Sulfoxide-Containing Aromatic Nitrogen Mustards as Hypoxia-Directed Bioreductive Cytotoxins

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Received September 29, 1999

A series of diaryl and alkylaryl sulfoxide-containing nitrogen mustards were synthesized and evaluated for their hypoxia-selective cytotoxicity against V-79 cells in vitro as well as for their metabolism profiles with the rat S-9 fractions. In general, the diaryl sulfoxides (**4**, **5**, and **7**−**9**) showed much greater hypoxia selectivity (11−27-fold) than the alkylaryl sulfoxides (∼3-fold) (**1** and **3**). The fused diphenyl sulfoxides (**10** and **11**), on the other hand, showed very low hypoxia selectivity (1.3−3-fold). Compound **10** was highly cytotoxic under both aerobic and anaerobic conditions, while **11** showed low cytotoxicity under both conditions. The bioreduction of **8** by the rat S-9 fraction under anaerobic conditions was inhibited by menadione and enhanced by benzaldehyde, acetaldehyde, or 2-hydroxypyrimidine suggesting the involvement of aldehyde oxidase in the reduction of the sulfoxides. Bioreductive metabolism studies of selected model sulfoxides suggested that diaryl sulfoxides are better substrates for aldehyde oxidase than alkylaryl sulfoxides.

Introduction

Solid tumors contain areas of low oxygen tension (hypoxia) generally thought to arise in solid tumors due to a poor and disorganized blood supply.[1] Although these microenvironmental properties induce tumor resistance to radiation therapy and to many conventional chemotherapeutic agents, an opportunity exists to exploit the hypoxic environment of solid tumors for targeting cytotoxic anticancer agents. Approaches to the use of bioreductive agents, which are selectively toxic to hypoxic cells upon enzymatic reduction, in the treatment of solid tumors continue to develop steadily.[1−5] Much of the work has been centered on nitroheterocyclics (e.g., RSU 1069), quinones (e.g., mitomycin C), and di-*N*-oxides (e.g., tirapazamine, SR4233). Bioreductive activation of these hypoxia-selective cytotoxins can be catalyzed by a number of enzymes such as cytochrome P-450, cytochrome P-450 reductase, DT-diaphorase (quinone-oxidoreductase), xanthine oxidase, and aldehyde oxidase.[6−10] It appears that several different enzymes participate to different extents with the various bioreductive agents.[11] Workman[11] suggests a distinct possibility that one can design a successful bioreductive drug possessing a promiscuous relationship with several reductases or tailored for a particular enzyme that might be hyperexpressed in a particular tumor.

Sulfoxides are known to be susceptible to bioreduction. Sulindac, a sulfoxide-containing nonsteroidal antiinflammatory drug, can undergo reduction to the sulfide or oxidation to the sulfone.[12] The oxidation is the dominant process under normal physiological conditions; however, the reduction process becomes significant under anaerobic conditions.[13] The reductive metabolite sulfide is largely responsible for the antiinflammatory activity of the parent drug.[14] The reduction of sulfoxides by mammalian systems can be catalyzed by aldehyde oxidase[15−18] and the thioredoxin enzyme system.[15,19] Under anaerobic conditions, aldehyde oxidase plus electron donors, or a combination of aldehyde oxidase and xanthine oxidase plus xanthine, catalyzes the reduction of sulfoxides (e.g., sulindac, diphenyl sulfoxide, phenothiazine sulfoxide, and dibenzyl sulfoxide) to their corresponding sulfides.[16−18] The sulfoxide-reducing activity of aldehyde oxidase is enhanced by the addition of electron donors, such as aldehydes or 2-hydroxypyrimidine.[18] The specificity of enzyme for sulfoxide reduction varies with sulfoxide structures. Both rat and rabbit liver aldehyde oxidases or the renal thioredoxin system, for example, can reduce sulindac. Diphenyl sulfoxide, however, is mainly reduced by aldehyde oxidase.[15]

We previously reported that 4-[bis(2-chloroethyl)-amino]-1-(methylsulfinyl)benzene (**1**), a sulfoxide-containing nitrogen mustard, showed some hypoxia-selective cytotoxicity against the Chinese hamster ovary (CHO) cell line.[20] It has been proposed that the cytotoxicity be generated by the more reactive sulfide **2** formed from **1** upon bioreduction as shown in Scheme 1.[21] An in vitro study with the rat S-9 fraction showed that **1** was bioreduced to **2** more significantly under anaerobic conditions in the presence of a NADPH-generating system. This suggested a possible involvement of the NADPH-dependent cytochrome P-450 reductase, which has been shown to catalyze the reduction of nitromin to nitrogen mustard,[22] in the reduction of the sulfoxide to the sulfide. These results provided the first evidence supporting the concept of enhanced bioreduction of the sulfoxide to the sulfide under hypoxic conditions.

In this report, a series of sulfoxide-containing alkylating agents were synthesized (Chart 1) and evaluated for their hypoxia-selective cytotoxicity. The correspond-

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10.1021/jm9904957 CCC: $19.00 © 2000 American Chemical Society
Published on Web 09/23/2000

Scheme 1

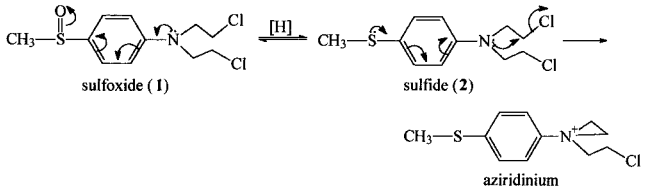
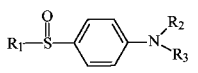
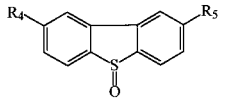


Chart 1



3: $R_1 = n\text{-}C_3H_7$, $R_2 = R_3 = C_2H_4Cl$
4: $R_1 = C_6H_5$, $R_2 = R_3 = C_2H_4Cl$
5: $R_1 = p\text{-}(C_2H_4Cl)_2NC_6H_4$, $R_2 = R_3 = C_2H_4Cl$
6: $R_1 = p\text{-}(C_2H_4Cl)NHC_6H_4$, $R_2 = C_2H_4Cl$, $R_3 = H$

7: $R_1 = p\text{-}O_2NC_6H_4$, $R_2 = R_3 = C_2H_4Cl$
8: $R_1 = p\text{-}H_2NC_6H_4$, $R_2 = R_3 = C_2H_4Cl$
9: $R_1 = p\text{-}(9\text{-acridinyl})NHC_6H_4$, $R_2 = R_3 = C_2H_4Cl$



10: $R_4 = H$, $R_5 = N(C_2H_4Cl)_2$
11: $R_4 = R_5 = N(C_2H_4Cl)_2$

ing sulfides and sulfones of some of these sulfoxides were also synthesized for comparison purposes. In addition, several model sulfoxides were studied to understand the relationship between the structures and their chemical/biochemical reduction properties.

