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Published on Web 06/03/2004

Oxidation of 2-Deoxyribose by Benzotriazinyl Radicals of Antitumor 3-Amino-1,2,4-benzotriazine 1,4-Dioxides

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Abstract: Tirapazamine (3-amino-1,2,4-benzotriazine 1,4-dioxide) is the lead bioreductive drug in clinical trials as an anticancer agent to kill refractory hypoxic cells of solid tumors. It has long been known that, upon metabolic one-electron reduction, tirapazamine induces lethal DNA double strand breaks in hypoxic cells. These strand breaks arise from radical damage to the ribose moiety of DNA, and in this pulse radiolysis and product analysis study we examine mechanistic aspects of the dual function of tirapazamine and analogues in producing radicals of sufficient power to oxidize 2-deoxyribose to form radicals, as well as the ability of the compounds to oxidize the resulting deoxyribose radicals to generate the strand breaks. Both the rate of oxidation of 2-deoxyribose and the radical yield increase with the one-electron reduction potentials of the putative benzotriazinyl radicals formed from the benzotriazine 1,4-dioxides. Subsequent oxidation of the 2-deoxyribose radicals by the benzotriazine 1,4-dioxides and 1-oxides proceeds through adduct formation followed by breakdown to form the radical anions of both species. The yield of the radical anions increases with increasing one-electron reduction potentials of the compounds. We have previously presented evidence that oxidizing benzotriazinyl radicals are formed following one-electron reduction of the benzotriazine 1,4-dioxides. The reactions reported in this work represent the kinetic basis of a short chain reaction leading to increased oxidation of 2-deoxyribose, a process which is dependent on the one-electron reduction potential of the benzotriazinyl radicals that are above a threshold value of ca. 1.24 V.

Introduction

Tirapazamine, 3-amino-1,2,4-benzotriazine 1,4-dioxide, **1**, (TPZ), is a bioreductive drug, currently in phase II/III clinical trials in combination with radiotherapy and also with cisplatin-based chemotherapy.[1−3] TPZ is selectively toxic to hypoxic cells,[4,5] and consequently it is a useful adjunct to radiotherapy and chemotherapy, which often fail to eliminate hypoxic cells within tumors.[6,7] TPZ is reduced by one-electron reductases[8−12] to form a radical which is a precursor to the induction of lethal double strand breaks in cellular DNA.[13] It has been proposed from work with radical scavengers[14] that the •OH radical, eliminated in a unimolecular reaction from one-electron reduced TPZ (**2**/**3**), is the cytotoxic species (Scheme 1), although spin-trap EPR experiments are inconclusive.[15] We have recently presented spectral and kinetic evidence that a unimolecular reaction occurs to eliminate water and form a benzotriazinyl radical, **5**.[16] This unimolecular reaction proceeds with a rate constant of $112 \pm 24\ s^{-1}$ at pH 7 for TPZ and is accelerated in analogues with electron-donating substituents on the A-ring. Spectral studies with benzotriazine 1,4-dioxide analogues (A) have shown that the same radical (B•) is formed on the one-electron oxidation of their equivalent benzotriazine 1-oxides (B), **4** (Scheme 1).[16]

The abstraction of a H-atom from the deoxyribose backbone of DNA is thought to be a prerequisite for the formation of lethal strand breaks.[17] Such a reaction by the •OH radical is well-established, and we have shown that the benzotriazinyl

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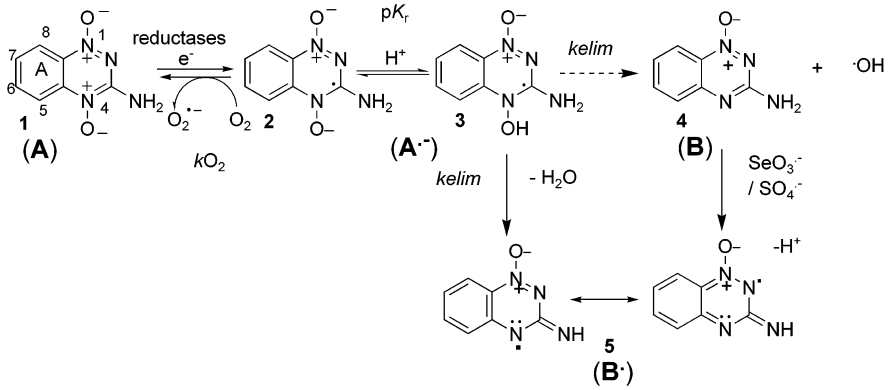
(1) Lee, D. J.; Trotti, A.; Spencer, S.; Rostock, R.; Fisher, C.; von Roemeling, R.; Harvey, E.; Groves, E. *Int. J. Radiat. Oncol., Biol., Phys.* **1998**, *42*, 811−815.
(2) Del Rowe, J.; Scott, C.; Werner-Wasik, M.; Bahary, J. P.; Curran, W. J.; Urtasun, R. C.; Fisher, B. *J. Clin. Oncol.* **2000**, *18*, 1254−1259.
(3) von Pawel, J.; von Roemeling, R.; Gatzemeier, U.; Boyer, M.; Elisson, L. O.; Clark, P. T. D.; Rey, A.; Butler, T. W.; Hirsh, V.; Olver, I.; Bergman, B.; Ayoub, J.; Richardson, G.; Dunlop, D.; Arcenas, A.; Vescio, R.; Viallet, J.; Treat, J. *J. Clin. Oncol.* **2000**, *18*, 1351−1359.
(4) Brown, J. M. *Brit. J. Cancer* **1993**, *67*, 1163−1170.
(5) Denny, W. A.; Wilson, W. R. *Expert Opin. Invest. Drugs* **2000**, *9*, 2889−2901.
(6) Nordsmark, M.; Overgaard, M.; Overgaard, J. *Radiother. Oncol.* **1996**, *41*, 31−40.
(7) Brizel, D. M.; Sibley, G. S.; Prosnitz, L. R.; Scher, R. L.; Dewhirst, M. W. *Int. J. Radiat. Oncol., Biol., Phys.* **1997**, *38*, 285−289.
(8) Walton, M. I.; Workman, P. *Biochem. Pharmacol.* **1990**, *39*, 1735−1742.
(9) Walton, M. I.; Wolf, C. R.; Workman, P. *Biochem. Pharmacol.* **1992**, *44*, 251−259.
(10) Fitzsimmons, S. A.; Lewis, A. D.; Riley, R. J.; Workman, P. *Carcinogenesis* **1994**, *15*, 1503−1510.
(11) Patterson, A. V.; Barham, H. M.; Chinje, E. C.; Adams, G. E.; Harris, A. L.; Stratford, I. J. *Brit. J. Cancer* **1995**, *72*, 1144−1150.
(12) Patterson, A. V.; Saunders, M. P.; Chinje, E. C.; Patterson, L. H.; Stratford, I. J. *Anti-Cancer Drug Des.* **1998**, *13*, 541−573.
(13) Biedermann, K. A.; Wang, J.; Graham, R. P.; Brown, J. M. *Br. J. Cancer* **1991**, *63*, 358−362.
(14) Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1996**, *118*, 3380−3385.
(15) Patterson, L. H.; Taiwo, F. A. *Biochem. Pharmacol.* **2000**, *60*, 1933−1935.
(16) Anderson, R. F.; Shinde, S. S.; Hay, M. P.; Gamage, S. A.; Denny, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 748−756.
(17) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis Ltd.: London, 1987.

Scheme 1. Formation of Radical Species from 1,2,4-Benzotriazine 1,4-Dioxides



radical **5** also abstracts an H-atom from the model compound 2-deoxyribose (dR) with a rate constant of $3.7 \pm 0.5 \times 10^6$ M^{-1} s^{-1} (eq 1).[16]

$$B^{\bullet} + dR \rightarrow B + dR^{\bullet} \quad (1)$$

Following the formation of similar sugar radicals on DNA, the key event in the generation of strand breaks is mediated by the formation (eq 2) and breakdown (eq 3) of an adduct formed between a sugar radical and a further benzotriazine 1,4-dioxide molecule.[18−20] This breakdown produces the one-electron reduced benzotriazine 1,4-dioxide ($\mathbf{A}^{\bullet-}$) and a labile sugar cation.

$$A + dR^{\bullet} \rightarrow [dR\text{-}A]^{\bullet} \quad (2)$$

$$[dR\text{-}A]^{\bullet} \rightarrow A^{\bullet-} + dR^{+} (\rightarrow \rightarrow \text{products} + H^{+}) \quad (3)$$

In this study, we investigate the redox properties of different benzotriazinyl radicals ($B^{\bullet}$) and report their reactivity with dR together with the reactions between deoxyribose radicals and both the benzotriazine 1,4-dioxide (A) and 1-oxide analogues (B).

