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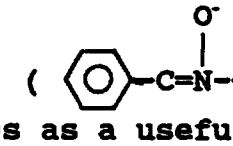
2,4-DISULFONYL PHENYL BUTYL NITRONE, ITS SALTS, AND THEIR USE AS PHARMACEUTICAL FREE RADICAL TRAPS

Background of the Invention

This invention relates to a particular nitrone compound and its salts and their advantageous use as pharmaceutical nitrone free radical trapping agents.

Background Information

Alpha-phenyl tert butyl nitrone



($C_6H_5-C(O^-)=N-C(CH_3)_3$) or "PBN") was identified in the 1970s as a useful analytical reagent to be used in conjunction with electron spin resonance ("ESR") to aid in the detection of free radicals. PBN was found to react with certain free radicals and generate a chemical species yielding a characteristic ESR spectrum and thus making it possible to determine the presence or absence of free radicals.

In the late 1970s and early 1980s the medical community began to focus on the roles played by free radicals in diseases such as heart attacks, strokes and the like. PBN was used increasingly *in vitro* to provide analytical evidence of the presence of free radicals in these settings. It was also later administered *in vivo* in animal models, again as an analytical adjunct in attempts to observe free radicals during ischemia simulations and the like.

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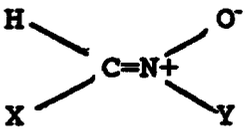
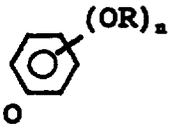
In the mid 1980s, the first possible therapeutic effects of PBN were implied when severe trauma ischemia animal tests showed that PBN-treated animals were more likely to survive than controls.

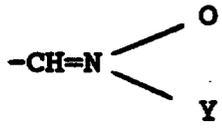
On May 2, 1991, our PCT patent application WO-91-05552 was published. This patent application, which in part corresponds to now-issued United States Patent Nos. 5,825,032 and 5,036,097, described PBN and a family of PBN derivatives defined by the formula



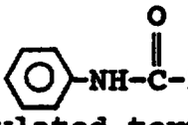
wherein

X is phenyl or 

where R is H, Z-C-, or Z; or



and n is a whole integer from 1 to 5 or



and Y is tert-butyl or a hydroxylated or acetylated tert-butyl or a substituted phenyl. These compounds were proposed as pharmaceutical agents to treat the aftermath of stroke and other conditions reported to be associated with free radical damage.

In 1992 we filed a second PCT patent application on PBN and related compounds and their medical use. This application, based on prior United States Patent Application Serial No. 716,952 (filed June 18, 1991), was published on December 23, 1992 as WO

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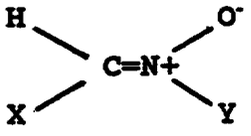
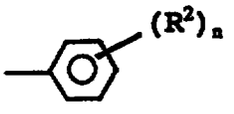
92/22290. This 1992 publication provided two extremely broad and general disclosures. First, it attempted to describe as many disease states as possible which were associated with free radicals. These ranged from CNS conditions (including stroke, aging, migraine, etc.) through peripheral organ disease (including atherosclerosis, bed sores, wounds, and muscle overexertion) through UV exposure, to mention but a few highlights. Second, it attempted to list as many potential spin traps as possible.

In addition to a whole range of non-PBN materials, this application greatly expanded the definition of potentially useful PBN compounds to include PBN, and derivatives thereof of the formula

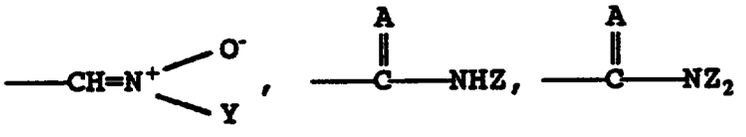


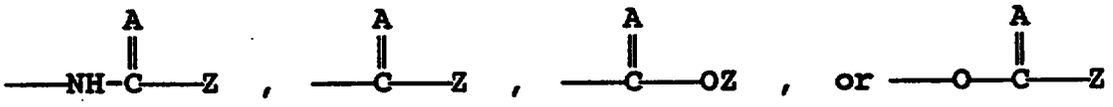
wherein

X is phenyl, imidazolyl, phenothiazinyl or 

n=1-5, preferably 1-3;

R^2 = independently (can vary within the molecule) halogen, alkyl, oxyalkyl, alkenyl, oxyalkenyl, OH, NH_2, NHZ, NZ_2, NO,





$-SO_3H$, $-OSO_3H$, SH, -S(alkyl), -S(alkenyl, and haloalkyl, specifically including $-CF_3$;

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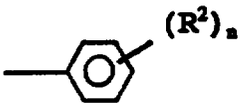
A = O or S; and