Chemistry

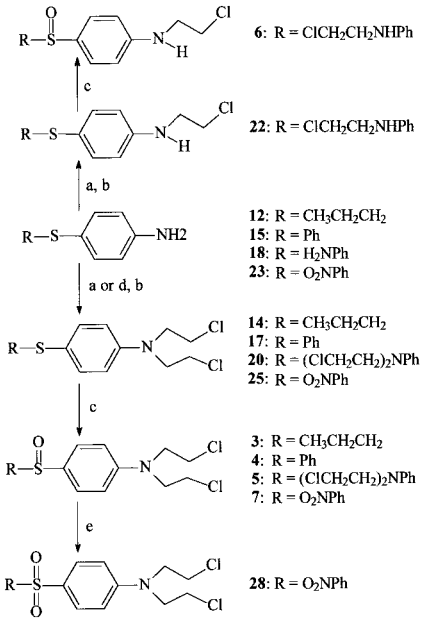
The (2-chloroethyl)aminophenyl- (**22**) and [bis(2-chloroethyl)]aminophenyl-substituted sulfides (**14**, **17**, **25**, **20**) and [bis(2-chloroethyl)]amino-substituted dibenzothiophenes (**32**, **34)** were synthesized by hydroxyethylation of the corresponding aminophenyl sulfides with ethylene oxide[23] or with 2-chloroethanol[21] followed by chlorination of the hydroxyl group (**21**, **13**, **16**, **24**, **19**, **31**, **33**) with phosphoryl chloride (Schemes 2, 4). The corresponding sulfoxides (**3**−**7**, **10**, **11**) were prepared by oxidizing the sulfides with hydrogen peroxide in trifluoroacetic acid (TFA)[24] or with *m*-chloroperbenzoic acid (mCPBA)[25] (Schemes 2, 4). The sulfone (**28**) was prepared by oxidation of the corresponding sulfide or sulfoxide with hydrogen peroxide using ammonium molybdate as catalyst[26] (Scheme 2).

The amino compounds were synthesized from the corresponding nitro compounds by reduction either with Sn/HCl[27] (**12**, **15)** or with Fe/HCl (**8**, **26**, **29**) (Scheme 3). The (acridin-9-ylamino)phenyl [bis(2-chloroethyl)-amino]phenyl sulfide (**27**), sulfoxide (**9**), and sulfone (**30**) were synthesized by condensation of 9-chloroacridine with **26**, **8**, and **29**, respectively (Scheme 3).[28]

Results and Discussion

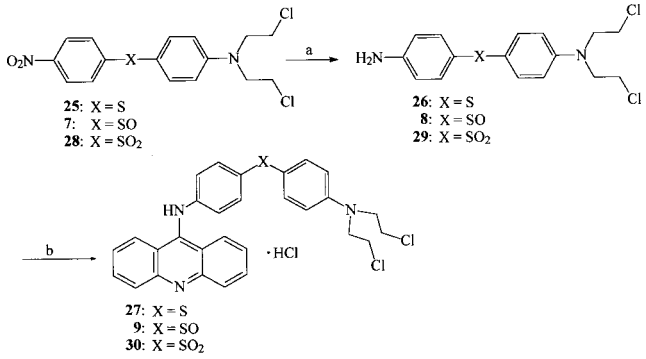
A selected series of substituted sulfinyl aryl nitrogen mustards (**3**−**11**) were synthesized as potential bioreductive cytotoxins. The cytotoxicity of aromatic nitrogen mustards is very dependent on the electronic properties of the substituents on the aromatic ring.[21,29] Both the parent sulfoxide nitrogen mustards and the oxidative product sulfones should be relatively unreactive due to electron-withdrawing effects of the substituents. On the other hand, the bioreductive product sulfides should be more reactive and provide higher cytotoxicity. To verify this, the cytotoxicity profile of selected sulfoxide compounds (**7**−**9)** was compared with that of the corresponding sulfides and sulfones. The comparative cytotoxicity was determined by SRB assay against Chinese hamster lung (V-79) cells under normal conditions (Table 1). The sulfide compounds (**25**−**27**), which do not require bioactivation for their cytotoxicity, were 10−14-fold more cytotoxic than their corresponding sulfoxides;

Scheme 2[a]



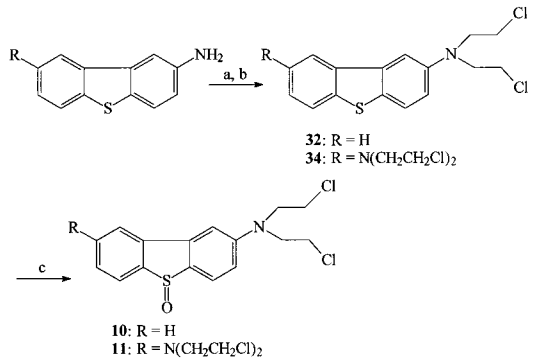
[a] Reagents: (a) $ClCH_2CH_2OH$, $CaCO_3$ for **14**, **17**, and **20**; (b) $POCl_3$; (c) H_2O_2, TFA; (d) ethylene oxide, AcOH for **25**; (e) H_2O_2, $(NH_4)_2MoO_4$.

Scheme 3[a]



[a] Reagents: (a) Fe, HCl; (b) 9-chloroacridine, catalytic HCl.

Scheme 4[a]



[a] Reagents: (a) $ClCH_2CH_2OH$, $CaCO_3$; (b) $POCl_3$; (c) H_2O_2, TFA for **10**; (d) mCPBA for **11**.

Table 1. Relative Aerobic Cytotoxicities of 4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfinyl)-1-nitrobenzene (**7**), 4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfinyl)phenylamine (**8**), and 4-{[4-(Acridin-9-ylamino)phenyl]sulfinyl}-1-[bis(2-chloroethyl)amino]benzene Hydrochloride (**9**) and Their Corresponding Sulfides and Sulfones Against V-79 Cells

compd	IC_{50} (μM)[a]	relative cytotoxicity[b]
25 (the corresponding *sulfide* of **7**)	2.25	1
7 (the *sulfoxide*)	21.88	0.1
28 (the corresponding *sulfone* of **7**)	26.73	0.08
26 (the corresponding *sulfide* of **8**)	2.49	1
8 (the *sulfoxide*)	30.03	0.08
29 (the corresponding *sulfone* of **8**)	26.90	0.09
27 (the corresponding *sulfide* of **9**)	1.93	1
9 (the *sulfoxide*)	26.20	0.07
30 (the corresponding *sulfone* of **9**)	22.24	0.09

[a] IC_{50}: concentration (μM) of drug required to reduce cell survival to 50% of controls in a SRB assay from mean of triplicate determinations of one experiment. [b] Relative cytotoxicity is expressed as the ratio of the IC_{50} values of the corresponding sulfide to sulfoxide or to sulfone.

Table 2. Hypoxia-Selective Cytotoxicity of Sulfoxide-Containing Aromatic Nitrogen Mustards

	IC_{90} (μM)[a]				IC_{90} (μM)[a]		
compd	air	N_2	hypoxic selectivity[b]	compd	air	N_2	hypoxic selectivity[b]
1	944	340	3	**7**	3680	182	20
3	953	319	3	**8**	2797	185	15
4	2012	189	11	**9**	4950	180	27
5	3094	184	17	**10**	210	161	1.3
6	1577	2208	0.7	**11**	4673	1522	3

[a] IC_{90}: drug concentration (μM) required to reduce cell survival to 10% of controls using V-79 cells in the clonogenic assay. [b] Hypoxic selectivity is expressed as the ratio of IC_{90} values in air and N_2 [IC_{90}(air)/IC_{90}(nitrogen)].

the cytotoxicities of the corresponding sulfones (**28**−**30**) were similar to those of the sulfoxides (less than 2-fold). Differences in partition behavior of the sulfides compared to the sulfoxides/sulfones may also contribute to the overall uptake and hence intracellular drug availability for cell damage.