Results

One-Electron Reduction Potentials of Benzotriazine 1,4-Dioxides (A), 1-Oxides (B), and Benzotriazinyl Radicals ($B^{\bullet}$). Redox equilibria were established (within a few tens of microseconds) between the one-electron reduced forms of compounds A and B and appropriate reference compounds, from which the one-electron reduction potentials, $E(A/A^{\bullet-})$ and $E(B/B^{\bullet-})$, were calculated, and the values are presented in Table 1. The one-electron reduction potentials of the benzotriazinyl radicals, $E(B^{\bullet}, H^{+}/B)$ produced upon the one-electron oxidation of the benzotriazine 1-oxides by the $SO_4^{\bullet-}$ radical, were similarly measured, and their values are presented in Table 1. Substitution of the 3-NH_2 group with the charged, solubilizing, 3-NHR group raises the potential of both $E(A/A^{\bullet-})$ and $E(B/B^{\bullet-})$ by ca. 30−60 mV but lowers the potential of $E(B^{\bullet}, H^{+}/B)$ by similar amounts. While the $E(A/A^{\bullet-})$ values of 6-substituted analogues of **1** exhibit a strong dependency on Hammett σ_p ($P < 0.0001$),[21] this is not the case for the 6-substituted analogues of **4** where the $E(B/B^{\bullet-})$ data correlate well with σ_m ($P = 0.0002$) and not σ_p ($P = 0.0062$). Regression analysis on the data for the six 6-substituted analogues of **1** and **4** gave the following equations:

$$E(A/A^{\bullet-})/\text{mV} = -460 \pm 7 + (260 \pm 14)\sigma_p$$

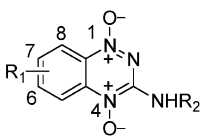
$$E(B/B^{\bullet-})/\text{mV} = -577 \pm 8 + (332 \pm 27)\sigma_m$$

This difference in the dependency of the one-electron reduction potentials on σ_p or σ_m indicates that the positions of the radical centers on the $A^{\bullet-}$ and $B^{\bullet-}$ species are different. Whereas the $A^{\bullet-}$ radical species may have its electron density spread over the 1-oxide and 4-oxide positions (with possible involvement of the adjacent C3 position), it seems likely for $B^{\bullet-}$ that the radical center is on the 1-oxide position. There is no simple dependency of the $E(B^{\bullet}, H^{+}/B)$ values on substituent constants. These values were measured by observing the kinetic equilibrium between the radicals and oxidizable reference compounds, but it is commonly observed that ρ has negative values for radical reaction rates when substituents are of positive σ or σ^{+}.[22]

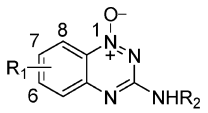
Oxidation of 2-Deoxyribose (dR) by Benzotriazinyl Radicals ($B^{\bullet}$). The reaction between the benzotriazinyl radicals ($B^{\bullet}$) and dR was studied for compounds of sufficient aqueous solubility by following the optical decay kinetics of the benzotriazinyl radicals in the presence of dR (0.1−4.0 mM). The benzotriazinyl radicals were produced quickly (<5 μs) by the one-electron oxidation of analogues of benzotriazine 1-oxides (0.5−1 mM) by the $SO_4^{\bullet-}$ radical, or, in the case of compound **16**, by the $SeO_3^{\bullet-}$ radical, as this compound reacts thermally with peroxodisulfate. Under these conditions there is nearly quantitative formation of the $B^{\bullet}$ as the $SO_4^{\bullet-}$ radical reacts with dR one order of magnitude slower in rate at (3.8×10^7 M^{-1} s^{-1})[23] than with the benzotriazine 1-oxides. The data were treated as previously described,[16] where plots of $1/t_{1/2}$ against initial radical concentration for a series of radiation doses give straight lines with intercepts dependent on the dR concentration. The first-order rate constants, determined from the intercepts of these plots, when plotted against the dR concentrations yield the second-order rate constants of the $B^{\bullet}$ with dR. These second-order rate constants are listed in Table 1 as $k(1)$ values. The

(18) Jones, G. D. D.; Weinfeld, M. *Cancer Res.* **1996**, *56*, 1584−1590.
(19) Daniels, J. S.; Gates, K. S.; Tronche, C.; Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1254−1257.
(20) Hwang, J.-T.; Greenberg, M. M.; Fuchs, T.; Gates, K. S. *Biochemistry* **1999**, *38*, 14248−14255.
(21) Hay, M. P.; Gamage, S. A.; Kovacs, M.; Pruijn, F. B.; Anderson, R. F.; Patterson, A. V.; Wilson, W. R.; Brown, J. M.; Denny, W. A. *J. Med. Chem.* **2003**, *46*, 169−182.
(22) Russell, G. A. In *Free Radicals*; Koch, J. K., Ed.; John Wiley & Sons: New York, 1973; Vol. 1, pp 275−331.
(23) O'Neill, P.; Davies, S. E. *Int. J. Radiat. Biol.* **1987**, *52*, 577−587.

Table 1. Compound Structures, One-Electron Reduction Potentials, and Kinetic Parameters



A (benzotriazine 1,4-dioxide)



B (benzotriazine 1-oxide)

No.	Class	R_1	R_2[a]	$E(A/A^{\bullet-}) \pm 0.01$/V	$10^{-9}\,k(2)/M^{-1}\,s^{-1}$	$10^{-4}\,k(3)/s^{-1}$
1	A	H	H	−0.456[b,c]	2.45 ± 0.13	6.7 ± 0.5
6	A	H	R′	−0.396	1.60 ± 0.10	4.46 ± 0.12
7	A	6-$N(CH_3)_2$	H	−0.668[c]	3.03 ± 0.18	6.92 ± 0.53
8	A	7-$N(CH_3)_2$	H	−0.535[c]	3.38 ± 0.83	7.98 ± 0.45
9	A	8-CH_3	H	−0.510[c]	2.21 ± 0.14	4.94 ± 0.06
10	A	6-OMe	H	−0.554[c]	2.90 ± 0.19	4.70 ± 0.10
11	A	6-OMe	R′	−0.500	1.27 ± 0.05	5.16 ± 0.23
12	A	6-CH_3	H	−0.493[c]	3.02 ± 0.13	6.16 ± 0.35
13	A	6-CH_3	R′	−0.440	1.69 ± 0.08	4.86 ± 0.11
14	A	6-CF_3	H	−0.335[c]	1.78 ± 0.05	6.94 ± 0.13
15	A	6-CF_3	R′	−0.263	1.57 ± 0.11	3.64 ± 0.17
16	A	6-SO_2CH_3	H	−0.258[c]	1.90 ± 0.05	5.84 ± 0.16

No.	Class	R_1	R^{2a}	$E(B/B^{\bullet-}) \pm 0.01$/V	$E(B^{\bullet}, H^+/B) \pm 0.01$/V	$10^{-6}\,k(1)/M^{-1}\,s^{-1}$	$10^{-9}\,k(2)/M^{-1}\,s^{-1}$	$10^{-4}\,k(3)/s^{-1}$
4	B	H	H	−0.568	1.32[d]	3.74 ± 0.5[d]	1.60 ± 0.06	7.47 ± 0.23
17	B	H	R′	−0.502	1.27	1.34 ± 0.15	1.59 ± 0.03	5.43 ± 0.05
18	B	6-$N(CH_3)_2$	H	−0.647	1.16	0.42 ± 0.03	ND[e]	ND[e]
19	B	7-$N(CH_3)_2$	H	−0.567	0.94[d]	<0.1	ND[e]	ND[e]
20	B	8-CH_3	H	−0.581	1.30	ND[e]	ND[e]	ND[e]
21	B	6-OMe	H	−0.533	1.30[d]	ND[e]	ND[e]	ND[e]
22	B	6-OMe	R′	−0.562	1.28	1.58 ± 0.20	1.14 ± 0.03	5.78 ± 0.09
23	B	6-CH_3	H	−0.586	1.30	3.10 ± 0.23	ND[e]	ND[e]
24	B	6-CH_3	R′	−0.527	1.28	1.75 ± 0.06	1.29 ± 0.07	4.87 ± 0.19
25	B	6-CF_3	H	−0.455	1.27	ND[e]	ND[e]	ND[e]
26	B	6-CF_3	R′	−0.424	1.24	1.04 ± 0.09	2.76 ± 0.13	7.52 ± 0.14
27	B	6-SO_2CH_3	H	−0.368	1.28	ND[e]	ND[e]	ND[e]

[a] R′ = $CH_2CH_2N^+H(CH_3)_2$. [b] ref 24. [c] ref 21. [d] ref 16. [e] ND = not determined due to insufficient solubility.