Z is a C_1 to C_6 straight, branched, alkyl or cyclic group; and

Y is a tert-butyl group that can be hydroxylated or acetylated at one or more positions; phenyl or



PBN was stated to be the most preferred compound at that time, being said to have no measurable effect on normal or uninjured cells, and a number of derivatives were also stated to be useful, including hydroxy derivatives, especially 2-, 3- or 4-hydroxyphenyl t-butyl nitrone and phenyl (mono-, di- or trihydroxy) tert-butyl nitrone; PBN esters, especially esters which release 2-, 3-, or 4-hydroxyphenyl t-butyl nitrone such as acetoxy derivative; 2-, 3-, or 4-carboxyphenyl t-butyl nitrone; phenyl hydroxybutyl nitrone; alkoxyl derivatives, especially alkoxyl derivatives which release 2-, 3-, or 4-hydroxyphenyl t-butyl nitrone, for example, the 2-, 3-, or 4-methoxyphenyl derivatives of PBN; and acetamide derivatives, especially acetamide derivatives which release 2-, 3-, or 4-aminophenyl t-butyl nitrone; diphenyl nitrone (PPN) and the analogous diphenyl nitrone derivatives; N-tert-butyl-α-(4-nitro-phenyl) nitrone; and N-tert-butyl-α-(2-sulfophenyl) nitrone.

Statement of the Invention

We have now discovered that one particular PBN derivative and its salts have unexpectedly superior pharmacological properties. Although this derivative, 2,4-disulfonyl PBN, falls within the broad family of materials generally described in the aforementioned WO 92/022290 publication, it is not specifically

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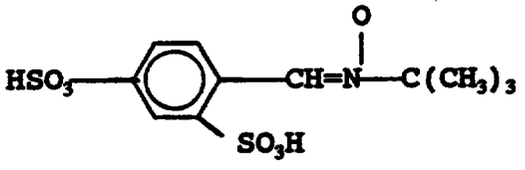
disclosed. Neither are its advantageous properties predicted.

The present compound with its two sulfonate groups was expected to exhibit improved water solubility but was also expected to exhibit poor transport across the blood/brain barrier because of its lipophobic character. However, when the present compound was made and tested in vivo, it showed an unexpected increase in efficacy as compared to PBN. This increase in efficacy occurred along with an increase in potency as compared to PBN. In direct contrast to this marked increase in potency and efficacy there was a marked and highly significant decrease in toxicity as compared to PBN.

These results were unexpected because in the general literature on structure/activity relationships within specific defined families of compounds therapeutic potency typically covaries with toxicity. Thus, most related compounds maintain their ratio of therapeutic potency to toxicity. In contrast, the compound of this invention deviates from this expected relationship when its potency increased and its toxicity decreased relative to closely related analogs.

Accordingly, in one aspect, the invention provides the PBN-disulfonyl compound



and its pharmaceutically acceptable salts.

In a second aspect, the invention provides intravenously- and orally-administrable pharmaceutical compositions having this compound or its salt as active ingredient.

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In a third aspect, this invention provides a method for treating a patient who is suffering from a condition involving acute oxidative damage to the central nervous system, such as a patient who has suffered a stroke, in which a pharmaceutical composition based on this compound or its salt is administered intravenously.

In a fourth aspect, this invention provides a method for treating a patient suffering from a condition characterized by protracted low grade oxidative stress upon the central nervous system and progressive loss of central nervous system function. In this method, a pharmaceutical composition based on this compound or its salt is administered intravenously or preferably orally.

Detailed Description of the Invention

This Detailed Description is arranged into the following sections:

Brief Description of Drawings.
The Compounds and Salts.
Compound Preparation.
Pharmaceutical Compositions.
Conditions Treated and Treatment Regimens.
Examples.

Brief Description of the Drawings

In this specification reference will be made to the accompanying drawings in which

Fig. 1 is a schematic flow chart of the reactions used to prepare the compound.

Figs. 2 (A, B and C) and 3 (A, B, C, and D) are two sets of graphs illustrating the undesirable change in animal body thermal regulatory ability which occurs as a function of dose level with a prior art nitrone radical trapping agent and contrasting this with the lack of such undesired toxic effect with the compound of the invention.

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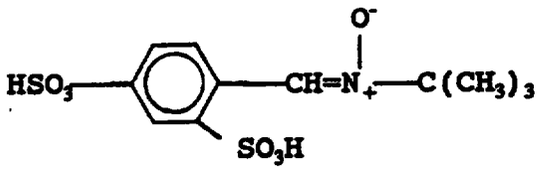
Fig. 4 (A, B, C, and D) is a set of four graphs demonstrating the superiority of the compound of the invention as compared to a closely related prior art nitrone compound in the treatment of gradual neurodegeneration conditions (such as Alzheimer's disease) as illustrated by their relative ability to interfere with beta amyloid protein's inactivation of key enzymes in solution.

