Novel S-Substituted Aminoalkylamino Ethanethiols as Potential Antidotes against Sulfur Mustard Toxicity

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Sulfur mustard (SM) is a highly toxic chemical warfare agent. A satisfactory treatment regimen is not yet available for this toxicant. In a search for an effective antidote against SM, a series of novel *S*-2(*ω*-aminoalkylamino)ethyl alkyl/aryl thioethers [$H_2N(CH_2)_nNHCH_2CH_2SR$], where R = alky, alicyclic, aryl, and heterocyclic substituents, have been designed and synthesized as candidate antidotes against SM toxicity. These compounds were screened for their protective efficacy through the oral route against dermally applied sulfur mustard in female mice measured on the basis of percent survival following percutaneous administration of SM. A number of compounds demonstrated significant protection.

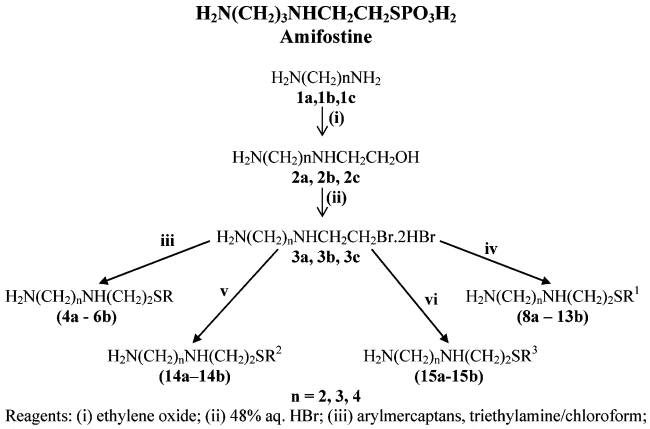
Introduction

Bis(2-chloroethyl)sulfide, commonly known as sulfur mustard (SM) or mustard gas, is an alkylating agent that causes serious blisters upon contact with human skin. SM is a frequently used chemical warfare agent, and several reports are available of its recent use.[1−4] SM forms the sulfonium ion in the body and alkylates DNA, leading to DNA strand breaks and cell death.[5] Eyes, skin, and the respiratory tract are the principal target organs of SM toxicity.[5−7] Several compounds have been evaluated for reducing the systemic toxicity of SM but with limited protection.[8−20] So far, no accepted antidote is available for SM and the treatment is symptomatic.

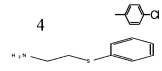
The fact that symptoms of SM exposure are similar to those caused by radiation[21−23] led us to investigate the protection offered by radioprotectors against SM. Amifostine or WR-2721 [*S*-2(3-(aminopropyl)amino)-ethylphosphorothioate] (Scheme 1), is a potent radioprotector. This compound has also been found useful in cancer treatment where it protects the normal cells against the chemotherapeutic agent including nitrogen mustard.[24−30] We reasoned, therefore, that amifostine might be a promising antidote against SM also. Amifostine and two of its homologues, which are essentially the S-substituted derivative of aminoalkylamino ethanethiol, were synthesized and evaluated against SM toxicity. Initial screening of these compounds showed that amifostine is impressively active by ip and oral routes,[31,32] though the protection was marginally less by the oral route. These observations encouraged us to synthesize a series of S-substituted aminoalkylamino ethanethiols as potential antidotes against sulfur mustard toxicity and to undertake their evaluation.

It is well-known that the presence of a phosphoric group reduces lipophilicity and therefore limits bioavailability of a compound. In view of this fact, we explored the possibility of other types of S-substituted aminoalkylamino ethanethiols than phosphorothioates as potential antidotes against SM. A survey of the literature revealed that most of the studies on these compounds are with reference to radioprotection. A number of compounds structurally related to aminoalkylamino ethanethiol have been synthesized but limited by variation at S substitution. The types that have received main attention and accounted for much method development are phosphorothioates, thiosulfates, and oc-

Scheme 1. General Scheme for the Synthesis of Thioethers



Reagents: (i) ethylene oxide; (ii) 48% aq. HBr; (iii) arylmercaptans, triethylamine/chloroform; (iv) alkylmercaptans, NaOMe/benzene; (v) 4-mercaptopyridine,NaOH/water; (vi) thionicotinamide, ethanol.

