Studies of Structure−Activity Relationship of Nitroxide Free Radicals and Their Precursors as Modifiers Against Oxidative Damage

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The protective effects of stable nitroxides, as well as their hydroxylamine and amine precursors, have been tested in Chinese hamster V79 cells subjected to H_2O_2 exposure at fixed concentration or exposure to ionizing radiation. Cytotoxicity was evaluated by monitoring the viability of the cells assessed by the clonogenic assay. The compounds tested at fixed concentration varied in terms of ring size, oxidation state, and ring substituents. Electrochemical studies were carried out to measure the redox midpoint potentials. The studies show that in the case of protection against H_2O_2 exposure, the protection was determined by the ring size, oxidation state, and redox midpoint potentials. In general the protection factors followed the order nitroxides > hydroxylamines > amines. Both the six-membered ring nitroxides and substituted five-membered ring nitroxides were efficient protectors. For six-membered ring nitroxides, the compounds exhibiting the lowest midpoint potentials exhibited maximal protection. In the case of X-radiation, nitroxides were the most protective though some hydroxylamines were also efficient. The amines were in some cases found to sensitize the toxicity of aerobic radiation exposure. The protection observed by the nitroxides was not dependent on the ring size. However, the ring substituents had significant influence on the protection. Compounds containing a basic side chain were found to provide enhanced protection. The results in this study suggest that these compounds are novel antioxidants which can provide cytoprotection in mammalian cells against diverse types of oxidative insult and identify structural determinants optimal for protection against individual types of damage.

Introduction

Reactive oxygen species (ROS) are byproducts of normal metabolic processes in aerobic environments.[1] These species can exert cytotoxicity by damaging critical cellular components necessary for viability.[2] Examples of the ROS are superoxide, hydrogen peroxide, and hydroxyl radicals produced by sources such as bioreductively activated xenobiotics and ultraviolet and ionizing radiation. Low-molecular-weight complexes of transition metals such as iron and copper can also catalyze the formation of some of these oxidizing species. In addition to exerting toxicity by oxidation, ROS have been also implicated in the activation of factors such as NF-κB via mechanisms involving redox reactions. Detoxification of these oxidants is provided by enzymatic and nonenzymatic mechanisms. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (Cat), and glutathione peroxidase (GPX) which catalytically detoxify oxidants, while nonenzymatic antioxidants are primarily reducing agents such as vitamin C and vitamin E which can scavenge oxidants by H atom donation in a stoichiometric manner.[3] Imbalances in the detoxification of ROS relative to their production lead to what is a widely accepted phenomenon called "oxidative stress". In most cases the defense provided by the enzymatic and nonenzymatic antioxidants is adequate. However, in acute situations, such as radiation-induced normal tissue toxicity, anthracycline-induced cardiomyopathy, and iron overload, supplementation with exogenous antioxidants may be necessary to minimize tissue damage. However, the targets and sites of free radical damage vary depending on the kind of stress. For example, organic peroxides exert cytotoxicity by damaging cell membranes, whereas ionizing radiation induces cytotoxicity by causing DNA double-strand breaks. Therefore, the sites of damage need to be identified, and appropriate antioxidant strategies need to be devised to effectively limit tissue injury depending on the type of insult. This has prompted a search for efficient antioxidants which can augment the normal antioxidant defense and provide defense against a stress imposed by a wide range of modalities in chronic as well as acute pathological conditions. Desired features in an effective antioxidant would include (a) ability to localize in subcellular compartments, (b) ability to react and scavenge with a wide range of reactive species, and (c) ability to be regenerated to the active form efficiently.

Stable nitroxide free radicals, which have been used as biophysical probes, have been identified as novel antioxidants. By undergoing one-electron-transfer reactions, nitroxides are readily reduced to the hydroxylamines[4,5] or oxidized to the oxoammonium cation.[6,7] Consequently, all three forms of the oxidation states

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might exist in a biological system. The nitroxide/oxoammonium pair behaves as an efficient and electrochemically reversible redox pair and is involved in the catalytic decomposition of superoxide[6,8] as well as inducing catalase-like activity in heme proteins.[9] Though not directly oxidizing, piperidin-1-oxyl nitroxides are often used as cooxidants in organic chemistry.[10] The hydroxylamine, on the other hand, can function as a typical reducing agent like vitamins C and E to scavenge oxidants,[11] while the nitroxide participates in radical−radical recombination reactions.[12] The metabolism of nitroxides in biological systems has been investigated in detail and found to be reduced to the corresponding hydroxylamines through enzyme-related mechanisms.[4] The oxidation of the hydroxylamines can also occur efficiently. Consequently, a distribution of nitroxide/hydroxylamine is achieved regardless of which form is administered in vivo. Sterically hindered five- and six-membered cyclic amines without H atoms at α-positions have the ability of undergoing oxidation to paramagnetic *N*-oxyl compounds.[13] The influence of the ring structure on the nitroxide/hydroxylamine distribution in cells and tissues has been studied. Nitroxides belonging to the six-membered piperidine ring structure have been shown to be rapidly converted to the hydroxylamine, unlike the five-membered pyrrolidine or pyrroline nitroxides.[4]

It was found that compared to nitrones, which trap short-lived radicals to form stable nitroxide adducts, several nitroxide free radicals possess better antioxidant properties and decrease the oxidative damage caused by reactive oxygen-centered free radicals ($HO^{\bullet}$, $O_2^{\bullet-}$, $HO_2^{\bullet}$).[8] Studies conducted in this laboratory have identified protective effects of nitroxides in several cellular and in vivo studies.[14] For example, nitroxides and hydroxylamines have been shown to protect mammalian cells exposed to superoxide, hydrogen peroxide (H_2O_2), organic hydroperoxides, and redox cycling anticancer agents.[15−18] A variety of sterically hindered pyrrolinecarboxamides were prepared and tested for antiarrhythmic effects.[19] It was recently demonstrated that one of these compounds, *N*-[(2,2,5,5-tetramethyl-3-pyrroline-3-carboxamido)propyl]phthalimide which is currently under clinical trial, was oxidized in vivo to stable nontoxic nitroxide free radical as detected by in vivo EPR spectroscopy in living animals.[20] Mechanisms explaining the protective effects include (a) SOD-mimic activity, (b) oxidizing redox active transition metals, (c) reducing hypervalent heme states, (d) radical−radical recombination, and (e) chain-breaking antioxidants. However, in the case of exposure to ionizing radiation, nitroxides were effective in inhibiting the aerobic cytotoxicity, whereas the hydroxylamines, which are reducing agents, were ineffective similar to other reducing agents such as vitamin C and vitamin E.[21] The thiol-based radioprotectors, where the sulfhydryl agent provides radioprotection either by radical scavenging or by chemical repair, inhibit radiation-induced DNA lesions.[22] The most effective thiol-based radioprotectors require the presence of basic functions (amino, amidino, or guanidino).[23,24] The enhanced protection afforded by thiols with basic functional groups has been attributed to the better affinity of the positively charged substituents on the thiol to the DNA thus allowing the scavenging of short-lived radicals generated in close proximity to DNA.

To study the structural requirements of nitroxide for general antioxidant effects as well as radioprotection, a systematic screening of these agents was performed using an in vitro assay. The effect of ring size, substituents, and oxidation state of the test compounds at a fixed concentration, in providing protection to mammalian cells exposed to oxidative damage induced by exposure to H_2O_2 or ionizing radiation, was evaluated using the clonogenic viability of Chinese hamster lung fibroblasts. The results indicate that the requirements of an agent to be effective in providing protection against H_2O_2-induced toxicity are predominantly influenced by the ring size, redox potential, and oxidation state. In the case of protection against ionizing radiation, the ring substituents and the oxidation state were the main determinants of the radiation modification.

Chemistry

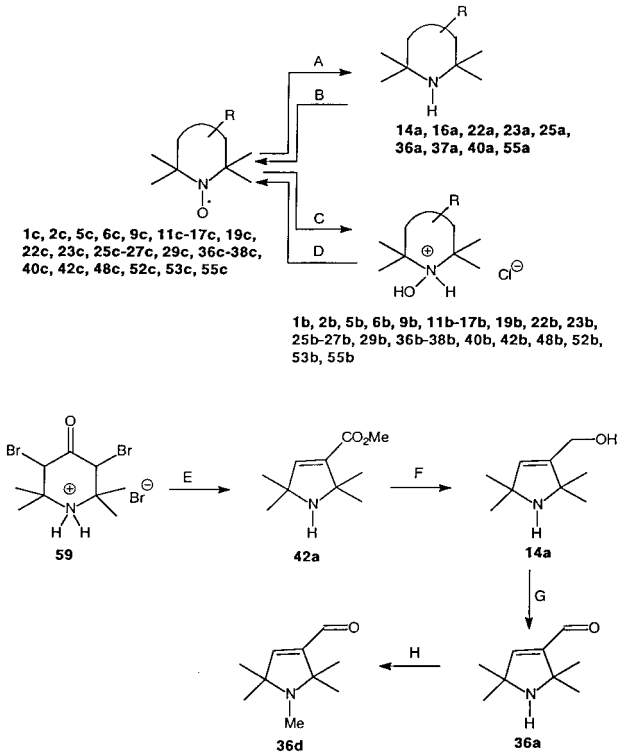
Generally speaking the structure of nitroxides can be modified by three methods: first, five- or six-membered ring can be constructed as it is required; second, the nitroxide function can be reduced partially, totally removed, or restored; third, the functional group on the ring can be altered as well. Nitroxides (**14c**, **16c**, **22c**, **23c**, **25c**, **36c**, **37c**, **40c**, **55c**) were reduced to secondary amines with Fe/AcOH[24] without alteration of other functional groups attached to the nitroxide ring. In a reverse process sterically hindered amine **55a** could be oxidized to nitroxide with H_2O_2 in the presence of Na_2WO_4.[13] The *N*-hydroxylamines (**1b**, **2b**, **5b**, **6b**, **9b**, **11b**−**17b**, **19b**, **22b**, **23b**, **25b**−**27b**, **29b**, **36b**−**38b**, **40b**, **42b**, **48b**, **52b**, **53b**, **55b**) could be obtained by refluxing nitroxides with EtOH saturated with HCl gas.[13] The *N*-hydroxylamines in basic or buffered media were oxidized to nitroxides with PbO_2 or MnO_2 accompanied by bubbling oxygen through the solution.

The synthesis of all pyrroline or piperidine nitroxides utilized 4-oxo-2,2,6,6-tetramethylpiperidine[13] as starting material. Favorskii rearrangement of 3,5-dibromo-4-oxo-2,2,6,6-tetramethylpiperidine (**59**) with sodium methoxide afforded the pyrroline methyl ester (**42a**), which could be reduced to allylic alcohol (**14a**) by SMEAH. This compound was readily oxidized to α,β-unsaturated aldehyde[26] **36a** which was methylated at its amino function to tertiary amine **36d** (Scheme 1).

