

assay was developed for simultaneous determinations of both compounds in plasma in the range of 10–1000 ng/ml. Rats (three animals/time pt.) were dosed with 10 mg/kg racemate CNS 5655 (**10**) using a 5 min iv infusion as a formulation of the drug 3 mg/ml in 40 mM acetate and 0.3 M mannitol and three control animals were dosed with vehicle alone.

The results indicated that after an initial build-up of sulfide, CNS 5161 (**3**) (C_{max} at 30 min), data for both sulfoxide and sulfide can be fit well with single exponential decays, which yield half-lives of 31 and 55 min, respectively. Based on area under curve (AUC) values of 93,000 for **10** and 4700 for **3**, about 5% of putative sulfoxide prodrug is reduced to the sulfide. The plasma levels of sulfide, achieved after the 10 mg/kg iv dose of CNS 5655 (**10**) are in the range of the plasma levels achieved by neuroprotective doses of CNS 5161 (**3**) in MCAO experiments (Fig. 3).

Results from preliminary pharmacokinetics experiments with the acetate salt of CNS 5788 (**15**) were strikingly similar to those found with CNS 5655 (**10**) administration. The half-life of CNS 5788 was about 32 min, the same as the 31 min, half-life found for CNS 5655. The CNS 5161 appeared to peak at 30 min and to then decline with a half-life of 73 min, compared to 55 min after CNS 5655 administration. The overall percentage conversion of CNS 5788 to CNS 5161 was ~6%, compared to the 5% seen previously with CNS 5655 administration. Figure 3 shows the plasma levels versus time curves for **15** (CNS 5788) and **10** (CNS 5655).

Conversion of a methylthio group in **3** (pK_a=9.04, log $D_{7.4}$=2.5) to the sulfoxide **10** or **15** (pK_a=8.63, log $D_{7.4}$=0.83) lowers the pK_a by 0.41 units (versus 0.5 predicted from electronic substituent effects) and the lipophilicity (log $D_{7.4}$) by 1.67 units. The difference in log $D_{7.4}$ may be reflected in the improved oral activity (25–33%) of **10** (CNS 5655) and **15** (CNS 5788) in the DBA/2 mouse anti-seizure test relative to the parent sulfide, **3** (Table 2).

A comparison of the biological activities of the sulfoxide-based channel blocker **10/15** with the parent sulfide **3** in various animal models is given in Table 2.

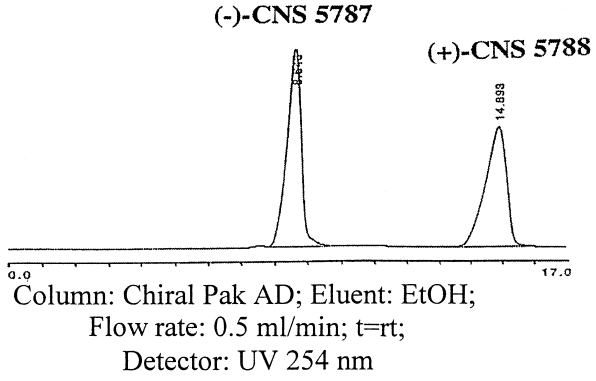


Figure 2. Preparative chiral HPLC chromatogram of **10**.

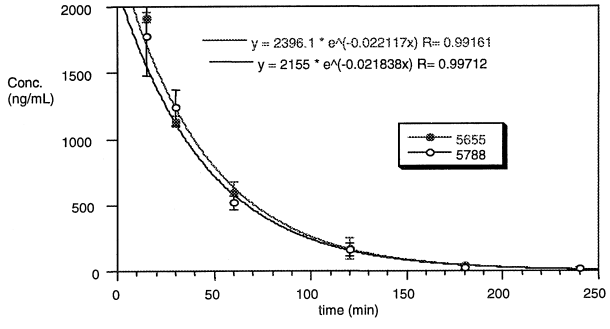


Figure 3. Drug levels of CNS 5161 after iv dosing of rats with CNS 5655 (**10**) and CNS 5788 (**15**).