Compound	*n*	*R/R¹/R²/R³*	*Compound*	*n*	*R/R¹/R²/R³*
4a	2		10a	2	-n-C_3H_7
4b	3		10b	3	-n-C_3H_7
4c	4		11a	2	-i-C_3H_7
5a	2		11b	3	-i-C_3H_7
5b	3		12a	2	-n- C_4H_9
5c	4		12b	3	-i- C_4H_9
6a	2		13a	2	-Cyclohexyl
6b	3		13b	3	-Cyclohexyl
6c	4		14a	2	
7			14b	3	
8a	2	-CH_3	15a	2	
8b	3	-CH_3			
9a	2	-C_2H_5	15b	3	
9b	3	-C_2H_5			

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casionally isothiourium derivatives and disulfides.[33−37] For effective modification of the compound, we envisaged thioethers as a target structure having an aminoalkylamino ethane backbone and focused our attention on varying the substitution at sulfur with a wide range of functional groups. The rationale behind the synthesis of these compounds was to develop new compounds with enhanced lipophilicity, sharing some properties of aminoalkyamino ethanethiols.

The toxicity of SM has been attributed to the reactivity of the intermediate sulfonium ion with various cell constituents.[5] SM is reported to have the potential to react with a number of compounds that are present in cells and tissues, such as thiols, carboxylic groups of proteins, aromatic nitrogen atoms (e.g., pyridine and imidazol), amino groups of amino acids, peptides, purines, pyrimidines, and sulfide sulfur (e.g., methionine). Any reagent having an affinity for the sulfonium ion can be expected to help prevent sulfur mustard injury. The various groups introduced at the thiol moiety of aminoalkylamino ethanethiol were guided by these observations and selected on the basis of reported SM chemistry. Mainly, two types of groups were selected: one that will simply influence the lipophilicity and the other that will have potential to contribute toward the desired activity by reacting with SM in addition to the modification in lipophilicity.

It has been observed that an increase in alkyl chain length increased the lipophilicity of the molecule, and hence, compounds with a gradual increase in carbon chain length at the sulfur atom were synthesized. For bioisosteric replacement of the phosphoric moiety, cyclohexyl, phenyl, and substituted phenyl groups were selected.[38] Furthermore, since SM has affinity for a ring nitrogen, S-heterocyclic substituted compounds with pyridine and nicotinamide moieties were also synthsised. In this paper we report the synthesis of a series of aminoalkylamino ethane thioethers and their evaluation against SM.

Chemistry

Thioethers and sulfides can be synthesized by a halide mercaptan reaction. Various methods have been reported in the literature.[39−43] These methods center on a common scheme in which a halide and mercaptan are allowed to react in the presence of a suitable base in different reaction media at varying temperatures. First, we took aminoalkylamino ethanethiol as a substrate and treated it with the halide of the required moiety. But this approach was abandoned because formation of disulfide and polymerization thwarted the preparation of the desired thioether. Then we investigated the alternative approach, i.e., reacting aminoalkylamino ethyl halide with the thiol of the required moiety, and finally compounds were synthesized by this method (Scheme 1).

We selected aminoalkylamino ethyl bromides (**3a**−**c)** as the key intermediates to synthesize all the target compounds because bromides were reported to be the most effective for the synthesis of thioethers and sulfides. These bromides were obtained by the method reported by Piper et al.[33] In the first step, diaminoalkanes (**1a**−**c**) were reacted with ethylene oxide to give the corresponding alcohols (**2a**−**c**). These alcohols on treatment with 48% aqueous HBr afforded the respective bromides in the form of the dihydrobromide salt as illustrated in Scheme 1.

Arylthioethers **4a**−**6c** were prepared by reacting bromides **3a**−**c** with aryl mercaptans using triethylamine as a base. This reaction afforded clean products in quantitative yields, and the compounds resulting from this reaction were conveniently purified further by crystallization. Compound **7** was obtained by reacting aminoethyl bromide with thiophenol using triethylamine as base.

This synthetic pathway, however, was applicable only to aromatic thioethers. Alkyl and cyclohexyl thioethers could not be obtained by this method. Compounds **8a**−**13b** were prepared by reacting the bromide salt with the corresponding thiols in the presence of sodium methoxide as base. Sodium methoxide was generated in situ by adding methanol to the reaction mixture containing sodium metal. During this reaction, a small amount of oxy ether was also formed as a byproduct, which was separated from the thioether by column chromatography. To increase the lipophilicity, we changed the groups from methyl to cyclohexyl.

The synthesis of 4-pyridyl thioethers **14a**,**b** was achieved by the reaction of the corresponding bromide with mercaptopyridine in the presence of NaOH as a base. Nicotinamide derivatives (**15a**,**b)** were prepared by heating bromides (**3a**−**c)** with thionicotinamide in ethylene glycol.

Biology

All the compounds synthesized as potential antidotes to SM toxicity were screened for their protective efficacy by oral route. To select the appropriate dose for protection, the LD_{50} values through the oral route for all the compounds were determined (Table 1).