Having shown that the sulfides are much more cytotoxic than the sulfoxides and sulfones, the sulfoxides (**1**, **3**−**11**) were tested for their hypoxia-selective cytotoxicity. A clonogenic assay with V-79 cells was used as described in the Experimental Section. The selection of this cell line was based on the literature information[30] that V-79 cells contain DT-diaphorase, NADPH cytochrome *c* reductase, and xanthine oxidase, where the latter two are known to be involved in the reduction of sulfoxides to the corresponding sulfides. The relative cytotoxicity profiles along with the hypoxia selectivity of the sulfoxides are summarized in Table 2. Illustrative data are shown in Figure 1 for a representative compound of high hypoxia selectivity (**7**) and of low hypoxia selectivity (**3**) of the compounds tested. In general, the alkylphenyl sulfoxide nitrogen mustards (**1**, **3**) showed low hypoxia selectivity (~3-fold), which was due to the combination of relatively high cytotoxicity under aerobic conditions and low cytotoxicity under hypoxic conditions. On the other hand, the diaryl sulfoxide nitrogen mustards (**4, 5**, **7**−**9**) were less cytotoxic under aerobic conditions and more cytotoxic under hypoxic conditions than the alkylphenyl sulfoxides and therefore showed greater hypoxia selectivity (11−27-fold). Compound **9**, an acridine-substituted diphenyl sulfoxide nitrogen mustard, showed the greatest hypoxia selectivity (27-fold). This high selectivity, however, stemmed primarily from the low cytotoxicity under aerobic conditions, and there was no further enhancement of cytotoxicity under hypoxic conditions. It is unclear whether the acridine moiety provided any contribution to the overall cytotoxicity profile through a possible DNA intercalation. Compound **6** was neither hypoxia-selective nor significantly cytotoxic. This was consistent with its structural inability to cross-link DNA upon bioreduction. The fused diphenyl sulfoxides, dibenzothiophen-5-one nitrogen mustards (**10**, **11**), showed low hypoxia selectivity (1.3−3-fold). Compound **10** was fairly cytotoxic under both aerobic (IC_{90} 210 μM) and anaerobic (IC_{90} 161 μM) conditions. On the other hand, **11** showed much lower cytotoxicity under both aerobic (IC_{90} 4673 μM) and anaerobic (IC_{90} 1522 μM) conditions.

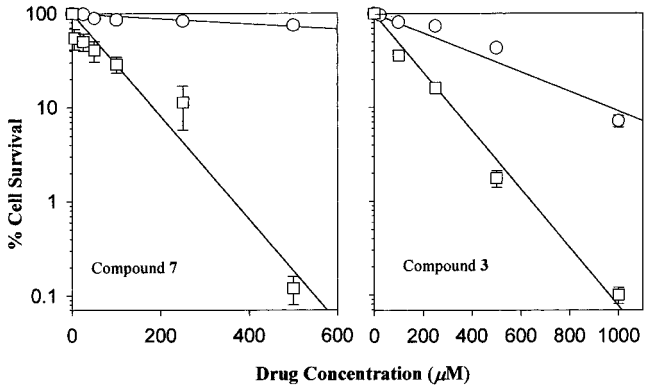


Figure 1. Survival of V-79 cells following treatment with **3** and **7**. Cells were subjected to aerobic or hypoxic conditions for 3 h. Survival was measured by using a clonogenic assay: (○) aerobic, (□) hypoxic. The data points represent the mean ± SE of at least triplicate determinations for each of three separate experiments.

It has been reported that the enzyme systems involved in sulfoxide reduction apparently show different substrate specificity.[15,31] To further understand the relationship between the hypoxia selectivity and the sulfoxide structures, selected model sulfoxide compounds such as methylphenyl sulfoxide, diphenyl sulfoxide, and dibenzothiophen-5-one were studied for their metabolism with emphasis on bioreduction. Selected sulfoxide nitrogen mustards were also included in the study for comparison purpose. The chemical reduction potential data of the model sulfoxides indicated (Table 3) that dibenzothiophen-5-one was the most easily reduced, followed by diphenyl sulfoxide and methylphenyl sulfoxide. On the other hand, diphenyl sulfoxide was the most easily bioreduced by the rat S-9 fraction, followed by methylphenyl sulfoxide and dibenzothiophen-5-one (Table 3). Dibenzothiophen-5-one was poorly reduced by aldehyde oxidase despite its low chemical redox potential. Diphenyl sulfoxide was further studied for its bioreduction profiles in detail (Table 3). Under aerobic conditions, the formation of diphenyl sulfone was observed only in the presence of NADPH, suggesting that NADPH-dependent enzymes were responsible for the oxidation. Under anaerobic conditions, diphenyl sulfoxide was reduced to diphenyl sulfide in the presence of benzaldehyde, 2-hydroxypyrimidine, or acetaldehyde. On the other hand, NADPH did not appear to be involved in catalyzing this reduction. In addition, the reduction was inhibited by menadione, a specific alde-

Table 3. Bioreduction Activity[a] and Reduction Potentials of Methylphenyl Sulfoxide, Diphenyl Sulfoxide, and Dibenzothiophen-5-one

enzyme activity[b]	methylphenyl sulfoxide	diphenyl sulfoxide	dibenzothiophen-5-one
N_2, control[c]	0	0	0
N_2, without cofactors	0	0	0
N_2, benzaldehyde	10.2 ± 0.5 (sulfide)	18.0 ± 0.5 (sulfide)	4.4 ± 0.7 (sulfide)
N_2, 2-hydroxypyrimidine		17.4 ± 0.9 (sulfide)	
N_2, acetaldehyde		6.0 ± 0.2 (sulfide)	
N_2, NADPH		0	
N_2, menadione[d]		0	
air, control[c]		0	
air, without cofactors		0	
air, benzaldehyde		0	
air, NADPH		11.6 ± 1.4 (sulfone)	
E^e (−V)	2.4	2.3	1.82

[a] The bioreduction experiments were conducted by incubation of the sulfoxides with rat liver S-9 fractions under aerobic (S-9 open to air) and anaerobic (S-9 pregassed with N_2) conditions, and the metabolites sulfide and sulfone were analyzed by HPLC. [b] Enzyme activity values are expressed as sulfide or sulfone formation in nmol/mg protein, and mean ± SD was from three experiments. The substance in parentheses was the metabolite detected. [c] The sulfoxides were incubated with boiled rat S-9 fraction as control in the presence of either NADPH under aerobic conditions or benzaldehyde under anaerobic conditions. [d] The S-9 fraction was preincubated with menadione for 30 min under aerobic conditions, then used in the presence of benzaldehyde. [e] Reduction potential (SCE) values were determined by differential pulse polarography.

Table 4. Formation of Sulfide Metabolites from Incubations of the Corresponding Sulfoxide Prodrugs with Rat S-9 Fractions

bioreduction (%)[a]	**1**[e]	**5**	**8**	**9**	**10**	**11**
N_2, control[b]	0	0	0	0	0	0
N_2, without cofactors	2.3	0	0	1.29 ± 0.15	0.46 ± 0.05	0.60 ± 0.05
N_2, benzaldehyde			5.00 ± 0.43	2.28 ± 0.08	1.68 ± 0.10	0.60 ± 0.01
N_2, 2-hydroxypyrimidine			4.50 ± 0.43	1.34 ± 0.06	2.02 ± 0.03	0.33 ± 0.01
N_2, acetaldehyde			4.42 ± 0.38	1.79 ± 0.04		
N_2, NADPH	4.3		0.37 ± 0.03	1.37 ± 0.03		
N_2, menadione[c]		0	0	1.07 ± 0.09		
air, control[b]	0	0	0	0		
air, without cofactors	1.0	0	0	0		
air, NADPH	2.6	0	0	0		
air, benzaldehyde		0	0	0		
E^d (−V)	2.60	2.63	2.55	2.63	2.03	1.96

[a] The bioreduction experiments were conducted by incubation of the sulfoxides with rat liver S-9 fractions under aerobic or anaerobic conditions, and the metabolites were analyzed by TLC densitometry. The values, representing percentage of sulfide formation from the corresponding sulfoxide, were from three experiments with the same batch of S-9 fractions. [b] See footnote *c* in Table 3. [c] See footnote *d* in Table 3. [d] See footnote *e* in Table 3. [e] The bioreduction data was determined by HPLC reported previously.[19]

hyde oxidase inhibitor. All these findings suggested that liver aldehyde oxidase, not other NADPH-dependent enzymes, was the enzyme involved in the reduction of diphenyl sulfoxide under anaerobic conditions. Under aerobic conditions, diphenyl sulfoxide was not reduced to the corresponding sulfide in the presence of benzaldehyde. This was consistent with the literature observation[32] that sulfoxide reduction by aldehyde oxidase is inhibited by oxygen. The feature that diphenyl sulfoxide is bioreduced to the sulfide by aldehyde oxidase only under anaerobic conditions may explain why the diaryl sulfoxide nitrogen mustards (**4, 5**, **7**−**9**) showed substantial hypoxia-selectivity characteristics as stated above.

