Anal.—Calcd. for $C_{22}H_{41}N_3O_3S_6$: C, 46.20; H, 7.23; N, 7.35; S, 33.64. Found: C, 45.97; H, 7.05; N, 7.05; S, 33.62.

Bis(2-amino-3-cyano-4-methyl-5-thienyl)sulfide—A mixture of 58 g. (1 mole) of acetone, 13.2 g. (0.2 mole) of malononitrile, and 6.4 g. (0.2 g. atom) of sulfur was treated dropwise with 20 ml. of triethylamine. The mixture was stirred at 30–35° for 7 hr. and allowed to stand at room temperature for 36 hr. After addition of 400 ml. of aqueous ethanol (1:1) and vigorous stirring, a tan, crystalline product was obtained which was extracted with boiling ethanol, giving 1.85 g. (3%) of product; m.p. 255–257°.

Anal.—Calcd. for $C_{12}H_{10}N_4S_3$: C, 47.03; H, 3.29; N, 18.28; S, 31.39. Found: C, 47.14; H, 3.38; N, 18.15; S, 31.28.

4-Amino-5,6-dimethylthiopheno[2,3-*d*]pyrimidine—A mixture of 3.04 g. (0.02 mole) of 2-amino-3-cyano-4,5-dimethylthiophene (5), 30 ml. of formamide, and two drops of acetic anhydride was refluxed at 160–165° for 2 hr. After being cooled, a solid product was isolated and recrystallized from dioxane, giving 1.48 g. (39%) of white crystals; m.p. 261–263°; IR (KBr) 1650 (NH_2), 3400 (NH_2) $cm.^{-1}$.

Anal.—Calcd. for $C_8H_9N_3S$: C, 53.47; H, 5.21; N, 23.22; S, 18.25. Found: C, 53.61; H, 5.01; N, 23.44; S, 17.94.

4-Amino-5,6-tetramethylenothiopheno[2,3-*d*]pyrimidine—A mixture of 3.56 g. (0.02 mole) of 2-amino-3-cyano-4,5-tetramethylenothiophene (5), 30 ml. of formamide, and two drops of acetic anhydride was refluxed at 165–170° for 2 hr. After being cooled, a solid product was isolated and recrystallized from dioxane, giving 0.7 g. (17%) of white product; m.p. 261–263°; IR (KBr) 1635 (NH_2), 3350 (NH_2) $cm.^{-1}$.

Anal.—Calcd. for $C_{10}H_{11}N_3S$: C, 58.23; H, 6.32; N, 21.15; S, 15.62. Found: C, 58.51; H, 5.94; N, 20.67; S, 16.01.

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N-Substituted Aminoethanethiols and *N*-Substituted Aminoethanethiol *S*-Sulfonic Acids as Radioprotective Agents

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Abstract □ Several *N*-substituted aminoethanethiols and *N*-substituted aminoethanethiol *S*-sulfonic acids were tested as potential radioprotective agents. 2-(2′-Carbamidoethylamino)-ethanethiol (I*a*), 2-(2′-carboxyethylamino)-ethanethiol (I*b*), 2-(2′-cyanoethylamino)-ethanethiol (I*c*), and 2-(2′-carbamidoethylamino)-ethanethiol *S*-sulfonic acid (II*a*) exhibited significant protective effects against ionizing radiation. Compound I*a* showed the highest activity and was selected for further radiation-protection test studies. The structure–activity relationships of this class of compounds are discussed.

Keyphrases □ Aminoethanethiols, *N*-substituted—radioprotective capacity, structure–activity relationships □ Aminoethanethiol *S*-sulfonic acids, *N*-substituted—radioprotective capacity, structure–activity relationships □ Radioprotective agents—aminoethanethiols, aminoethanethiol *S*-sulfonic acids, *N*-substituted

A recent list of the various types of compounds that show radioprotective properties has appeared and their structure–activity relationships have been discussed (1). Aminoalkylthiols constitute the most effective class of radioprotective agents. The initial discovery that 2-mercaptoethylamine (MEA) offered protection to mice against ionizing radiation (2) led to the synthesis of several hundred derivatives of this compound. Structural requirements necessary for radioprotective activity have evolved from the test results on these compounds and have been summarized (1). This effect was not observable when one or two alkyl substituents were placed on the carbon containing the thiol function of MEA (3, 4).[1] Subsequently, it was found that some *N*-substituted aminoethanethiols and *N*-substituted aminoethanethiol *S*-sulfonic acids, prepared in this laboratory, showed significant protection against ionizing radiation. In this report the radioprotection test results on these compounds are presented, and their structure–activity relationships are discussed.