Secondary amines (**18c**−**20c**, **25c**, **28c**) were obtained by sodium borohydride reduction of nonisolated Schiff's bases synthesized from aldehydes[26,27] (**36c**, **37c**). The secondary amine could be alkylated with methyl iodide in the presence of NaH to yield tertiary amines (**26c**, **29c**). Tertiary amine **10c** in which the nitrogen atom is attached to a secondary carbon atom could be achieved by reductive alkylation with sodium cyanoborohydride[28,29] starting from TEMPON (**39c**) and dibutylamine. Assessing the fact that paramagnetic ethers exhibited excellent NMR relaxitivity,[30] several of them, such as **15c**, were synthesized by alkylating the corresponding alcohol **14c** (Scheme 2).

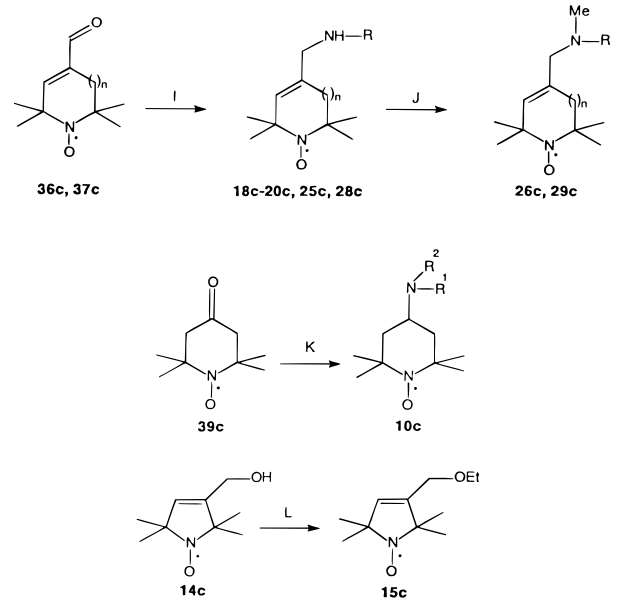
The most convenient method for obtaining paramagnetic tertiary amines (**17c**, **21c**, **22c**) was the alkylating of secondary amines with allylic bromide **60**.[31] This paramagnetic alkylating agent was used to get am-

Scheme 1[a]



[a] Reagents: (A) Fe powder, AcOH, 3 h, 40−50 °C; (B) 30% H_2O_2, MeOH, 3 days; (C) HCl/EtOH, 30 min; (D) K_2CO_3, $CHCl_3$, then MnO_2, 30 min; (E) MeONa, MeOH, 1 h; (F) SMEAH, toluene, 40 °C, 1 h; (G) MnO_2, $CHCl_3$, 30 min; (H) MeI, acetone, reflux, 4 h.

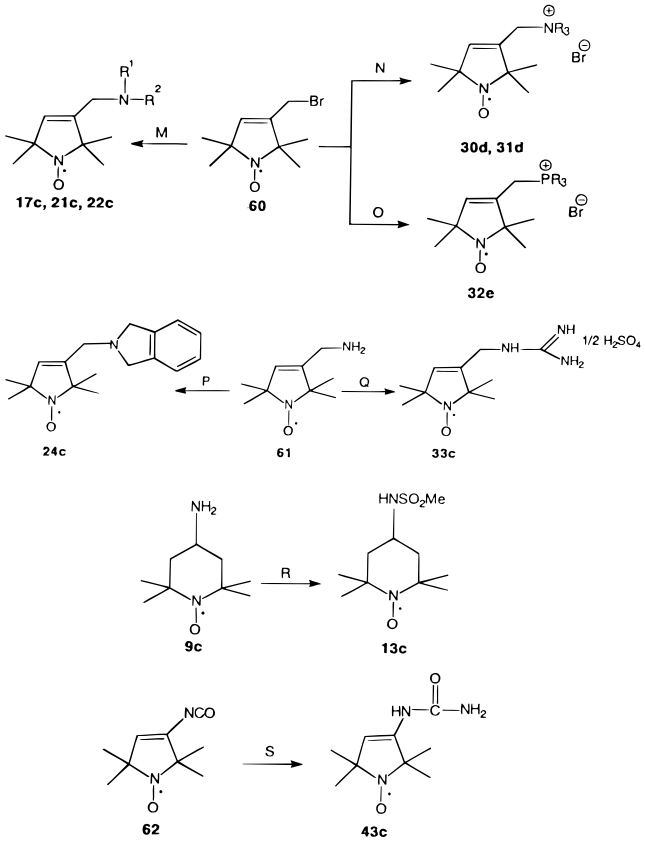
Scheme 2[a]



[a] Reagents: (I) (i) 1-aminoadamantane, cat. *p*-toluenesulfonic acid, toluene, reflux, 12 h, (ii) $NaBH_4$, EtOH, 0 °C then reflux for 1 h; (J) NaH, THF, reflux, 1 h then MeI, reflux 6 h; (K) dibutylamine hydrochloride, $NaCNBH_3$, MeOH, reflux, 48 h; (L) NaH, THF, rt, 1 h then EtBr, DMF, reflux, 2 h.

monium (**30d**, **31d**) and phosphonium (**32e**) salts from tertiary amines and triphenylphosphines.[32] The isoindolyl derivative **24c** was obtained most conveniently by alkylating allylic amine **61**[33] with α,α′-dibromo-*o*-xylene. Attractive challenging target was to achieve spin-labeled guanidine **33c** and urea **43c** from amine **61** and *S*-methylisothiouronium salt and from isocyanate **62**[33]

Scheme 3[a]



[a] Reagents: (M) pyrrolidine, $CHCl_3$, reflux, 3 h; (N) *n*-tributylamine, acetone, reflux, 6 days; (O) triphenylphosphine, acetone, reflux, 3 h; (P) *o*-dibromoxilol, K_2CO_3, $CHCl_3$, reflux, 3 h; (Q) *S*-methylisothiouronium sulfate, EtOH, reflux, 3 h; (R) Et_3N, $MeSO_2Cl$, CH_2Cl_2, rt, 3 h; (S) NH_3, cyclohexane.

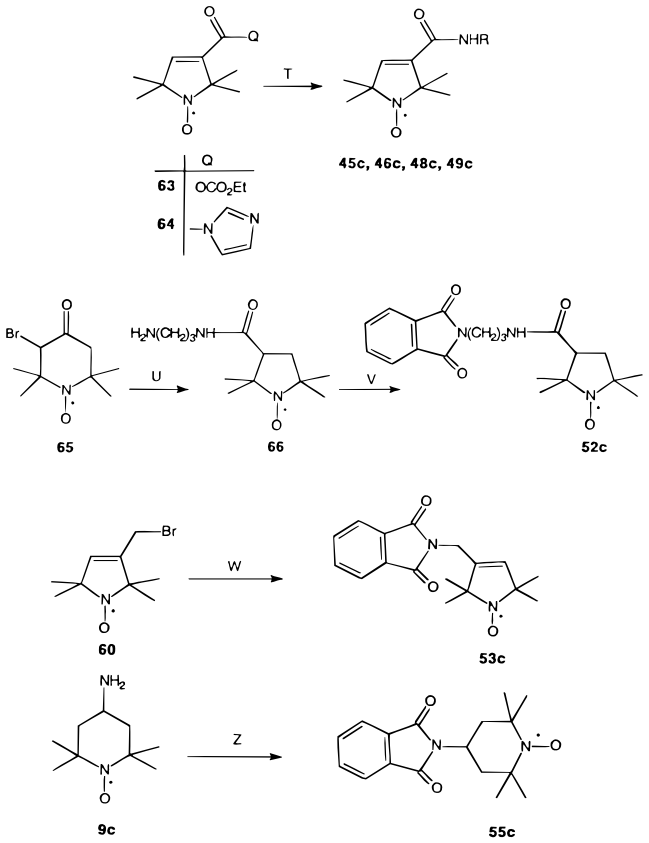
and ammonia. The investigation of these compounds is well-supported so that parent compounds, i.e., guanidine and urea, often appear in bioactive molecules and drugs. Besides the amines which proved the most efficient agents, we synthesized their sulfonylated **13c** derivative. Compound **13c** was obtained with standard procedure, e.g., reacting amine **9c** with methanesulfonyl chloride in the presence of triethylamine (Scheme 3).

Paramagnetic amides (**45c**, **46c**, **48c**, **49c**) were achieved by acylating the corresponding amines with paramagnetic mixed anhydride ester **63**[34] or 1-acylimidazole **64**. To avoid catalytic hydrogenation of double bond in pyrrolines, pyrrolidine derivative **66** was obtained by reaction of 1-oxyl-3-bromo-4-oxo-2,2,6,6-tetramethylpiperidine[35] (**65**) with 1,3-diaminopropane. Fusing compound **66** with phthalimide without solvent at 120 °C yielded compound **52c** while ammonia gas was released.[36] This method had to be used in synthesis of **55c** from **9c**, since in this case the alkylation did not give the desired product, while alkylation of phthalimide with allylic bromide **60** furnished **53c** in good yield (Scheme 4).

Results and Discussion

The compounds evaluated in this study as potential modifiers of cytotoxicity of both ionizing radiation and H_2O_2 are listed in Tables 1−10. Figure 1 shows the general structure of the compounds tested. For each ring type, three possible oxidation states may exist:

Scheme 4[a]



[a] Reagents: (T) 3-aminopropanol, $CHCl_3$, reflux, 30 min; or 1,3-diaminopropane, THF, 0 °C → rt, 1 h; (U) 1,3-diaminopropane, H_2O/THF, rt, 2 h; (V) phthalimide, 120 °C, 1 h; (W) phthalimide K_2CO_3, KOH, 18-K-6, dioxane, reflux, 4 h; (Z) phthalimide, 120 °C, 50 min.

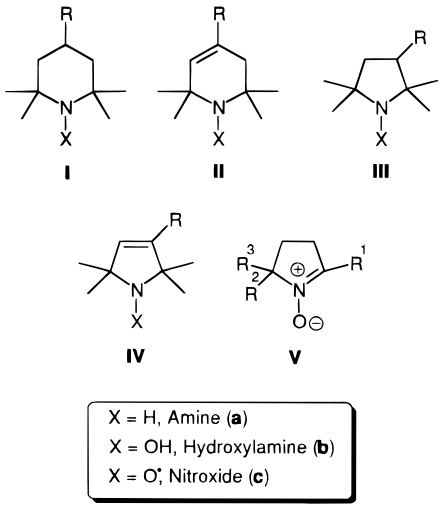


Figure 1. General chemical structures of the nitroxides studied.

namely, the fully reduced secondary amine (type **a**), the hydroxylamine (type **b**), and the nitroxide (type **c**). In some cases the redox midpoint potential of the nitroxide/oxoammonium cation redox pair was estimated.

