Communications to the Editor

Discovery of (2*S*)-1-(4-Amino-2,3,5-trimethylphenoxy)-3-{4-[4-(4-fluorobenzyl)phenyl]-1-piperazinyl}-2-propanol Dimethanesulfonate (SUN N8075): A Dual Na^+ and Ca^{2+} Channel Blocker with Antioxidant Activity

Hirokazu Annoura,* Kyoko Nakanishi, Tetsuya Toba, Naohiro Takemoto, Seiichi Imajo, Atsuko Miyajima, Yoshiko Tamura-Horikawa, and Shigeki Tamura

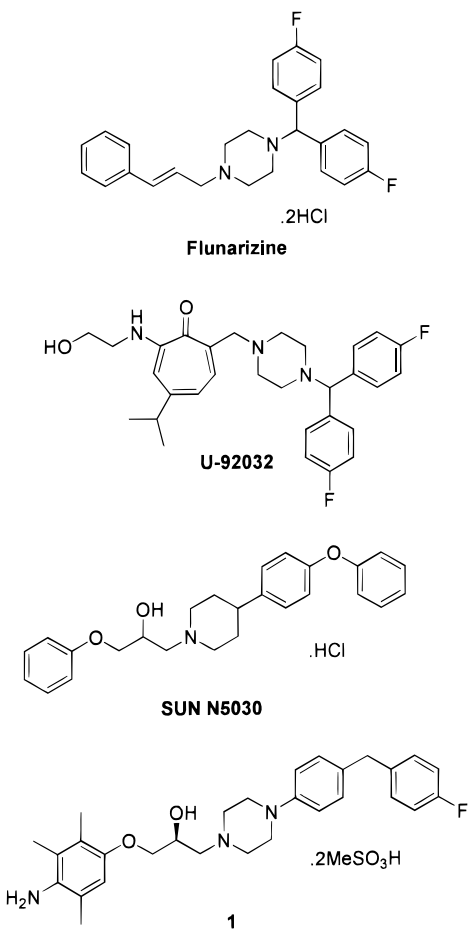
Suntory Biomedical Research Limited, 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan

Received March 28, 2000

Introduction. Stroke is a substantial public health problem and one of the leading causes of death in major industrialized countries. An effective and widely applicable treatment of cerebral ischemia would have an enormous public health impact; however, no such treatment has been found as yet.[1] Pathogenesis of progressive and delayed death of nerve cells that occurs in cerebral injury and cerebrovascular diseases, such as stroke and trauma, is considered to be caused by a rise in intracellular Ca^{2+} concentration to a pathological level due to ATP depletion following the failure of intracellular energy-dependent ion homeostasis.[2] This phenomenon is known as Ca^{2+} overload which induces functional disorders in the mitochondria, randomly activates various Ca^{2+}-dependent enzymatic reactions, and invites further Ca^{2+} overload. It has been demonstrated that aberrant activation of Na^+ and Ca^{2+} channels is extensively involved in the Ca^{2+} overload pathway and the accumulation of intracellular Na^+ ions results in rapid Ca^{2+} overload by the reverse operation of Na^+/Ca^{2+} exchange mechanism.[2,3] In addition, the Ca^{2+}-dependent activation of nitric oxide (NO) synthase, phospholipase A_2, and xanthine oxidase causes a marked increase in the generation of reactive oxygen species such as superoxide anion radical ($O^{2-\bullet}$), hydrogen peroxide (H_2O_2), hydroxy radical ($OH^\bullet$), and peroxynitrite ($ONOO^-$) on reperfusion of ischemia, imparting irreparable damage to the cell membrane through extensive lipid peroxidation.[4] These complex biochemical events mutually act as aggravating factors and repeat in a vicious amplifying cycle that ultimately leads to cell death.

Although clinical trials of quite a few neuroprotectants, including *N*-methyl-D-aspartate (NMDA) receptor antagonists,[5] AMPA receptor antagonists,[6] Na^+ and/or Ca^{2+} channel blockers,[3c,7] and antioxidants,[8] are currently underway, most of them interfere with only one of the pathways in the ischemic cascade and the benefits of such therapies have not yet been established.[9] It has recently been demonstrated that a combination of compounds, each possessing a different mechanism of action in preventing cell death, exhibits synergistic effects in ischemic animal models.[10] Consequently, a novel neuroprotectant with multiple mechanisms of action may provide clinically significant efficacy. We previously reported a structurally novel class of arylpiperidines, represented by SUN N5030, as Na^+ and Ca^{2+} channel blockers with reduced affinity for dopamine D_2 receptors[11] (Chart 1). In this communication, we report that our continuous endeavor for the development of agents for acute ischemic stroke based on molecular modification of flunarizine[3b,c,12] as a prototype has resulted in the discovery of a candidate: (2*S*)-1-(4-amino-2,3,5-trimethylphenoxy)-3-{4-[4-(4-fluorobenzyl)phenyl]-1-piperazinyl}-2-propanol dimethanesulfonate (**1**; SUN N8075). This compound possesses not only neuronal Na^+ and T-type[12] Ca^{2+}-channel-blocking effects but also antioxidant activity, which governs potent neuroprotective activity in an in vivo transient middle cerebral artery occlusion (MCAO) model. A survey of literature has revealed that U-92032 endowed with similar multiple mechanisms of action against ischemia was reported by the Upjohn group.[7b] The pharmacophore for the blockade of ion channels and