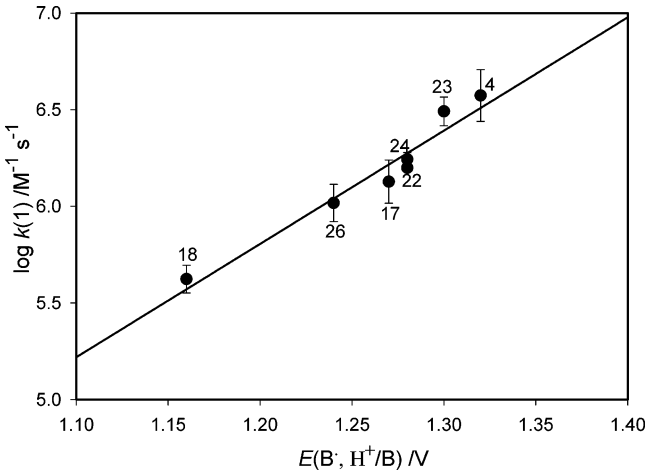


Figure 1. Dependence of the rate constants for the oxidation of 2-deoxyribose, dR, by benzotriazinyl radicals on $E(B^{\bullet}, H^+/B)$.

magnitude of the $k(1)$ values increase with more positive $E(B^{\bullet}, H^+/B)$ values (Figure 1). Regression analysis of the data gave the following equation:

$$\log k(1)/M^{-1}\,s^{-1} = (5.86 \pm 0.62)E(B^{\bullet}, H^+/B) - (1.23 \pm 0.79) \quad n = 7$$

One-Electron Reduction of 1,2,4-Benzotriazines (A and B) by Deoxyribose Radicals. Deoxyribose radicals, dR•, were found to react with all the benzotriazine 1,4-dioxide and 1-oxide compounds of this study. The reactions were studied in N_2O-saturated solutions containing excess dR (20 mM) to quantitatively scavenge the •OH radicals, and the rate constants for a series of concentrations of each compound (10−300 μM) with dR• were determined by monitoring the formation of transient absorption spectra. Two distinct kinetic phases were observed for each compound: an initial phase that is dependent on substrate concentration and a second phase that is independent of substrate concentration. Representative spectral and kinetic data for compounds **1** and **4** are presented in Figures 2 and 3. The absorption spectra present at the end of each phase were determined in solutions containing ≥150 μM of the compounds to kinetically separate the spectra. The time-resolved spectra have broadly similar characteristics with small differences in absorption at a limited number of wavelengths (Figure 2). The observed rate constant data at low concentrations of the compounds were used to determine second-order rate constants, $k(2)$, of the first phase (Figure 3), while the second phase proceeds at rate constants that are independent of concentration and equal to the observed plateau in rate constant, $k(3)$. These data are presented in Table 1 and are consistent with the formation of a transient adduct between a dR• radical and each compound, followed by the breakdown of the adduct to form a new species. The absorption spectra at the end of the second phase is the same as that produced upon the one-electron reduction of **1** by the $CO_2^{\bullet-}$ species[24] and the reduction of **4**

(24) Laderoute, K.; Wardman, P.; Rauth, A. M. *Biochem. Pharmacol.* **1988**, *37*, 1487−1495.

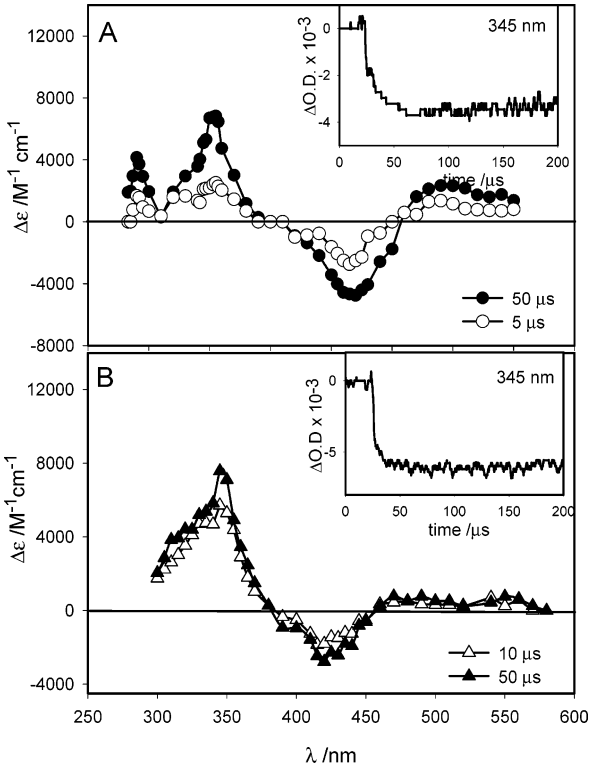


Figure 2. Time-resolved spectra following reaction of dR• radicals with **1** (150 μM) (A) and **4** (300 μM) (B). The N_2O-saturated solutions contained substrate and 2-deoxyribose (20 mM) at pH 7.0 (5 mM phosphate). Inserts in panel A and B display the oscilloscope trace for solution containing 75 μM of **1** and 300 μM of **4**, respectively.

(Figure 4), and hence they can be assigned as the radical anions $A^{\bullet-}$ and $B^{\bullet-}$. The pK_a of the radical anion of **4** was determined from its changes in absorbance with pH (Figure 4, insert) to be 9.73 ± 0.10, which differs considerably from that measured (6.19 ± 0.10[16,24]) for the radical anion of **1**. The apparent yield of overall electron transfer from the dR• radicals, formed in N_2O-saturated solution, to each of the compounds was determined at concentrations corresponding to the plateau region in Figure 3 ($\geq$150 μM) by comparing each yield to that formed by electron transfer from the $CO_2^{\bullet-}$ species (0.68 μM·Gy^{-1}) to methyl viologen radicals absorbing at 600 nm. The yield of electron transfer from dR• radicals to the compounds is dependent on E(A/A$^{\bullet-}$) or E(B/B$^{\bullet-}$) (Figure 5), with:

$$G\ /\mu\text{M}\cdot\text{Gy}^{-1} = (0.65 \pm 0.09)E(\text{A/A}^{\bullet-})E(\text{B/B}^{\bullet-}) + (0.62 \pm 0.04) \quad n = 9$$

However, these spectral studies alone cannot provide certainty as to the exact amount of electron transfer that has taken place since the absorption spectra of the adduct intermediates and radical anions are similar.

Conductivity Studies. Further information on the degree of electron transfer from dR• radicals was sought using conductivity detection. The pK values of the radical species of one-electron reduced 1,4-dioxide benzotriazines are known to be <7,[16,24] and hence the counterion to the radical anions should be detectable in basic solution as the loss of OH^- conductance due to neutralization. Little overall conductance change was observed for the reaction between dR• and **1** at pH 4.5 as the radical anion, **2**, is protonated forming radical **3**. Losses in conductance were observed for a subset of compounds in solutions at pH

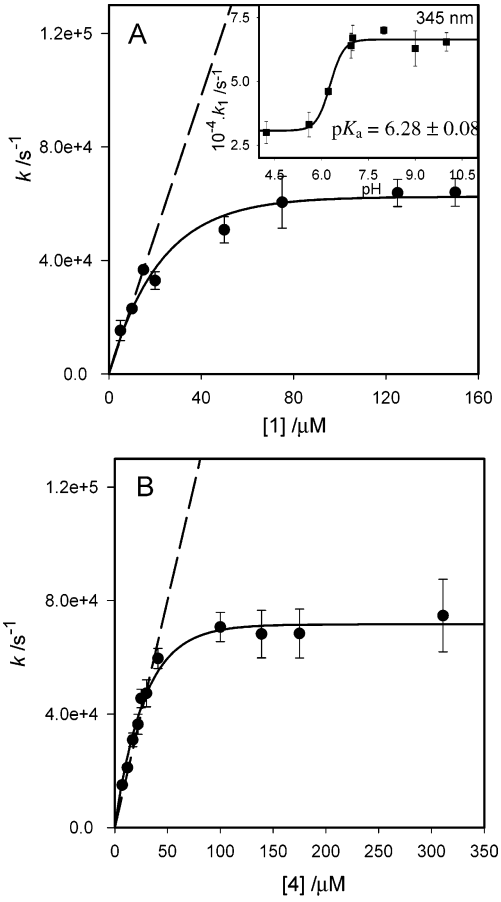


Figure 3. Rate of reduction of **1** (A) and **4** (B) by dR• radicals as a function of the substrate concentration. The N_2O-saturated solutions contained variable substrate concentration and 2-deoxyribose (20 mM) at pH 7.0 (5 mM phosphate). Dashed line indicates the rate of reduction, k(2), of **1**/**4** by 2-deoxyribose radicals. Insert in A displays the effect of pH on k(2), the rate of breakdown of the adduct formed between **1** and dR• radicals.

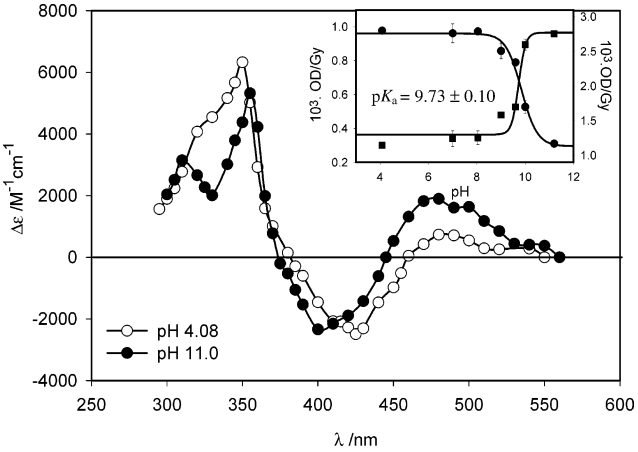


Figure 4. Absorption spectra of the radical anion of **4** (100 μM) following the one-electron reduction by $CO_2^{\bullet-}$ at high and low pH in N_2O-saturated solutions containing sodium formate (0.15 M). Insert displays the changes in absorbance with pH at 470 nm (■) and 330 nm (●) from which the pK_a value of the radical was determined.