Hydrogen Peroxide Treatment. The mean surviving fraction of V79 cells following a 1-h exposure to H_2O_2 alone (500 μM) was 0.14 ± 0.016. Possible modulation of H_2O_2-mediated cytotoxicity by the test compounds was evaluated using a fixed final concentration of 100 μM, present during the 1-h H_2O_2 exposure. This concentration was selected for several reasons. First, based on our experience with Tempol (**5c**), a final concentration of 100 μM provides partial protection against H_2O_2 cytotoxicity, whereas higher concentrations (1000 μM) completely protect.[15] A final concentration of 100 μM of each compound would, therefore, afford comparison to our lead compound and allow identification of agents with greater protective properties than Tempol (**5c**). Second, because numerous compounds were evaluated, only small quantities were synthesized making the use of higher concentrations prohibitive. The protection afforded by the compounds against H_2O_2-induced cytotoxicity is shown in Tables 1−10 and Figure 2 (Supporting Information). The results shown in Figure 2 indicate that the test compounds exhibited a broad range in modifying the cytotoxic effects of H_2O_2. Most agents tested were effective in providing protective effects against exposure. In comparison to Tempol (**5c**) which gave a PF of 2.5, 12 compounds yielded greater PFs. The most efficient protector identified by the screen was Tempo (**2c**) which provided a PF of 3.5. With respect to structure and the protective effects against H_2O_2 exposure, the following observations can be made.

The efficacy of stable nitroxides as well as the corresponding hydroxylamines in reversing the free radical-mediated damage induced by oxidative stress prompted this study to identify structural requirements of this class of compounds for optimal antioxidant effects. The model chosen to screen for the efficacy of the antioxidant effects of the test compounds was by exposing Chinese hamster V79 cells to hydrogen peroxide for a fixed duration of time and assessing the clonogenic viability in the absence and presence of a fixed concentration of the compound to be tested. Hydrogen peroxide is produced cellularly as a result of incomplete reduction of oxygen to water or also produced by phagocytic cells as well as by redox cycling xenobiotic drugs. The damage associated with exposure to hydrogen peroxide is understood to be mediated by free radical generation presumably by the generation of $^{\bullet}OH$ radical or an equally strong oxidant catalyzed by redox active transition-metal complexes (eq 1).

$$H_2O_2 + Fe^{2+}\text{−chelate} \rightarrow {}^{\bullet}OH + {}^{-}OH + Fe^{3+}\text{−chelate} \quad (1)$$

$$^{\bullet}OH + LH \rightarrow L^{\bullet} + H_2O \text{ (initiation)} \quad (2)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \text{ (propagation)} \quad (3)$$

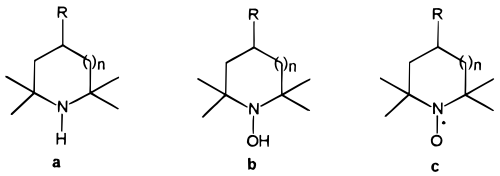
$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \text{ (propagation)} \quad (4)$$

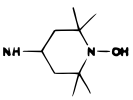
$$LOOH \rightarrow LO^{\bullet} + {}^{\bullet}OH \text{ (propagation)} \quad (5)$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{products (termination)} \quad (6)$$

Earlier studies have shown that both free radical scavengers as well as metal chelators such as desferal can significantly inhibit both the cytotoxicity as well as the oxidation of cellular components. Thus cellular incubation of H_2O_2 represents a valid modality of oxidant stress and has been widely used to screen for agents to reverse this damage. The damage is associ-

Table 1. Alcohols, Amines, and Amides



compd	*n*	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
1b	0	H	C	78	170	$C_8H_{17}NO{\cdot}HCl$	179.69	43	2.3 (0.52)	0.70 (0.10)
1c	0	H				$C_8H_{16}NO$	142.22	50	0.9 (0.06)	4.60 (0.40)
2b	1	H	C	67	180 dec	$C_9H_{19}NO{\cdot}HCl$	193.72	43	1.5 (0.20)	0.68 (0.10)
2c	1	H				$C_9H_{18}NO$	156.25	44	3.5 (0.28)	4.20 (0.00)
3c	0	OH				$C_8H_{16}NO_2$	158.22	13	1.4 (0.14)	2.90 (1.40)
4c	0	CH_2OH				$C_9H_{18}NO_2$	172.25	26	1.1 (0.00)	4.7 (0.20)
5a	1	OH				$C_9H_{19}NO$	157.26	44	0.4 (0.00)	0.90 (0.08)
5b	1	OH	C	65	>240	$C_9H_{19}NO_2{\cdot}HCl$	209.72	43	NT[b]	1.05 (0.04)
5c	1	OH				$C_9H_{18}NO_2$	172.25	13	2.5 (0.23)	5.10 (0.08)
6b	1	OCH_2CH_3	C	63	120	$C_{11}H_{23}NO_2{\cdot}HCl$	237.77	43	1.6 (0.30)	NT
6c	1	OCH_2CH_3				$C_{11}H_{23}NO_2$	200.30	30	1.9 (0.50)	NT
7c	0	NH_2				$C_8H_{17}N_2O$	157.24	44	1.1 (0.00)	12.9 (0.02)
8c	0	CH_2NH_2				$C_9H_{19}N_2O$	171.26	44	0.7 (0.00)	13.7 (0.00)
9b	1	NH_2	C	74	>230	$C_9H_{20}N_2O{\cdot}2HCl$	245.19	43	1.0 (0.00)	1.10 (0.04)
9c	1	NH_2				$C_9H_{19}N_2O$	171.26	44, 13	2.8 (0.14)	18.5 (0.31)
10c	1	$N(n\text{-}Bu)_2$	K	35	oil	$C_{17}H_{35}N_2O$	283.48	43	2.9 (0.64)	NT
11b	1		C	61	>230	$C_{18}H_{37}N_3O_2.3HCl$	436.89	43	1.9 (0.30)	1.40 (0.13)
12b	1	$NHCOCH_3$	C	73	128−130	$C_{11}H_{22}N_2O_2{\cdot}HCl$	250.77	43	1.8 (0.00)	0.85 (0.20)
13b	1	$NHSO_2CH_3$	C	78	182−184	$C_{10}H_{22}N_2O_3S{\cdot}HCl$	286.82	43	NT	0.77 (0.05)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

ated with DNA single- and double-strand breaks (in the presence of specific chelators) as well as damage to other cellular organelles.

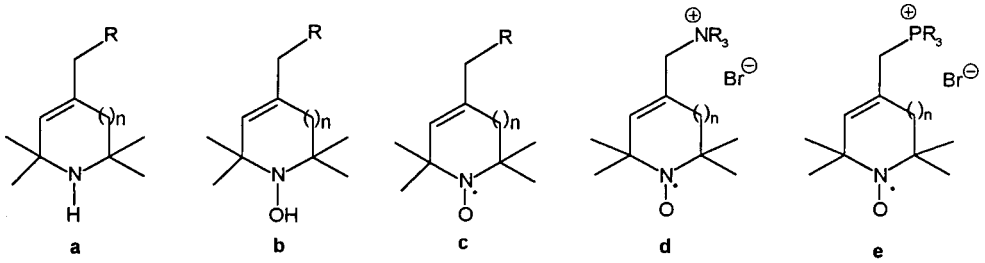
In this study nitroxides of four different ring classes were evaluated such as the six-membered piperidine class (**I**), the six-membered 1,2,5,6-tetrahydropyridine class (**II**), the saturated five-membered pyrrolidine class (**III**), the unsaturated five-membered pyrroline class (**IV**), and nitrones (**V**) (Figure 1). Electrochemical studies on these agents show that while the hydroxylamines undergo an irreversible oxidation around 200 mV vs NHE, at higher potentials, a reversible redox couple consistent with the nitroxide and the corresponding one-electron oxidation product, the oxoammonium cation, exists around in the range 700−1100 mV for the compounds tested. The nitroxide/oxoammonium redox couple has been shown in earlier studies to mediate enzyme mimetic reactions catalytically, such as SOD-mimicking activity.[6]

$$RRNO^{\bullet} + H^{+} + HO_2^{\bullet} \rightarrow RRNO^{+} + H_2O_2 \quad (7)$$

$$RRNO^{+} + O_2^{-\bullet} \rightarrow RRNO^{\bullet} + O_2 \quad (8)$$

The catalytic efficiencies of superoxide dismutation were found to depend on the ease of oxidation of the nitroxide to the corresponding oxoammonium cation, which represents the rate-limiting step. The catalytic rate constants were found to be inversely correlated to the redox midpoint potential of the nitroxide/oxoammonium couple.[8] The nitroxide/oxoammonium couple has also been postulated to induce catalase-like activity in heme proteins.[9] Either of these roles can confer antioxidant activity in the nitroxides. In addition, nitroxides have been shown to prevent the generation of oxidants via Fenton reactions (eq 1) by maintaining the metals in the oxidized state.[15]

Table 2. Alcohols and Amines



a b c d e

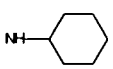
compd	*n*	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
14a	0	OH	A, F	64, 78	66-69	$C_9H_{17}NO$	155.24	43	0.9 (0.06)	1.50 (0.30)
14b	0	OH	C	69	157-159	$C_9H_{17}NO_2$·HCl	207.70	43	3.1 (0.34)	0.92 (0.07)
14c	0	OH				$C_9H_{16}NO_2$	170.23	25	1.8 (0.00)	2.6 (0.07)
15b	0	OCH_2CH_3	C	62	89-92	$C_{11}H_{21}NO_2$·HCl	235.75	43	3.2 (0.61)	NT[b]
15c	0	OCH_2CH_3	L	57	oil	$C_{11}H_{20}NO_2$	198.29	43	1.3 (0.20)	NT
16a	0	NMe_2	A	58	oil	$C_{11}H_{22}N_2$	182.31	43	NT	0.95 (0.18)
16b	0	NMe_2	C	71	hygrosc.	$C_{11}H_{22}N_2O$·2HCl	271.23	43	NT	16.0 (1.02)
16c	0	NMe_2				$C_{11}H_{21}N_2O$	197.30	32	2.1 (0.43)	13.9 (0.70)
17b	0	NEt_2	C	74	189-191	$C_{13}H_{26}N_2O$·2HCl	299.28	43	NT	0.96 (0.03)
17c	0	NEt_2	M	82	oil	$C_{13}H_{25}N_2O$	225.35	43	NT	17.2 (1.20)
18c	0	NH-*n*-Bu	I	64	oil	$C_{13}H_{25}N_2O$	225.35	43	2.5 (0.21)	3.96 (1.75)
19b	0	NH-*t*-Bu	C	68	> 250	$C_{13}H_{26}N_2O$·2HCl	299.28	43	2.1 (0.12)	1.20 (0.00)
19c	0	NH-*t*-Bu	I	67	56-60	$C_{13}H_{25}N_2O$	225.35	43	2.0 (0.35)	19.3 (3.15)
20c/Ts	0		I	60	186 dec	$C_{15}H_{27}N_2O$·$C_7H_8O_3S$	423.59	43	NT	4.40 (0.40)
21c	0	$N(n\text{-}Bu)_2$	M	72	oil	$C_{17}H_{33}N_2O$	281.46	43	3.2 (0.14)	NT
22a	0		A	47	> 240	$C_{13}H_{24}N_2$·2HCl	281.27	43	1.0 (0.00)	0.34 (0.00)