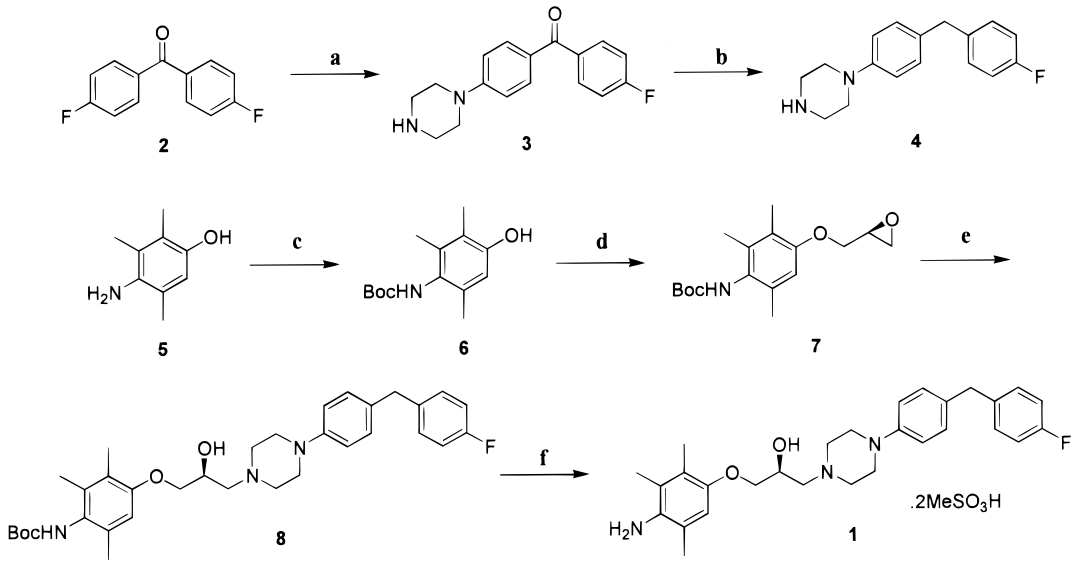
Chart 1



* To whom correspondence should be addressed. Tel: +81-75-962-8481. Fax: +81-75-962-6448. E-mail: Hirokazu_Annoura@suntory.co.jp.

10.1021/jm000143w CCC: $19.00
Published on Web 08/19/2000

Scheme 1[a]



[a] Reagents and conditions: (a) piperazine (3 equiv), Et_3N (2 equiv), MeCN, reflux, 12 h, 66%; (b) Et_3SiH (2.3 equiv), concd H_2SO_4 (2.2 equiv), TFA, rt, 2 h, 79%; (c) $(Boc)_2O$, THF, 80 °C, 2 h, 96%; (d) NaH, (*S*)-glycidyl 3-nitrobenzenesulfonate, DMF, rt, 2 h, 90%; (e) **4**, 2-propanol, reflux, 6 h, 90%; (f) TFA, CH_2Cl_2, rt, 2 h, then $MeSO_3H$ (2.05 equiv), MeOH, rt, 65%.

Table 1. Biological Activity of **1**[a]

	IC$_{50}$ (μM)				anticonvulsant effects on audiogenic seizures in DBA/2 mice ($n = 6$)[i] ED$_{50}$ (mg/kg; ip)
entry	anti-veratridine[b]	T-type Ca^{2+} currents[c]	lipid peroxidation[d]	D_2[h]	
1	0.42	2.0	0.31	>10	6.5
flunarizine	0.29	2.2	>50[e]	0.228	11.4
U-92032	0.36	0.3	20.0[f]	1.69	14.5
α-tocopherol	NT[j]	NT[j]	>100[g]	NT[j]	NT[j]