As reported previously,[20] compound **1**, an alkylphenyl-substituted sulfoxide nitrogen mustard, was reduced to its sulfide **2** in the presence of NADPH (Table 4) suggesting that an NADPH-dependent enzyme may be involved in the bioreduction. The fact that this reduction took place under both aerobic and anaerobic conditions may explain why this compound was more cytotoxic under aerobic conditions and less hypoxia-selective than the diaryl counterparts. The metabolism data of some selected sulfoxide nitrogen mustards is summarized in Table 4. Compounds **5**, **8**, and **9** are diphenyl-substituted sulfoxide nitrogen mustards and all showed substantial hypoxia selectivity. Under anaerobic conditions, both **8** and **9** were significantly reduced to their corresponding sulfides (**26** and **27**, respectively) in the presence of benzaldehyde, 2-hydroxypyrimidine, or acetaldehyde. The bioreduction was inhibited in the presence of menadione, indicating that aldehyde oxidase is involved in the reduction. In addition, **9** was also reduced in the presence of NADPH or without cofactor, and the bioreduction was not completely inhibited by menadione, suggesting some additional enzyme(s) may be involved in the reduction. Regardless, both **8** and **9** were reduced exclusively under anaerobic conditions. Despite numerous attempts, we were unable to detect the sulfide metabolite (**20**) from **5**. The reason is unknown. Interestingly, however, **5** produced 7-fold further enhanced hypoxia-selective cytotoxicity against V-79 cells in the presence of benzaldehyde (0.25 mM) under hypoxic conditions. Benzaldehyde, at this concentration, had no effect on the controls (both aerobic and hypoxic). This finding suggested that V-79 cells contain aldehyde oxidase and a significantly enhanced reduction of the sulfoxide to the cytotoxic sulfide takes place in the presence of a suitable electron donor for aldehyde oxidase. Compounds **10** and **11** are fused diphenyl-substituted sulfoxide nitrogen mustards. Compound **10** was reduced to its sulfide **32** in the presence of benzaldehyde or 2-hydroxypyrimidine, indicating involvement of aldehyde oxidase in the reduction. The reason **10** was highly toxic under aerobic conditions is not clear. Compound **11** was not significantly reduced to its sulfide **34** in the presence of benzaldehyde or 2-hydroxypyrimidine, suggesting that **11** may be not a good substrate

Table 5. Formation of **8** from Incubation of **7** with Rat S-9 Fractions

	bioreduction (%)[a]	
incubation conditions	N_2	air
boiled S-9[b]	0	0
without cofactors	1.75 ± 0.09	1.14 ± 0.14
NADPH	42.10 ± 4.33	16.00 ± 1.00
NADH	9.00 ± 0.43	9.75 ± 0.66
benzaldehyde	17.93 ± 0.62	1.05 ± 0.17
2-hydroxypyrimidine	8.50 ± 0.75	
acetaldehyde	12.92 ± 0.52	
menadione[c]	1.60 ± 0.03	

[a] The bioreduction experiments were conducted by incubation of the sulfoxides with rat liver S-9 fractions under aerobic (air) or anaerobic (N_2) conditions, and the metabolites were analyzed by TLC densitometry. The values, representing percentage of sulfide formation from sulfoxide, were from three experiments with the same batch of S-9 fractions. [b] See footnote *c* in the Table 3. [c] See footnote *d* in the Table 3.

of aldehyde oxidase. This might be a possible reason to explain the relative lack of hypoxia-selective cytotoxicity of **11**. The bioreductivity of the sulfoxides did not parallel their chemical reduction potentials. Compound **11**, for example, was the most easily reduced chemically ($E = -1.96$ V), but it was apparently a poor substrate for aldehyde oxidase and one of the least hypoxia-selective compounds (Table 4).

Compound **7** contains both nitro and sulfoxide groups in the molecule. The metabolism profiles of **7** are summarized in Table 5. Under our experimental conditions, reduction of the nitro group to the amine metabolite (**8**) was observed in most cases. The reductions of sulfoxide group however occurred only in the presence of NADPH under aerobic conditions. Compound **7** was significantly reduced to **8** in the presence of NADPH under both aerobic and anaerobic conditions in the amount of 16% and 42% bioreduction, respectively. NADH, albeit less effective, was also involved under both conditions. These suggest that the NADPH/NADH-dependent enzyme system is responsible for reduction of the nitro group in **7**. Compound **7** was also reduced to **8** in the presence of benzaldehyde, 2-hydroxypyrimidine, and acetaldehyde in the amount of 17.93%, 8.50%, and 12.92%, respectively. These reductions were observed only under hypoxic conditions and were inhibited by menadione. These findings indicate that aldehyde oxidase may also be involved in the reduction of the nitro group in **7**. The metabolism study of **7** suggests compound **7** might be the prodrug of **8**. The nitro group is first reduced to form **8**, followed by reduction of the sulfoxide to form the reactive sulfide nitrogen mustard **26**.

In conclusion, these results support that properly designed sulfoxide-containing nitrogen mustards can serve as potential hypoxia-directed bioreductive cytotoxins. Diaryl sulfoxide nitrogen mustards showed much greater hypoxia-selective in vitro cytotoxicity than alkylaryl sulfoxide mustards. Metabolism study with rat S-9 fraction showed that bioreduction of compound **8** under anaerobic conditions can be inhibited by menadione, an inhibitor of aldehyde oxidase, and enhanced by electron donors of aldehyde oxidase (e.g., benzaldehyde, acetaldehyde, and 2-hydroxypyrimidine). This suggests that aldehyde oxidase may be involved in the reduction of the sulfoxides. Diphenyl sulfoxide is more easily reduced with rat S-9 fraction under anaerobic conditions than methylphenyl sulfoxide, suggesting that diaryl sulfoxides may be the better substrates for aldehyde oxidase than alkylaryl sulfoxides.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. The following instruments were used: IR, Perkin-Elmer model 281; ^{1}H NMR, Varian EM-360L CW, 60 MHz (TMS as internal standard). Silica gel GF plates (Analtech) were used for TLC (250 μm, 2.5 × 10 cm) and preparative TLC (1000 μm, 20 × 20 cm). Silica gel (40 μm; Baker) was used for flash column chromatography. All chemicals and solvents were reagent grade and were purchased from commercial vendors.