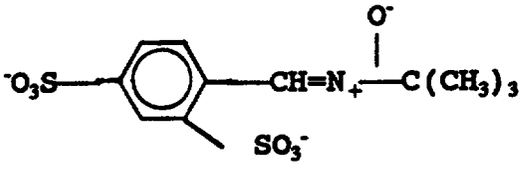
< Fig 5 & 6

The Compound and Salts

The compound of this invention is 2,4-disulfonyl α-phenyl tertiary butyl nitrone. It is also referred to informally herein as "2,4-disulfonyl PBN" or "PBN 2,4-disulfonate." It exists in an acid form



as a solid and in solution in low pH conditions. It also exists at higher pHs in an ionized salt form which can be shown as



or as

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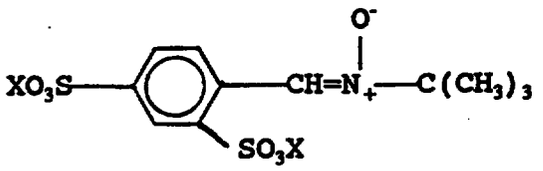

where X is a pharmaceutically acceptable cation. Most commonly, this cation is a monovalent material such as sodium, potassium or ammonium, but it can also be a multivalent cation in combination with a pharmaceutically acceptable monovalent anion, for example calcium with a chloride, bromide, iodide, hydroxyl, nitrate, sulfonate, acetate, tartrate, oxalate, succinate, palmoate or the like anion; magnesium with such anions; zinc with such anions or the like. When these combinations of a polyvalent cation and a monovalent anion are illustrated in structural formulae, herein, the monovalent anion is identified as "Y".

Among these materials, the free acid and the simple sodium, potassium or ammonium salts are most preferred with the calcium and magnesium salts also being preferred but somewhat less so.

Compound Preparation

As detailed in Figure 1 and demonstrated in Example 1, the compound of this invention can be prepared by a two step reaction sequence. In the first step, commercially available tertiary butyl nitrate (2-methyl-2-nitropropane) is converted to the corresponding n-hydroxyl amine using a suitable catalyst such as an activated zinc/acetic acid catalyst or an aluminum/mercury amalgam catalyst. This reaction can be carried out in 0.5 to 12 hours and especially about 2 to 6 hours or so at a temperature of about 15 to 100°C in a liquid reaction medium such as alcohol/water mixture in the case

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of the zinc catalyst or an ether/water mixture in the case of the aluminum amalgam catalyst.

In the second step, the freshly formed hydroxylamine is reacted with 4-formyl-1,3-benzenedisulfonic acid, typically with a slight excess of the amine being used. This reaction can be carried out at similar temperature conditions. This reaction is generally complete in 10 to 24 hours.

The product so formed is the free acid and is characterized by a molecular weight of 89 g/mole. It is a white powdery material which decomposes upon heating. It is characterized by a solubility in water of greater than 1 gram/ml and a ^1H NMR spectrum in D_2O of 8.048 ppm (dd, 8.4, 1.7 Hz); 8.836 ppm (d, 8.4 Hz); 8.839 ppm (d, 1.7 Hz); 8.774 ppm (s).

The various salts can be easily formed by admixing the free acid in aqueous medium with two equivalents of the appropriate base, for example, KOH for the potassium salt, and the like.

Pharmaceutical Compositions

The compound (including its salts) can be formulated into pharmaceutical compositions suitable for oral or intravenous injection administration

The compositions for oral administration can take the form of liquid solutions or suspensions, powders, tablets, capsules or the like. In such compositions, the PBN 2,4-disulfonate or its salt is usually a minor component (0.1 to say 50% by weight) with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form. A liquid form may include a suitable aqueous or nonaqueous vehicle with buffers, suspending dispensing agents, colorants, flavors and the like.

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A solid form may include, for example, any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, sugar, methyl salicylate, or orange flavoring.

In the case of injectable compositions, they are commonly based upon injectable sterile saline or phosphate-buffered saline or other injectable carriers known in the art. Again the active nitrone is typically a minor component, often being from about 0.05 to 10% by weight with the remainder being the injectable carrier and the like.

Conditions Treated and Treatment Regimens

The conditions treated with the 2,4-disulfonyl PBN generally fall into two groups. The first includes conditions involving acute intense oxidative damage to a region of the central nervous system. Examples of these conditions include stroke, conditions associated with stroke, concussion and subarachnoid hemorrhage. In this setting, the compound is administered in manners designed to get the drug into the patient's bloodstream as quickly and directly as possible. This usually means intravenous administration.