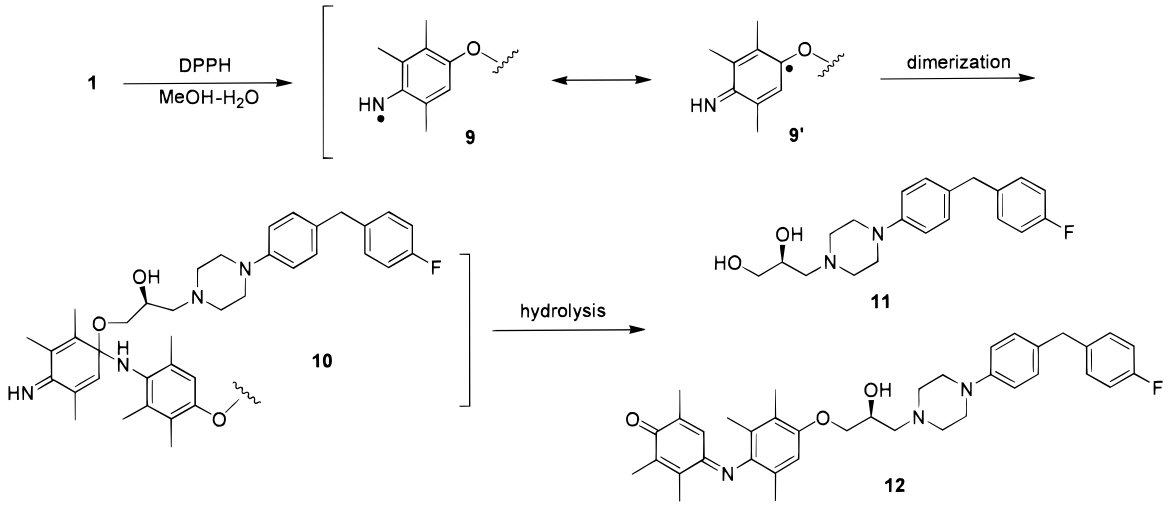
[a] Each value represents multiple determinations (≥2) with a deviation of less than 20%. [b] Determined as inhibitory effects upon the veratridine-induced depolarization in rat cerebrocortical synaptosomes using a membrane potential sensitive fluorescent dye, rhodamine 6G.[14] [c] Determined as inhibitory effects on low-threshold (T-type) Ca^{2+} currents in primary cultured rat cerebrocortical neurons using whole-cell voltage-clamp recording technique.[12a] [d] Determined as inhibitory effects on automatic oxidation in plasma membrane of rat brain homogenates by measuring malondialdehyde and 4-hydroxyalkenals using peroxidized lipid colorimetric assay kit.[17] [e] Results reported in refs 7c and 17b are 64.1 and 85 μM, respectively. [f] Result reported in ref 7c is 13.5 μM. [g] Result reported in ref 17b is >100 μM. [h] Determined in competition experiments with [^{3}H]raclopride.[20] [i] Compounds were administered intraperitoneally to DBA/2 mice ($n = 6$) 20 min prior to auditory stimulation of at least 90 dB for 1 min.[22a] [j] NT, not tested.

the mechanism of action for the antioxidant activity of **1** are also discussed.

Chemistry. Compound **1** was synthesized using the pathway shown in Scheme 1. Treatment of commercially available 4,4′-difluorobenzophenone with piperazine (3 equiv) in the presence of triethylamine in acetonitrile gave **3** in 66% yield. Reduction of **3** with Et_3SiH (2.3 equiv) in the presence of sulfuric acid (2.2 equiv) and trifluoroacetic acid (TFA) produced the requisite 1-[4-(4-fluorobenzyl)phenyl]piperazine **(4)** in 79% yield. Phenoxy-2,3-epoxypropane **(7)**, the coupling partner of **4**, was synthesized from 2,3,5-trimethyl-4-aminophenol **(5)**.[13] Thus, chemoselective protection of the amino group in **5** with di-*tert*-butyl dicarbonate gave **6** as a single product in 96% yield. None of the corresponding *O*-butoxycarbonylated isomer was detected during this reaction. Treatment of **6** with sodium hydride produced the phenolate anion, which was quenched with (*S*)-glycidyl 3-nitrobenzenesulfonate to give **7** in 90% yield. Coupling reaction between **7** and **4** in 2-propanol proceeded smoothly to give **8** in 90% yield, without concomitant loss of optical purity. Deprotection of the Boc group in **8** by exposure to trifluoroacetic acid followed by treatment with methanesulfonic acid (2.05 equiv) furnished **1** as a dimethanesulfonate in 65% yield with >98.5% ee after recrystallization from 2-propanol/water (20:1). Its optical purity was determined by normal phase HPLC using a Daicel Chiralpak OD column (*n*-hexane:2-propanol:diethylamine = 600:400:1) and was found to increase to >99% ee upon repeated recrystallization. Judging from the ^{19}F NMR (500 MHz, DMSO-d_6), the signal attributable to trifluoroacetic acid could not be detected. Therefore, complete salt exchange occurred from the initially formed trifluoroacetate into the methanesulfonate during the final deprotecting step, presumably due to the higher acid dissociation constant of methanesulfonic acid ($pK_a = -1.2$) as compared with that of trifluoroacetic acid ($pK_a = 0.2$).