In conclusion, a sulfoxide based NMDA ion channel blocker, **15** (CNS 5788), that shows good neuroprotection, reduced side effects and improved oral activity has been identified and CNS 5788 may have potential for the treatment of stroke and other ischemia related disorders. The interesting properties of CNS 5788 (**15**) led to the studies on a chiral synthesis, which has been communicated separately.[20]

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8. To a suspension of 2-chloro-5-methylthiobenzoic acid (**4**, 20 g) in *t*-butanol (75 mL) triethyl amine (21 mL) was added followed by the dropwise addition of diphenylphosphoryl azide (24 mL) at room temperature. The reaction mixture was slowly heated to reflux for 6 h, cooled to room temperature, and solvent was removed under reduced pressure. The crude reaction mixture was dissolved in tetrahydrofuran (50 mL) followed by the addition of aq HCl (1:1, 50 mL) and was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure and treated with NaOH (25%) to bring the pH to 12 with cooling in ice water bath. The product was repeatedly extracted into ethyl acetate (4×50 mL) and the organic layer was washed with water (20 mL). Concentration of the organic layer led to the isolation of 2-chloro-5-methylthioaniline, **5** (13.6 g, 79%); TLC: hexanes/EtOAc (4:1); R_f 0.58; ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 4.1 (br., 2H, NH_2), 6.55 (m, 1H), 6.65 (m, 1H), 7.15 (m, 1H).
9. To a solution of *N*-methyl-3-methylthioaniline (**7**, 10 g) and trifluoroacetone (1 mL) in chloroform (50 mL) hydrogen peroxide (41 mL, 5%) solution was added dropwise over a period of 15 min at room temperature. The reaction mixture was stirred overnight (~36 h) and the disappearance of the starting material was observed on TLC (hexanes/EtOAc, 4:1). The reaction mixture was diluted with water (20 mL) and repeatedly extracted with chloroform (3×20 mL). Combined organic extracts were washed with sodium thiosulfate solution (10%, 25 mL), dried and concentrated to afford the reaction mixture. The residue was chromatographed on silica gel using gradually a mixtures of hexanes/ethyl acetate initially 4:1, and later 3:2. These elutions remove any traces of the unreacted starting material (if any) and most of the sulfone formed. Finally elution with ethyl acetate and later ethyl acetate containing 5% methanol eluted the desired sulfoxide **8** and the fractions were combined, concentrated under reduced pressure to yield the sulfoxide (**8**) as an oil 10 g (90%); TLC $CHCl_3$/MeOH (19:1); R_f 0.48; ^{1}H NMR ($CDCl_3$) δ 2.69 (s, 3H, S(O)Me), 2.86 (s, 3H, NMe), 6.67 (dd, 1H), 6.81 (dd, 1H), 6.92 (d, 1H), 7.27 (m, 1H).
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11. A solution of 3-methylsulfinyl-*N*-methylaniline hydrochloride (**9**, 2.8 g), 2-chloro-5-methylthiophenylcyanamide (**6**, 1.8 g) in dichloromethane (25 mL) and toluene (25 mL). The reaction mixture was slowly heated in an oil bath to 115–120 °C with a distillation setup for collecting the solvent distilled during the reaction. After the complete distillation of dichloromethane the reaction mixture was maintained at 115–120 °C for 1 h, cooled to room temperature. The residue, after the solvent removal, was purified by silica gel column chromatography using, initially, chloroform and gradually to chloroform containing 5% methanol as eluents. The almost colorless product, CNS 5655 **10**, obtained upon concentration of fractions, was dried under high vacuum. 1.90 g (53%); mp 156–160 °C; TLC $CHCl_3$/MeOH 4:1; R_f 0.33; ^{1}H NMR (CD_3OD) δ 2.46 (s, 3H, SMe), 2.82 (s, 3H, S(O)Me), 3.53 (s, 3H, NMe), 7.19 (s, 1H), 7.21 (d, 1H), 7.40 (dd, 1H), 7.65 (m, 1H), 7.70 (m, 2H), 7.81 (d, 1H).
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