10.5 ± 0.1 on the time scale seen for eq 3 above, (Figure 3). In these experiments, the dR• radicals were produced in N_2O-saturated solutions containing dR (20 mM), and each compound (150 μM) was adjusted to alkaline pH with sodium hydroxide. The measured losses in conductance can be compared to that in N_2O-saturated basic solutions of DMSO because of the

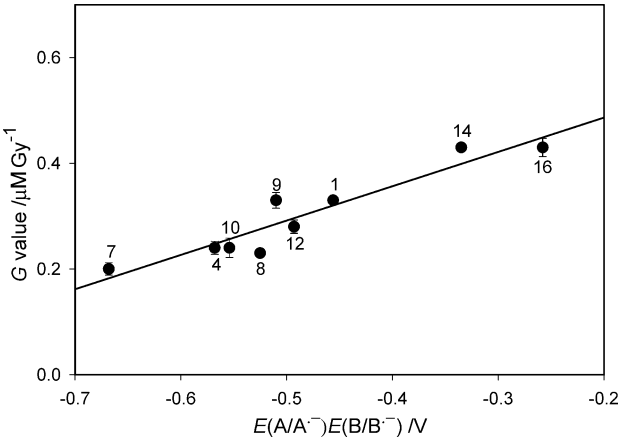


Figure 5. Dependence of the yield of radical anions formed on reaction of TPZ analogues with dR• on E(A/A•−) or E(B/B•−). The yield of reducing radicals was determined by electron transfer to methyl viologen (150 μM) at 600 nm relative to electron transfer from the $CO_2^{\bullet-}$ species.

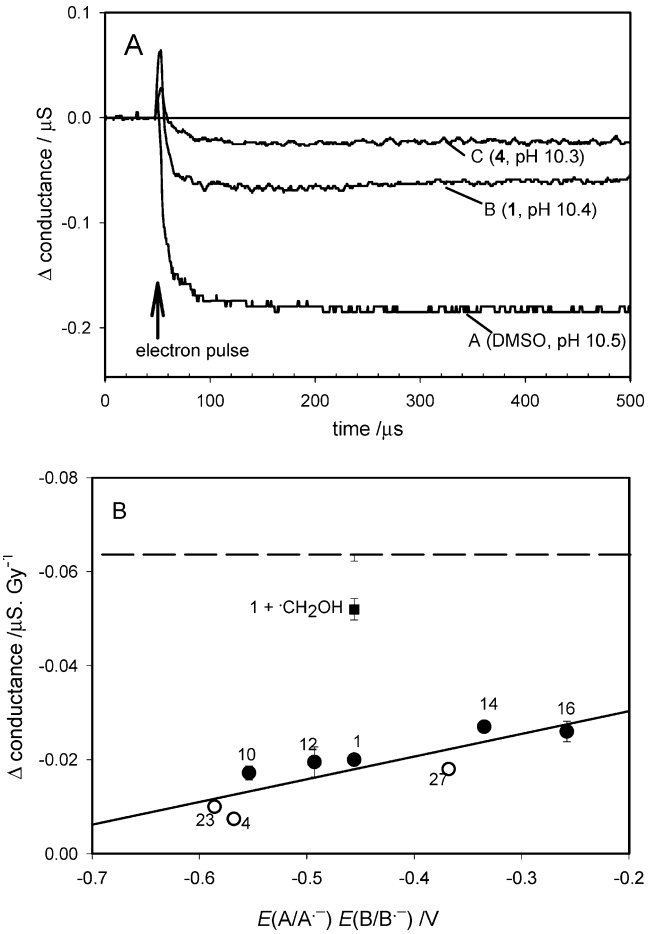


Figure 6. (A) Changes in conductance following pulse radiolysis (2.5 Gy) of N_2O-saturated basic solutions of the compounds (150 μM) and 2-deoxyribose (50 mM) relative to that seen for dimethyl sulfoxide (1 mM). (B) Conductivity yields as in A for benzotriazine 1,4-dioxides and 1-oxides.

formation of sulfenic acid,[16,25] which under our experimental conditions amounts to -0.063 μS·Gy^{-1} (Figure 6A). The loss in conductance, measured at the end of eq 3, is dependent on E(A/A•−) (Figure 6B), increasing to -0.027 ± 0.001 μS·Gy^{-1} for **14** and **16**. The maximum possible yield of protons arising from eqs 2 and 3 for electron transfer from the dR• radical is expected to be somewhat less than the yield of protons arising

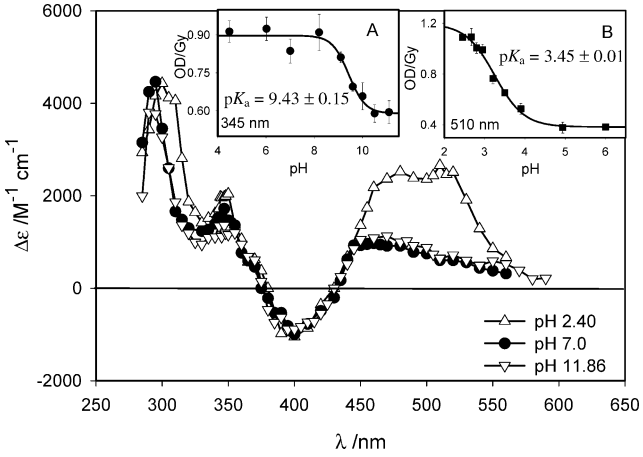


Figure 7. Radical spectra produced (at 20 μs) following pulse radiolysis (2.5 Gy) of **4** (150 μM) in N_2-saturated solutions of potassium peroxodisulfate (15 mM), 2-methyl-2-propanol (0.5 M), N_2 at different pH. Inserts A and B display the changes in absorbance with pH from which the pK_a values of the radicals were determined.

from •OH radicals reacting with DMSO since a proportion of the produced radical sites on dR will not be reducing in nature. For comparison purposes, the change in conductance upon the reaction of hydroxymethyl radicals with **1** at pH 10.4 was measured as -0.052 ± 0.002 μS·Gy^{-1}, which corresponds to a radiation chemical yield of protons being formed of 0.52 μM·Gy^{-1} (7% of the •OH radical yield of 0.56 μM·Gy^{-1} reacting with methanol form the nonreducing $CH_3O^{\bullet}$ radical[26]). The observed change in conductance upon the reaction of dR• radicals with **1** is -0.020 ± 0.001 μS·Gy^{-1}, which corresponds to a radiation chemical yield of protons produced through eqs 2 and 3 of 0.38 ± 0.01 μM·Gy^{-1}. This yield corresponds well with that measured in the optical studies above 0.34 ± 0.01 μM·Gy^{-1}, which gives support to the mechanism of adduct formation followed by breakdown to form the radical anion. The benzotriazinyl radical, formed upon the one-electron oxidation of **4**, gave different absorption spectra at pH 12, 7, and 2.5 (Figure 7). Using differences in the absorption bands at 510 and 345 nm, we determined radical pK_a values of 3.45 $\pm$ 0.01 and 9.43 $\pm$ 0.15 (Figure 7, inserts). The second-order decay kinetics of the benzotriazinyl radical was unaffected by increasing ionic strength at pH 7, implying an uncharged species, while at pH 12 an increase in decay rate was seen (data not shown).

The observed losses in conductance upon the reaction of dR• radicals with the benzotriazine 1-oxides at pH 10.3 are considerably smaller than that measured for the benzotriazine 1,4-dioxides of similar one-electron reduction potential. The small change in conductance of -0.007 ± 0.001 μS·Gy^{-1} for **4**, compared to a measured yield of 0.23 ± 0.01 μM·Gy^{-1} for electron transfer, is understood by the one-electron reduced product of the reaction being mainly protonated.

Temperature Studies. Thermodynamic parameters for the reaction between the benzotriazinyl radical **5** and dR, eq 1, and the formation of the radical anion **3** and oxidized dR, eq 3, were obtained from measuring the effect of temperature on these

(25) Asmus, K.-D.; Janata, E. In *The Study of Fast Processes and Transient Species by Electron Pulse Radiolysis*; Baxendale, J. H., Busi, F., Eds.; D. Reidel Publishing Company: Dordrecht, The Netherlands, 1982; Vol. 86, pp 91−113.
(26) Asmus, K.-D.; Mockel, H.; Henglein, A. *J. Phys. Chem.* **1973**, *77*, 1218−1221.