Table 2 (Continued)

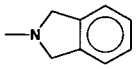
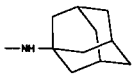
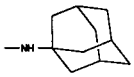
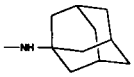
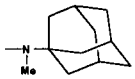
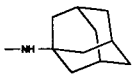
compd	*n*	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
22b	0		C	67	219-221	$C_{13}H_{24}N_2O\cdot 2HCl$	297.27	43	NT	28.3 (0.20)
22c/Ts	0		M	78	129-132	$C_{13}H_{23}N_2O$ $C_7H_8O_3S$	395.54	43	NT	21.4 (1.15)
23a	0		A	45	oil	$C_{14}H_{26}N_2$	222.37	43	NT	0.95 (0.00)
23b	0		C	70	210-212	$C_{14}H_{26}N_2O\cdot 2HCl$	311.30	43	NT	30.0 (5.40)
23c	0					$C_{14}H_{25}N_2O\cdot$ $C_7H_8O_3S$	409.56	32	NT	19.1 (3.50)
24c	0		P	68	89-90	$C_{17}H_{23}N_2O$	271.38	43	NT	3.90 (0.00)
25a	0		A	62	116-118	$C_{19}H_{32}N_2$	288.48	43	1.9 (0.60)	1.40 (0.13)
25b	0		C	70	215-216	$C_{19}H_{32}N_2O\cdot 2HCl$	377.40	43	2.5 (0.43)	1.80 (0.07)
25c	0		I	72	109-111	$C_{19}H_{31}N_2O$	303.47	43	3.3 (0.63)	NT
26b	0		C	67	210-212	$C_{20}H_{34}N_2O\cdot 2HCl$	391.42	43	2.6 (0.92)	NT
26c	0		J	62	110-112	$C_{20}H_{33}N_2O$	317.49	43	3.3 (0.50)	NT
27b	1		C	66	>200	$C_{20}H_{34}N_2O.2HCl$	391.42	43	2.1 (0.07)	NT
28c	0	$NHCH_2Ph$	I	59	oil	$C_{16}H_{23}N_2O$	259.39	43	2.0 (0.07)	5.20 (1.10)
29c	0	$N(Me)CH_2Ph$	J	56	oil	$C_{17}H_{25}N_2O$	273.42	43	2.0 (0.35)	NT
30d	0	$\oplus N(Et)_3\ Br^{\ominus}$	N	66	238-240 dec	$C_{15}H_{30}BrN_2O$	334.32	43	NT	1.20 (0.00)
31d	0	$\oplus N(n\text{-}Bu)_3\ Br^{\ominus}$	N	63	180-181	$C_{21}H_{42}BrN_2O$	418.48	43	1.1 (0.35)	0.69 (0.02)
32e	0	$\oplus P(Ph)_3\ Br^{\ominus}$	O	71	224-226	$C_{27}H_{30}BrNOP$	495.42	32, 43	NT	0.72 (0.07)
33c	0	$-CH_2NHC(NH)NH_2$	Q	55	180 dec	$C_{10}H_{19}N_4O$ $1/2\ H_2SO_4$	260.34	43	NT	1.00 (0.00)

Table 2 (Continued)

compd	n	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
34c	0	$-CH_2SC(NH)NH_2$				$C_{11}H_{20}N_3OS$	338.46	31	2.2	7.50
						CH_4O_3S			(0.29)	(1.10)
35c	0	$-CH_2SeC(NH)NH_2$				$C_{11}H_{20}N_3OSe$	370.17	46	1.3	NT
						HBr			(0.12)	

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

$$RRNO^{\bullet} + H^{+} + Fe^{2+}-\text{chelate} \rightarrow RRNOH + Fe^{3+}-\text{chelate} \quad (9)$$

The hydroxylamines, on the other hand, were found to behave like classic reducing agents such as ascorbate and tocopherol by being oxidized to the corresponding nitroxide by the oxidant in the detoxifying reaction.

$$RRNOH + {}^{\bullet}OH \rightarrow RRNO^{\bullet} + H_2O \quad (10)$$

However, unlike ascorbate, tocopherol, and other phenolic antioxidants, the product (nitroxide) is also capable of further detoxification modalities such radical−radical recombination reactions and interrupting chain propagation of lipid peroxidation reactions.[37]

$$RRNO^{\bullet} + L^{\bullet} \rightarrow RRNOL \quad (11)$$

For a given ring size, the effect of the midpoint redox potential of the nitroxide/oxoammonium catalytic couple has also been examined for its influence on the protective effects. On the basis of the results obtained in this study, some observations can be made.

Structure−Activity Relationships. The compounds of type **a** (amines) were in general ineffective in providing protection or in some cases actually enhanced the cytotoxicity of H_2O_2 exposure. The nitroxides and hydroxylamines were in general protective.

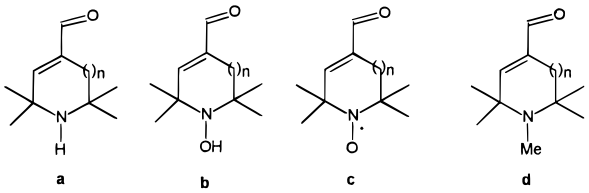
The well-known six-membered nitroxides (**2c**, **5c**, **9c**, **39c**) were investigated earlier and showed better protecting activity then the corresponding five-membered nitroxides (**1c**, **3c**, **7c**, **38c**). Redox behavior alone might not be able to account for this behavior since no significant differences in their midpoint potentials were found. However, on the basis of several earlier reports which found six-membered nitroxides more reactive with radicals and are more likely to be converted to the corresponding hydroxylamine than the corresponding five-membered counterparts,[4] the enhanced ability of the pideridine class of nitroxides can be attributed to their reactivities. This has been explained in terms of the access to the nitroxide moiety in the ring to reactants. In the case of six-membered ring nitroxides, the "boat" or "chair" conformations of the piperidine ring provide easy access to the nitroxide moiety to participate in electron-transfer reactions involved in antioxidant effects.

The protecting activity is also influenced by ring saturation and functional groups; for instance, the protecting activity of substituted pyrroline nitroxides (**21c**, **25c**, **26c**) approaches the efficiency of six-membered nitroxides. This is a remarkable fact because of the lower toxicity of five-membered ring maintaining the similar H_2O_2 protecting activity.[38,39] Saturated five-membered alcohol **4c** exhibited a smaller protecting activity than its unsaturated derivative **14c**. Among saturated (**50c**, **51c**) and unsaturated (**41c**, **44c**) acid derivatives, there is no significant difference in H_2O_2 protecting activity. In the range of well-available unsaturated acid derivatives, we observed that ester **42c** has better protecting activity than the corresponding acid **41c**. Among all the compounds which exhibit the best protecting activity are amines **9c**, **10c**, **21c**, **25c**, and **26c**; however, quaternary salts **30d**, **31d**, **33c**, **34c**, and **35c** show small protecting activity. The amine-substituted five-membered and six-membered nitroxides (**9c**, **10c**, **25c 26c**) show good protecting activity. The order of amines was not found to have significant influence on protecting activity as was observed in the case of **25c** and **26c**.

Among the six-membered piperidines, where the nitroxide and the hydroxylamines were compared at similar concentrations, nitroxides were found to be better protectors. However, the protection was inversely proportional to the midpoint potentials of the nitroxide/oxoammonium couple for the piperidines (Figure 3A). The nitroxides which were easily oxidizable provided better protection. However, in the case of five-membered nitroxides such as the pyrrolidines and the pyrrolines such a correlation was absent (Figure 3B). However, in this case, several hydroxylamines exhibited better protection than the corresponding nitroxides suggesting that a H atom donation might be the predominant operating factor in these cases, similar to antioxidants such as ascorbate, thiols, etc.

Ionizing Radiation Treatment. The mean surviving fraction of V79 cells following a 12-Gy dose of radiation alone was 0.02 ± 0.002. Possible modulation of radiation-mediated cytotoxicity by the test compounds was evaluated using a fixed final concentration of 10 mM, present during the 10 min prior to radiation exposure. This concentration was selected for several reasons. From prior results obtained with Tempol, a final concentration of 10 mM provides partial protection against radiation-induced cytotoxicity. A final concentration of 10 mM of each compound would therefore afford comparison to our lead compound and allow identification of agents with greater protective properties than Tempol. As stated above, because numerous compounds were evaluated, only small quantities were synthesized making the use of higher concentrations prohibitive. Additionally, our earlier studies showed

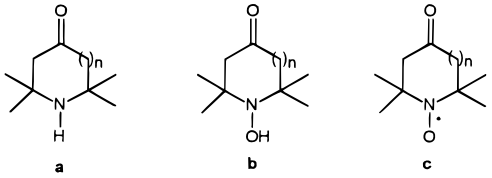
Table 3. Aldehydes



compd	*n*	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
36a	0	A, G	62, 89	oil	$C_9H_{15}NO$	153.22	43	0.9 (0.06)	0.07 (0.02)
36b	0	C	69	153−158	$C_9H_{15}NO_2$·HCl	205.68	43	2.7 (0.29)	0.13 (0.30)
36c	0	D	91	77−79	$C_9H_{14}NO_2$	168.22	26, 43	1.3 (0.00)	2.70 (0.23)
36d	0	H	85	oil	$C_{10}H_{17}NO$	167.25	43	NT[b]	0.60 (0.10)
37a	1	A	45	oil	$C_{10}H_{17}NO$	167.25	43	NT	0.40 (0.00)
37b	1	C	77	194−197	$C_{10}H_{17}NO_2$·HCl	219.71	43	NT	1.50 (0.15)
37c	1				$C_{10}H_{16}NO_2$	182.24	27	0.8 (0.00)	6.10 (0.25)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