Results and Discussion. The inhibitory effect of compound **1** on Na^+ channels stimulated by the neurotoxin veratridine was evaluated in rat cerebrocortical synaptosomes using the voltage-sensitive fluorescent dye rhodamine 6G.[14] The effect of **1** on low-threshold (T-type) Ca^{2+} currents in primary cultured rat cerebrocortical neurons was examined using a whole-cell voltage-clamp recording technique.[12a] The results are given as IC$_{50}$ values as shown in Table 1. Compound **1** blocks both Na^+ and T-type Ca^{2+} channels with potency equal to that of the reference standards flunarizine and U-92032.[7b] Compound **1** showed concentration-dependent block of T-type Ca^{2+} currents induced by a depolarizing pulse to −40 mV from a holding potential (V_H) of

Scheme 2



−100 mV. We also investigated the effect of **1** on neurotoxin binding sites of Na^+ channels. Compound **1** displaced the binding of the site 2 ligand, [^{3}H]batrachotoxin,[15b] with high affinity (IC_{50} = 0.75 μM) but had no effect on the site 1 ligand, [^{3}H]saxitoxin, binding (IC_{50} > 10 μM).[16] [Each value represents replicate determinations, and tetrodotoxin was used as a reference compound: IC_{50} = 0.0063 μM (site 1) and >10 μM (site 2).] The neurotoxin receptor site 2 of Na^+ channels is believed to be localized on a region involved in voltage-dependent activation and inactivation and is allosterically linked to the transmembrane pore of the channels.[15a,c] The Na^+ channel site 2 blockers work in a voltage-dependent manner, implying event- and site-specific block of Na^+ channels without primary hemodynamic adverse effects in ischemic diseases.[3b,c]

Antioxidant activity was measured by lipid peroxidation-suppressing effect in rat cerebrocortical membranes using a BIOXYTECH/LPO-586 peroxidized colorimetric assay kit.[17] Surprisingly, compound **1** exhibits lipid peroxidation-suppressing effects with a potency greater than that of flunarizine, U-92032, or α-tocopherol. These results prompted us to investigate the mechanism of action for antioxidant activity at the molecular level by reacting **1** with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical.[18a,b] Compound **1** dissolved in methanol/pH = 6.86 standard buffer solution (1:1, v/v) and when subjected to DPPH radical unexpectedly produced propanediol **11** and quinone monoimine **12**. Both structures, **11** and **12**, were elucidated by ^{1}H NMR, IR, and HRMS spectra. In the ^{1}H NMR (400 MHz, $CDCl_3$) of **12**, the signal assignable to the hydrogen on the quinone monoimine moiety appeared at δ 6.38 (singlet). In addition, the signal attributable to the hydrogen of the trimethylphenoxy ring was at δ 6.64 (singlet), showing a shift to lower field than that of the parent compound **1** (δ 6.57) due to the electron-withdrawing effect of the quinone monoimine moiety. As shown in Scheme 2, the degradation pathway is proposed to be triggered by abstraction of the hydrogen atom from the amino group in the 2,3,5-trimethyl-4-aminophenoxy moiety followed by disproportionation of the generated anilino radical **9** and cyclohexadienyl radical **9′** to give the dimerization product **10**, which is subsequently hydrolyzed to produce compounds **11** and **12**.

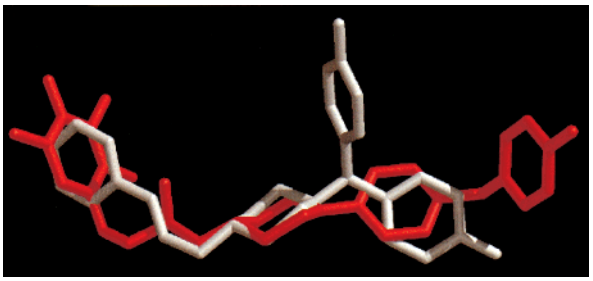


Figure 1. Overlay of energetically allowed conformers of **1** (red) and flunarizine (white).

These results suggest that the aminophenoxy moiety in **1** is the essential pharmacophore for antioxidant activity and that the mechanism of action is similar to that of α-tocopherol.[18c] The direct evidence for radical-scavenging action was obtained on the ESR spectrum where the intensity of the signal of DPPH radical decreased dose-dependently upon the addition of **1**. With increasing evidence that the excess production of reactive oxygen species affects ion homeostasis and is a major causative factor in cell death,[4] it is tempting to speculate that the ion channel-blocking and antioxidant properties of **1** may act synergistically against cerebral ischemia.[10] The dopamine D_2 receptor binding affinity was assessed in a competitive binding assay using [^{3}H]-raclopride in rat striatum membranes.[19] In sharp contrast to flunarizine, compound **1** has no binding affinity for dopamine D_2 receptors (IC_{50} > 10 μM). These differences clearly demonstrate that **1** not only has structural features distinctly different from those of flunarizine and related compounds but also may have a decreased clinical risk for extrapyramidal side effects.[20]

To elucidate the common pharmacophore and/or the special arrangement of essential parts for inhibition of ion channels and influence for binding affinity at dopamine D_2 receptors, a systematic conformation search and a similar structure search by root-mean-square (rms) fitting were carried out for **1** and flunarizine. Structures were minimized with MM2 parameters implemented by Macromodel.[21] As depicted in Figure 1, the corresponding pharmacophoric groups between **1** (red) and flunarizine (white) closely overlap, with the exception of each distal benzene ring. This

implies that the benzene ring directly connected to the piperazine ring in **1** likely occupies the same binding site of the ion channels as flunarizine, whereas the other benzene ring of the diphenylmethane moiety likely interferes with dopamine D_2 receptor binding.