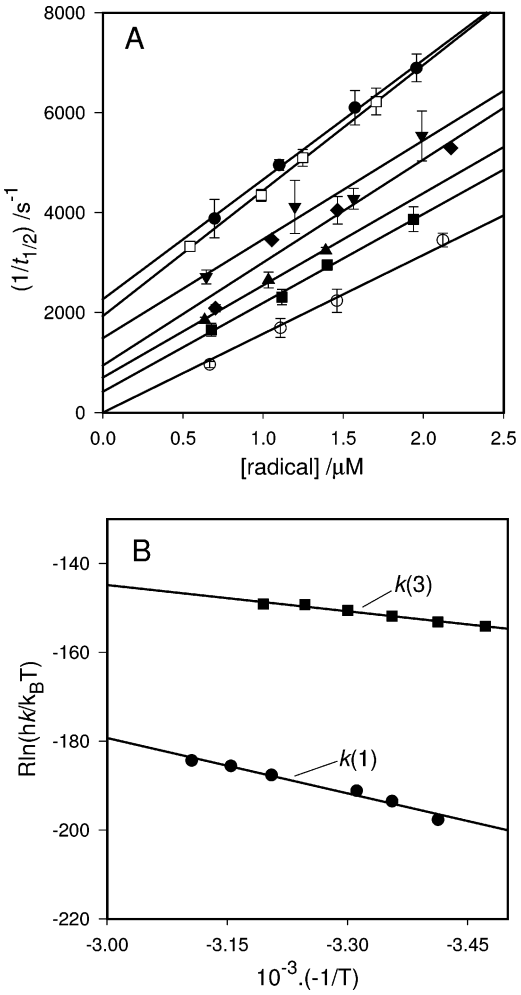


Figure 8. (A) Temperature dependence of the reciprocal of the first half-life of **5** on the initial radical concentration, formed with increasing radiation dose, for 10 (○), 20 (■), 25 (▲), 30 (◆), 40 (▼), 45 (□), and 50 °C (●). Radicals produced on the oxidation of **4** (500 μM) by the $SO_4^{\bullet-}$ radical, produced in a deaerated solution of peroxodisulfate (15 mM) and 2-methylpropanol-2-ol (0.2 M) at pH 7.0 (5 mM phosphate) in the presence of 300 μM 2-deoxyribose. (B) Eyring plots for k(1) (●), first-order rate constants derived from the intercepts of plots from part A, and k(3) (■), the rate of breakdown of adduct formed on the one-electron reduction of **1** by 2-deoxyribose radicals, are plotted as a function of temperature. The N_2O-saturated solution contained **1** (75 μM) and 2-deoxyribose (20 mM) at pH 7.0 (5 mM phosphate).

reactions. Equation 1 was studied as described above by producing **5** on oxidizing **4** with the $SO_4^{\bullet-}$ radical in the presence of dR (300 μM) and constructing kinetic plots of $1/t_{1/2}$ for the loss in absorption at 345 nm vs initial radical concentrations at each temperature (Figure 8A). The rate constant data, derived from the intercepts of the plots, were then treated by Arrhenius (not shown) and Eyring plots (Figure 8B), k(1), to obtain the thermodynamic parameters presented in Table 2. Equation 3 was studied in N_2O-saturated solution containing excess dR (20 mM) and **1** (75 μM) by monitoring the rate of buildup in absorption at 560 nm with increasing temperature. The Eyring plot of these data is presented in Figure 8B, k(3), and the obtained parameters are given in Table 2. The activation energies of these two reactions are small and indicative of radical reactions, while the negative entropy changes result in the reactions being slower than normal. The large negative activation entropy associated with eq 3 of -85.8 J K^{-1} mol^{-1} is of the order expected for the hydration and separation of a proton in the transition state.[27]

Product Analysis Following γ-Radiolysis. A subset of the compounds was serially irradiated at a concentration of 100 μM in N_2O-saturated solutions at pH 7 containing dR (20 mM), and the samples were analyzed by HPLC for the loss of the compounds and formation of their two-electron reduced products. The radiation chemical yield values were calculated from plots of concentration lost or gained against accumulated radiation dose, and these values are presented in Table 3. The radiation chemical losses of **1** and **12** are both greater than the yield of the dR$^\bullet$ radicals, indicating that a short chain reaction occurs. There is no evidence of such a chain reaction for analogues **8** and **15**, which contain strongly electron-donating and electron-withdrawing substituents, respectively. The occurrence of a chain reaction appears to be related to a threshold in the reduction potential of the benzotriazinyl radicals, E(B$^\bullet$,H$^+$/B), lying in the region >1.24 to ≤ 1.30 V. The expected maxima in the yields of the two-electron reduced products, the benzotriazine 1-oxides, are equal to half the loss in the parent benzotriazine 1,4-dioxides. The measured yields of the benzotriazine 1-oxides formed from **1**, **12**, and **15** are slightly less than half the loss of the parent compounds, which may indicate that the dR$^\bullet$ radicals may undergo minor attack at other sites. Only a small loss in the benzotriazine 1-oxide **4** and consequent formation of the fully reduced norbenzotriazine (**31**, SR 4330) is observed, possibly indicating that only a minor proportion of the dR$^\bullet$ radicals can reduce this compound.

Discussion

The reaction between deoxyribose radicals, dR$^\bullet$, and benzotriazine compounds has been studied using both optical and conductivity detection. The dR$^\bullet$ was produced by $^\bullet$OH radical attack on deoxyribose, resulting in relatively indiscriminant H-atom abstraction that, in the absence of added oxidants or reductants, forms a range of fragmentation products.[28] Both a comparatively inert alkyl radical (H-atom abstraction from C_2') and a number of reducing α-hydroxyalkyl radicals are produced. It is likely that the benzotriazinyl radical **5** abstracts an H-atom more selectively than the $^\bullet$OH radical from deoxyribose (eq 1), decreasing the proportion of radicals at C_2' since the C−H bond strength at this position is greater than those of the other positions.[29] It is clear that radicals are formed on deoxyribose since steady-state radiolysis experiments show **1** is lost through a short chain reaction when irradiated in the presence of excess deoxyribose.[30]

Nitroaryl radiosensitizers, such as substituted nitrobenzenes and the nitroimidazoles, have been reported to undergo a variety of reactions with the radicals of DNA model compounds.[31−34] Kinetic, esr, and spectral evidence point to the formation of

(27) Issacs, N. S. *Physical Organic Chemistry*, 2nd ed.; Longman Scientific & Technical: Harlow, U.K., 1995.
(28) Hartmann, V.; von Sonntag, C.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1970**, *25B*, 1394−1404.
(29) Miaskiewicz, K.; Osman, R. *J. Am. Chem. Soc.* **1994**, *116*, 232−238.
(30) Wardman, P.; Dennis, M. F.; Everett, S. A.; Patel, K. B.; Stratford, M. R. L.; Tracy, M. In *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives*; Rice-Evans, C. A., Halliwell, B., Lund, G. G., Eds.; Portland Press: London, 1995; Vol. 61, pp 171−194.
(31) McMillan, M.; Norman, R. O. C. *J. Chem. Soc. B* **1968**, 590−597.
(32) Jagannadham, V.; Steenken, S. *J. Am. Chem. Soc.* **1984**, *106*, 6542−6551.
(33) Steenken, S.; Jagannadham, V. *J. Am. Chem. Soc.* **1985**, *107*, 6818−6826.
(34) Wardman, P. *Radiat. Phys. Chem.* **1984**, *24*, 293−305.

Table 2. Thermodynamic Parameters for the Oxidation of 2-Deoxyribose by the Benzotriazinyl Radical of TPZ $k(1)$ and the Breakdown of the Adduct Formed upon One-Electron Reduction of Tirapazmine by 2-Deoxyribose Radicals $k(3)$ at pH 7.0

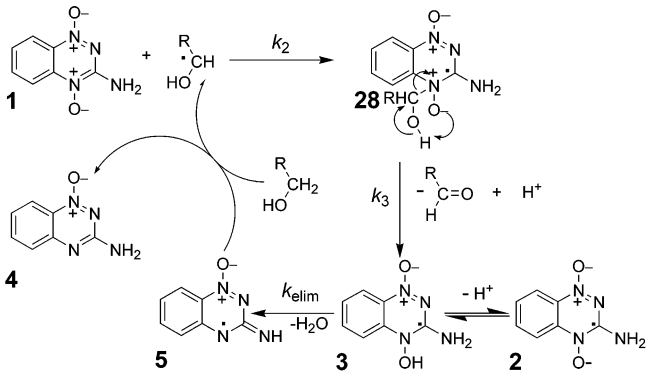
	E_a/kJ mol^{-1}				
reaction	Arrhenius Plot	Eyring Plot	A/s^{-1}	$\Delta H^{\#}$/kJ mol^{-1}	$\Delta S^{\#}$/J K^{-1} mol^{-1}
$k(1)$	$+44.1 \pm 3.4$	$+44.0 \pm 3.7$	$2.5 \pm 0.1 \times 10^{10}$	$+41.6 \pm 3.5$	-54.6 ± 11.0
$k(3)$	$+22.1 \pm 1.1$	$+22.2 \pm 1.1$	$5.5 \pm 0.7 \times 10^{8}$	$+19.7 \pm 1.0$	-85.8 ± 3.0

Table 3. Radiation Chemical Yield, G, for the Loss of Compounds on Reaction with 2-Deoxyribose Radicals and the Production of Two-Electron Reduced Products (B and C)

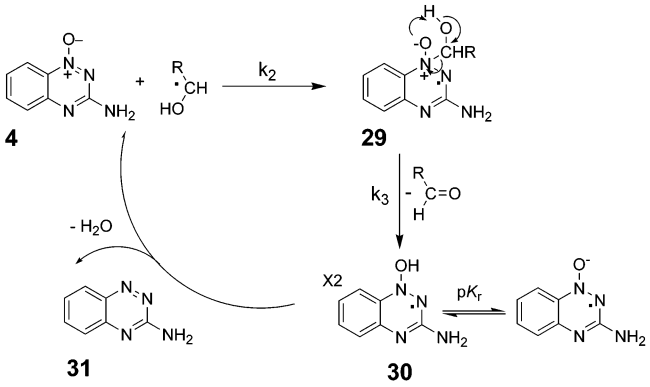
compound number/R	E(A/A$^{\bullet-}$) ± 0.01/V	E(B$^{\bullet}$,H$^+$/B) ± 0.01/V	G(–A)/M·Gy^{-1}	G(+B)/M·Gy^{-1}
8, 7-N(CH$_3$)$_2$	−0.535	0.94	-0.34 ± 0.01	0.16 ± 0.01
12, 6-CH$_3$	−0.493	1.30	-0.77 ± 0.02	0.23 ± 0.02
1, H	−0.456	1.32	-0.67 ± 0.06	0.28 ± 0.02
14, 6-CF$_3$	−0.335	1.27	-0.40 ± 0.01	ND[a]
15, 6-CF$_3$ 3-NHR′	−0.263	1.24	-0.31 ± 0.01	0.14 ± 0.01
	E(B/B$^{\bullet-}$)/V		$G(-\mathrm{B})/\mu$M·Gy^{-1}	G(+C)/M·Gy^{-1}
4, H	0.568 ± 0.01		0.06 ± 0.02	0.04 ± 0.01

[a] ND = not determined because of insufficient solubility.