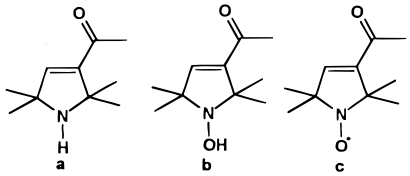
Table 4. Ketones



compd	*n*	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
38b	0	C	68	151−153	$C_8H_{15}NO_2$·HCl	193.67	43	1.1 (0.00)	1.0 (0.20)
38c	0				$C_8H_{14}NO_2$	156.20	13	0.9 (0.00)	10.2 (0.45)
39a	1				$C_9H_{17}NO$	155.24	47	0.5 (0.00)	1.10 (0.10)
39b	1				$C_9H_{17}NO_2$·HCl	207.70	35	NT[b]	0.68 (0.15)
39c	1				$C_9H_{16}NO_2$	170.23	13, 47	1.1 (0.00)	7.10 (0.85)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

Table 5. Ketones

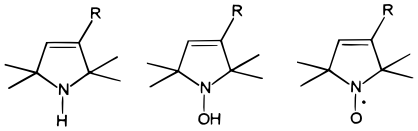


compd	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
40a	A	57	oil	$C_{10}H_{17}NO$	167.25	43	NT[b]	0.63 (0.13)
40b	C	80	181−183	$C_{10}H_{17}NO_2$·HCl	219.71	43	NT	0.15 (0.02)
40c				$C_{10}H_{16}NO_2$	182.24	48, 49	NT	4.80 (0.20)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

that nitroxides, but not the hydroxylamines, were radioprotective under aerobic conditions.[21] The protection afforded by the compounds against radiation-induced cytotoxicity is shown in Tables 1−10 and Figure 3. The studies show that the protective effects of the test compounds varied from protection factors of 1 to

Table 6. Acids, Esters, and Amides

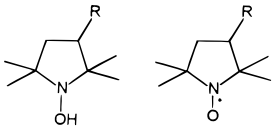


a **b** **c**

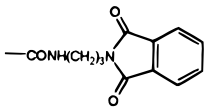
compd	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
41c	CO_2H				$C_9H_{14}NO_3$	184.24	13	1.0 (0.00)	NT[b]
42a	CO_2Me	E	82	oil	$C_{10}H_{17}NO_2$	183.25	43	1.3 (0.35)	NT
42b	CO_2Me	C	64	143−145	$C_{10}H_{17}NO_3 \cdot HCl$	235.71	43	2.7 (0.46)	NT
42c	CO_2Me				$C_{10}H_{16}NO_3$	198.24	13	1.5 (0.29)	0.94 (0.08)
43c	$NHCONH_2$	S	70	189−190	$C_9H_{16}N_3O_2$	198.24	43	1.2 (0.00)	NT
44c	$CONH_2$				$C_9H_{15}N_2O_2$	183.23	13	1.0 (0.00)	10.7 (3.80)
45c	$CONH(CH_2)_2OH$	T1	62	99−100	$C_{11}H_{19}N_2O_3$	227.28	43	1.2 (0.04)	NT
46c	$CONH(CH_2)_3OH$	T1	84	95−96	$C_{12}H_{21}N_2O_3$	241.31	43	1.1 (0.14)	NT
47c	$CONHCH_2CO_2H$				$C_{11}H_{17}N_2O_4$	241.26	33	0.9 (0.20)	0.98 (0.02)
48a	$CONH(CH_2)_3NH_2$				$C_{12}H_{23}N_3O$	225.33	19	NT	0.52 (0.07)
48b	$CONH(CH_2)_3NH_2$	C	66	hygrosc	$C_{12}H_{23}N_3O_2 \cdot 2HCl$	314.26	43	1.0 (0.12)	NT
48c	$CONH(CH_2)_3NH_2$	T1, T2	73, 55	104−108	$C_{12}H_{22}N_3O_2$	240.33	43	1.1 (0.17)	NT
49c	$CONH(CH_2)_3NMe_2$	T1	59	94−96	$C_{14}H_{26}N_3O_2 \cdot C_2H_2O_4$	358.41	43	NT	15.0 (4.20)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

Table 7. Acids, Amides, and Imides



b **c**

compd	R	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
50c	CO_2H				$C_9H_{16}NO_3$	186.23	13	1.0 (0.00)	NT[b]
51c	$CONH_2$				$C_9H_{17}N_2O_2$	185.25	13	1.7 (0.00)	11.2 (1.80)
52b		C	68	dec	$C_{20}H_{27}N_3O_4 \cdot HCl$	409.91	43	NT	1.26 (0.00)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

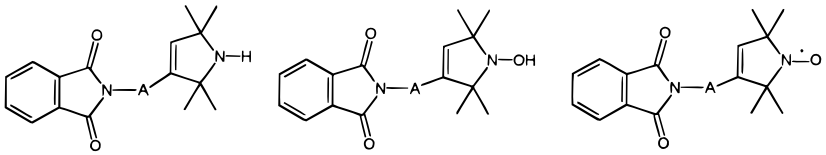
30 (Figure 4 in Supporting Information). In comparison to Tempol (**5c**) which gave a PF of 2.2, 19 compounds yielded greater PFs. In some cases the agents provided sensitization of radiation effects with sensitization factors ranging between 1 and 0.1. A correlation between the protective effects and the structure and oxidation state was made to evaluate the determinant factors governing the radiation-modifying effects.

Among the compounds studied, the nitroxides were found to be the most effective in providing the protection followed by the hydroxylamines under identical concentrations. Though the nitroxides and hydroxylamines were both effective in reversing the toxicity mediated by H_2O_2, the results obtained from radiation studies differ presumably as a result of kinetic reasons as well as differences in the sites of damage.

The possible chemical reactions involved in the protective reactions for nitroxides are as follows. If TH is a target molecule which is a critical biomolecule such as DNA, abstraction of the H atom by the radiation-induced species ($X^\bullet$) causes the initial lesion.

$$TH + X^\bullet \rightarrow T^\bullet + XH \text{ (radical formation)} \quad (12)$$

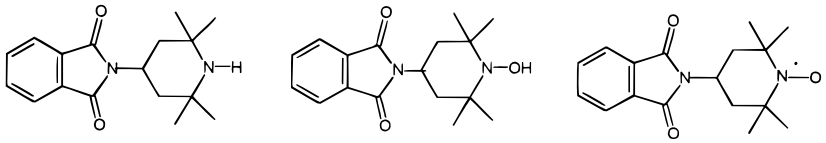
Table 8. Imides



compd	A	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
53b	CH_2	C	78	118−120	$C_{17}H_{20}N_2O_3$·HCl	336.82	43	3.3 (0.52)	0.49 (0.03)
54a	$(CH_2)_3NHCO$				$C_{20}H_{25}N_3O_3$·HCl	391.90	19	1.1 (0.23)	1.90 (0.23)
54b	$(CH_2)_3NHCO$				$C_{20}H_{25}N_3O_4$·HCl	407.90	19	2.2 (0.23)	1.30 (0.17)

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula.

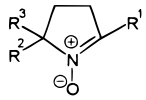
Table 9. Imides



compd	method	yield, %	mp, °C	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
55a	A	54	128−129	$C_{17}H_{22}N_2O_2$	286.37	43	0.8 (0.00)	1.70 (0.29)
55b	C	72	>235	$C_{17}H_{22}N_2O_3$·HCl	338.83	43	1.5 (0.14)	NT[b]

[a] Analyses for C, H, N, S, Br, and Cl are within ±0.4% of the expected value for the formula. [b] NT, not tested.

Table 10. Nitrones



compd	R^1	R^2	R^3	formula[a]	mol wt	ref	H_2O_2 prot	radiat prot
56	H	CH_3	CH_3	$C_6H_{11}NO$	113.15	44	0.9 (0.17)	0.93
57	CH_3	H	CH_3	$C_6H_{11}NO$	113.15	51	1.1 (0.09)	0.91
58	CH_3	CH_3	CH_3	$C_7H_{13}NO$	127.18	52	0.8 (0.12)	1.10

In the presence of oxygen or other hypoxic cell radiation sensitizers such as nitric oxide, the damage on the molecule is fixed by radical−radical recombination reactions.

$$T^{\bullet} + O_2 \rightarrow TOO^{\bullet} \quad (13)$$

These intermediates lead to further breakdown products leading to single- and double-strand breaks in DNA. However, thiol-based radioprotectors have been shown to be effective H atom donors and compete with O_2 and restore the damage.

$$T^{\bullet} + RSH \rightarrow TH + RS^{\bullet} \quad (14)$$

The thiyl radical, being a weak oxidant, is presumed to be minimally involved in mediating further biologic damage.

If $X^{\bullet}$ represents the species generated by ionizing radiation which mediate biological damage, then protection by radical scavenging can proceed by:

$$H^+ + X^{\bullet} + RRNO^{\bullet} \rightarrow XH + RRNO^+ \text{ (electron transfer)} \quad (15)$$

$$X^{\bullet} + RRNO^{\bullet} \rightarrow RRNOX \text{ (radical−radical recombination)} \quad (16)$$

Protection by chemical repair is also feasible with nitroxides:

$$H^+ + T^{\bullet} + RRNO^{\bullet} \rightarrow TH + RRNO^+ \quad (17)$$

Thus, nitroxides can afford radioprotection by radical scavenging as well as by chemical repair.