Next, we investigated the effect of **1** on audiogenic seizures in DBA/2 mice to confirm in vivo activity and brain permeability.[22a,b] Anticonvulsant and neuroprotective activities are mutually related in several voltage-dependent Na^+ channel blockers.[22c] Compound **1** exhibits potent anticonvulsant effects following systemic (ip) administration with an ED_{50} of 6.5 mg/kg. The neuroprotective activity of **1** was assessed in a transient MCAO model.[23] The middle cerebral artery (MCA) occluded for 60 min and 24 h after reperfusion neuronal damage was quantified by staining brains with 2,3,5-triphenyltetrazolium chloride[24] (TTC). The relative area of infarction to the whole area of the corresponding cerebrum is calculated using a computerized image analysis system and was shown in percent (vide infra). Compound **1** was administered intravenously immediately after both MCAO and reperfusion (each 3 mg/kg). Consequently, **1** significantly reduced infarct size in all treated groups compared to vehicle-treated rats [4.77 ± 0.84% (n = 14) vs 14.87 ± 1.24% (n = 15) (mean ± SEM), $^{**}p < 0.01$ (Student's t-tests)], amounting to 67.9% reduction in mean infarct volume, whereas U-92032 effected only miner reduction (<15%). In this model, rectal temperature was found to increase during transient MCAO to above 38.5 °C and **1** slightly reduced the ischemic hyperthermia within 1 °C. Mild hypothermia immediately after reperfusion reduces infarct size and improves cerebral outcome.[25] These results indicate that **1** has a pronounced neuroprotective efficacy against neuronal damage induced by transient focal ischemia in rats. Interestingly, **1** at the effective doses had no effects on systemic blood pressure and heart rate in anesthetized rats. These studies will be described in more detail in subsequent papers.

In conclusion, we describe a novel neuroprotectant, **1**, that blocks both neuronal Na^+ and T-type Ca^{2+} channels but which does not affect dopamine D_2 receptors. Compound **1** is also a powerful antioxidant, significantly more potent than the standard. Since compound **1** (SUN N8075) has complementarily multiple beneficial effects against ischemia and remarkable neuroprotective activity in the transient MCAO model, this compound may have greater clinical efficacy for the treatment of acute ischemic stroke compared to candidates with a single mechanism of action in preventing neuronal cell death or damage.[9,10] The structure–activity relationships (SAR) of this series of compounds will be reported in due course.[26]

Acknowledgment. We thank Dr. T. Ohno for his valuable suggestions. Mr. K. Namikawa is thanked for his assistance in measuring ESR spectra.

Supporting Information Available: Experimental details for the synthesis of compounds **1**, **3**, **4**, **6**–**8**, **11**, and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Sandercock, P.; Willems, H. Medical Treatment of Acute Ischaemic Stroke. *Lancet* **1992**, *339*, 537–539. (b) Sila, C. A. Prophylaxis and Treatment of Stroke. *Drugs* **1993**, *45*, 329–337.

(2) (a) Farber, J. L. The Role of Calcium in Cell Death. *Life Sci.* **1981**, *29*, 1289–1295. (b) Meyer, F. B. Calcium, Neuronal Hyperexcitability and Ischemic Injury. *Brain Res. Rev.* **1989**, *14*, 227–243. (c) Boddeke, E.; Hugtenburg, J.; Jap, W.; Heynis, J.; Van Zwieten, P. New Anti-ischaemic Drugs: Cytoprotective Action with No Primary Haemodynamic Effects. *Trends Pharmacol. Sci.* **1989**, *10*, 397. (d) Choi, D. W. Calcium, Still Center-stage in Hypoxic-ischemic Neuronal Death. *Trends Neurosci.* **1995**, *18*, 58–60. (e) Kristiàn T.; Siesjö, B. K. Calcium-related Damage in Ischemia. *Life Sci.* **1996**, *59*, 357–367. (f) Kristiàn T.; Siesjö, B. K. Calcium in Ischemic Cell Death. *Stroke* **1998**, *29*, 705–718.

(3) (a) Boddeke, E.; Hugtenburg, J.; Jap, W.; Heynis, J.; Van Zwieten, P. New Anti-ischaemic Drugs: Cytoprotective Action with No Primary Haemodynamic Effects. *Trends Pharmacol. Sci.* **1989**, *10*, 397–400. (b) Pauwels, P. J.; Leysen, J. E.; Janssen, P. A. J. Ca^{2+} and Na^+ Channels Involved in Neuronal Cell Death. Protection by Flunarizine. *Life Sci.* **1991**, *48*, 1881–1893. (c) Urenjak, J.; Obrenovitch, T. P. Pharmacological Modulation of Voltage-gated Na^+ Channels: A Rational and Effective Strategy against Ischemic Brain Damage. *Pharmacol. Rev.* **1996**, *48*, 21–67.