Intravenous dose levels for treating these conditions range from about 0.1 mg/kg/hour to at least 10 mg/kg/hour, all for from about 1 to about 120 hours and especially 24 to 96 hours. A preloading bolus of from about 10 to about 500 mg may also be administered to achieve adequate steady state levels.

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An unexpected and key advantage of 2,4-disulfonyl PBN is that it can be administered at vastly higher levels than are possible with PBN itself. As will be shown in the Examples, doses of up to 1000 mg/kg/hour and higher or intravenous bolus doses of from 10 to 2500 mg/kg have been demonstrated to be possible with PBN-2,4-disulfonyl or its salts while with PBN itself death or acute toxicity results from such doses. With 2,4 disulfonyl PBN there is an unexpected positive continuance of the dose/response curve in these high dose levels with the clear message that intense heavy dosing immediately post stroke or other trauma may in many cases provide a major positive impact upon recovery.

The second group of conditions which respond favorably to 2,4-disulfonyl PBN treatment are conditions characterized by protracted low grade oxidative stress upon the central nervous system and gradual progressive central nervous system function loss. These conditions include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), multi-infarct dementia, retinopathy and the like. Each of these conditions is characterized by a progressive loss of function. 2,4-disulfonyl-PBN or its salts, when administered orally or intravenously, can slow and possibly reverse the loss of function. If intravenous administration is desired, similar levels to those used with acute conditions but at the lower end of the ranges are generally used.

In these cases, the regimen for treatment may stretch over many months or years so oral dosing is preferred for patient convenience and tolerance. With oral dosing, one to three oral doses per day, each from about 0.02 to about 50 mg/kg are called for with preferred doses being from about 0.04 to about 5.0 mg/kg.

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Of course, one can administer 2,4-disulfonyl PBN as the sole active agent or one can administer it in combination with other agents.

EXAMPLES

Example 1

Synthesis of 2,4-disulfonylphenyl-N-t-butylnitrone (Compound "I" in subsequent Examples). This preferred synthesis is based on the work by R.H. Hinton and E.G. Janzen (J. Org. Chem. 57:2646-2651, 1992). As shown in Fig. 1 it involves the condensation of an aldehyde with a hydroxylamine. The hydroxylamine is unstable and is prepared fresh on the day of use using an activated zinc catalyst. The synthesis is as follows:

Prerequisite Chemicals

1. 95% Ethanol
2. 2-Methyl-2-nitropropane
3. Zinc dust
4. Glacial acetic acid
5. Diethyl ether
6. Saturated sodium chloride
7. Magnesium Sulfate, Anhydrous solid
8. 4-Formyl-1,3-benzenesulfonic acid (MW 310.21 g/mole), disodium salt, hydrate
9. Methanol
10. Dichloromethane

Procedure

A. Preparation of N-t-Butylhydroxylamine

1. A 500 mL three neck round bottom flask is equipped with a magnetic stir bar, thermometer adapter, thermometer, and addition funnel.

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2. 95% ethanol (350 mL) was added to the flask and cooled to 10°C in an ice bath.
3. 2-Methyl-2-nitropropane (6.18 g, 0.060 mole), and zinc dust (5.89 g, 0.090 mole) were added in single portions.
4. Glacial acetic acid (10.8 g, 0.180 mole) was placed in the addition funnel and added dropwise at such a rate with vigorous stirring to maintain the temperature below 15°C.
5. The ice bath was removed and mixture was stirred for 3 hrs at room temperature.
6. The solvent was stripped from the mixture, leaving t-butylhydroxylamine, zinc acetate, and water.
7. Dichloromethane (50 mL) was added and the mixture filtered through a Buchner funnel.
8. The zinc acetate cake left on the filter paper was washed with 2 x 25 mL dichloromethane.
9. Water was separated from the filtrate in a separatory funnel and the organic layer dried over magnesium sulfate.
10. The magnesium sulfate was removed by filtering through fluted filter paper, then dichloromethane stripped off by rotary evaporation.

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11. The product (100% yield = 5.34 g), a viscous liquid, was dissolved in methanol (50 mL) for use in part B.

B. Preparation of 2,4-disulfonylphenyl-N-t-butylnitrone

1. A 3-neck 250 ml round bottom flask was set up with a stir bar, a gas dispersion tube, an addition funnel, and a Friedrichs condenser cooled with recirculating ice water.
2. To the flask were added 200 mL of methanol, 4-formyl-1,3-benzenedisulfonic acid (9.31 g, 30 mmoles) and N-t-butylhydroxylamine (25 mL of the methanol solution from part A, 30 mmoles theoretical).
3. The reaction was heated to reflux with a heating mantle while bubbling the reaction with nitrogen with stirring.
4. The mixture was refluxed for 2 hours.
5. The remainder of hydroxylamine from part A was added.
6. Refluxing was continued with nitrogen bubbling for at least 18 hours, but not more than 24 hours.
7. The hot reaction mixture was filtered on a Buchner funnel, and the solid washed with hot methanol.
8. The methanol was stripped off by rotary evaporation to a yellow, viscous oil.

