# 2-Mercaptoacetamidine and Derivatives as Antiradiation Agents[†,‡]

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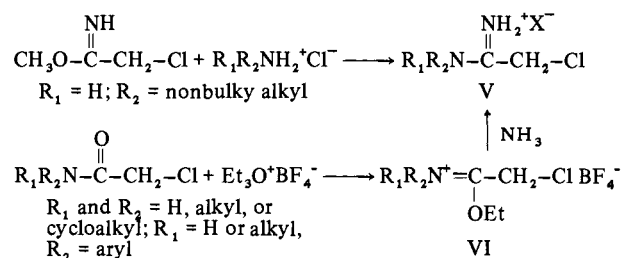
$N$-Substituted-2-mercaptoacetamidines and functional derivatives, including the corresponding disulfides, Bunte salts, and phosphorothioates, have been synthesized and found as a class to be highly effective antiradiation agents. Variations in the N substituent include alkyl, cycloalkyl, cycloalkylalkyl, aralkyl, hydroxyalkyl, cycloalkyloxyalkyl, aryloxyalkyl, thioethers, and heteroaralkyl groups. A synthesis other than the conventional ones was needed and developed for the key intermediate, $N$-substituted-ethyl 2-chloroacetimidate salt, in which the substituent possessed a bulky carbon skeleton. Treatment of 2-chloroacetamides with Meerwein's reagent gave ethyl 2-chloroacetimidate fluoroborates (VI) ($R_1$ or $R_2$ = H) or (2-chloro-1-ethoxyethylidene)methylammonium tetrafluoroborates (VI) ($R_1$ and $R_2 \neq$ H). This allowed displacement of alkoxide by $NH_3$ rather than by a bulky amine. Half-life determinations in EtOH were made of purified samples of VI in an attempt to correlate stability with successful preparation of 2-chloroacetamidine (V). Use of 1-amino-2-propanol in the Pinner amidine synthesis resulted in a rearrangement to give an unusual diester, mercaptoacetic acid, 2-amino-1-methylethyl ester, $S$-thiosulfate ester (VII). Antiradiation data for 84 compds are presented. Survival rates of 90–100% in the 30-day test were obtained for many compds given either ip or po at doses well below toxic levels. Some compds were extremely potent, being effective at doses below 10 mg/kg ip and below 50 mg/kg po. Sodium hydrogen $S$-{$N$-[(3,5-dimethyl-1-adamantyl)methyl]amidino}methyl phosphorothioate (**84**) given ip resulted in 93% survival at 8 mg/kg and 60% survival at 4 mg/kg (1/31 ip LD$_{50}$). 2,2′-Dithiobis{$N$-[(1-adamantyl)methyl]acetamidine}·dihydrochloride (**75**) given po resulted in 80% survival at 50 mg/kg (1/6 po LD$_{50}$) and 53% at 25 mg/kg.

A series of N- and S-substituted derivatives of 2-mercaptoacetamidine, $YSCH_2C(=NH)NHR$, has been synthesized as potential antiradiation agents.[1-6] Based on this structure, a class of $\alpha$-amidinium Bunte salts (Y = $SO_3H$) has been reported[2,4] to possess radiation-protective properties in mice. Selected thiols, disulfides, and phosphorothioates, based on the mercaptoamidine structure, have been prepared.[4,5] Synthetically, the most accessible members of this group have been Bunte salts, $S$-($N$-substituted amidino)methyl hydrogen thiosulfates (I); those now reported are shown in Table I. 2-Mercaptoacetamidines (shown as their salts by II, Table II) and the corresponding disulfides (III) (Table III) and phosphorothioates (IV) (Table IV) were prepared to evaluate their effectiveness as antiradiation agents. The work was concentrated on variations of the group R in I–IV and on comparisons of the protective action of compds bearing the same substituent R on the amidine N of I–IV in similar biological tests.

**Chemistry.** Most of the substances reported in this paper were prepared by methods previously elaborated.[1-3,5] Syntheses hinged on the availability of 2-chloroacetamidine salts (V) for displacement with a S-bearing nucleophile to form I–IV. The most useful mode of generating V consisted of allowing chloroacetonitrile to react with MeOH containing NaOMe to generate, *in situ*, methyl 2-chloroacetimidate, $CH_3OC(=NH)CH_2Cl$, which was converted by an amine hydrochloride to V (X = Cl) (Scheme I; characterized examples, Table V).[2,4] Selective displacement of methoxide