(4) (a) Mattson, M. P. Modification of Ion Homeostasis by Lipid Peroxidation: Roles in Neuronal Degeneration and Adaptive Plasticity. *Trends Neurosci.* **1998**, *21*, 53–57. (b) Love, S. Oxidative Stress in Brain Ischemia. *Brain Pathol.* **1999**, *9*, 119–131. (c) Lipton, P. Ischemic Cell Death in Brain Neurons. *Physiol. Rev.* **1999**, *79*, 1431–1568.

(5) (a) Carter, C. J.; Llyd, K. G.; Zivkovic, B.; Scatton, B. Ifenprodil and SL 82.0715 as Cerebral Antiischemic Agents. III. Evidence for Antagonistic Effects at the Polyamine Modulatory Site within the *N*-Methyl-D-aspartate Receptor Complex. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 475–482. (b) Subramaniam, S.; Donevan, S. D.; Rogawski, M. A. Block of the *N*-Methyl-D-asparatate Receptor by Remacemide and its Des-glycine Metabolite. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 161–168. (c) Davis, S. M.; Albers, G. W.; Diener, H. C.; Lees, K. R.; Norris, J. Termination of Acute Stroke Studies Involving Selfotel Treatment. ASSIST Steering Committed. *Lancet* **1997**, *349*, 32. (d) Davis, S. M.; Lees, K. R.; Albers, G. W.; Diener, H. C.; Markabi, S.; Karlsson, G.; Norris, J. Selfotel in Acute Ischemic Stroke: Possible Neurotoxic Effects of an NMDA Antagonist. *Stroke* **2000**, *31*, 347–354.

(6) (a) Sheardown, M. J.; Nielsen, E. O.; Hansen, A. J.; Jacobsen, P.; Honore, T. 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxaline: A Neuroprotectant for Cerebral Ischemia. *Science* **1990**, *247*, 571–574. (b) Shimizu-Sasamata, M.; Kawasaki-Yatsugi, S.; Okada, M.; Sakamoto, S.; Yatsugi, S.; Togami, J.; Hatanaka, K.; Ohmori, J.; Koshiya, K.; Usuda, S.; Murase, K. YM90K: Pharmacological Characterization as a Selective and Potent α-Amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate Receptor Antagonist. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 84–92.

(7) (a) Brown, C. M.; Calder, C.; Alps, B. J.; Spedding, M. The Effect of Lifarizine (RS-87476), a Novel Sodium and Calcium Channel Modulator, on Ischaemic Dopamine Depletion in the Corpus Striatum of the Gerbil. *Br. J. Pharmacol.* **1993**, *109*, 175–177. (b) Ito, C.; Im, W. B.; Takagi, H.; Takahashi, M.; Tsuzuki, K.; Liou, S.-Y.; Kunihara, M. U-92032, a T-type Ca^{2+} Channel Blocker and Antioxidant, Reduces Neuronal Ischemic Injuries. *Eur. J. Pharmacol.* **1994**, *257*, 203–210. (c) Wilder, B. J.; Campbell, K.; Ramsay, R. E.; Garnett, W. R.; Pellock, J. M.; Henkin, S. A.; Kugler, A. R. Safety and Tolerance of Multiple Doses of Intramuscular Fosphenytoin Substituted for Oral Phenytoin in Epilepsy or Neurosurgery. *Arch. Neurol.* **1996**, *53*, 764–768. (d) Diener, H. C. Multinational Randomized Controlled Trial of Lubeluzole in Acute Ischaemic Stroke. European and Australian Lubeluzole Ischaemic Stroke Study Group. *Cerebrovasc. Dis.* **1998**, *8*, 172–181. (e) Oka, M.; Itoh, Y.; Ukai, Y.; Kimura, K. Blockade by NS-7, a Neuroprotective Compound, of Both L-type and P/Q-type Ca^{2+} Channels Involving Depolarization-stimulated Nitric Oxide Synthase Activity in Primary Neuronal Culture. *J. Neurochem.* **1999**, *72*, 1315–1322.

(8) (a) Fleishaker, J. C.; Peters, G. R. Pharmacokinetics of Tirilazad and U-89678 in Ischemic Stroke Patients Receiving a Loading Regimen and Maintenance Regimen of 10 mg/kg/day of Tirilazad. *J. Clin. Pharmacol.* **1996**, *36*, 809–813. (b) Ohkawa, S.; Fukatsu, K.; Miki, S.; Hashimoto, T.; Sakamoto, J.; Doi, T.; Nagai, Y.; Aono, T. 5-Aminocoumarans: Dual Inhibitors of Lipid Peroxidation and Dopamine Release with Protective Effects against Central Nervous System Trauma and Ischemia. *J. Med. Chem.* **1997**, *40*, 559–573. (c) Yamaguchi, T.; Sano, K.; Takakura, K.; Saito, I.; Shinohara, Y.; Asano, T.; Yasuhara, H. Ebselen in Acute Ischemic Stroke: A Placebo-controlled, Double-blind Clinical Trial. Ebselen Study Group. *Stroke* **1998**, *29*, 12–17.