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9. Hot 1:1 ethanol:acetone (200 mL) was added and the mixture heated to dissolve the oil.
10. The solution was cooled to crystallize the product.
11. The product was collected on a Buchner funnel and dried under vacuum overnight.
12. The reaction typically gives 75% yield of I, a white powder.

Example 2

Alternate Synthesis of 2,4-disulfonylphenyl-N-t-butylnitrone (Compound I). This is an earlier-developed method which used to prepare samples of the compound used in several of the experiments reported in the Examples of this specification. The product of this Example is identical in all ways to the product of Example 1. This synthesis method is as follows:

Prerequisite Chemicals

1. Aluminum Foil, cut into 5 cm wide strips and rolled in a ca. 1 cm diameter cylinder
2. Mercury (II) Chloride (9.68 g in 476 ml water)
3. Ethanol
4. Ether (6 L)
5. Pure Water
6. 2-Methyl-2-nitropropane
7. Sodium Hydroxide, 2 M (80 g in 1 L water)
8. Magnesium Sulfate, Anhydrous solid
9. 4-Formyl-1,3-benzenesulfonic acid (MW 310.21 g/mole)

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Procedure

A. Preparation of N-t-Butylhydroxylamine

1. Aluminum foil cylinders were dipped into $HgCl_2$ solution for 15-30 seconds, then dipped in ethanol, then dipped in ether and then placed into a 5L flask containing 500 ml of diethyl ether and 21.4 ml of water.
2. The flask was fitted with a 250 ml pressure-equalizing dropping funnel, a mechanical stirrer, a nitrogen inlet, and a Friedrichs condenser cooled with recirculated ice water.
3. The mixture was stirred for 10 minutes.
4. 2-Methyl-2-nitropropane (71.68 g, 75.5 ml) was added using the dropping funnel at such a rate as to maintain a vigorous reflux.

NOTE: Addition must be completed in less than 20 minutes or the yield drops significantly.

5. As the addition proceeded, ether was added in 500 ml portions. This was done to maintain as high a concentration of product as possible without the formation of a gel. Up to 2 L of ether can be added with no deleterious effects on the yield.
6. Once addition of 2-methyl-2-nitropropane was complete, the reaction was stirred for an additional 30 minutes.

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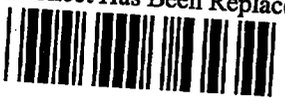

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7. The resulting gray suspension was suction filtered in 3 batches to remove aluminum salts.
8. Each filter cake was washed with 1 L of ether.
9. The combined either layers were washed with 300 ml of 2 M NaOH, then dried ($MgSO_4$), and concentrated in vacuo to leave a soft white solid.
10. The solid melts just above room temperature, but could be dried further in a vacuum oven (no more than a few minutes), leaving 38 to 45 g of solid.
11. The solid can be used as is or was purified by recrystallization from pentane.
12. Molecular weight – 89 g/mole.

B. Preparation of 2,4-disulfonylphenyl-N-t-butylnitrone

1. A 250 ml flask was equipped with a stir bar and a Friedrichs condenser cooled with recirculated ice water.
2. The flask was charged with 71.8 ml of methanol, 14.5 g of 4-formyl-1,3-benzenedisulfonic acid (46.7 mmoles, 1 eq.), and 5.0 g of N-t-butylhydroxylamine (56.2 mmoles, 1.2 eq.).
3. The mixture was refluxed overnight.
4. The reaction product was transferred to round-bottom flask and rotovaped to dry.

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5. The solid residue was mashed with ether, the ether was decanted off (yellow).
6. Step 5 was repeated.
7. Product ("I") was crystallized from methanol following a hot methanol filtration to remove insoluble precipitates and recrystallized twice from methanol.

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Example 3

A series of experiments were carried out to compare in vivo the efficacy of 2,4 disulfonyl PBN ("I"), PBN, and two monosulfonate PBN compounds as agents for protecting against neuron loss following brain ischemia and reperfusion injury. The test procedure is that reported by W. Cao, J.M. Carney, A. Duchon, R.A. Floyd and M. Chevion as "Oxygen free radical involvement in ischemia and reperfusion injury to brain, Neuroscience Letters, 88 (1988), 233. In the experiments a test compound was administered to groups of six gerbils i.p. as a single dose 30 min before 5 min bilateral carotid occlusion. the density of neuronal nuclei in a 100 micron was measured. Two controls were present - controls which received no test compound and controls which received no test compound and no brain ischemia. As illustrated in Table 1. the compound of the invention showed unexpected advantages as compared to the prior art materials. First, it was seen that at low dose levels, such as 3.2 mg/kg, compound I was 2-3 times as potent at preventing neuronal loss. At high does levels it was seen that I was able to achieve complete protection against neuronal loss as the test brains showed neuronal densities identical to the non-ischemic controls. The prior materials were either toxic at these dose levels or showed significantly lower degrees of protection. These results show a clear increase in potency for neural protection for compound I compared to PBN and two closely related analogs and an unexpected decrease in toxicity compared to PBN.