4-Propylthiophenylamine (12). To a boiling suspension of 4-nitro-1-propylthiobenzene[33,34] (11.2 g, 56.8 mmol) in a solution of 37% HCl (35 mL) in water (30 mL) were added tin turnings (11.0 g) upon which the suspension became a clear light brown solution. The solution was heated under reflux with vigorous stirring for 15 min. Activated charcoal (0.10 g) was added and the mixture was allowed to continue refluxing for an additional 15 min. The mixture was filtered hot and the filtrate was cooled and diluted with cold water (100 mL). Aqueous NaOH (40%) was added till the mixture became strongly alkaline. The resulting gray product was filtered and purified with charcoal give 10.1 g of white solid which was further purified on a flash column using hexane/EtOAc (4:1) as eluent to give 8.13 g (85.6% yield) of **12** as a yellow oil: TLC R_f 0.37 in hexane/EtOAc (4:1); ^{1}H NMR ($CDCl_3$) δ 1.00 (t, J = 6 Hz, 3H), 1.32−1.93 (m, 2H), 2.75 (t, J = 6 Hz, 2H), 3.58 (s, 2H), 6.58 (d, J = 8 Hz, 2H), 7.24 (d, J = 8 Hz, 2H).

2-[(2-Hydroxyl)(4-propylthiophenyl)amino]ethan-1-ol (13). 4-Propylthiophenylamine (**12**) (8.12 g, 48.6 mmol) and 4 equiv of 2-chloroethanol (14.3 mL, 17.15 g, 196 mmol) were added to a suspension of $CaCO_3$ (10.0 g, 99.9 mmol) in water (250 mL). The cloudy mixture was heated under reflux with vigorous stirring, protected from light, for 24 h. An additional 4 equiv of 2-chloroethanol, $CaCO_3$ (10.0 g) were added in two 2-equiv portions over the following 48 h. The reaction mixture was then cooled, adjusted to pH 7.0 with 10% aqueous NaOH, and extracted with EtOAc (4 × 200 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to give 8.18 g of brown oil. The crude material was purified on a flash column with eluent EtOAc/hexane (from ratio 2:1 to 3:1) to give 3.80 g (30.7% yield) of **13** as a light brown oil: TLC R_f 0.46 in EtOAc/hexane (3:1); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, J = 6 Hz, 3H), 1.32−1.68 (m, 2H), 2.74 (t, J = 6 Hz, 2H), 3.15−3.94 (m, 8H), 6.55 (d, J = 8 Hz, 2H), 7.30 (d, J = 8 Hz, 2H).

Bis(2-chloroethyl)(4-propylthiophenyl)amine (14). A solution of **13** (3.80 g, 14.9 mmol) in $POCl_3$ (9.0 mL) was heated under reflux for 40 min, then the hot reaction mixture was added, with stirring, to a mixture of ice/water (150 mL). The mixture was extracted with EtOAc (4 × 100 mL) and washed with 10% $NaHCO_3$ solution (3 × 200 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to give 3.23 g (70.0% yield) of **14** as a brown oil: TLC R_f 0.84 in hexane/EtOAc (2:1); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 6 Hz, 3H), 1.32−1.82 (m, 2H), 2.74 (t, J = 6 Hz, 2H), 3.32−3.75 (m, 8H), 6.60 (d, J = 8 Hz, 2H), 7.34 (d, J = 8 Hz, 2H). Anal. ($C_{13}H_{19}Cl_2NS$) C, H, Cl, N, S.

4-[Bis(2-chloroethyl)amino]-1-(propylsulfinyl)benzene (3). To a solution of **14** (1.00 g, 3.42 mmol) in 5 mL TFA was added H_2O_2 in TFA (1.0 mL, 1 equiv), prepared by adding 30% H_2O_2 (8.6 mL) to TFA (16.4 mL), while stirring at 0 °C. The reaction was allowed to proceed for 2 h. The ice bath was removed and the reaction was allowed to continue for an additional 30 min. TFA was evaporated to give 0.78 g (74.2% yield) of **3** as a light yellow solid: mp 45−48 °C; TLC R_f 0.41 in EtOAc/hexane (3:1); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 6 Hz, 3H), 1.40−1.84 (m, 2H), 2.85 (t, J = 6 Hz, 2H), 3.38−3.79 (m, 8H), 6.45 (d, J = 8 Hz, 2H), 7.20 (d, J = 8 Hz, 2H). Anal. ($C_{13}H_{19}Cl_2NOS$) C, H, Cl, N, S.

2-[(2-Hydroxyethyl)(4-phenylthiophenyl)amino]ethan-1-ol (16) was prepared from 4-phenylthiophenylamine (**15**), obtained from reduction of 4-nitro-1-phenylthiobenzene according to the procedure for **12**, on a 50.0-mmol (10.1-g) scale as in the procedure for **13** to give 9.60 g of the crude product as a brown oil. The oil was purified on a flash column using eluent EtOAc/hexane (from ratio 1:1 to 2:1) to give 2.26 g (15.6% yield) of **16** as a brown oil: TLC R_f 0.25 in EtOAc/hexane (2:1); ^{1}H NMR ($CDCl_3$) δ 3.26−4.00 (m, 8H), 6.52−6.77 (m, 4H), 7.19 (s, 5H).

Bis(2-chloroethyl)(4-phenylthiophenyl)amine (17) was prepared from **16** on a 7.82-mmol (2.26-g) scale in the procedure for **14** to give 2.11 g of the crude product as a light brown oil. The crude was purified on a flash column using hexane/EtOAc (4:1) as eluent to give 2.03 g (79.7% yield) of **17** as a light brown oil: TLC R_f 0.69 in hexane/EtOAc (4:1); ^{1}H NMR ($CDCl_3$) δ 3.32−3.78 (m, 8H), 6.40−7.48 (m, 4H), 7.11 (s, 5H). Anal. ($C_{16}H_{17}Cl_2NS$) C, H, Cl, N, S.

4-[Bis(2-chloroethyl)amino]-1-(phenylsulfinyl)benzene (4) was prepared from **17** on a 3.07-mmol (1.00-g) scale in the procedure for **3** to give 0.84 g of the crude product as a dark brown viscous oil. The crude oil was purified on a flash column using EtOAc/hexane (3:1) to give 0.71 g (67.6% yield) of **4** as an ivory-colored crystalline product: mp 122−124 °C; TLC R_f 0.68 in EtOAc/hexane (3:1); ^{1}H NMR ($CDCl_3$) δ 3.36−3.77 (m, 8H), 6.51−7.59 (m, 4H), 7.20 (s, 5H). Anal. ($C_{16}H_{17}Cl_2NOS$) C, H, Cl, N, S.

2-[(4-{4-[Bis(2-hydroxyethyl)amino]phenylthio}phenyl)-(2-hydroxyethyl)amino]ethan-1-ol (19) was prepared from 4-(4-aminophenylthio)phenylamine[27] (**18**) (6.50 g 30.0 mmol) and 12 equiv of 2-chloroethanol as in the similar procedure for **13** to give 6.60 g crude product. The crude was purified on a flash column to give 3.15 g (26.8% yield) of **19** as an off-white solid: mp 135−137 °C; TLC R_f 0.60 in EtOH/EtOAc (1:4); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 3:1) δ 3.16−3.68 (m, 16H), 6.60 (d, J = 9 Hz, 4H), 7.60 (d, J = 9 Hz, 4H).

Bis(2-chloroethyl)[4-{4-[bis(2-chloroethyl)amino]-phenylthio}phenyl]amine (20) was prepared from **19** on a 3.82-mmol (1.50-g) scale in the procedure for **14** to give 1.24 g of the crude product as a light brown oil. The crude was purified on a silica gel column to give 1.20 g (67.3% yield) of **20** as an off-white solid: mp 65−66 °C; TLC R_f 0.51 in hexane/EtOAc (5:1); ^{1}H NMR ($CDCl_3$) δ 3.46−3.89 (m, 16H), 6.58 (d, J = 9 Hz, 4H), 7.28 (d, J = 9 Hz, 4H). Anal. ($C_{20}H_{24}Cl_4N_2S$) C, H, Cl, N, S.