To establish the preferred time of administration of drug, the compounds were administered as pretreatment (30 min), simultaneous treatment (0 min), and posttreatment (30 min). After the preliminary experiments, it was observed that 0.2 LD_{50} of the compound **4a** given 30 min prior to SM application yielded the maximum protection. The other compounds were given orally at a molar equivalent dose of **4a**. The results of the protective efficacies of the compounds at 30 min pretreatment are summarized in Table 1.

Results and Discussion

The results of evaluation of the compounds through the oral route in vivo are summarized in Table 1. It is evident from the table that most of the compounds have given protection against SM. Bioisosteric replacement of the phosphoric moiety of amifostine by phenyl and cyclohexyl groups proved to be quite useful; these compounds gave better protection than amifostine. The introduction of substitution at the para position of the phenyl ring gave interesting results. An increase in activity was observed for the tolyl derivatives (**4a**, **4b** → **5a**, **5b**). In the case of chlorophenyl derivatives, however, the activity remained the same for the aminoethylamino analogue (**4a** → **6a**) but changed drastically for the (aminopropyl)amino analogue **(4b** → **6b).** To find out the importance of the aminoalkylamino ethane backbone, we prepared aminoethylphenyl sulfide

Table 1. Protective Efficacy of S-Substituted Aminoalkylamino Ethane Thiols through Oral Route against SM (38.7 mg/kg Percutaneously)

compd	analogue	LD_{50} (mg/kg) (oral route)	% survival after 7 days	% survival after 14 days
	S-2-(3-(aminopropylamino)ethyl phosphorothioate (amifostine)	1049	75	25
4a	*S*-2(2-aminoethylamino)ethyl phenyl sulfide dihydrochloride	1247	100	75
4b	*S*-2(3-(aminopropylamino)ethyl phenyl sulfide dihydrochloride	1345	75	0
4c	*S*-2(4-aminobutylamino)ethyl phenyl sulfide dihydrochloride	952	100	0
5a	*S*-2(2-aminoethylamino)ethyl tolyl sulfide dihydrochloride	1902	100	100
5b	*S*-2(3-(aminopropylamino)ethyl tolyl sulfide dihydrochloride	1131	100	75
6a	*S*-2(2-aminethylamino)ethyl chlorophenyl sulfide dihydrochloride	1131	100	75
6b	*S*-2(3-aminpropylamino)ethyl chlorophenyl sulfide dihydrochloride	1588	100	75
7	aminoethylphenyl sulfide hydrochloride	2262	100	25
8a	*S*-2(2-aminoethylamino)ethylmethyl sulfide dihydrochloride	4525	100	0
8b	*S*-2(3-(aminopropylamino)ethyl methyl sulfide dihydrochloride	3200	50	0
9a	*S*-2(2-aminoethylamino)ethyl ethyl sulfide dihydrochloride	3200	100	50
9b	*S*-2(3-(aminopropylamino)ethyl ethyl sulfide dihydrochloride	4525	100	25
10a	*S*-2(2-aminoethylamino)ethyl propyl sulfide dihydrochloride	4525	100	75
10b	*S*-2(3-aminopropylamino)ethyl propyl sulfide dihydrochloride	2263	75	0
11a	*S*-2(2-aminoethylamino)ethyl isopropyl sulfide dihydrochloride	4525	100	50
11b	*S*-2(3-(aminopropylamino)ethyl isopropyl sulfide dihydrochloride	2263	100	25
12a	*S*-2(2-aminoethylamino)ethyl butyl sulfide dihydrochloride	4525	100	75
12b	*S*-2(3-(aminopropylamino)ethyl butyl sulfide dihydrochloride			
13a	*S*-2(2-aminoethylamino)ethyl cyclohexyl sulfide dihydrochloride	1131	100	100
13b	*S*-2(3-(aminopropylamino)ethyl cyclohexyl sulfide dihydrochloride	1131	100	75
14a	*S*-2(2-aminoethylamino)ethyl 4-pyridyl sulfide dihydrochloride	4525	100	50
14b	*S*-2(3-(aminopropylamino)ethyl 4-pyridyl sulfide dihydrochloride			
15a	*S*-2(2-aminoethylamino)ethyl isothionicotinamide dihydrobromide	4525	100	0
15b	*S*-2(3-(aminopropylamino)ethyl isothionicotinamide dihydrobromide	4525	50	0
	SM only		50	0

(**7**) by removing the aminoalkylamino part of the phenyl analogue. The resultant compound **7** lost its activity, indicating that this backbone was essential but not sufficient for activity. Initially, three homologues (n = 2, 3, 4) were prepared for each type of derivative. The screening of these compounds, however, showed a general trend where ethyl homologues ($n = 2$) were the most effective and the least toxic followed by propyl and butyl homologues. Hence, for subsequent compounds n was restricted to 2 and 3 only.