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TABLE 1.

	Neuronal nuclei/100 micron field			
	PBN	*2-sulfo*	*3-sulfo*	I.
Non-ischemic control	4.21 (.43)	4.21 (.43)	4.21 (.43)	4.21 (.43)
Ischemic control	0.58 (.28)	0.58 (.28)	0.58 (.28)	0.58 (.28)
3.2 mg/kg	0.43 (.18)	0.73 (.34)	0.35 (.21)	1.43 (.31)
10 mg/kg	1.13 (.39)	0.68 (.31)	0.81 (.40)	2.57 (.25)
32 mg/kg	1.83 (.21)	0.73 (.31)	1.63 (.35)	3.53 (.41)
50 mg/kg	3.11 (.29)	1.01 (.61)	1.63 (.35)	4.11 (.43)
100 mg/kg	3.68 (.71)	0.93 (.53)	1.93 (.39)	4.18 (.49)
320 mg/kg	3.78 (.43)	1.11 (.41)	1.78 (.40)	4.23 (.39)
1000 mg/kg	Toxic	0.98 (.43)	1.58 (.38)	4.11 (.41)
3200 mg/kg	Toxic	-	-	4.18

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Example 4

A series of experiments were conducted in which compound I was compared to PBN and two sulfonate analogs in post-ischemia treatment. The general method described in example 1 was used but the test compounds were administered i.p. as a single dose 30 min after reperfusion following 5 min ischemia. The results are summarized in Table 2. They show that the compound of the invention is again more potent at low doses and more potent and less toxic at high doses. Also again, toxicity interferes with the prior art materials ability to go to high doses at which levels the compound of the invention provides dramatically effective therapy.

TABLE 2.

	Neuronal nuclei/100 micron field			
	PBN	*2-sulfo*	*3-sulfo*	I.
Non-ischemic control	4.18 (.59)	4.18 (.59)	4.18 (.59)	4.18 (.59)
Ischemic control	0.85 (.19)	0.85 (.19)	0.85 (.19)	0.85 (.19)
32 mg/kg	1.09 (.31)	ND	ND	1.83 (.41)
50 mg/kg	1.85 (.49)	0.68 (.31)	0.73 (.34)	2.73 (.39)
100 mg/kg	2.11 (.51)	0.78 (.23)	1.09 (.48)	3.41 (.37)
320 mg/kg	2.25 (.43)	0.81 (.31)	0.93	3.55 (.48)
1000 mg/kg	Toxic	ND	ND	3.68 (.39)

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Example 5

Compound I was compared with PBN to determine their relative effectiveness for protection of neuronal loss when administered i.v. 60 min after reperfusion onset following 5 min ischemia in gerbils using the general test method described in Example 1.. The results are summarized in Table 3. and illustrate that compound I is of significantly greater therapeutic benefit in a clinical treatment setting following injury to the brain.

TABLE 3.

N=6 per group	mg/kg single dose			
	0.0	0.5	1.0	10
Saline, no ischemia	4.11 (.28)	-	-	-
saline, ischemia	0.93 (.17)	-	-	-
PBN	-	0.83 (.23)	1.07 (.29)	1.23 (.31)
I.	-	1.25 (.19)	1.75 (.28)	2.43 (.31)

Neither PBN nor I. had an effect on neuronal density in control gerbils without brain injury.

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Example 6

Brain injury can manifest itself as behavioral changes. In this experiment, young adult (3-4 months of age) gerbils were tested to determine their ability to perform an 8-arm maze test 24 hours following an ischemic event as described in Example 1. As compared to nonischemic animals, when untreated they committed many more errors. PBN and compound I were administered to some of the test animals. As detailed in Table 4. gerbils treated with high doses of compound I had error levels indistinguishable from those of nonischemic animals. PBN was less effective. This shows that compound I can protect against the loss of temporal/spatial short term memory following ischemia (24 hours post) errors in 8-arm radial maze test of young gerbils following 5 min ischemia.

TABLE 4.