The hydroxylamines can mediate both electron-transfer and chemical repair reactions and provide

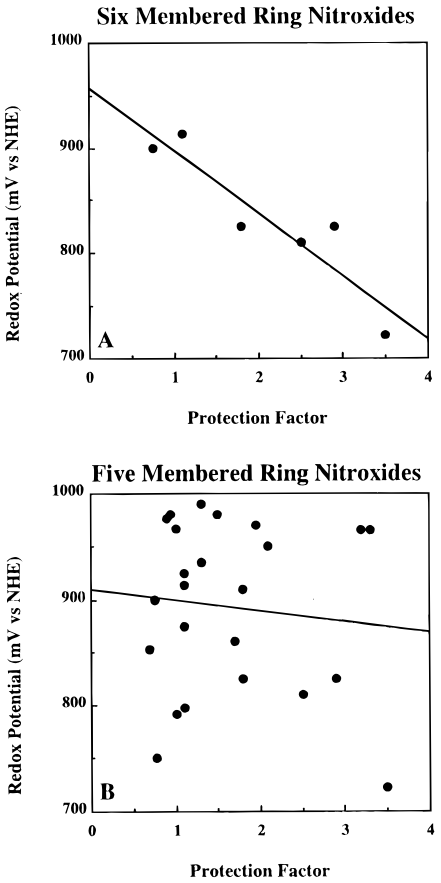


Figure 3. Protection factors of hydrogen peroxide-induced cytotoxicity as a function of redox potential of selected compounds: (A) six-membered ring nitroxides and (B) five-membered ring nitroxides.

protective effects.

$$X^{\bullet} + RRNOH \rightarrow HX + RRNO^{\bullet} \text{ (electron-transfer)} \quad (18)$$

$$T^{\bullet} + RRNOH \rightarrow TH + RRNO^{\bullet} \text{ (chemical repair)} \quad (19)$$

The fully reduced amines were found to provide radiosensitization in some cases. These effects are consistent with the reactions listed below:

$$X^{\bullet} + RRNH \rightarrow XH + RRN^{\bullet} \quad (20)$$

$$X^{\bullet} + TH \rightarrow T^{\bullet} + XH \quad (21)$$

$$RRN^{\bullet} + TH \rightarrow T^{\bullet} + RRNH \text{ (damage)} \quad (22)$$

$$T^{\bullet} + RRN^{\bullet} \rightarrow RRN\text{-}T \text{ (damage fixation)} \quad (23)$$

The ring size was not found to be of significant effect in determining the radiation response. DNA binding characteristics of the nitroxide-based radioprotectors have been evaluated by nonequilibrium dialysis of DNA with individual nitroxides. These studies show that the positively charged nitroxides had higher degrees of association with DNA as compared to the negatively charged nitroxides. However, since the binding to DNA should be significantly similar for a given ring substituent, nitroxide and hydroxylamine should accumulate at the DNA to similar extent. In a recent study, Milligan et al.[40] show that a diradical intermediate with lifetime of 1 μs is involved in the formation of dsbs. Hence agents which can scavenge such intermediates should effectively inhibit the radiation-induced cytotoxicity. While both hydroxylamine and nitroxides are effective radical scavengers, the mechanisms and the kinetics are expected to be different.

Structure−Activity Relationships. The unsubstituted base compounds (**1c**, **2c**) afforded modest radioprotective activity similar to that found for Tempol (**5c**). Effective radioprotection from nitroxides was observed when the substituent contained an amino group in the side chain. The protection however, was not found to dependent on the order of the amine; primary amines (**7c**−**9c**), secondary amines (**19c**), or tertiary amines (**16c**, **17c**, **22c**, **23c**, **49c**) were all found to have significant radioprotecting activity. The positively charged isothiouronium salt (**34c**) also exhibited remarkable radioprotective activity.

Only **c** type nitroxide compounds or in some cases **b** type hydroxylamines showed X-ray protecting activity. The latter compounds, which are *N*-hydroxy derivatives, could be active because of the possible propensity to undergo spontaneous oxidation back to nitroxide. In addition it was found that upon reducing the nitroxide free radical function to amine, the protecting activity disappeared. This effect was independent of the type of side chain or other functional group.

In addition to the amino substituents, five- and six-membered nitroxide compounds (**c** type) containing a formyl function (**36c**, **37c**) were found to have protecting activity, but in their amine oxidation state (**a** type), they exhibited significant radiosensitization effects (**36a**, **37a**).

Oxidation state of the ring appears to be a critical factor in influencing the protective effects of the compounds. The fully reduced amines (compounds type **a**) predominantly exhibited sensitizing effects, whereas the nitroxides and hydroxylamines exhibited protective effects. Among the nitroxides tested, a comparison of the protective effects with the corresponding redox midpoint potentials shows that there is no significant correlation between the observed protection and the redox potentials. This indicates that even for an amine-based ring oxidation state to be an efficient sensitizer, DNA seems to be the likely target, and therefore, accumulation at DNA to sufficient concentrations confers effective radiosensitizing properties.

Conclusions

The results from the present study show that the nitroxide-based antioxidants represent a novel class of recycling antioxidants which have both catalytic as well as stoichiometric scavenging effects. A general screen of their effectiveness against two types of damage such as H_2O_2 exposure and ionizing radiation shows that while they are effective in both cases, the structural requirements are distinctly different. For protection against H_2O_2 exposure, the kinetic effects predominate, and both six-membered ring and substituted five-membered ring nitroxides were found to be effective. In the case of ionizing radiation, accumulation of the agent at the site of damage was critical. In this case, nitrox-

ides with basic side groups were most effective. With respect to toxicity, none of the agents evaluated in the present study exhibited cytotoxicity. The toxicity of only a few selected nitroxides has been evaluated in mice where sufficient concentrations of the nitroxides for antioxidant and radioprotective purposes were achieved without untoward toxicity.[41,42] The results from this screen will hopefully provide optimal candidates for more intensive evaluation as agents with respect to toxicity and for chemical modification of damaging agents such as H_2O_2 and ionizing radiation.

Experimental Section

The syntheses of the compounds are described by way of typical examples: the methods are denoted in the same way as in Schemes 1−4. The structural and molecular formulas and physical properties of the compounds are summarized in Tables 1−10.

Melting points (uncorrected) were determined on a Boetius micromelting point apparatus. Elemental analyses (C, H, N, S) were obtained on a Carlo-Erba EA 1110 apparatus or (Hal) determined titrimetrically by Schöniger's method. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode (70 eV, direct inlet) or with thermospray technique. Samples were analyzed in the bypass mode; 10 μL of the sample solution in CH_3OH was introduced via the thermospray interface. The mobile phase was CH_3OH/H_2O, 1:1, solution containing 0.1 M NH_4OAc. The capillary tip temperature was 230 °C, the electrode voltage was 180 V, and the source temperature was 210 °C. The ESR spectra were obtained from 10^{-5} M solution, using a BRUKER 300-E spectrometer. All the monoradicals exhibited three equidistant lines with a_N = 14.7−15.5 G. Flash column chromatography was performed with silica gel 60 (230−400 mesh) (Merck).

Method A. 3-[*N*-(1-Adamantyl)aminomethyl]-2,2,5,5-tetramethyl-3-pyrroline (25a). The nitroxide **25c** (3.04 g, 10 mmol) was dissolved in AcOH (10 mL); then Fe powder (2 g, 36 mmol) was added. The reaction mixture was stirred at 40−50 °C for 3 h, diluted with water (10 mL), and filtered from unreacted Fe. The filtrate was basified by adding solid K_2CO_3, then extracted with $CHCl_3$ (3 × 20 mL), dried ($MgSO_4$), and filtered again, and the solvent was evaporated. The residue was flash-chromatographed on silica gel (Merck 60) with $CHCl_3$−Et_2O and evaporated to dryness. The product was crystallized from Et_2O−hexane, to give the base **25a** (1.78 g, 62%) as off-white crystals: mp 116−118 °C; EI (*m*/*z*, %) 288 (M^+, 2), 273 (65), 135 (100), 122 (81). Anal. ($C_{19}H_{32}N_2$) C, H, N.

Preparation of hydrochloride salt: The base (0.86 g, 3 mmol) was acidified with HCl in EtOH to pH 3 and then diluted with Et_2O to start crystallization (0.92 g, 85%). The analytical sample of the salt of **25a/HCl** was recrystallized from EtOH−Et_2O (mp 226 °C dec). Anal. ($C_{19}H_{34}N_2Cl_2$) C, H, N, Cl.

Compounds **14a**, **16a**, **22a**, **23a**, **36a**, **37a**, **40a**, and **55a** were prepared following a procedure similar to method A.

Method B. 1-Oxyl-4-(*N*-phthalimid-1-yl)-2,2,6,6-tetramethylpiperidine (55c). To a solution of amine **55a** (2.86 g, 10 mmol) in methanol (10 mL) was added 30% H_2O_2 solution (5 mL, 50 mmol), and the solution was allowed to stand for 3 days. Then the reaction mixture was evaporated to one-half volume and extracted with $CHCl_3$. The organic phase was dried ($MgSO_4$) and evaporated. The product was crystallized from $CHCl_3$−hexane to give nitroxide **55c** (2.17 g, 72%) as pale-yellow crystals: mp 134−136 °C; EI (*m*/*z*, %) 301 (M^+, 2), 287 (20), 147 (85), 76 (100). Anal. ($C_{17}H_{21}N_2O_3$) C, H, N.

Method C. 1-Hydroxy-3-[*N*-(1-adamantyl)aminomethyl]-2,2,5,5-tetramethyl-3-pyrroline (25b). The paramagnetic compound **25c** (0.61 g, 2 mmol) was dissolved in EtOH saturated with HCl gas (10 mL), then refluxed until the color of nitroxide disappeared (about 30 min), and diluted with Et_2O to start crystallization. The crystalline hydrochloride **25b** that separated on refrigeration was filtered off, washed with Et_2O, and dried (0.53 g, 70%) as off-white crystals: mp 215−216 °C; TSP $[M + H]^+$ 304. Anal. ($C_{19}H_{32}N_2O$·2HCl) C, H, N, Cl.

Compounds **1b**, **2b**, **5b**, **6b**, **9b**, **11b**−**17b**, **19b**, **22b**, **23b**, **26b**, **27b**, **29b**, **36b-38b**, **40b**, **42b**, **48b**, **52b**, **53b**, and **55b** were prepared following a procedure similar to method C.

Method D. 1-Oxyl-3-formyl-2,2,5,5-tetramethyl-3-pyrroline (36c). *N*-Hydroxylamine hydrochloride salt **36b** (2.06 g, 10 mmol) was dissolved in saturated K_2CO_3 solution (15 mL) and extracted with $CHCl_3$. To the organic phase were added drying agent ($MgSO_4$) and catalytic amount of MnO_2 (50 mg), and this mixture was aerated for 30 min. Then the reaction mixture was filtered and evaporated. The crystalline product **36c** was filtered off and evaporated with hexane (1.53 g, 91%) as yellow crystals: mp 77−79 °C; EI (*m*/*z*, %) 168 (M^+, 35), 138 (41), 123 (100), 95 (88). Anal. ($C_9H_{14}NO_2$) C, H, N.

Method E. Methyl (2,2,5,5-Tetramethyl-3-pyrrolin-3-yl)carboxylate (42a). 2,2,6,6-Tetramethyl-3,5-dibromo-4-piperidone hydrobromide[13] (**59**) (39.39 g, 0.1 mol) was added during about 1 h to a stirred methanol solution of sodium methoxide (30%) (57 mL, 0.3 mol) at room temperature; then the formed NaBr was filtered off, the filtrate evaporated to dryness, and the residual pale oil distilled under reduced pressure (1.3 mmHg, 56−58 °C) to yield the pure product **42a** (15.0 g, 82%) as an oil: EI (*m*/*z*, %) 183 (M^+, 1), 168 (100), 136 (49), 108 (64). Anal. ($C_{10}H_{17}NO_2$) C, H, N.