**Scheme I**

$$\begin{array}{ccc}
\overset{\displaystyle NH}{\underset{\displaystyle \parallel}{CH_3O-C-CH_2-Cl}} + R_1R_2NH_2^+Cl^- & \longrightarrow & \overset{\displaystyle NH_2^+X^-}{\underset{\displaystyle \parallel}{R_1R_2N-C-CH_2-Cl}} \\
R_1 = H; R_2 = \text{nonbulky alkyl} & & V
\end{array}$$

$$\uparrow NH_3$$

$$\begin{array}{ccc}
\overset{\displaystyle O}{\underset{\displaystyle \parallel}{R_1R_2N-C-CH_2-Cl}} + Et_3O^+BF_4^- & \longrightarrow & R_1R_2N^+=C-CH_2-Cl\ BF_4^- \\
R_1 \text{ and } R_2 = H, \text{ alkyl, or} & & \overset{\displaystyle |}{OEt} \\
\text{cycloalkyl}; R_1 = H \text{ or alkyl,} & & \\
R_2 = \text{aryl} & & VI
\end{array}$$

on the imidate C, in the presence of the reactive Cl, fortuitously provided a successful synthesis of V in this and earlier studies. However, this synthesis of V failed for a number of primary amines in which the amino group was attached to either a tertiary carbon atom (*e.g.*, *tert*-butylamine or 1-adamantanamine) or to a bulky carbon skeleton. A new method was developed for these members of V. This involved treatment[7] of the corresponding 2-chloroacetamide with Meerwein's reagent giving ethyl 2-chloroacetimidate fluoroborates (VI) ($R_1$ or $R_2$ = H) or (2-chloro-1-ethoxyethylidene)methylammonium tetrafluoroborates (VI, $R_1$ and $R_2 \neq$ H) (Scheme I; characterized examples, Table VI).

This second approach to V from VI using $NH_3$ as the displacing nucleophile provided a general synthesis of the 2-chloroacetamidine (V), seemingly without limitations. The imidate ester VI is, of course, a highly reactive compound. Imidate VI ($R_1$ = 1-adamantyl; $R_2$ = $CH_3$) derived from $N$-methyl-1-adamantanamine on reaction with $NH_3$ in EtOH soln resulted in solvolysis of the imidate, giving only $N$-methyl-1-adamantanamine as an identifiable product. Stability studies (Table VI) at 25° in MeOH revealed a half-life of only 1.3 min for this imidate. Half-life values of 40–440

Table I. S-[(N-Substituted amidino)methyl] Hydrogen Thiosulfates, $RNHC(=NH_2^+)CH_2S_2O_3^-$ (1)