Attempts were also made to see the effects of increased lipophilicity of the compounds, by gradually increasing the chain length of the alkyl groups at sulfur, on the protective efficacy. Except for the methyl and ethyl analogues, all the alkyl analogues were effective. The lack of efficacy of methyl and ethyl analogues may be due to the poor pharmacokinetic profile originating from the lower lipophilicity of methyl and ethyl groups. Among the propyl and isopropyl analogues, propyl was more effective. Installation of the heterocyclic moiety was not useful because heterocyclic analogues were significantly less effective.

It is well-known that SM causes toxicity by reacting with important biomolecules that are present in cells and tissues.[5] Critical reactions of SM are complete within minutes of absorption through the formation of an intermediate sulfonium ion. The adduct formed with SM is quite stable under physiological conditions, and hence, reversal of reaction is very difficult (i.e., regeneration of biomolecules causing the reversal of injury is difficult).

A compound that prevents SM toxicity by preferentially binding with it and thus removing SM from the site of action is called a scavenger. This suggests that for a scavenger to prevent SM toxicity, it should be present in the critical target organs before SM. This condition can be achieved by two means: one is the administration of the scavenger before SM so that it has sufficient time to reach cells and tissue; the other requires that the rate of distribution of the compound be fast enough to reach the critical target organs before SM. The second requirement is difficult to achieve even if the drug is given simultaneously. Most probably, the compounds tested in these studies are acting as scavengers because they are able to reduce the SM toxicity only when given prior to SM application. The simultaneous administration is not providing any protective effect because it is not able to reach the target organs before SM, and once SM has caused injury, the compound is not of much use even if it is present in high concentrations.

Conclusion

Novel S-substituted aminoalkylamino ethane thiols have been made and evaluated. These studies clearly demonstrate that most of the compounds exhibited good to excellent in vivo efficacy against SM. In particular, compounds **4a**, **5a**, **5b**, **6a**, **6b**, **10a**, **12a**, **13a**, and **13b** are the most effective. These compounds are being pursued further for detailed investigations. In depth pharmacological studies of the most effective compound and antidotal efficacy of other compounds will be reported in due course.

Experimental Section

General Chemistry Methods. All reactions described below were performed using laboratory grade materials and solvents under a dry atmosphere. All solvents were distilled prior to use or stored over molecular sieves. Melting points were determined in an open capillary and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 1700 FTIR spectrophotometer in KBr. The NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrophotometer. Chemical shifts are expressed as δ values (ppm) relative to TMS as the internal standard for ^{1}H NMR. The mass spectral

analysis was performed on a TSQ 7000 mass spectrometer. In most of the cases, the compounds were identified by recording their pseudomolecular ion $(M + H)^+$ under electrospray ionization (ESI) because, being salts, most compounds could not be analyzed by the conventional electron impact (EI) mass spectrometry. Electrospray MS analysis was performed using methanol−water (50:50) with 1% acetic acid. Nitrogen was used as a sheath gas, and the ESI needle was held at 4.5 eV.

***S*-2(*ω*-Aminoalkylamino)ethanol (2a−c).** These compounds were prepared according to the literature procedure.[33]

***S*-2(*ω*-Aminoalkylamino)ethyl Bromide Dihydrobromide (3a−c).** These compounds were prepared according to the literature procedure.[33]

Synthesis of *S*-2(*ω*-Aminoalkylamino)ethyl Aryl Sulfide Dihydrochloride. General Procedure. Thiophenol (0.028 mol) and triethylamine (12 mL) were taken in chloroform at 0 °C, and aminoalkylamino ethyl bromide dihydrobromide (0.025 mol) was added to it in portions. The reaction mixture was stirred at 0−5 °C for 3 h and then refrigerated overnight. The compound was washed with water and extracted with chloroform. The solvent was removed under reduced pressure, and the contents were washed with chilled petroleum ether (40−60 °C) (50 mL) followed by chilled ether (20 mL). The compound thus obtained was dissolved in ethanol and treated with HCl to get a white crystalline hydrochloride salt of the compound. The resulting crystals were collected by filtration. The compound was purified by recrystallization from ethanolic solution by the addition of acetone.