Method F. 3-(Hydroxymethyl)-2,2,5,5-tetramethyl-3-pyrroline (14a). To a solution of ester **42a** (1.83 g, 10 mmol) in toluene (10 mL) was added SMEAH (70% solution in toluene, 6.4 mL, 23 mmol) dropwise in argon atmosphere. The reaction mixture was kept at 40 °C for 1 h and then added to a NaOH solution (20%, 20 mL). THF (10 mL) was also added, and the organic phase was separated, dried ($MgSO_4$), and evaporated. The crystalline product **14a** was filtered and washed with hexane (1.21 g, 78%) as pale-yellow crystals: mp 66−69 °C; EI (*m*/*z*, %) 155 (M^+, 1), 140 (40), 122 (18), 110 (100). Anal. ($C_9H_{17}NO$) C, H, N.

Method G. 3-Formyl-2,2,5,5-tetramethyl-3-pyrroline (36a). A stirred solution of **14a** (1.55 g, 10 mmol) in $CHCl_3$ (30 mL) was refluxed with active MnO_2 (5 g, 56 mmol) until oxidation of alcohol **14a** was completed and then filtered, the filtrate evaporated to dryness, and the residual solidified when cooled to give the aldehyde **36a** (1.36 g, 89%) as a pale-yellow oil: EI (*m*/*z*, %) 153 (M^+, 1), 138 (48), 110 (100), 95 (44). Anal. ($C_9H_{15}NO$) C, H, N.

Method H. 3-Formyl-1,2,2,5,5-pentamethyl-3-pyrroline (36d). A solution of aldehyde **36a**[25] (0.77 g, 5 mmol) and MeI (1.42 g, 10 mmol) in acetone (10 mL) was refluxed for 4 h and then evaporated to dryness. The residual solid was dissolved in water, basified with solid K_2CO_3, extracted with $CHCl_3$ (3 × 20 mL), dried ($MgSO_4$), and evaporated to dryness. The oily residue was flash-chromatographed on silica gel (Merck 60) to get the pure compound **36d** (0.71 g, 85%): EI (*m*/*z*, %) 167 (M^+, 11), 152 (41), 124 (100), 109 (42). Anal. ($C_{10}H_{17}NO$) C, H, N.

Method I. 1-Oxyl-3-[*N*-(1-adamantyl)aminomethyl]-2,2,5,5-tetramethyl-3-pyrroline (25c). A solution of aldehyde **36c**[26] (1.68 g, 10 mmol) and 1-aminoadamantane (1.66 g, 11 mmol) in toluene was refluxed in the presence of catalytic amount of *p*-toluenesulfonic acid, in a flask equipped with a Dean−Stark water separator. After the separation of the calculated amount of water (about 12 h), the mixture was concentrated under reduced pressure (water pump). The remaining Schiff base was dissolved in EtOH (20 mL) and added slowly to a stirred suspension of $NaBH_4$ (0.57 g, 15 mmol) in EtOH (30 mL) at 0 °C; then the mixture refluxed for 1 h. It was diluted with water (50 mL) and evaporated in a vacuum to about one-half volume, and the oily aqueous residue was extracted with $CHCl_3$ (3 × 50 mL). The extract was dried ($MgSO_4$), filtered, and concentrated. The crude base **25c** was recrystallized from a mixture of Et_2O and hexanes (2.18 g, 72%) as yellow crystals: mp 109−111 °C; EI (*m*/*z*, %) 303 (M^+, 10), 273 (34), 164 (48), 135 (100). Anal. ($C_{19}H_{31}N_2O$) C, H, N.

The base was converted into its tosylate salt. To the base in acetone was added an equivalent amount of *p*-toluene-

sulfonic acid in acetone (10 mL), and the mixture was cooled. The precipitated salt **25c/Ts** was filtered off, washed with Et_2O, and dried to give **25c/Ts (**3.76 g, 79%) as yellow crystals: mp 250−251 °C. Anal. ($C_{19}H_{31}N_2O{\cdot}C_7H_8O_3S$) C, H, N, S.

Compounds **18c**−**20c**, and **28c** were prepared following a procedure similar to method I.

Method J. 1-Oxyl-3-[*N*-methyl-*N*-(1-adamantyl)aminomethyl]-2,2,5,5-tetramethyl-3-pyrroline (26c). To a stirred solution of **25c** (608 mg, 2 mmol) in dry tetrahydrofuran (THF) (20 mL) in argon atmosphere was added NaH (60 mg, 2.5 mmol), and the mixture refluxed for 1 h. After MeI (284 mg, 2.0 mmol) was added to the reaction mixture and refluxed for 6 h, the cooled reaction mixture was washed with saturated NaCl solution (10 mL), and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed (hexanes−Et_2O) to separate the nonmethylated starting compound. The pure compound **26c** was recrystallized from $CHCl_3$−hexanes (394 mg, 62%) as yellow crystals: mp 110−112 °C; EI (*m*/*z*, %) 317 (M^+, 2), 287 (5), 165 (55), 135 (100). Anal. ($C_{20}H_{33}N_2O$) C, H, N.

Compound **29c** was prepared following a procedure similar to method J.

Method K. 1-Oxyl-4-(*N*,*N*-*n*-dibutylamino)-2,2,6,6-tetramethylpiperidine (10c). To solution of ketone **39c** (TEMPONE)[44] (1.70 g, 10 mmol) and di-*n*-butylamine hydrochloride (6.63 g, 40 mmol) in dry methanol (100 mL) was added $NaCNBH_3$ (3.77 g, 60 mmol), and the reaction mixture was refluxed for 48 h and finally evaporated to dryness. The residue was taken up in water (30 mL), acidified by adding diluted H_2SO_4 (5%), and extracted with $CHCl_3$ (3 × 20 mL) to remove the unreacted ketone. The aqueous phase was basified by adding solid K_2CO_3, extracted again with $CHCl_3$ (3 × 20 mL), then dried, evaporated, and purified by flash chromatography to give the product **10c** (0.99 g, 35%) as a deep-red thick oil: TSP 284 $(M + H)^+$. Anal. ($C_{17}H_{35}N_2O$) C, H, N.

Method L. 1-Oxyl-3-(ethoxymethyl)-2,2,5,5-tetramethyl-3-pyrroline (15c). To a suspension of NaH (0.24 g, 10 mmol) in dry THF (15 mL) was added alcohol **14c**[26] (1.70 g, 10 mmol) dropwise in dry THF (10 mL), and the mixture was stirred at room temperature for 1 h. After that EtBr (1.09 g, 10 mmol) in dry DMF (10 mL) was added dropwise, and the reaction mixture was refluxed for 2 h. Then the cooled reaction mixture was extracted with brine (20 mL), and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was flash-chromatographed (hexanes−Et_2O) to give the pure product **15c** (1.13 g, 57%) as a yellow oil: EI (*m*/*z*, %) 198 (M^+, 10), 138 (9), 122 (26), 107 (100). Anal. ($C_{11}H_{20}NO_2$) C, H, N.

Method M. 1-Oxyl-3-(*N*-pyrrolidinylmethyl)-2,2,5,5-tetramethyl-3-pyrroline (22c). A solution of 1-oxyl-3-(bromomethyl)-2,2,5,5-tetramethyl-3-pyrroline[31] (**60**) (2.33 g, 10 mmol) and pyrrolidine (2.13 g, 30 mmol) in $CHCl_3$ (30 mL) was refluxed for 3 h and then evaporated, and the residue was diluted with water (20 mL) and extracted with Et_2O (3 × 20 mL). The extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography (hexanes−Et_2O) to give the base **22c** (1.74 g, 78%) as a red oil. Anal. ($C_{13}H_{23}N_2O$) C, H, N.

The pure base **22c** was converted to its tosylate salt (3.08 g, quantitatively) in the same way as described in method I: yellow crystals; mp 129−132 °C; TSP 224 $[M + H]^+$. Anal. ($C_{13}H_{23}N_2O{\cdot}C_7H_8O_3S$) C, H, N, S.

Compounds **17c** and **21c** were prepared following a procedure similar to method M.

Method N. 1-Oxyl-3-(tributylammoniumylmethyl)-2,2,5,5-tetramethyl-3-pyrroline Bromide (31d). A solution of 1-oxyl-3-(bromomethyl)-2,2,5,5-tetramethyl-3-pyrroline[31] (**60**) (2.33 g, 10 mmol) and the excess of *n*-tributylamine (3.71 g, 20 mmol) was heated in acetone (10 mL) for 6 days and then diluted with Et_2O to start crystallization. The solid residue was recrystallized from acetone−Et_2O to give the pure compound **31d** (2.64 g, 63%) as yellow crystals: mp 180−181 °C; EI (*m*/*z*, %) 338 (M^+, 1), 281 (2), 202 (8), 142 (100). Anal. ($C_{21}H_{42}BrN_2O$) C, H, N, Br.

Compound **30d** was prepared following a procedure similar to method N.

Method O. 1-Oxyl-3-(triphenylphosphoniumylmethyl)-2,2,5,5-tetramethyl-3-pyrroline Bromide (32e). A solution of 1-oxyl-3-(bromomethyl)-2,2,5,5-tetramethyl-3-pyrroline[31] (**60**) (2.33 g, 10 mmol) and triphenylphosphine (2.62 g, 10 mmol) was heated in acetone (10 mL) for 3 h and then diluted with Et_2O to start crystallization. The solid residue was recrystallized from $CHCl_3$−Et_2O to give the pure compound **32e** (3.51 g, 71%) as pale-brown crystals: mp 224−226 °C; TSP 416 $(M + H)^+$. Anal. ($C_{27}H_{30}BrNOP$) C, H, N, Br.

Method P. 1-Oxyl-*N*-(2-isoindolinylmethyl)-2,2,5,5-tetramethyl-3-pyrroline (24c). A solution of 1-oxyl-3-(aminomethyl)-2,2,5,5-tetramethyl-3-pyrroline[33] (**61**) (1.69 g, 10 mmol), and *o*-dibromoxilol (2.64 g, 10 mmol), and K_2CO_3 (2.76 g, 20 mmol) in $CHCl_3$ (50 mL) was stirred and refluxed for 3 h and then evaporated, and the residue was diluted with water (20 mL) and extracted with Et_2O (3 × 20 mL). The extract was dried ($MgSO_4$), filtered, and concentrated. The residue was purified by chromatography ($CHCl_3$−Et_2O) to give the base **24c** (1.85 g, 68%) as pale-yellow crystals: mp 89−90 °C; TSP 272 $(M + H)^+$. Anal. ($C_{17}H_{23}N_2O$) C, H, N.