[1] Subsequent antiradiation test results have shown that 2-mercapto-2-methylaminopropane hydrochloride, when administered at 90 mg./kg. i.p. using CMCTW as vehicle, gave 67% survival to mice irradiated with 825 r. (See footnotes to Table II for explanation of test data.)

Table I—Radiation-Protective Activities of *N*-Substituted Aminoethanethiols

$HSCH_2CH_2\overset{+}{N}H_2RA^-$

Compound I	R	A	Vehicle of Administration	pH of Preparation	Approx. LD_{50}, mg./kg.	Drug[a] Dose, mg./kg.	Radiation[b] Dose, r	No. Mice	Mortality by Days[c]	30-Day Survival, %[d]
a	$CH_2CH_2CONH_2$	Tos	Saline[e]	5.5	> 900	500	1100	15	000000/00000/00003/10000/00000/00000	73
			Saline	5.5		250	1100	15	000000/00000/00122/00000/00000/00000	67
			Saline	5.7		900	1000	10	000000/00000/00000/00000/00000/00000	100
			Saline	5.6		450	1000	10	000000/00000/00100/00000/00000/00000	90
			Saline	5.7		175	1000	15	000000/00023/24100/3	0
b	$CH_2CH_2CO_2H$	Tos	Water	6.9	>1200	1200	1000	15	000000/00000/00000/10010/00000/00000	87
			Water	6.9		600	1000	15	000000/00002/03411/00000/00000/00000	27
			Saline	7.8		300	800	19	000000/00012/55311/00000/00000/00000	5
			PEG[f]	5.5		593	800	6	000000/01100/02100/01	0
c	CH_2CH_2CN	Tos	Saline	5.2	500	300	825	15	000001/00001/11001/01100/10000/00000	47
			Saline	5.2		150	825	15	000000/00022/4223	0
d	$CH_2CH_2CO_2C_2H_5$	HSO_4	Water	6.8	1700	600	800	15	000000/00003/23213/01	0
e	$CH_2CH_2CO_2C_2H_5$	Tos	Water	6.2	1700	1000	825	15	001000/00010/30120/01010/11000/00000	20
			Water	6.2		500	825	15	000000/00002/54103	0
f	$CH_2CH(CH_3)CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2$	2Cl	Water	6.7	200	100	825	15	000000/00022/42201/11	0
g	$CH_2CH(CH_3)CO_2(CH_2)_2\overset{+}{N}HC(CH_3)_3$	2Cl	Water	5.9	700	400	825	13	100000/00003/5211	0
h	$CH(CH_3)CH_2CO_2C_2H_5$	Tos	Water	5.5	2000	1300	825	15	001000/00011/41320/01000/00000/00000	7
			Water	5.5		650	825	15	000000/00011/54020/101	0
i	$CH_2CH(CH_3)CO_2CH_3$	Tos	Water	6.0	2200	1000	825	13	000001/00004/21311	0
j	$CH_2CH(CH_3)CO_2CH_2CH(CH_3)_2$	Tos	Water	6.5	1000	500	825	15	000000/00002/27201/1	0
k	$CH(CH_3)CH_2CO_2CH_3$	Tos	Water	6.3	1500	1000	825	15	000000/00000/03523/00010/00000/00000	7
			Water	6.3		500	825	15	000000/00011/35310/1	0

[a] Compound administered intraperitoneally as 0.5–10% solution 15 min. before irradiation. [b] 800–825 r (X-rays); 1000–1100 r (γ-rays). [c] The number of animals dying on Days 0 through 30. [d] Control mice did not survive 30 days. [e] Physiological saline solution. [f] Polyethylene glycol.

Table II—Radiation-Protective Activities of *N*-Substituted Aminoethanethiol *S*-Sulfonic Acids