***S*-2(2-Aminoethylamino)ethyl Phenyl Sulfide Dihydrochloride (4a).** Yield 72%, mp 192−194 °C. IR: 1438, 1025, 807, 736.7, 689 cm^{-1}. ^1H NMR (CD_3OD): δ 3.33 (8H, m), 7.24−7.56 (5H, m). MS (ESI *m/z*): 197 $(M + H)^{+\bullet}$. Anal. ($C_{10}H_{16}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Phenyl Sulfide Dihydrochloride (4b).** Yield 75%, mp 248−250 °C. IR: 1438, 1039, 1025, 899, 737, 689 cm^{-1}. ^1H NMR (CD_3OD): δ 2.22 (2H, m), 3.08 (2H, t) 3.19−3.34 (6H, m), 7.24−7.51 (5H, m). MS (ESI *m/z*): 211 $(M + H)^{+\bullet}$. Anal. ($C_{11}H_{18}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(4-Aminobutylamino)ethyl Phenyl Sulfide Dihydrochloride (4c).** Yield 75%, mp 243−245 °C. IR: 1481, 1438, 1026, 738, 689 cm^{-1}. ^1H NMR (CD_3OD): δ 1.76 (4H, m), 2.59 (2H, t), 3.30−3.42 (6H, m), 7.22−7.49 (5H, m). MS (ESI *m/z*): 225 $(M + H)^{+\bullet}$. Anal. ($C_{12}H_{20}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl 4-Tolyl Sulfide Dihydrochloride (5a).** Yield 70%, mp 200−204 °C. IR: 1494, 1088, 1027, 806 cm^{-1}. ^1H NMR (CD_3OD): δ 2.20 (3H, s), 3.20 (2H, t, $J = 7$ Hz), 3.39 (6H, m), 7.13 (2H, d), 7.32 (2H, d). MS (ESI *m/z*): 211 $(M + H)^{+\bullet}$. Anal. ($C_{11}H_{18}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl 4-Tolyl Sulfide Dihydrochloride (5b).** Yield 72%, mp 236−38 °C. IR: 1496, 1166, 1087, 985, 817, 784 cm^{-1}. ^1H NMR (CD_3OD): δ 1.91 (2H, m), 2.23 (3H, s), 2.89 (2H, t, $J = 7$ Hz), 2.96−3.33 (6H, m), 7.12-(2H, d), 7.32 (2H, d). MS (ESI *m/z*): 225 $(M + H)^{+\bullet}$. Anal. ($C_{12}H_{20}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(4-Aminobutylamino)ethyl 4-Tolyl Sulfide Dihydrochloride (5c).** Yield 74%, mp 246−47 °C IR: 1493, 1459, 1052, 1034, 804 cm^{-1}. ^1H NMR (CD_3OD): δ 1.70 (4H, m), 2.22 (3H, s), 2.89 (2H, t), 2.94−3.29 (6H, m), 7.14 (2H, d), 7.24 (2H, d). MS (ESI *m/z*): 239 $(M + H)^{+\bullet}$. Anal. ($C_{13}H_{22}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl 4-Chlorophenyl Sulfide Dihydrochloride (6a).** Yield 70%, mp 238−39 °C. IR: 1478, 1092, 1006, 815 cm^{-1}. ^1H NMR (CD_3OD): δ 3.1 (2H, t, $J = 7$ Hz), 3.20−3.26 (6H, m), 7.28 (2H, d), 7.39 (2H, d). MS (ESI *m/z*) 231/233 $(M + H)^{+\bullet}$. Anal. ($C_{10}H_{15}ClN_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl 4-Chlorophenyl Sulfide Dihydrochloride (6b).** Yield 70%, mp 261−62 °C. IR: 1477, 1096, 1012, 808 cm^{-1}. ^1H NMR (CD_3OD): δ 2.21 (2H, m), 2.89 (2H,t), 3.12−3.26 (6H, m), 7.27 (2H, d)-7.40 (2H, d). MS (ESI *m/z*): 245/247 $(M + H)^{+\bullet}$. Anal. ($C_{11}H_{17}ClN_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(4-Aminobutylamino)ethyl 4-Chlorophenyl Sulfide Dihydrochloride (6c).** Yield 70%, mp 274−275 °C. IR: 1476, 1094, 1013,871 cm^{-1}. ^1H NMR (CD_3OD): δ 1.86 (4H, t), 2.95 (2H, t), 2.99−3.33 (6H, m), 7.26 (2H, d), 7.38 (2H, d). MS (ESI *m/z*): 259/261 $(M + H)^{+\bullet}$. Anal. ($C_{12}H_{19}ClN_2S\cdot 2HCl\cdot H_2O$) C, H, N.