(9) (a) Koroshetz, W. J.; Moskowitz, M. A. Emerging Treatments for Stroke in Humans. *Trends Pharmacol.* **1996**, *17*, 227–233. (b) Onal, M. Z.; Fisher, M. Acute Ischemic Stroke Therapy. A

Clinical Overview. *Eur. Neurol.* **1997**, *38*, 141−154. (c) De Keyser, J.; Sulter, G.; Luiten, P. G. Clinical Trials with Neuroprotective Drugs in Acute Ischaemic Stroke: Are We Doing the Right Thing? *Trends Neurosci.* **1999**, *22*, 535−540.

(10) (a) Auer, R. N. Combination Therapy with U74006F (Tirilazad mesylate), MK-801, Insulin and Diazepam in Transient Forebrain Ischaemia. *Neurol. Res.* **1995**, *17*, 132−136. (b) Stuiver B. T.; Douma, B. R.; Bakker R.; Nyakas, C.; Luiten, P. G. In vivo Protection against NMDA-induced Neurodegeneration by MK-801 and Nimodipine: Combined Therapy and Temporal Course of Protection. *Neurodegeneration* **1996**, *5*, 153−159. (c) Aronowski, J.; Strong, R.; Grotta, J. C. Combined Neuroprotection and Reperfusion Therapy for Stroke. Effect of Lubeluzole and Diaspirin Cross-Linked Hemoglobin in Experimental Focal Ischemia. *Stroke* **1996**, *27*, 1571−1576. (d) Onal, M. Z.; Li, F.; Tatlisumak, T.; Locke, K. W.; Sandage, B. W., Jr.; Fisher, M. Synergistic Effects of Citicoline and MK-801 in Temporary Experimental Focal Ischemia in Rats. *Stroke* **1997**, *28*, 1060−1065.

(11) Annoura, H.; Nakanishi, K.; Uesugi, M.; Fukunaga, A.; Miyajima, A.; Tamura-Horikawa, Y.; Tamura, S. A Novel Class of Na^+ and Ca^{2+} Channel Dual Blockers with Highly Potent Antiischemic Effects. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2999−3002. Compounds described in this report have not showed the other additional effects including antioxidant activity ($IC_{50} > 50\ \mu M$).

(12) (a) Takahashi, K.; Akaike, N. Calcium Antagonist Effects on Low-threshold (T-type) Calcium Current in Rat Isolated Hippocampal CA1 Pyramidal Neurons. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 169−175. (b) Perez-Reyes, E.; Cribbs, L. L.; Daud, A.; Lacerda, A. E.; Barclay, J.; Williamson, M. P.; Fox, M.; Rees, M.; Lee, J.-H. Molecular Characterization of a Neuronal Low-voltage-activated T-type Calcium Channel. *Nature* **1998**, *391*, 896.

(13) Caldwell, W. T.; Thompson, T. R. Nuclear Methylation of Phenols. A New Synthesis of Intermediates in the Preparation of Antisterility Factors. *J. Am. Chem. Soc.* **1939**, *61*, 765−767.

(14) Aiuchi, T.; Matsunaga, M.; Daimatsu, T.; Nakaya, K.; Nakamura, Y. Effect of Glucose and Pyruvate Metabolism on Membrane Potential in Synaptosomes. *Biochim. Biophys. Acta* **1984**, *771*, 228−234.

(15) (a) Creveling C. R.; McNeal E. T.; Lewandowski, G. A.; Rafferty, M.; Harrison, E. H.; Jacobson, A. E.; Rice, K. C.; Daly, J. W. Local Anesthetic Properties of Opioids and Phencyclidines: Interaction with the Voltage-dependent, Batrachotoxin Binding Site in Sodium Channels. *Neuropeptides* **1985**, *5*, 253−256. (b) Brown, G. B. ^{3}H-Batrachotoxinin-A benzoate Binding to Voltage-sensitive Sodium Channels: Inhibition by the Channel Blockers Tetrodotoxin and Saxitoxin. *J. Neurosci.* **1986**, *6*, 2064−2070. (c) Wasserstrom, J. A.; Liberty, K.; Kelly, J.; Santucci, P.; Myers, M. Modification of Cardiac Na^+ Channels by Batrachotoxin: Effects on Gating, Kinetics, and Local Anesthetic Binding. *Biophys. J.* **1993**, *65*, 386−395.

(16) Catterall, W. A.; Morrow, C. S.; Hartshorne, R. P. Neurotoxin Binding to Receptor Sites Associated with Voltage-sensitive Sodium Channels in Intact, Lysed, and Detergent-solubilized Brain Membranes. *J. Biol. Chem.* **1979**, *254*, 11379−11387.