Scheme 2. One-Electron Reduction of Benzotriazine 1,4-Dioxides by α-Hydroxyalkyl Radicals



Scheme 3. One-Electron Reduction of Benzotriazine 1-Oxides by α-Hydroxyalkyl Radicals



radical intermediates between both carbon-centered sugar radicals and pyrimidine radicals with an oxygen of the nitro group to form a nitroxyl radical. The formation of such an adduct with an oxygen of benzotriazine 1,4-dioxides is hard to envisage as no satisfactory Lewis structures can be drawn. Furthermore, our kinetic evidence that the α-hydroxyalkyl radicals of deoxyribose form adducts with the benzotriazine 1,4-dioxides and 1-oxides, which break down at rates independent of the OH^- concentration and the nature of the substituents on the benzotriazine, is in stark contrast to the adducts formed between α-hydroxyalkyl radicals and the nitro group of nitrobenzene compounds.[32] It is likely that a different mechanism operates for the benzotriazine 1,4-dioxides in that α-hydroxyalkyl radicals add mainly to the N4 position to form a radical adduct, **28** (eq 2), followed by an intramolecular pathway of elimination, which is non OH^- catalyzed (eq 3), to form the radical anion, **2**, which protonates at pH 7 to form **3** (Scheme 2).

Similarly, one-electron reduction of the benzotriazine 1-oxides by dR$^{\bullet}$ radicals can be envisaged as an initial addition to the N1 position to form radical **29** (eq 2), followed by elimination (eq 3) to form radical **30**. The prototropic property of **30** (pK_a = 9.73) is considerably different from that of **2**/**3** (pK_a = 6.18) and may indicate that a different radical center, such as N2, is involved (Scheme 3) or that **30** quickly rearranges to a C3 radical, and the 1-oxide moiety possesses a higher pK than that of the dioxide species, **3**.

Evidence for the existence of a chain reaction, following reduction of the compounds by dR$^{\bullet}$ radicals, is the observed greater loss of the compounds (Table 3) over the yield of electron transfer from the dR$^{\bullet}$ radicals in the pulse radiolysis experiments (Figure 4). Whereas short chain reactions occur following the reduction of the benzotriazine 1,4-dioxides **1** and **12**, this is not the case for **8** and **14** nor for the benzotriazine 1-oxide, **4**. These data imply that the benzotriazinyl radicals formed following the reduction of **1** and **12** can abstract a H-atom from dR to propagate a chain. The apparent shortness of the chain (two to three cycles) may well arise from the compounding effects of the two-step reaction where only a small fraction of reducing dR$^{\bullet}$ radicals are formed upon the reaction of the benzotriazinyl radicals with dR compared to the $^{\bullet}$OH radical. The reduction potentials of the benzotriazinyl radicals, E(B$^{\bullet}$, H$^+$/B), may well be a controlling factor as to whether a chain reaction with dR takes place with a threshold ≥1.24 V. The consistently lower yields of the benzotriazine 1-oxides (B) compared to the loss of the benzotriazine 1,4-dioxides (A) of the order ≤0.5 (Table 3) is evidence against the release of the $^{\bullet}$OH radical from the one-electron reduced benzotriazine 1,4-dioxides (Scheme 1) as then the concentration of the benzotriazine 1-oxides produced should equal the concentration of the benzotriazine 1,4-dioxides that is consumed. The occurrence

of the radical chain reaction, as modeled in the present study, may well underlie the reported high efficacy of compounds **1** and **12** to act as hypoxia-selective bioreductive drugs compared to all 32 other analogues tested in vitro.[21] Also, the targeting of **1** to the DNA of hypoxic cells, by appending a DNA binding moiety, results in a great increase in potency,[40] which has been correlated to the enhanced conversion of DNA radical damage to double strand breaks.[41] The results from the present study provide a mechanistic model for how tirapazamine interacts with the ribose component of DNA following its one-electron reduction.

Experimental Section

Materials. All reagents used were of analytical grade. Sodium formate, sodium hydroxide, perchloric acid, and phosphate buffers were obtained from Merck, and potassium thiocyanate was obtained from Riedel-de Haen. All other reagents were obtained from Aldrich Chemical Co. All solutions were prepared in water purified by the Millipore "Milli-Q" system. Solution pH values were adjusted using the phosphate salts (5 mM) and either NaOH or $HClO_4$ when necessary. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined on an Electrothermal 2300 melting point apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C spectra. Spectra were obtained in $CDCl_3$ unless otherwise specified and are referenced to Me_4Si. Chemical shifts and coupling constants were recorded in units of parts per million and hertz, respectively. Solutions in organic solvents were dried with anhydrous Na_2SO_4. Solvents were evaporated under reduced pressure on a rotary evaporator. Thin-layer chromatography was carried out on aluminum-backed silica gel plates (Merck 60 F_{254}) with visualization of components by UV light (254 nm) or exposure to I_2. Column chromatography was carried out on silica gel (Merck 230−400 mesh). All compounds designated for testing were analyzed at >99% purity by reverse-phase HPLC using an Agilent 1100 liquid chromatograph, an Alltima C_{18} (5 μ) stainless steel column (150 mm × 3.2 mm i.d.). and an Agilent 1100 diode array detector. Chromatograms were run using various gradients of aqueous (0.045 M ammonium formate and formic acid at pH 3.5) and organic (80% MeCN/MilliQ water) phases. DCM refers to dichloromethane, DME refers to dimethoxyethane, DMF refers to dry dimethylformamide, ether refers to diethyl ether, EtOAc refers to ethyl acetate, MeOH refers to methanol, and pet. ether refers to petroleum ether, boiling point range was 40−60 °C. All solvents were freshly distilled. Compounds **1**, **4**, and **31** were prepared as previously described.[35] Compounds **7**−**9**, **10**, **12**, **14**, **16**, **20**, **21**, **23**, and **25** were prepared as previously described.[21]

3-Chloro-6-methoxy-1,2,4-benzotriazine 1-Oxide (32). Sodium nitrite (7.14 g, 103.4 mmol) was added in portions to a stirred solution of 6-methoxy-1,2,4-benzotriazin-3-amine 1-oxide (**21**) (9.94 g, 51.7 mmol) in trifluoroacetic acid (50 mL) at 5 °C, and the solution was stirred at 20 °C for 1 h. The solution was poured into ice water, filtered, washed with water (2 × 50 mL), and dried. The solid was suspended in $POCl_3$ (80 mL), DMF (two drops) was added, and the mixture was stirred at 100 °C for 3 h. The solution was poured into ice water, stirred for 20 min, and filtered. The solid was dissolved in DCM (150 mL) and dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with 5% EtOAc/DCM, to give chloride **32** (7.42 g, 68%) as a pale yellow solid: mp (EtOAc/DCM) 196−199 °C; ^{1}H NMR δ 8.30 (d, J = 9.6 Hz, 1H), 7.32 (dd, J = 9.6, 2.7 Hz, 1H), 7.19 (d, J = 2.7 Hz, 1H), 4.01 (s, 3H); ^{13}C NMR δ 166.3, 157.8, 150.2, 128.9, 123.9, 121.9, 105.7, 56.5. Anal. Calcd for $C_8H_6ClN_3O_2$: C, 45.4; H, 2.9; N, 19.9; Cl, 16.8. Found C, 45.2; H, 2.6; N, 19.9; Cl, 16.9%.

3-Chloro-6-methyl-1,2,4-benzotriazine 1-Oxide (33). Similarly, reaction of 6-methyl-1,2,4-benzotriazin-3-amine 1-oxide (**23**) (9.05 g, 51.4 mmol) gave chloride **33** (7.86 g, 78%) as a pale yellow solid: mp (EtOAc/DCM) 156−158 °C; ^{1}H NMR δ 8.29 (d, J = 8.8 Hz, 1H), 7.74 (d, J = 1.7 Hz, 1H), 7.56 (dd, J = 8.8, 1.7 Hz, 1H), 2.61 (s, 3H). Anal. Calcd for $C_8H_6ClN_3O$: C, 49.1; H, 3.1; N, 21.5. Found: C, 49.2; H, 3.4; N, 21.5%.