Synthesis of Aminoethylphenyl Sulfide Hydrochloride (7). Compound **7** was prepared by the same procedure as described for **4a** using aminoethyl bromide in place of **3a**. Yield 73%, mp 100−102 °C. IR: 1471, 1094, 754, 696 cm^{-1}. ^1H NMR (CD_3OD): δ 3.12 (2H, t), 3.23 (2H, t), 3.30 (3H, m), 7.29 (1H, t), 7.38 (2H, t), 7.49 (2H, d). MS (ESI *m/z*): 154 $(M + H)^{+\bullet}$. Anal. ($C_8H_{11}NS.HCl\cdot H_2O$) C, H, N.

Synthesis of *S*-2(*ω*-Aminoalkylamino)ethyl Alkyl Sulfide Dihydrochloride. General Procedure. Aminoalkylaminoethyl bromide hydrobromide (0.025 mol) and alkane thiols (0.027 mol) was taken in benzene (50 mL) in a two-necked round-bottom flask equipped with a magnetic stirring bar, calcium chloride guard tube, and an ice-filled cooling bath. For methyl analogues, sodium methanethiolate was used. Sodium metal (2 g) was added to the flask followed by the addition of anhydrous methanol (10 mL) dropwise through a pressure-equalizing dropping funnel. The mixture was stirred continuously at 0−5 °C. The progress of the reaction was monitored by TLC. After the completion of the reaction, ether (50 mL) was added to it. The white precipitate, which was mostly inorganic salt, was removed by filtration under reduced pressure, and the solid was further washed with an ether−chloroform mixture (80:20). The combined filtrate was evaporated to dryness on a water bath. The residue was treated with methanolic HCl, and acetone was added to it until the precipitation was complete. The white salt thus obtained was filtered and dried. The salt was then dissolved in 10 mL of water, and sodium hydroxide (3 g) was added to it for converting the hydrochloride salt into the free amine. The amine was then extracted with ether and again converted into the HCl salt as described above. This process was repeated until it gave a single spot from TLC.

***S*-2(2-Aminoethylamino)ethyl Methyl Sulfide Dihydrochloride (8a).** Yield 50%, mp 196 °C. IR: 1458, 1025, 816 cm^{-1}. ^1H NMR (CD_3OD): δ 2.19 (3H, s), 2.80 (2H, t), 3.42 (9H, m). MS (CI *m/z*): 135 $(M + H)^{+\bullet}$. Anal. ($C_5H_{14}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Methyl Sulfide Dihydrochloride (8b).** Yield 45%, mp 252 °C. IR: 1449, 1174, 1050, 990, 902, 784 cm^{-1}. ^1H NMR (CD_3OD): δ 2.11 (2H, m), 2.84 (2H, t), 3.30 (3H,m) 3.06 (2H, t), 3.16 (2H, t), 3.27 (2H, t). MS (CI *m/z*): 149 $(M + H)^{+\bullet}$. Anal. ($C_6H_{16}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl Ethyl Sulfide Dihydrochloride (9a).** Yield 52%, mp 194−196 °C. IR: 1460, 1022, 980, 814 cm^{-1}. ^1H NMR (CD_3OD): δ 1.10 (3H, t), 2.65 (2H, m), 2.90 (2H, t), 3.35 (9H, m). MS (CI *m/z*): 149 $(M + H)^{+\bullet}$. Anal. ($C_6H_{16}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Ethyl Sulfide Dihydrochloride (9b).** Yield 50%, mp 256−258 °C. IR: 1461, 1173, 991, 908, 785 cm^{-1}. ^1H NMR (CD_3OD): δ 1.28 (3H, t), 2.22 (2H, m), 2.60 (2H, m), 2.88 (2H, t), 3.08 (2H, t), 3.12 (2H, t), 3.23 (2H,t), 3.34 (3H, m). MS (CI *m/z*): 163 $(M + H)^{+\bullet}$. Anal. ($C_7H_{18}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl Propyl Sulfide Dihydrochloride (10a).** Yield 60%, mp 196−198 °C. IR: 1459, 1377, 1924, 1026, 811 cm^{-1}. ^1H NMR (CD_3OD): δ 1.10 (3H, t), 1.67 (2H, m), 2.60 (2H, t), 2.88 (2H, t), 3.29−3.42 (9H, m). MS (CI *m/z*): 163 $(M + H)^{+\bullet}$. Anal. ($C_7H_{18}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Propyl Sulfide Dihydrochloride (10b).** Yield 60%, mp >160 °C (dec). IR: 1455, 1400, 11293, 1050, 992, 109, 779 cm^{-1}. ^1H NMR (CD_3OD): δ 1.03 (3H, t), 1.62 (2H, m), 2.12 (2H, m), 2.56 (2H, t), 2.89 (2H, t), 3.18 (2H, t), 3.20 (2H, t), 3.25 (2H, t), 3.34 (3H, m). MS (CI *m/z*): 177 $(M + H)^{+\bullet}$. Anal. ($C_8H_{20}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl Isopropyl Sulfide Dihydrochloride (11a).** Yield 52%, mp 192−194 °C. IR: 1461,