N=6 per group	mg/kg/hr for 24 hours				
	0.0	1.0	32	50	100
Control	4.1 (.38)	-	-		-
Postischemic	37.6 (4.85)	-	-		-
PBN	-	29.8 (7.27)	18.19 (5.83)	6.23 (.71)	5.83 (.49)
I.	-	14.63 (3.81)	7.19 (.81)	4.28 (.29)	4.11 (.19)

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Example 7

The ability of the compound of the invention to reduce infarct volume following an ischemic event was determined. As detailed in Table 5. it was observed that while PBN and compound I were both effective at low doses, at high doses I gave the best protection and PBN was toxic. Table 5 shows the infarct volume observed when test compound was administered i.v. 60 min after middle cerebral occlusion and continued for 24 hours in C57BL/6J mice.

TABLE 5.

	Infarct Volume in mm^3			
Posttreatment (mg/kg/hr)	0.0	1.0	10	100
Control, no ischemia	0	-	-	-
Saline, ischemia	23 (2)	-	-	-
PBN	-	17.7 (2.8)	13.8 (2.3)	Toxic
I.	-	16.8 (1.7)	12.7 (3.93)	8.3 (.71)

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Example 8

In this study, compound I and PBN were compared for their ability to impart lethality protection (% survived) in aged gerbils (18-24 months of age, n=12/group) from 10 min ischemia when given 30 min before ischemia. As shown in Table 6. compound I was superior at all dose levels and achieved complete protection at high levels where PBN was only partially effective.

TABLE 6.

Pretreatment (mg/kg)	0.0	10	32	100	320
Saline	11	-	-	-	-
PBN	-	42	50	75	92
I.	-	50	75	100	100

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Example 9

An important advantage of the compound of this invention as compared to the art-taught compound, PBN, is its markedly diminished toxicity. As detailed in Table 7. acute lethality in C57BL/6L mice was determined based upon varying sizes of single i.p. doses of nitrone. PBN showed significant toxicity at 560 mg/kg dose levels. Compound I showed no toxicity at doses nearly twenty times as great.

TABLE 7.

	mg/kg				
% Survival n = 20 mice	320	560	1000	3000	10000
PBN	100	25	0	0	0
I.	100	100	100	100	100

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Example 10

Another undesirable systemic effect which has been observed in vivo with nitrone radical traps is a depression in body temperature. This toxicity can have serious health consequences and also can complicate diagnosis of other conditions. As detailed in Figs. 2 and 4, the compound of this invention was administered to mice and gerbils at levels as high as 1000 mg/kg with no measurable temperature decrease. In contrast, the compound of the art, PBN, gave up to an 8°C decrease in body temperature at a does of only 500 mg/kg.

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Example 11

The compound of the invention was tested to determine its effectiveness in the treatment of conditions characterized by protracted low grade oxidative stress upon the central nervous system and gradual progressive central nervous system function loss by testing its effectiveness in a model for Alzheimer's disease ("AD"). This model has the following basis: Recent studies have demonstrated that there is an age-associated increase in protein oxidation and loss of enzyme activities in the brain of aged individuals. Tissue cultures of fibroblasts from aged individuals and red blood cells of different ages both show an exponential increase in protein carbonyl content (a measure of protein oxidation) and a decrease in marker enzyme activities. Brain protein oxidation progressively increases over the life span of the individual.

The role of abnormal amyloid precursor peptide processing and metabolism in AD has also been explored in a number of different models. *In vitro* studies using embryonic hippocampal neuronal and neuronal/glial cultures have demonstrated that βAP 1-40 produces cytotoxicity over an extended period of co-incubation. When this peptide is infused into rat brains, lesions are produced. Some of the proposed breakdown fragments of βAP are also neurotoxic [e.g. βAP (25-35)]. The neurotoxicity appears to be both mediated via glutamate receptors, and also by non-glutamate receptors mechanisms. Confocal microscopy studies of neuronal cultures have demonstrated that exposure to βAP (1-40) results in oxidative stress [Dichlorofluorescein and increased intracellular free calcium Fura-2].

In our model it has been demonstrated that βAP fragments can directly inactivate glutamine synthetase (GS) and creatine kinase (CK) in tissue extracts and in

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cultured hippocampal neurons and glia (See A and B in Fig. 4). A and B of Fig.4 present the dose-related inactivation of glutamine synthetase and creatine kinase by AP (25-35). Cytosolic fractions from gerbil neocortex were prepared and enzyme activities determined. Samples were incubated in the presence of different concentrations of the peptide for 10 min prior to assay. Solid symbols represent the effects of the naturally occurring 25-35 fragment. Open circles indicate that the reverse sequence (32-25) had no effect on enzyme activity. Open triangles indicate that the scrambled amino acid sequence also had no effect on enzyme activities, compared to the effect of 25-35. Each point is the mean (+/- s.e.) of 5 observations. βAP derived and other cellular sources of free radicals are an important determinant of the initiation and progression of AD.