1-[Bis(2-chloroethyl)amino]-4-[{4-[bis(2-chloroethyl)-amino]phenyl}sulfinyl]benzene (5) was prepared from **20** on a 1.99-mmol (0.93-g) scale in the procedure for **3** to give 0.75 g (78.0% yield) of **5** as an off-white solid: mp 130.5−132 °C; TLC R_f 0.26 in EtOAc/hexane (3:2); ^{1}H NMR ($CDCl_3$) δ 3.40−3.95 (m, 16H), 6.70 (d, J = 9.6 Hz, 4H), 7.28 (d, J = 9.6 Hz, 4H). Anal. ($C_{20}H_{24}Cl_4N_2OS$) C, H, Cl, N, S.

2-[(4-{4-[Bis(2-hydroxyethyl)amino]phenylthio}phenyl)-amino]ethan-1-ol (21) was prepared from 4-(4-aminophenylthio)phenylamine (**18**) (5.00, 23.1 mmol) and 6 equiv of 2-chloroethanol as in the similar procedure for **13** to give 6.23 g of a brown oil. The crude oil was purified on a flash column using EtOAc/hexane (5:1) as eluent to give 0.77 g (11.0% yield) of **21** as a pale yellow oil: TLC R_f 0.23 in EtOAc/hexane (4:1); ^{1}H NMR ($CDCl_3$) δ 2.92−3.45 (m, 8H), 6.53 (d, J = 9 Hz, 4H), 7.12 (d, J = 9 Hz, 4H).

(2-Chloroethyl)[4-{4-[(2-chloroethyl)amino]phenylthio}-phenyl]amine (22) was prepared from **21** on a 21.4-mmol (0.75-g) scale as in the procedure for **14** to give 0.63 g of the crude product as a golden yellow oil. The crude oil was purified on preparative TLC plates using hexane/EtOAc (3:1) as eluent to give 0.46 g (52.3% yield) of **22** as a dark brown oil: TLC R_f 0.43 in hexane/EtOAc (3:1); ^{1}H NMR ($CDCl_3$) δ 3.18−3.68 (m, 8H), 6.70 (d, J = 9 Hz, 4H), 7.22 (d, J = 9 Hz, 4H). Anal. ($C_{16}H_{18}Cl_2N_2S$) C, H, Cl, N, S.

1-[(2-Chloroethyl)amino]-4-[{4-[(2-chloroethyl)amino]-phenyl}sulfinyl]benzene (6) was prepared from **22** on a 0.91-mmol (0.31-g) scale as in the procedure for **3** to give 0.29 g of the crude product as a light gray viscous oil. The crude oil was purified on preparative TLC plates using EtOAc/hexane (1:1) as eluent to give 0.23 g (70.1% yield) of **6** as a white crystalline solid: TLC R_f 0.35 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.22−3.75 (m, 8H), 6.78 (d, J = 9 Hz, 4H), 7.33 (d, J = 9 Hz, 4H). Anal. ($C_{16}H_{18}Cl_2N_2OS$) C, H, Cl, N, S.

2-{(2-Hydroxyethyl)[4-(4-nitrophenylthio)phenyl]-amino}ethan-1-ol (24). To a solution of 4-amino-4′-nitrodiphenyl sulfide (**23**) (25.0 g, 102 mmol) in THF (200 mL) and AcOH (250 mL) was added 10% of ethylene oxide in THF (315 mL, 715 mmol) 45-mL portions over the following 7 days at room temperature. The mixture was then neutralized with Na_2CO_3/H_2O and extracted with EtOAc for three times. The EtOAc extract was washed with water, dried over anhydrous sodium sulfate, and then evaporated to remove EtOAc. The crude product was crystallized with EtOAc to give 19.5 g of yellow product **24**. The mother liquid was evaporated, and the residue was purified on a flash column using CH_2Cl_2/CH_3COCH_3 (8:2) as eluent, then recrystallized with EtOAc/hexane to obtain 7.8 g of **24** (80.4% total yield): mp 99.5−100.5 °C; TLC R_f 0.41 in CH_2Cl_2/CH_3COCH_3 (8:2); ^{1}H NMR ($CDCl_3$) δ 3.63−3.90 (m, 8H), 6.75 (d, J = 9 Hz, 2H), 7.00−7.45 (m, 4H), 8.00 (d, J = 9 Hz, 2H). Anal. ($C_{16}H_{18}N_2O_4S$) C, H, N, S.

Bis(2-chloroethyl)[4-(4-nitrophenylthio)phenyl]-amine (25) was prepared from **24** on a 29.9-mmol (10.0-g) scale as in the similar procedure for **14** to give 8.90 g of **25** (80.2% yield) as a yellow crystalline after crystallization with EtOAc/hexane: mp 112.5−113.5 °C; TLC R_f 0.51 in hexane/EtOAc (5:1); ^{1}H NMR ($CDCl_3$) δ 3.60−3.80 (m, 8H), 6.70 (d, J = 9.6 Hz, 2H), 6.95−7.45 (m, 4H), 8.00 (d, J = 9.6 Hz, 2H). Anal. ($C_{16}H_{16}Cl_2N_2O_2S$) C, H, N, Cl, S.

4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfinyl)-1-nitrobenzene (7) was prepared from **25** on a 36.4-mmol (13.5-g) scale as in the procedure for **3** to give 13.7 g (97.1% yield) of **7** as yellow crystalline after crystallization with EtOAc/hexane: mp 107−108 °C; TLC R_f 0.45 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.60−3.77 (m, 8H), 6.70 (d, J = 9.6 Hz, 2H), 7.25−7.80 (m, 4H), 8.30 (d, J = 9.6 Hz, 2H). Anal. ($C_{16}H_{16}Cl_2N_2O_3S$) C, H, Cl, N, S.

4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfonyl)-1-nitrobenzene (28). To a solution of **7** (1.00 g, 2.58 mmol) in 130 mL acetone and 13 mL of water was added a mixture of 25.8 mL 30% (wt) of hydrogen peroxide (252 mmol) and 3.9 mL 0.3 M of ammonium molybdate. The reaction mixture was stirred at room temperature for 5 h and evaporated in vacuo at 30 °C. The residue was partitioned between CH_2Cl_2 and water. The water layer was extracted with CH_2Cl_2. The organic extract was dried over anhydrous $MgSO_4$ and evaporated to give the residue which was recrystallized with EtOAc/hexane to give 1.00 g (96.0% yield) of **28** as light yellow crystals: mp 132.5−133.5 °C; TLC R_f 0.75 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.63−3.80 (m, 8H), 6.71 (d, J = 9.6 Hz, 2H), 7.70−8.40, (m, 6H). Anal. ($C_{16}H_{16}Cl_2N_2O_4S$) C, H, Cl, N, S.