1082, 1026, 810 cm^{-1}. ^1H NMR (CD_3OD): δ 1.21 (6H, d), 2.94 (2H, t), 3.10 (1H, m), 3.31−3.46 (9H, m). MS (ESI *m/z*): 163 $(M + H)^{+\bullet}$. Anal. ($C_7H_{18}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Isopropyl Sulfide Dihydrochloride (11b).** Yield 39%, mp 259−262 °C (dec). IR: 1457, 1398, 1154, 1050, 992 cm^{-1}. ^1H NMR (CD_3OD): δ 1.21 (6H, d), 2.22 (2H, m), 2.88 (2H, t), 3.08 (1H, m), 3.17 (2H, t), 3.23 (2H, t), 3.29 (2H, t), 3.32 (3H, m). MS (ESI *m/z*): 177 $(M + H)^{+\bullet}$. Anal. ($C_8H_{20}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl Butyl Sulfide Dihydrochloride (12a).** Yield 48%, mp 194−195 °C. IR: 1463, 1029, 812, 744 cm^{-1}. ^1H NMR (CD_3OD): δ 0.84 (3H, t), 1.38 (2H, m), 1.53 (2H, m), 2.53 (2H, t), 2.82 (2H, t), 3.22 (6H, m), 3.35 (3H, m). MS (CI *m/z*): 177 $(M + H)^{+\bullet}$. Anal. ($C_8H_{20}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Butyl Sulfide Dihydrochloride (12b).** Yield 52%, mp >262 °C (dec). IR: 1466, 1029, 813, 746 cm^{-1}. ^1H NMR (CD_3OD): δ 0.82 (3H, t), 1.22−1.34 (2H, t), 1.52 (2H, m), 2.03 (2H, m), 2.55 (2H, t), 2.76 (2H, t), 3.06 (2H, t), 3.12 (2H, t), 3.16 (2H, t), 3.28 (3H, m). MS (CI *m/z*): 191 $(M + H)^{+\bullet}$. Anal. ($C_9H_{22}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(2-Aminoethylamino)ethyl Cyclohexyl Sulfide Dihydrochloride (13a).** Yield 41%, mp 193−195 °C. IR: 1452, 1344, 1032, 956, 762 cm^{-1}. ^1H NMR (CD_3OD): δ 1.32−1.47 (2H, m), 1.32−1.47 (2H, m), 2.08 (4H, m), 2.62 (1H, m), 2.93 (2H, t), 3.39 (6H, m), 3.42 (3H, m). MS (CI *m/z*) 203 $(M + H)^{+\bullet}$. Anal. ($C_{10}H_{22}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Cyclohexyl Sulfide Dihydrochloride (13b).** Yield 40%, mp >255 °C (dec). IR: 1455, 1000, 763 cm^{-1}. ^1H NMR (CD_3OD): δ 1.33 (2H, m), 1.52−1.73 (4H, m), 2.06 (4H, m), 2.16 (2H, m), 2.82 (1H, m), 2.91 (2H, t), 3.06 (2H, t), 3.17 (2H, t), 3.28 (2H, t), 3.31 (3H, m). MS (CI *m/z*): 217 $(M + H)^{+\bullet}$. Anal. ($C_{11}H_{24}N_2S\cdot 2HCl\cdot H_2O$) C, H, N.

Synthesis of *S*-2-(*ω*-Aminoalkylamino)ethyl 4-Pyridyl Sulfide Trihydrochloride. General Procedure. 4-Mercaptopyridine (25 mmol) and aminoalkylaminoethyl bromide dihydrobromide (25 mmol) were mixed together in a flask. A solution of NaOH (75 mmol) dissolved in 10 mL of water was added. The reaction starts immediately. The reaction mixture was heated at 90−95 °C for 10 min. The compound was extracted with chloroform. After solvent removal, the residue was dissolved in ethanol and treated with concentrated HCl. The hydrochloride salt formed was further purified by recrystallization from ethanol−acetone.