3-Chloro-6-trifluoromethyl-1,2,4-benzotriazine 1-Oxide (34). Similarly, reaction of 6-trifluoromethyl-1,2,4-benzotriazin-3-amine 1-oxide (**25**) (1.15 g, 6.6 mmol) gave chloride **34** (375 mg, 33%) as a pale yellow solid: mp (DCM/pet. ether) 118−120 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 8.52−8.57 (m, 2H), 8.15 (dd, J = 9.0, 1.8 Hz, 1H); ^{13}C NMR [$(CD_3)_2$SO] δ 156.6, 146.6, 135.8, 135.5 (q, J = 33 Hz), 126.6 (q, J = 3 Hz), 126.1 (q, J = 4 Hz), 126.6 (q, J = 274 Hz), 122.0. Anal. Calcd for $C_8H_3ClF_3N_3O$: C, 38.5; H, 1.2; N, 16.8; F, 22.8. Found: C, 38.5; H, 1.1; N, 16.7; F, 14.4%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (17).** *N*,*N*-Dimethylethanediamine (0.66 mL, 6.0 mmol) was added to a stirred solution of 3-chloro-1,2,4-benzotriazine 1-oxide[35] **35** (438 mg, 2.4 mmol) in DME (50 mL), and the solution was stirred at reflux temperature for 2 h. The solution was cooled, the solvent was evaporated, and the residue was partitioned between dilute aqueous NH_3 (100 mL) and DCM (100 mL). The organic fraction was dried, and the solvent was evaporated. The residue was purified by chromatography, eluting with a gradient (0−10%) of MeOH/DCM, to give 1-oxide **17** (514 mg, 91%) as a yellow solid: mp (MeOH/EtOAc) 121−123 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 8.13 (dd, J = 8.6, 1.1 Hz, 1H), 7.78 (ddd, J = 8.5, 7.0, 1.1 Hz, 1H), 7.72 (br s, 1H), 7.57 (br d, J = 8.5 Hz, 1H), 7.33 (ddd, J = 8.6, 7.0, 1.3 Hz, 1H), 3.41−3.45 (m, 2H), 2.45−2.50 (m, 2H), 2.20 (s, 6H); ^{13}C NMR [$(CD_3)_2$SO] δ 158.8, 148.3, 135.6, 129.9, 125.9, 124.4, 129.8, 57.6, 45.1 (2), 38.6. Anal. Calcd for $C_{11}H_{15}N_5O$: C, 56.6; H, 6.5; N, 30.0. Found: C, 56.8; H, 6.6; N, 30.4%.

***N*^{1}-(6-Methoxy-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (22).** Similarly, reaction of *N*,*N*-dimethylethanediamine (1.33 mL, 12.1 mmol) and chloride **32** (0.85 g, 4.04 mmol) gave the amine **22** (0.72 g, 68%), which was dissolved in HCl-saturated MeOH. The solvent was evaporated, and the residue was crystallized as a tan solid: mp (MeOH/EtOAc) 236−239 °C; ^{1}H NMR [$(CD_3)_2$SO] δ 10.68 (br s, 1H), 8.07 (d, J = 9.3 Hz, 1H), 8.03 (br s, 1H), 6.95−6.99 (m, 2H), 3.92 (s, 3H), 3.70−3.76 (m, 2H), 3.30−3.35 (m, 2H), 2.81 (d, J = 4.9 Hz, 6H); ^{13}C NMR [$(CD_3)_2$SO] δ 164.9, 159.0, 150.4, 125.4, 121.6, 117.3, 104.3, 55.2, 55.2, 42.3 (2), 35.8. Anal. Calcd for $C_{12}H_{18}ClN_5O_2$: C, 48.1; H, 6.1; N, 23.4; Cl, 11.8. Found: C, 48.3; H, 6.1; N, 23.6; Cl, 11.9%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-methyl-1-oxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (24).** Similarly, reaction of *N*,*N*-dimethylethanediamine (705 μL, 6.6 mmol) and chloride **33** (518 mg, 2.7 mmol) gave 1-oxide **24** (603 mg, 92%) as a yellow solid: mp (MeOH/EtOAc) 143−145 °C; ^{1}H NMR δ 8.11 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 1.7 Hz, 1H), 7.07 (dd, J = 8.8, 1.7 Hz, 1H), 5.89 (br s, 1H), 3.50−3.56 (m, 2H), 2.52−2.56 (m, 2H), 2.45 (s, 3H), 2.26 (s, 6H); ^{13}C NMR δ 159.2, 149.1, 146.9, 129.2, 126.9, 125.3, 120.1, 57.5, 45.1 (2), 38.7, 22.0. Anal. Calcd for $C_{12}H_{17}N_5O$: C, 58.3; H, 6.9; N, 28.3. Found: C, 58.5; H, 7.1; N, 28.6%.

***N*^{1}-(6-Trifluoromethyl-1-oxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (26).** Similarly, reaction of *N*,*N*-dimeth-

(35) Mason, J. C.; Tennant, G. *J. Chem. Soc., B* **1970**, 911−916.
(36) Anderson, R. F.; Denny, W. A.; Li, W.; Packer, J. E.; Tercel, M.; Wilson, W. R. *J. Phys. Chem.* **1997**, *101*, 9704−9709.
(37) Wardman, P. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1637−1755.
(38) Jonsson, M.; Lind, J.; Reitberger, T.; Eriksen, T. E.; Merenyi, G. *J. Phys. Chem.* **1993**, *97*, 11278−11282.
(39) Jonsson, M.; Lind, J.; Eriksen, T. E.; Merenyi, G. *J. Am. Chem. Soc.* **1994**, *116*, 1423−1427.
(40) Delahoussaye, Y.; Hay, M. P.; Pruijn, F. B.: Denny, W. A.; Brown, J. M. *Biochem. Pharmacol.* **2003**, *65*, 1807−1815.
(41) Anderson, R. F.; Harris, T. A.; Hay, M. P.; Denny, W. A. *Chem. Res. Toxicol.* **2003**, *16*, 1477−1483.

ylethanediamine (0.5 mL, 4.6 mmol) and chloride **34** (305 mg, 1.2 mmol) gave the amine **26** (367 mg, 100%) as a yellow solid: mp (MeOH/DCM) 159−161 °C; ^{1}H NMR δ 8.33 (d, J = 8.9 Hz, 1H), 7.87 (br s, 1H), 7.40 (dd, J = 8.9, 1.5 Hz, 1H), 6.24 (br s, 1H), 3.53−3.58 (m, 2H), 2.57−2.60 (m, 2H), 2.28 (s, 6H); ^{13}C NMR δ 159.4, 148.5, 136.8 (q, J = 33 Hz), 132.0, 127.1, 124.3 (q, J = 4 Hz), 121.9, 120.0 (q, J = 3 Hz), 57.3, 45.0 (2), 38.7. Anal. Calcd for $C_{12}H_{14}F_3N_5O$: C, 47.8; H, 4.7; N, 23.3. Found: C, 48.0; H, 4.4; N, 23.1%.

6-(Methylsulfonyl)-1,2,4-benzotriazin-3-amine 1-Oxide (27). Hydrogen peroxide (70%, 0.55 mL, ca. 11 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (1.6 mL, 11.0 mmol) in DCM (10 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 6-(methylsulfanyl)-1,2,4-benzotriazin-3-amine 1-oxide[21] (**36**) (230 mg, 1.1 mmol) in $CHCl_3$ (20 mL) at 5 °C, and the mixture was stirred at 5 °C for 15 min. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL). The precipitate was purified by chromatography and eluted with 5% MeOH/DCM to give 1-oxide **27** (50 mg, 19%) as a yellow powder: mp (MeOH/DCM) 288−292 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 8.36 (d, J = 8.9 Hz, 1H), 8.04 (d, J = 1.9 Hz, 1H), 7.78 (dd, J = 8.9, 1.9 Hz, 1H), 7.72 (br s, 2H), 3.41 (s, 3H); ^{13}C NMR [$(CD_3)_2SO$] δ 160.8, 148.4, 146.5, 138.1, 125.2, 122.0, 120.3, 42.7. Anal. Calcd for $C_8H_8N_4O_3S$: C, 40.0; H, 3.4; N, 23.3. Found: C, 40.7; H, 3.3; N, 22.7%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (6).** Hydrogen peroxide (70%, 0.7 mL, ca. 14.5 mmol) was added dropwise to a stirred solution of trifluoroacetic anhydride (2.1 mL, 14.5 mmol) in DCM (20 mL) at 5 °C. The mixture was stirred at 5 °C for 5 min, warmed to 20 °C, stirred for 10 min, and cooled to 5 °C. The mixture was added to a stirred solution of 1-oxide **17** (338 mg, 1.5 mmol) and trifluoroacetic acid (223 μL, 2.9 mmol) in $CHCl_3$ (20 mL) at 5 °C, and the mixture was stirred at 20 °C for 6 h. The solution was carefully diluted with dilute aqueous NH_3 solution (20 mL), and the mixture was extracted with $CHCl_3$ (5 × 50 mL). The organic fraction was dried, and the solvent was evaporated. The residue was purified by chromatography and eluted with a gradient (0−10%) of MeOH/DCM to give 1,4-dioxide **6** (252 mg, 70%) as red needles: mp (MeOH/EtOAc) 153−156 °C; ^{1}H NMR δ 8.32 (d, J = 8.7 Hz, 1H), 8.29 (d, J = 8.7 Hz, 1H), 7.84 (ddd, J = 8.7, 7.1, 1.3 Hz, 1H), 7.48 (ddd, J = 8.7, 7.1, 1.2 Hz, 1H), 7.45 (br s, 1H), 3.60−3.65 (m, 2H), 2.58−2.62 (m, 2H), 2.29 (s, 6H); ^{13}C NMR δ 149.9, 138.4, 135.6, 130.4, 127.0, 121.6, 117.4, 57.4, 45.2 (2), 38.9. Anal. Calcd for $C_{11}H_{15}N_5O_2\cdot 1/4H_2O$: C, 52.1; H, 6.2; N, 27.6. Found: C, 52.1; H, 6.0; N, 27.5%.