(17) (a) Botsoglou, N. A.; Fletouris, D. J.; Papageorgiou, G. E.; Vassilopoulos, V. N.; Mantis, A. J.; Trakatellis, A. G. Rapid, Sensitive, and Specific Thiobarbituric Acid Method for Measuring Lipid Peroxidation in Animal Tissue, Food, and Feedstuff Samples. *J. Agric. Food Chem.* **1994**, *42*, 1931−1937. (b) Kubo, K.; Yoshitake, I.; Kumada, Y.; Shuto, K.; Nakamizo, N. Radical Scavenging Action of Flunarizine in Rat Brain in Vitro. *Arch. Int. Pharmacodyn. Ther.* **1984**, *272*, 283−295.

(18) (a) Yoshida, T.; Mori, K.; Hatano, T.; Okumura, T.; Uehara, I.; Komagoe, K.; Fujita, Y.; Okuda, T. Studies on Inhibition Mechanism of Autoxidation by Tannins and Flavonoids. V. Radical-Scavenging Effects of Tannins and Related Polyphenols on 1,1-Diphenyl-2-picrylhydrazyl Radical. *Chem. Pharm. Bull.* **1989**, *37*, 1919−1921. (b) Burton, G. W.; Ingold, K. U. Vitamin E: Application of the Principles of Physical Organic Chemistry to the Exploration of its Structure and Function. *Acc. Chem. Res.* **1986**, *19*, 194−201. (c) Valgimigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. Solvent Effects on the Antioxidant Activity of Vitamine E. *J. Org. Chem.* **1999**, *64*, 3381−3383.

(19) Kohler, C.; Hall, H.; Ogren, S. O.; Gawell, L. Specific in vitro and in vivo Binding of ^{3}H-Raclopride. A Potent Substituted Benzamide Drug with High Affinity for Dopamine D-2 Receptors in the Rat Brain. *Biochem. Pharmacol.* **1985**, *34*, 2251−2259.

(20) (a) Montastruc, J. L.; Llau, M. E.; Rascol, O.; Senard, J. M. Drug-induced Parkinsonism: A Review. *Fundam. Clin. Pharmacol.* **1994**, *8*, 293−306. (b) Daniel, J. R.; Mauro, V. F. Extrapyramidal Symptoms Associated with Calcium-channel Blockers. *Ann. Pharmacother.* **1995**, *29*, 73−75.

(21) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. MacroModel-An Integrated Software System for Modeling Organic and Bioorganic Molecules Using Molecular Mechanics. *J. Comput. Chem.* **1990**, *11*, 440−467.

(22) (a) De Sarro, G. B.; Meldrum, B. S.; Nisticò, G. Anticonvulsant Effects of Some Calcium Entry Blockers in DBA/2 mice. *Br. J. Pharmacol.* **1988**, *93*, 247−256. (b) Eigyo, M.; Shiomi, T.; Inoue, Y.; Sakaguchi, I.; Ishibashi, C.; Murata, S.; Koyabu, K.; Matsushita, A.; Adachi, I.; Ueda, M. Pharmacological Studies on a New Dihydrothienopyridine Calcium Antagonist, S-312-d. 5th Communication: Anticonvulsant Effects in Mice. *Jpn. J. Pharmacol.* **1994**, *65*, 175−177. (c) Rataud, J.; Debarnot, F.; Mary, V.; Pratt, J.; Stutzmann, J.-M. Comparative Study of Voltage-sensitive Sodium Channel Blockers in Focal Ischaemia and Electric Convulsions in Rodents. *Neurosci. Lett.* **1994**, *172*, 19−23.

(23) Koizumi, J.; Yoshida, Y.; Nakazawa, T.; Ooneda, G. Experimental Studies of Ischemic Brain Edema 1. A New Experimental Model of Cerebral Embolism in Rats in which Recirculation can be Introduced in the Ischemic Area. *Jpn. J. Stroke* **1986**, *8*, 1−8.

(24) Bederson, J. B.; Pitts, L. H.; Germano, S. M.; Nishimura, M. C.; Davis, R. L.; Barkowski, H. M. Evaluation of 2,3,5-Triphenyltetrazolium Chloride as a Stain for Detection and Quantification of Experimental Cerebral Infarction in Rats. *Stroke* **1986**, *17*, 1304−1308.

(25) (a) Ridenour, T. R.; Warner, D. S.; Todd, M. M.; McAllister, A. C. Mild Hypothermia Reduces Infarct Size Resulting from Temporary but Not Permanent Focal Ischemia in Rats. *Stroke* **1992**, *23*, 733−738. (b) Weinrauch, V.; Safar, P.; Tisherman, S.; Kuboyama, K.; Radovsky, A. Beneficial Effect of Mild Hypothermia and Detrimental Effect of Deep Hypothermia after Cardiac Arrest in Dogs. *Stroke* **1992**, *23*, 1454−1462.

(26) Annoura, H.; Nakanishi, K.; Tamura, S. Arylpiperidinopropanol and Arylpiperazinopropanol Derivatives and Pharmaceuticals Containing The Same. PCT Int. Appl. WO 99/23072, 1999; Chem. Abstr. **1999**, *130*, 338123p.

JM000143W