***S*-2(2-Aminoethylamino)ethyl 4-Pyridyl Sulfide Trihydrochloride (14a).** Yield 42%, mp 205−206 °C. IR: 1479, 1218, 1106, 828, 780 cm^{-1}. ^1H NMR (CD_3OD): δ 3.29 (2H, m), 3.35 (2H, m), 3.42 (2H, m), 3.57 (2H, m), 7.73 (2H, m), 8.38 (2H, m). MS (ESI *m/z*): 198 $(M + H)^{+\bullet}$. Anal. ($C_9H_{15}N_3S\cdot 3HCl\cdot H_2O$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl 4-Pyridyl Sulfide Trihydrochloride (14b).** Yield 42%, mp 245−260 °C. IR: 1468, 1201, 1170, 916, 785 cm^{-1}. ^{1}HNMR (CD_3OD): δ 1.83 (2H, m), 3.33 (2H, m), 3.39 (2H, m), 3.52 (2H, m), 3.64 (2H, m), 7.75 (2H, m), 8.39 (2H, m). MS (ESI *m/z*): 212 $(M + H)^{+\bullet}$. Anal. ($C_{10}H_{17}N_3S\cdot 3HCl\cdot H_2O$) C, H, N.

Synthesis of *S*-2(*ω*-Aminoalkylamino)ethyl Isothionicotinium Sulfide Trihydrobromide. General Procedure. Aminoalkylamino ethyl bromide (0.025 mol) and thionicotinamide (0.025 mol) were placed in ethylene glycol (15 mL) in a flask. The reaction mixture was heated on water bath for half an hour. Acetone was added to precipitate the compound. The isothionicotinium salt thus formed settled as a solvated syrup at the bottom of the flask. This solvated syrup was then stirred with methanol when it solidified gradually into a yellow solid, which was purified by crystallization.

***S*-2(2-Aminoethylamino)ethyl Isothionicotinium Sulfide Trihydrobromide (15a).** Yield 62%, mp 239−240 °C. IR: 1451, 1399, 1190, 980, 946, 678 cm^{-1}. ^1H NMR (CD_3OD): δ 3.41 (2H, t), 3.55 (1H, t), 3.96 (2H, t), 5.18 (2H, t), 8.22 (1H, t), 8.91 (1H, t), 9.24 (1H, d), 9.36 (1H, d). MS (ESI *m/z*): 225 $(M + H)^{+\bullet}$. Anal. ($C_{10}H_{16}N_4S\cdot 3HBr$) C, H, N.

***S*-2(3-(Aminopropylamino)ethyl Isothionicotinium Sulfide Trihydrobromide (15b).** Yield 60%, mp 221−222 °C. IR: 1474, 1208, 824, 673 cm^{-1}. ^1H NMR (CD_3OD): δ 2.20 (2H, m), 3.12 (2H, t), 3.44 (2H, t), 3.93 (2H, t), 5.19 (2H, t), 8.21 (1H, t), 8.90 (1H, d), 9.31 (1H, d), 9.72 (1H, s). MS (ESI *m/z*): 239 $(M + H)^{+\bullet}$. Anal. ($C_{11}H_{18}N_4S\cdot 3HBr$) C, H, N.

General Biology Methods. Randomly bred adult female Swiss mice obtained from the animal house of Defence Research & Development Establishment, weighing between 25 and 28 g body weight, were used in the present study. The Institute's Ethical Committee approval was obtained for these studies. The animals were housed in polypropylene cages (five per cage) with dust-free husk as bedding material and were provided with food (Lipton India Ltd.) and water ad libitum. Freshly prepared solutions of the compounds were administered orally for the estimation of LD_{50}. The compound was administered as a single oral dose, and the animals were observed for mortality for a period of 14 days. The LD_{50} was estimated by the moving average method of Gad and Weil using three to four log doses, consisting of four animals per dose.[44]

After the preliminary experiments, it was observed that 0.2 LD_{50} of compound **4a** given 30 min prior to SM application yielded the maximum protection. The other compounds were given orally as a molar dose of **4a**.

For the protection studies, 38.7 mg/kg SM (LD_{50} of SM is 8.1 mg/kg by percutaneous route)[32] dissolved in 3 N poly(ethylene glycol) was applied topically on the back of the mice ($n = 4$ for each test compound) after closely clipping the hair. The dose of 38.7 mg/kg was selected (which is more than 4-fold LD_{50}) such that the unprotected animals died in 8−10 days after percutaneous administration of SM. A freshly prepared solution of the test compounds in distilled water was administered orally 30 min before SM application (using an autopipet), and the animals were observed for 14 days for mortality. In the mouse model, the animals died before 14 days after SM administration and no death occurred as a result of SM beyond this. Hence, the percent mortality was calculated at 7 and 14 days after SM administration.

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