***N*^{1}-(6-Methoxy-1,4-dioxido-1,2,4-benzotriazin-3-yl)-*N*^{2},*N*^{2}-dimethyl-1,2-ethanediamine (11).** Similarly, oxidation of 1-oxide **22** (597 mg, 2.3 mmol) gave 1,4-dioxide **11** (424 mg, 67%) as a red solid, which was dissolved in HCl-saturated MeOH. The solvent was evaporated, and the residue was crystallized to give the hydrochloride: mp (MeOH/EtOAc) 170−174 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.57 (br s, 1H), 8.45 (br s, 1H), 8.17 (d, J = 9.6 Hz, 1H), 7.39 (d, J = 2.6 Hz, 1H), 7.22 (dd, J = 9.6, 2.6 Hz, 1H), 4.01 (s, 3H), 3.78−3.82 (m, 2H), 3.33−3.37 (m, 2H), 2.82 (d, J = 4.5 Hz, 6H); ^{13}C NMR [$(CD_3)_2SO$] δ 165.6, 150.1, 139.7, 125.7, 123.4, 119.4, 92.5, 56.8, 54.9, 42.3 (2), 36.0. Anal. Calcd for $C_{12}H_{18}ClN_5O_3\cdot 11/2H_2O$: C, 42.1; H, 6.2; N, 20.4. Found: C, 42.0; H, 5.9; N, 20.0%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-methyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (13).** Simliarly, oxidation of 1-oxide **24** (566 mg, 2.3 mmol) gave 1,4-dioxide **13** (207 mg, 34%) as a red solid: mp (MeOH/EtOAc) 187−189 °C; ^{1}H NMR δ 8.19 (d, J = 9.0 Hz, 1H), 8.05 (d, J = 1.7 Hz, 1H), 7.44 (br s, 1H), 7.29 (dd, J = 9.0, 1.7 Hz, 1H), 3.58−3.64 (m, 2H), 2.57−2.61 (m, 2H), 2.56 (s, 3H), 2.28 (2); ^{13}C NMR δ 149.9, 148.0, 138.2, 129.3, 128.8, 121.4, 116.0, 57.4, 45.2 (2), 38.8, 22.3. Anal. Calcd for $C_{12}H_{17}N_5O_2$: C, 54.7; H, 6.5; N, 26.6. Found: C, 54.3; H, 6.7; N, 28.8%.

***N*^{1},*N*^{1}-Dimethyl-*N*^{2}-(6-trifluoromethyl-1,4-dioxido-1,2,4-benzotriazin-3-yl)-1,2-ethanediamine (15).** Similarly, oxidation of 1-oxide **26** (220 mg, 0.73 mmol) gave 1,4-dioxide **15** (187 mg, 80%) as a purple solid: mp (MeOH/EtOAc) 179−183 °C; ^{1}H NMR δ 8.59 (br s, 1H), 8.45 (d, J = 9.1 Hz, 1H), 7.65 (dd, J = 9.1, 1.7 Hz, 1H), 7.63 (br s, 1H), 3.63−3.67 (m, 2H), 2.60−2.65 (m, 2H), 2.30 (s, 6H); ^{13}C NMR δ 150.4, 137.9, 137.0 (q, J = 33 Hz), 131.1, 123.4, 122.6 (q, J = 3 Hz), 122.6 (q, J = 272 Hz), 116.0 (q, J = 4 Hz), 57.3, 45.2 (2), 38.9. Anal. Calcd for $C_{12}H_{14}F_3N_5O_2$: C, 45.4; H, 4.5; N, 22.1. Found: C, 45.3; H, 4.4; N, 21.9%.

Methods. Pulse radiolysis experiments were carried out using a 4 MeV linear accelerator and optical detection system as described previously.[36] A temperature-controlled reaction cell was used to obtain thermodynamic parameters and a conductivity cell to measure conductance changes as previously described.[16] Steady-state radiolysis experiments were performed using a ^{60}Co γ-source delivering a dose rate of 17 Gy min^{-1}. Product analysis was by HPLC (Hewlett-Packard Series 1100 coupled to a photodiode array detector) utilizing a reverse-phase Alltima C-8 column (5μ, 150 mm) and a solvent consisting of 80% acetonitrile in water and 0.45 M formate buffer (pH = 4.5) at a flow rate of 0.5 mL min^{-1}. A linear gradient was used in the mobile phase.

The radiolysis of water produces well-characterized radical species and molecular products (concentrations in micromolar per absorbed dose of 1 Gy (J Kg^{-1}) given in parentheses).

$$H_2O \rightsquigarrow e^-_{aq}(0.28) + {}^\bullet OH(0.28) + H^\bullet(0.06) + H_2(0.04) + H_2O_2(0.07) + H_3O^+(0.28)$$

One-electron reduction of substrates, **A** and **B**, for kinetic, redox, and spectral studies were carried out in (i) deaerated solutions containing propan-2-ol (0.2 M) to convert the $^\bullet$OH radicals to reducing propan-2-oxyl radicals, (ii) deaerated solutions containing 2-methylpropan-2-ol (≤1 M) to aid solubility of the 1-oxide compounds and scavenge the $^\bullet$OH radicals, leaving the e^-_{aq} as the reducing species, and (iii) N_2O-saturated solutions (to quantitatively convert the $e^{\bullet -}_{aq}$ to $^\bullet$OH radicals) containing either sodium formate (0.15 M) or 2-deoxyribose (20 mM) to form reducing $CO_2^{\bullet -}$ or dR$^\bullet$ radicals

$$e^-_{aq} + A/B \rightarrow A^{\bullet -}/B^{\bullet -} \quad (4)$$

$$^\bullet OH + (CH_3)_2CHOH \rightarrow (CH_3)_2C^\bullet OH + H_2O \quad (5)$$

$$(CH_3)_2C^\bullet OH + A/B \rightarrow (CH_3)_2CO + A^{\bullet -}/B^{\bullet -} + H^+ \quad (6)$$

$$^\bullet OH + (CH_3)_3COH \rightarrow {}^\bullet CH_2(CH_3)_2COH + H_2O \quad (7)$$

$$N_2O + e^-_{aq} \rightarrow {}^\bullet OH + OH^- + N_2 \quad (8)$$

$$^\bullet OH/H^\bullet + HCOO^-/dR \rightarrow H_2O/H_2 + CO_2^{\bullet -}/dR^\bullet \quad (9)$$

$$CO_2^{\bullet -}/dR^\bullet + A/B \rightarrow A^{\bullet -}/B^{\bullet -} + CO_2/dR^+(dR^\bullet + H^+) \quad (10)$$

One-electron oxidation of benzotriazine 1-oxides (B) was carried out by reaction with either the selenite radical ($SeO_3^{\bullet -}$) or sulfate radical ($SO_4^{\bullet -}$) produced by scavenging the e^-_{aq} by sodium selenate (50 mM) or sodium peroxidodisulfate (25 mM) in deaerated solutions containing 2-methylpropan-2-ol (0.2 M) to scavenge the $^\bullet$OH radicals.

$$e^-_{aq} + SeO_4^{2-}/S_2O_8^{2-} + H_2O \rightarrow SeO_3^{\bullet -}/SO_4^{\bullet -} + 2OH^- + /SO_4^{2-} \quad (11)$$

$$SeO_3^{\bullet -}/SO_4^{\bullet -} + B \rightarrow B^\bullet + H^+ + SeO_3^{2-}/SO_4^{2-} \quad (12)$$

The one-electron reduction potentials of the compounds $E(A/A^{\bullet-})$ and $E(B/B^{\bullet-})$ were determined at pH 7.0 (5 mM phosphate buffer) by establishing redox equilibria between three mixtures of the one-electron reduced compounds and the reference compounds methyl viologen ($E(MV^{2+}/MV^{\bullet+}) = -447 \pm 7$ mV) or triquat ($E(TQ^{2+}/TQ^{\bullet+}) = -548 \pm 7$ mV) and calculating ΔE values from the equilibrium constants, K_e, using the Nernst equation, as described in the literature.[37]

$$MV^{+\bullet}/TQ^{+\bullet} + A/B \overset{K_e}{\rightleftharpoons} MV^{2+}/TQ^{2+} + A^{\bullet-}/B^{\bullet-} \quad (13)$$

$$DMB^{+\bullet}/AN^{+\bullet} + B \overset{K_e}{\rightleftharpoons} B^{+\bullet} + DMB/AN \quad (14)$$

Similarly, the one-electron reduction potentials of the benzotriazinyl radicals were determined using mixtures of the oxidized benzotriazine 1-oxides and the reference compound 1,2-dimethoxybenzene ($E(DMB^{+\bullet}/DMB) = 1.30 \pm 0.1$ V[38]) or, in the case of compound **17**, aniline ($E(AN^{+\bullet}/AN) = 1.08$ V[39]).

Acknowledgment. This work was supported by Grant 00/462 from the Health Research Council of New Zealand and by Grant CA82566 from the NCI.

JA048740L