As demonstrated in C and D in Fig. 4, compound 1 and PBN each show the ability to protect GS and CK against the effects of βAP fragments. C and D of Fig. 4 present the protective effects of co-incubation of the cytosolic fractions with BAP 25-35 (0.4 mg/ml) in combination with different concentrations of PBN (open circles) or compound I (closed circles). Each point is the mean (+/- s.e.) of 3 observations. As can be seen in C and D, compound I gives complete protection and in fact might even be able to reverse the effects of oxidation. In contrast, PBN's effectiveness is quite limited as it is asymptotically leveling out at a substantially incomplete level of protection.

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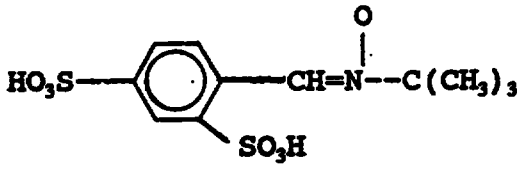
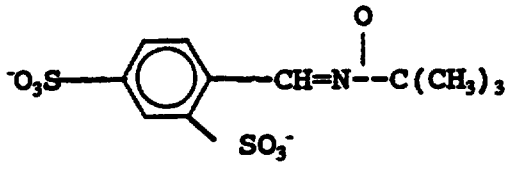
What is claimed is:

1. 2,4-disulfonyl α-phenyl tertiary butyl nitrone.

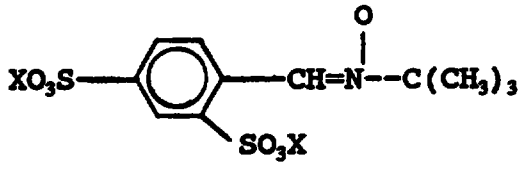
2. A compound selected from the group consisting of



3. A pharmaceutically acceptable salt of



4. The salt of claim 3 having the formula:



wherein X is selected from the group consisting of Na, K, NH_4, Ca, Mg, CaY and MgY, wherein
Y is a pharmaceutically acceptable monovalent anion.

5. A pharmaceutical composition comprising the compound of claim 1 in a pharmaceutically acceptable intravenous injectable carrier.

6. A pharmaceutical composition comprising the compound of claim 2 in a pharmaceutically acceptable intravenous injectable carrier.

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7. A pharmaceutical composition comprising the compound of claim 3 in a pharmaceutically acceptable intravenous injectable carrier.

8. A pharmaceutical composition comprising the compound of claim 4 in a pharmaceutically acceptable intravenous injectable carrier.

9. A pharmaceutical composition comprising the compound of claim 1 in a pharmaceutically acceptable oral carrier.

10. A pharmaceutical composition comprising a compound of claim 2 in a pharmaceutically acceptable oral carrier.

11. A pharmaceutical composition comprising a compound of claim 3 in a pharmaceutically acceptable oral carrier.

12. A pharmaceutical composition comprising a compound of claim 4 in a pharmaceutically acceptable oral carrier.

13. A method for treating a patient who has suffered a stoke comprising intravenously administering to said patient an effective stroke treating amount of at least about 0.2 mg/kg/hour of the pharmaceutical composition of claim 5.

14. A method for treating a patient who has suffered a stoke comprising intravenously administering to said patient an effective stroke treating amount of at least about 0.2 mg/kg/hour of the pharmaceutical composition of claim 6.

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15. A method for treating a patient who has suffered a stoke comprising intravenously administering to said patient an effective stroke treating amount of at least about 0.2 mg/kg/hour of the pharmaceutical composition of claim 7.

16. A method for treating a patient who has suffered a stoke comprising intravenously administering to said patient an effective stroke treating amount of at least about 0.2 mg/kg/hour of the pharmaceutical composition of claim 8.

17. A method for treating a patient suffering from a progressive central nervous system function loss condition comprising orally administering to said patient an effective progressive central nervous system function loss treating amount of at least about 0.1 mg/kg day of the pharmaceutical composition of claim 9.

18. A method for treating a patient suffering from a progressive central nervous system function loss condition comprising orally administering to said patient an effective progressive central nervous system function loss treating amount of at least about 0.1 mg/kg day of the pharmaceutical composition of claim 10.

19. A method for treating a patient suffering from a progressive central nervous system function loss condition comprising orally administering to said patient an effective progressive central nervous system function loss treating amount of at least about 0.1 mg/kg day of the pharmaceutical composition of claim 11.

20. A method for treating a patient suffering from a progressive central nervous system function loss

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condition comprising orally administering to said patient an effective progressive central nervous system function loss treating amount of at least about 0.1 mg/kg day of the pharmaceutical composition of claim 12.

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