4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfinyl)-phenylamine (8). Ion powder (19.1 g, 100 mesh) was activated by refluxing it with 1.5 mL of distilled water and 1 drop of concentrated hydrochloric acid for 0.5 h under vigorous stirring. Ethanol (68.4 mL) and **7** (13.7 g, 35.3 mmol) were added into the mixture at 40−50 °C. The reaction mixture was slowly heated until reflux, then vigorously stirred for 15 mim. Several drops of 10% NaOH was added to the reaction mixture to pH 8. The hot reaction mixture was then filtered, and the residue was washed with EtOH for 3 times. After evaporation of EtOH, the residue was diluted with water, and extracted with EtOAc for 3 times. The EtOAc extract was dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude was purified on a flash column using EtOAc/CH_2Cl_2 (6:4) as eluent, then recrystallized with EtOAc/hexane to give 9.09 g (72.1% yield) of **8** as an off-white crystalline: mp 136.5−138 °C; TLC R_f 0.49 in EtOAc; ^{1}H NMR ($CDCl_3$) δ 3.60−3.73 (m, 8H), 6.60−6.75 (m, 4H), 7.20−7.50 (m, 4H). Anal. ($C_{16}H_{18}N_2Cl_2OS$) C, H, Cl, N, S.

[4-(4-Aminophenylthio)phenyl]bis(2-chloroethyl)-amine (26) was prepared from **25** (2.00 g, 5.39 mmol) according to the procedure for **8** to give 1.17 g (63.8% yield) of

26 as a light brown solid: mp 77−79 °C; TLC R_f 0.64 in CH_2Cl_2/Me_2CO (49:1); ^{1}H NMR ($CDCl_3$) δ 3.45−3.75 (m, 8H), 6.50−6.70, (m, 4H), 7.10−7.30 (m, 4H). Anal. ($C_{16}H_{18}Cl_2N_2S$) C, H, Cl, N, S.

4-({4-[Bis(2-chloroethyl)amino]phenyl}sulfonyl)-phenylamine (29) was prepared from **28** (1.00 g, 2.48 mmol) according to the procedure for **8** to give 0.76 g (70.1% yield) of **29** as an off-white solid after flash column purification using CH_2Cl_2/EtOAc (95:5) as eluent: mp 153.5−155 °C; TLC R_f 0.41 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 2:1) δ 3.55−3.80 (m, 8H), 6.50−6.80 (m, 4H), 7.35−7.75 (m, 4H). Anal. ($C_{16}H_{18}Cl_2N_2O_2S$) C, H, Cl, N, S.

{4-[4-(Acridin-9-ylamino)phenylthio]phenyl}bis(2-chloroethyl)amine Hydrochloride (27). A solution of **26** (0.52 g, 1.53 mmol) and 9-chloroacridine[35] (0.30 g, 1.40 mmol) in *N*-methyl-2-pyrrolidinone (13.5 mL) was treated with a few drops of concentrated HCl and then stirred at room temperature for 1.5 h. The mixture was diluted with EtOAc, and the resulting precipitate was collected and washed with EtOAc to give a crude product. This crude product was mixed with 10 mL of *N*-methyl-2-pyrrolidinone and stirred for 1 h. Ethyl acetate (250 mL) was gradually added for complete precipitation, and the resulting precipitate was collected and washed with EtOAc to give 0.70 g (89.4% yield) of **27** as an orange solid: mp 261−262 °C dec; TLC R_f 0.73 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:4) δ 3.70−3.90 (m, 8H), 6.80 (d, J = 9 Hz, 2H), 7.20−7.60 (m, 8H), 7.95−8.35 (m, 6H). Anal. ($C_{29}H_{26}$ Cl_3N_3S) C, H, Cl, N, S.

4-{[4-(Acridin-9-ylamino)phenyl]sulfinyl}-1-[bis(2-chloroethyl)amino]benzene hydrochloride (9) was prepared from **8** (1.00 g, 2.81 mmol) and 9-chloroacridine (0.55 g, 2.57 mmol) according to the procedure for **27** to give 1.30 g (88.4% yield) of **9** as an orange solid: mp 250−251 °C dec; TLC R_f 0.81 in EtOAc; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:4) δ 3.70−3.85 (m, 8H), 6.88 (d, J = 10.2 Hz, 2H), 7.45−4.60 (m, 8H), 8.00−8.40 (m, 6H). Anal. ($C_{29}H_{26}Cl_3N_3OS$) C, H, Cl, N, S.

4-{[4-(Acridin-9-ylamino)phenyl]sulfonyl}-1-[bis(2-chloroethyl)amino]benzene hydrochloride (30) was prepared from **29** (0.38 g, 1.02 mmol) and 9-chloroacridine (0.20 g, 0.94 mmol) according to the procedure for **27** to give 0.52 g (94.7% yield) of **30** as an orange solid: mp 270.5−271.5 °C dec; TLC R_f 0.34 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 1:3) δ 3.65−3.90 (m, 8H), 6.88 (d, J = 8.4 Hz, 2H), 7.40−8.40 (m, 14H). Anal. ($C_{29}H_{26}Cl_3N_3O_2S$) C, H, Cl, N, S.

2-[Bis(2-hydroxyethyl)]aminodibenzothiophene (31) was prepared from 2-aminodibenzothiophene[36] (4.10 g, 20.5 mmol) and 2.8 equiv of 2-chloroethanol according to the procedure for **13** to give 6.00 g of **31** as the crude oil. This oil was used to synthesize **32** without further purification: TLC R_f 0.54 in EtOAc; ^{1}H NMR ($CDCl_3$/DMSO-d_6, 6:1) δ 3.43−4.10 (m, 8H), 6.75−8.30 (m, 7H).

2-[Bis(2-chloroethyl)amino]dibenzothiophene (32) was prepared from **31** on a 24.0-mmol (6.00-g) scale as in the procedure for **14** to give 5.80 g of a brownish solid. This crude product was recrystallized with CH_2Cl_2/hexane to give 3.50 g (45.5% yield from 2-aminodibenzothiophene) of **32** as a light brownish solid: mp 101−102 °C; TLC R_f 0.49 in EtOAc/hexane (1:10); ^{1}H NMR ($CDCl_3$/DMSO-d_6, 10:1) δ 3.40−4.05 (m, 8H), 6.60−8.20 (m, 7H). Anal. ($C_{16}H_{15}Cl_2NS$) C, H, Cl, N, S.

2-[Bis(2-chloroethyl)amino]dibenzothiophen-5-one (10) was prepared from **32** on a 3.04-mmol (1.00-g) scale in the procedure for **3** to give a brown oil which was purified on a flash column eluting with EtOAc/CH_2Cl_2 from ratio 1:20 to 1:4 to give 0.55 g (53.0% yield) of **10** as a brown solid: mp 170−172 °C dec; TLC R_f 0.31 in EtOAc/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 3.65−5.30 (m, 8H), 6.30−7.65 (m, 7H). Anal. ($C_{16}H_{15}$-Cl_2NOS) C, H, Cl, N, S.

2,8-Bis[bis(2-hydroxyethyl)amino]dibenzothiophene (33) was prepared from 2,8-diaminodibenzothiophene[36] (0.70 g, 3.25 mmol) and 30 equiv of 2-chloroethanol in the procedure for **13** to give 1.38 g of **33** as a dark brown thick oil. This oil was used to synthesize **34** without further purification: TLC R_f 0.26 in EtOAc/EtOH (9:1); ^{1}H NMR ($CDCl_3$) δ 2.90−5.05 (m, 16H), 6.73−8.22 (m, 6H).

2,8-Bis[bis(2-chloroethyl)amino]dibenzothiophene (34) was prepared from **33** on a 3.53-mmol (1.38-g) scale in the procedure for **14** to give 0.44 g (29.1% yield from 2,8-diaminodibenzothiophene) of **34** as a light brown solid: mp 160−163 °C dec; TLC R_f 0.58 in EtOAc/hexane (2:8); ^{1}H NMR ($CDCl_3$) δ 3.10−4.45 (m, 16H), 6.85−7.95 (m, 6H). Anal. ($C_{20}H_{22}Cl_4N_2S$) C, H, Cl, N, S.