$^-O_3S_2CH_2CH_2\overset{+}{N}H_2R$

Compound II	R	Vehicle of Administration	pH of Preparation	Approx. LD_{50}, mg./kg.	Drug[a] Dose, mg./kg.	Radiation[b] Dose, r	No. Mice	Mortality by Days[c]	30-Day Survival, %[d]
a	$CH_2CH_2CONH_2$	Water	5.5	1200	800	1000	15	000000/00001/02000/00000/01101/00100	53
		Water	5.5		400	1000	15	000000/00210/46001/00000/00000/00000	7
b	$CH_2CH_2CONHCH(CH_3)_2$	Saline[e]	5.6	400	250	1000	15	000000/00012/04321/2	0
c	$CH_2CH_2CONHC(CH_3)_2$	Water	6.0	350	200	1000	15	000000/00012/35012/01	0
d	$CH_2CH(CH_3)CONH_2$	CMCTW[f]	5.7	750	600	1000	15	100000/00002/04011/00000/00000/00000	40
		CMCTW	5.7		300	1000	15	000000/10012/07021/1	0
e	$CH(CH_3)CH_2CN$	CMCTW	5.6	850	600	1000	15	000000/00001/12211/11100/02000/00000	13
		CMCTW	5.6		300	1000	15	000000/00001/03533	0
f	$CH(C_6H_5)CH_2CN$	CMCTW	5.5	> 800	200	1000	15	000000/00000/375	0
g	$CH_2CH(CH_3)CO_2CH_3$	Water	5.9	>1000	800	1000	15	000001/00011/5601	0
h	$CH_2CO_2C_2H_5$	Water	5.5	>2000	2000	1000	15	000000/00001/63300/11	0

[a] Compound administered intraperitoneally as 0.5–10% solution 15 min. before irradiation. [b] 800–825 r (X-rays); 1000–1100 r (γ-rays). [c] The number of animals dying on Days 0 through 30. [d] Control mice did not survive 30 days. [e] Physiological saline solution. [f] 0.3% methylcellulose and 0.1% polysorbate 80.

METHOD

The synthesis of all the compounds listed in Tables I and II has been reported previously (5, 6). The irradiation was performed utilizing either a 300-kvp. GE Maxitron Unit, dose rate in air 45 r/min., or a ^{60}Co irradiator, which contained a 1200-c. source, with dose rate between 100–50 r/min. (7). Female mice of the Walter Reed Bagg Swiss or Inbred Charles River (ICR) strain, 5–6 weeks old and weighing 21–25 g., were used. Forty mice were exposed to whole body lethal irradiation. Equal numbers of control mice injected with only the vehicle used for the particular drug evaluation were irradiated simultaneously. The mice were exposed in a perforated Lucite dish which rotated continuously during exposure. A 30-day period for survival was observed. All control animals died before the 21st day following exposure. Survival of treated mice was interpreted as good (>45% survival), fair (25–44% survival), slight (1–24% survival), and none (0% survival).[2]

RESULTS AND DISCUSSION

Structure–activity relationship studies on MEA and its derivatives have established that the presence of a basic function and a free thiol group or function readily convertible to a free thiol *in vivo* is necessary for high radioprotective effect. Alkylation of the amino group of MEA gives compounds with varied activities. Simple *N,N*-dialkyl derivatives of MEA exhibited little or no effect, whereas significant effects were observed in *N*-(2′-phenethylamino)-ethanethiol and *N*-(2′-thienylethylamino)-ethanethiol (8). Good radioprotection was also found in several other *N*-substituted aminoethanethiols. The results are summarized in Table I. Significant activity was observed in the case of the 2-carbamidoethyl derivative (I*a*), 2-carboxyethyl derivative (I*b*), or 2-cyanoethyl derivative (I*c*), and the activity was considerably reduced with the 2-carbethoxyethyl derivatives (I*d* and I*e*). This reduction of activity could possibly be connected with the ester function, since several other ester derivatives (I*f–k*) offered either slight activity or no effect at all.

A similar structure–activity relationship was also observed in the *N*-substituted aminoethanethiol *S*-sulfonic acids: good activity when the *N*-alkyl group was 2-carbamidoethyl (II*a*) and reduced activity when a methyl group was placed alpha to the amide function, *N*-alkyl equal 2-carbamidopropyl (II*d*). Placement of alkyl substituents on the amide nitrogen, Compounds II*b* and II*c*, resulted in a complete loss of activity. With the exception of Compound II*e*, which showed only slight activity, the remaining compounds were inactive.

The good radioprotective property of Compounds I*a–c* and II*a*, with their relatively low toxicity, has created additional interest in these agents. In particular, the amide (I*a*) that showed the highest activity was selected for further radioprotection studies.

The *S*-triphenylmethyl derivative (III) of I*a* was tested for potential latent antiradiation activity but proved to be completely inactive.

$$(C_6H_5)_3CSCH_2CH_2NHCH_2CH_2CONH_2$$

III

[2] These antiradiation screening tests were performed at the Walter Reed Army Institute of Research, Washington, D.C., under the direction of J. P. Jacobus.

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