Method Q. 1-Oxyl-3-(guanidinomethyl)-2,2,5,5-tetramethyl-3-pyrroline Hydrogen Sulfate Salt (33c). A suspension of 1-oxyl-3-(aminomethyl)-2,2,5,5-tetramethyl-3-pyrroline[33] (**61**) (340 mg, 2 mmol) and *S*-methylisothiouronium sulfate (278 mg, 1 mmol) in EtOH (10 mL) was refluxed for 3 h, then the hot reaction mixture was filtered, and the filtrate was diluted with Et_2O to precipitate off-white crystalline product **33c** (287 mg, 55%) as yellow crystals: mp >180 °C dec; TSP 212 $(M + H)^+$. Anal. ($C_{10}H_{19}N_4O{\cdot}{}^1/_2H_2SO_4$) C, H, N, S.

Method R. 1-Oxyl-4-(methylsulfonylamino)-2,2,6,6-tetramethylpiperidine (13c). To a stirred solution of amino compound **9c**[28] (342 mg, 2 mmol) and triethylamine (TEA) (303 mg, 3 mmol) in dry CH_2Cl_2 (20 mL) was added methanesulfonyl chloride (252 mg, 2.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h, then washed with saturated aqueous NaCl solution (10 mL), dried ($MgSO_4$), and evaporated to dryness. The solid residue was recrystallized from $CHCl_3$−hexane to give the pure orange compound **13c** (409 mg, 82%) as yellow crystals: mp 141−143 °C; EI (*m*/*z*, %) 249 (M^+, 3), 235 (23), 124 (61), 84 (100). Anal. ($C_{10}H_{21}N_2O_3S$) C, H, N, S.

Method S. 1-Oxyl-3-ureido-2,2,5,5-tetramethyl-3-pyrroline (43c). To a cyclohexane solution of the freshly prepared isocyanate **62**[33] (0.91 g, 5 mmol) was introduced ammonia gas. The precipitated solid urea **43c** was filtered off and recrystallized from $CHCl_3$−Et_2O to give a pure compound (0.69 g, 70%) as yellow crystals: mp 189−190 °C; EI (*m*/*z*, %) 198 (M^+, 7), 168 (41), 110 (58), 41 (100). Anal. ($C_9H_{16}N_3O_2$) C, H, N.

Method T1. 1-Oxyl-*N*-(3-hydroxypropyl)-3-carboxamido-2,2,5,5-tetramethyl-3-pyrroline (46c). To a solution of 1-oxyl-3-[(ethoxycarbonyl)oxycarbonyl]-2,2,5,5-tetramethyl-3-pyrroline[34] (**63**) (2.56 g, 10 mmol) in dry $CHCl_3$ (20 mL) was added 3-aminopropanole (0.75 g, 10 mmol), and the mixture refluxed for 30 min. Then evaporation to dryness and recrystallization from Et_2O−hexane gave **46c** (2.03 g, 84%) as yellow crystals: mp 95−96 °C; EI (*m*/*z*, %) 241 (M^+, 2), 211 (32), 136 (32), 110 (100). Anal. ($C_{12}H_{21}N_2O_3$) C, H, N.

Compounds **45c**, **48c**, and **49c** were prepared following a procedure similar to method T1.

Method T2. 1-Oxyl-*N*-(3-aminopropyl)-3-carboxamido-2,2,5,5-tetramethyl-3-pyrroline (48c). To a stirred solution of 1,3-diaminopropane (2.22 g, 30 mmol) in dry THF (50 mL) was added 1-oxyl-3-(*N*-imidazolylcarbonyl)-2,2,5,5-tetramethyl-3-pyrroline[26] (**64**) (2.56 g, 10 mmol) at 0 °C. Then the reaction mixture was warmed to room temperature, 1 h later evaporated, and flash-chromatographed with $CHCl_3$−Et_2O. The first band was the biradical (0.43 g; mp 177−178 °C), and the second yellow band was the monoradical **48c** (1.32 g, 55%) as yellow crystals: mp 104−108 °C; EI (*m*/*z*, %) 240 (M^+, 8), 226 (18), 136 (65), 41 (100). Anal. ($C_{12}H_{22}N_3O_2$) C, H, N.

Method U. 1-Oxyl-3-[*N*-(3-aminopropyl)-3-carboxamido]-2,2,5,5-tetramethylpyrrolidine (66). To a stirred solution of 1,3-diaminopropane (2.22 g, 30 mmol) in water−THF (2:1, 20 mL) was added 1-oxyl-3-bromo-4-oxo-2,2,6,6-tetramethylpiperidine[35] (**65**) (2.49 g, 10 mmol) in portions. The solution was extracted after stirring for 2 h with $CHCl_3$. The organic phase was dried, evaporated, and purified by flash chromatography to give the product **66** (1.50 g, 62%) as yellow crystals: mp 102−105 °C; EI (*m*/*z*, %) 242 (M^+, 18), 212 (11), 156 (54), 41 (100). Anal. ($C_{12}H_{24}N_3O_2$) C, H, N.

Method V. *N*-[(1-Oxyl-2,2,5,5-tetramethylpyrrolidine-3-carboxamido)propyl]phthalimide (52c). A mixture of equivalent amounts of 1-oxyl-3-[*N*-(3-aminopropyl)-3-carboxamido]-2,2,5,5-tetramethylpyrrolidine[19] (**66**) (2.42 g, 10 mmol) and phthalimide (1.47 g, 10 mmol) was heated at 120 °C without any solvent until the evolution of ammonia had ceased (about 60 min). After cooling the melt was dissolved in $CHCl_3$ (20 mL) and flash-chromatographed on silica gel (eluted with $CHCl_3$−Et_2O). The pure product was crystallized from $CHCl_3$−hexane to give **52c** (2.15 g, 58%) as yellow crystals: mp 118−120 °C; EI (*m*/*z*, %) 372 (M^+, 15), 358 (9), 342 (89), 41 (100). Anal. ($C_{20}H_{26}N_3O_4$) C, H, N.

Method W. 1-Oxyl-3-(*N*-phthalimid-1-ylmethyl)-2,2,5,5-tetramethyl-3-pyrroline (53c). A vigorously stirred solution of phthalimide (1.47 g, 10 mmol), 1-oxyl-3-(bromomethyl)-2,2,5,5-tetramethyl-3-pyrroline[31] (**60**) (2.33 g, 10 mmol), powdered K_2CO_3 (2 g), powdered KOH (100 mg), and 18-crown-6 (300 mg) in dioxane (30 mL) was refluxed for 4 h and filtered, and the filtrate was evaporated to dryness. Residue was flash-chromatographed with $CHCl_3$−Et_2O and then recrystallized from $CHCl_3$−hexane to give **53c** (2.18 g, 73%) as yellow crystals: mp 127−129 °C; EI (*m*/*z*, %) 299 (M^+, 13), 285 (27), 269 (100), 213 (42). Anal. ($C_{17}H_{19}N_2O_3$) C, H, N.

Method Z. 1-Oxyl-4-(*N*-phthalimid-1-yl)-2,2,6,6-tetramethylpiperidine (55c). A mixture of equivalent amounts of 1-oxyl-4-amino-2,2,6,6-tetramethylpiperidine[44] (**9c**) (1.71 g, 10 mmol) and phthalimide (1.47 g, 10 mmol) was heated at 120 °C without any solvent until the evolution of ammonia had ceased (about 50 min). After cooling the melt was dissolved in $CHCl_3$ (20 mL) and flash-chromatographed on silica gel (eluted with $CHCl_3$−Et_2O). The pure product was crystallized from $CHCl_3$−hexane to give **55c** (2.01 g, 67%) as yellow crystals: mp 134−136 °C; EI (*m*/*z*, %) 301 (M^+, 2), 287 (20), 147 (85), 76 (100). Anal. ($C_{17}H_{21}N_2O_3$) C, H, N.

Electrochemistry. Cyclic voltammetry was performed on the nitroxides in phosphate-buffered saline using a glassy carbon working electrode and a platinum auxiliary electrode. All other conditions are similar to that reported.[6]

Cell Culture. Chinese hamster V79 cells were grown in F12 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Survival was assessed in all studies by the clonogenic assay. The plating efficiency ranged between 80% and 90%. Stock cultures of exponentially growing cells were trypsinized, rinsed, and plated (5×10^5 cells/dish) into a number of 100-mm Petri dishes and incubated for 16 h at 37 °C prior to experimental protocols. Nitroxides were added to exponentially growing cells in complete F12 medium (final concentration, 100 μM) at room temperature immediately prior to treatment with H_2O_2 (final concentration, 500 μM) for 1 h. For radiation studies, nitroxides were added to exponentially growing cells in complete F12 medium (final concentration, 10 mM) at room temperature 10 min prior to a single radiation dose of 12 Gy. Plates were irradiated at room temperature with a cobalt-60 irradiator at a dose rate of 5.0 Gy/min. Full electron equilibrium was ensured for all irradiations. The time required for irradiation (at room temperature) was approximately 10 min. Immediately after treatment, cells were rinsed, trypsinized, counted, and plated for macroscopic colony formation. Using these conditions, none of the nitroxides exerted cytotoxicity alone. Each nitroxide/H_2O_2 or nitroxide/radiation determination was plated in triplicate, and experiments were repeated a minimum of two times. Plates were incubated for 7 days; colonies were then fixed with methanol/acetic acid (3:1) and stained with crystal violet. Colonies containing >50 cells were scored.

The results for H_2O_2 treatment were formulated to express a protection factor (PF, derived by dividing the surviving fraction of nitroxide plus H_2O_2 treatment by the surviving fraction of H_2O_2 treatment alone). A PF > 1.0 means that the agent provided protection against H_2O_2 cytotoxicity. PF values were also derived for radiation studies (PF, derived by dividing the surviving fraction at 12 Gy of nitroxide treatment by the surviving fraction of 12 Gy of treatment alone). PFs > 1.0 mean that the agent provided radiation protection, and values < 1.0 mean that radiation sensitization occurred. The PFs from individual experiments were pooled to find the mean PF ± standard deviation. Each nitroxide was then compared to the untreated irradiated control (PF = 1.0) using a two-tailed paired Students *t*-test to determine statistical significance.[45]

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Supporting Information Available: Graphical presentation of protection factors against H_2O_2- and radiation-induced cytotoxicity (Figures 2 and 4) (2 pages). Ordering information is given on any current masthead page.

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