2,8-Bis[bis(2-chloroethyl)amino]dibenzothiophen-5-one (11) 50−60% *m*-chloroperbenzoic acid (mCPBA) (0.29 g, 1.08 mmol) in 6 mL of CH_2Cl_2 was added dropwise to a solution of **34** (0.5 g, 1.08 mmol) in 8 mL of CH_2Cl_2 with stirring at 0 °C in an ice bath, and the reaction mixture was stirred for additional 10 min at 0 °C. The reaction solution was then washed sequentially with water (2 × 50 mL), saturated aqueous Na_2SO_3 (4 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and brine (2 × 50 mL). After drying over anhydrous Na_2SO_4, the solvent was removed to give 0.51 g of the crude product. The crude material was purified on a flash column using EtOAc/CH_2Cl_2 (1:9) as eluent to give 0.34 g (65.4% yield) of **11** as a light brown solid: mp 185−187 °C dec; TLC R_f 0.28 in EtOAc/CH_2Cl_2 (3:7); ^{1}H NMR ($CDCl_3$) δ 3.00−4.30 (m, 16H), 6.55−8.21 (m, 6H). Anal. ($C_{20}H_{22}Cl_4N_2OS$) C, H, Cl, N, S.

In Vitro Cytotoxicity Assessment. 1. Clonogenic Assay. The published procedure of Fracasso and Sartorelli[37] with some modifications was used. A confluent flask of Chinese hamster lung transformed V-79 transformed cells was scraped to collect the monolayer and the cells were counted by trypan blue dye exclusion. Glass milk dilution bottles were then seeded with 500 000 cells in 10 mL of medium (MEM supplemented with 10% fetal bovine serum, Pen G sodium, streptomycin sulfate, amphotericin, and essential and nonessential amino acids and vitamins) and incubated for 20 h in a 95% air/5% humidified atmosphere. One set of six bottles was sealed with sterilized rubber septa and fitted with 21-gauge needles for gas inflow and outflow. Each bottle was gassed individually, all outlet tubings were connected to individual flow meters (average flow rate = 0.15 L/min) with additional tubings immersed in water to allow visible monitoring of gas flow as well as to prevent back flow of air into the cultures. To produce hypoxia, cultures were gassed continuously for 2 h at 37 °C (maintained in a water bath) with a humidified mixture of 95% N_2/5% CO_2. Normally aerated cultures were maintained in air until drug treatment. At this time, appropriate dilutions of the drug in ethanol/water or DMF were added directly to the cultures, without breaking the hypoxia, by injecting 0.25 mL of drug through the rubber septa. Cells in the aerobic set were exposed to drug for 3 h under normal aerated conditions. The hypoxic set was gassed 10 min after injection of the drug, needles were pulled out, and the sealed bottles were incubated for 3 h in a normally aerated incubator. After drug exposure, the cells were washed twice with 5 mL sterile HBSS and treated with 2.5% trypsin (Gibco Labs) for 2−3 min. Cells were collected by centrifugation, resuspended in 5 mL fresh media, and counted by trypan blue dye exclusion. Appropriate dilutions were made, and 500 cells were plated in triplicate in a total of 10 mL medium. Dishes were incubated for 6−8 days to allow colony formation. Colonies were then rinsed twice with 0.9% saline, fixed with ethanol, stained with crystal violet and counted. Results are reported as the number of colonies surviving chemical treatment per number of colonies in the solvent-treated control. The IC_{50} or IC_{90} values were determined by semilogarithmically plotting the drug concentration versus cell viability as determined by the number of colonies surviving the treatments.

2. Sulforhodamine B (SRB) Assay. 100 μL of Chinese hamster lung V-79 cells (about 7 × 10^3 cells/100 μL) was preincubated for 24 h; 100 μL media, which contains variable concentrations of drugs, was added to each well and then the plate was incubated under the oxic conditions for 3 h. The cells were washed 5 times with HBSS and 200 μL of fresh media was added. The plate was incubated for 48 h. The subsequent TCA-fixation and SRB assay were done as reported[38] with a slight modification. The bound dye was solubilized with 10 mM Tris buffer for 5 min, and then 10-μL aliquots of the solution

were spotted on a TLC plate. Optical density (OD) was determined by the use of UNISCAN video densitometer with the visible light.

Determination of Chemical Reduction Potential by Polarography. The reduction potential of the sulfoxides compounds was determined by *differential pulse polarography*. Metrohm E626 polarecord and an E505 polarographic stand, which consisted of a dropping mercury electrode (cathode), a saturated calomelelectrode (SCE.), and a platinum wire as auxiliary electrode (anode), were used to measure all polarograms. The testing compounds (2 mM) and TBAB (tetrabutylammonium bromide; 0.1 M) as an electrolyte were dissolved in 50 mL of anhydrous DMF. The solution was purged with N_2 for 10 min and then the potential was scanned from −1.00 to −3.00 V. The peak potential was record on the polarogram, and each reduction potential value was based on the average of three readings. All the results were compared with 0.1 M of TBAB in DMF as a blank.

In Vitro Metabolism Study. The rat liver S-9 fraction was prepared essentially as previously reported.[20,39] The incubation was carried out in a 20-mL vial containing 2−4 mL of rat liver S-9 fraction, 5−10 μmol of testing compound (0.1 M in EtOH or DMF), cofactors (2 equiv for NADPH or NADH, 1 equiv for benzaldehyde, 2-hydroxypyrimidine, or acetaldehyde), and phosphate buffer to a final volume 3−6 mL. The boiled S-9 fraction and the S-9 fraction without adding any cofactors were used as controls. For the aldehyde inhibition study, the S-9 fraction was preincubated with 1 equiv menadione for 30 min under aerobic conditions prior adding testing compounds and benzaldehyde. Aerobic experiments were performed by leaving the vials open to the atmosphere during incubation. Anaerobic incubations were performed by purging the incubation vials with nitrogen for 10 min prior to the injecting the testing compounds. Incubations were done at 37 °C for 30 min in a Dubnoff metabolic shaking incubator.

HPLC or TLC densitometry was used to determine the metabolites. For HPLC determination, the incubation was terminated by addition of 2 mL EtOH. The supernatant was filtered through a syringe filter (0.2 μm, HT Tuffryn membrane) and directly used for HPLC. HPLC analysis was done on a Waters μ-Bondapak C-18 analytical column (3.9 × 150 mm) with isocratic elution (MeCN/H_2O, 10:25) and gradient system (MeCN/H_2O) at flow rate 2 mL/min. For TLC determination, the incubation was terminated by addition of 6 mL CH_2Cl_2. The resulting mixture was centrifuged (3000 rpm, 15 min), and the denatured protein precipitate was extracted with 5 mL of CH_2Cl_2 3 times. The combined supernatants were dried with anhydrous sodium sulfate and evaporated. The residue was then reconstituted with 0.5 mL of CH_3Cl. The metabolites were quantitatively determined on TLC plates (silica gel GF plates, 250 μm; Analtech) with a Uniscan Video densitometer (Analtech) under 254-nm UV light. The standard curves were obtained by using standard sulfides incubated with boiled S-9 fraction following the same experimental conditions as that for sulfoxides. The substrate activities are expressed as their corresponding sulfide metabolite formation/mg protein or percentage bioreduction.

Acknowledgment. We are grateful to Mr. K.-K. Min and Dr. J.-K. Kim of Medicinal Research Center, CKD Corp., Korea, for some of the cytotoxicity testing. This work was supported by the National Cancer Institute, NIH, under Grant CA 63618-01.

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JM9904957