| No. | R | Method[a] | Recrystn solvents | Yield, % | Mp, °C dec | Formula | Analyses | Intraperitoneal data $LD_{50}$, ca. mg/kg | Drug dose, mg/kg | Survival, % | Rating[c] | Peroral data $LD_{50}$, ca. mg/kg | Drug dose, mg/kg | Survival, % | Rating[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | H[d] | | | | | | | 87 | 50 | 100 | + | 300 | 100 | 73 | + |
| | | | | | | | | | 25 | 55 | | | 75 | 33 | |
| 2 | $i\text{-}C_5H_{11}$ | A | 95% EtOH | 15 | 114–115 | $C_7H_{16}N_2O_3S_2$ | C, H, N, S | 63 | 30[e] | 87 | + | 175 | 70[e] | 0 | 0 |
| 3 | $cyclo\text{-}C_3H_5$ | A | EtOH–$H_2O$ | 90 | 194 | $C_5H_{10}N_2O_3S_2$ | C, H, N, S | 150 | 90 | 93 | + | >600 | 600 | 0 | 0 |
| 4 | $cyclo\text{-}C_5H_9$ | C | $H_2O$ | 77 | 161–167 | $C_7H_{14}N_2O_3S_2$ | C, H, N, S | 85 | 25 | 0 | 0 | | | | |
| 5 | $cyclo\text{-}C_5H_9\text{-}CH_2$ | A | $H_2O$ | 50 | 145–151 | $C_8H_{16}N_2O_3S_2$ | C, H, N, S | 40 | 15[e] | 70 | + | 80 | 50[e] | 0 | 0 |
| 6 | $cyclo\text{-}C_6H_{11}$ | A | EtOH–$H_2O$ | 33 | 160–162 | $C_8H_{16}N_2O_3S_2$ | C, H, N | 75 | 50 | 67 | + | 1600 | 720 | 0 | 0 |
| 7 | $cyclo\text{-}C_6H_{11}\text{-}CH_2$ | A | 95% EtOH | 38 | 165–167 | $C_9H_{18}N_2O_3S_2$ | C, H, N, S | 45 | 20[e] | 93 | + | 180 | 100 | 20 | 0 |
| 8 | $cyclo\text{-}C_6H_{11}\text{-}(CH_2)_2$ | A | EtOH | 14 | 143–145 | $C_{10}H_{20}N_2O_3S_2$ | C, H, N, S | 20 | 10[e] | 100 | + | 112 | 50[e] | 93 | + |
| | | | | | | | | | 5[e] | 40 | | | 25[e] | 27 | |
| 9 | $cyclo\text{-}C_6H_{11}\text{-}(CH_2)_3$ | A | EtOH | 21 | 135–137 | $C_{11}H_{22}N_2O_3S_2$ | C, H, N, S | 11 | 5[e] | 64 | + | 180 | 30[e] | 60 | ++ |
| 10 | $cyclo\text{-}C_6H_{11}\text{-}(CH_2)_4$ | A | 95% EtOH | 17 | 140–143 | $C_{12}H_{24}N_2O_3S_2$ | C, H, N, S | 22 | 3 | 36 | 0 | | | | |
| 11 | $cyclo\text{-}C_7H_{13}$ | A | MeOH | 24 | 157–162 | $C_9H_{18}N_2O_3S_2$ | C, H, N, S | 65 | 25[e] | 87 | + | 175 | 100[e] | 0 | 0 |
| 12 | $cyclo\text{-}C_7H_{13}\text{-}CH_2$ | A | $H_2O$ | 82 | 150–152 | $C_{10}H_{20}N_2O_3S_2$ | C, H, N, S | 30 | 15[e] | 100 | ++ | 150 | 50[e] | 73 | ++ |
| | | | | | | | | | 7.5[e] | 40 | | | | | |
| 13 | $cyclo\text{-}C_7H_{13}\text{-}(CH_2)_2$ | A | MeCN | 52 | 157–158 | $C_{11}H_{22}N_2O_3S_2$ | C, H, N, S | 17 | 10[e] | 20 | 0 | 40 | 20[e] | 36 | 0 |
| 14 | $cyclo\text{-}C_8H_{15}$ | A | EtOH–$H_2O$ | 69 | 163–165 | $C_{10}H_{20}N_2O_3S_2$ | C, H, N, S | 35 | 20 | 93 | + | 450 | 200 | 13 | 0 |
| | | | | | | | | | 10 | 53 | | | | | |
| 15 | $cyclo\text{-}C_8H_{15}\text{-}CH_2$ | A | $i$-PrOH–$Et_2O$ | 26 | 152–154 | $C_{11}H_{22}N_2O_3S_2$ | C, H, N, S | 19 | 10[e] | 67 | ++ | 200 | 50 | 47 | 0 |
| | | | | | | | | | 5[e] | 93 | | | | | |
| 16 | endo-2-Norbornyl[f] | | | | | | | 55 | 30 | 87 | + | 150 | 75 | 0 | 0 |
| 17 | exo-2-Norbornyl[f] | | | | | | | 55 | 36 | 87 | + | 250 | 100 | 0 | 0 |
| 18 | endo-2-Norbornylmethyl[f] | | | | | | | 47 | 20 | 87 | ++ | 144 | 50 | 40 | 0 |
| | | | | | | | | | 10 | 73 | | | | | |
| 19 | 2-Bornyl[f] | | | | | | | 30 | 15 | 93 | ++ | 225 | 50 | 87 | +++ |
| | | | | | | | | | 8 | 50 | | | 25 | 27 | |
| 20 | cis-Myrtanyl[f] | | | | | | | 63 | 8 | 67 | +++ | 380 | 200 | 20 | 0 |
| 21 | 1-Adm | B | $i$-PrOH–$Et_2O$ | 27 | 199–200 | $C_{12}H_{20}N_2O_3S_2$ | C, H, N, S | 55 | 25 | 80 | + | >600 | 200 | 7 | 0 |
| 22 | 2-Adm | B | MeOH | 46 | 185–187 | $C_{12}H_{20}N_2O_3S_2\cdot 0.25H_2O$ | C, H, N, S[g] | 38 | 20[e] | 100 | ++ | 280 | 200 | 0 | 0 |
| | | | | | | | | | 10[e] | 100 | | | | | |
| 23 | 1-Adm-$CH_2$[f] | | | | | | | 25 | 10 | 93 | ++ | >300 | 200 | 0 | 0 |
| | | | | | | | | | 5 | 50 | | | | | |
| 24 | 1-Adm-$(CH_2)_2$ | A | MeOH, EtOH | 11 | 174–178 | $C_{14}H_{24}N_2O_3S_2$ | C, H, N, S | 40 | 10 | 27 | 0 | >600 | 600 | 7 | 0 |
| 25 | 1-Adm-$(CH_2)_3$ | A | $i$-PrOH–$Et_2O$ | 56 | 164–168 | $C_{15}H_{26}N_2O_3S_2$ | C, H, N, S | 8 | 2.5 | 40 | + | >600 | 600 | 13 | 0 |
| 26 | 1-Adm-$(CH_2)_5$ | A | MeOH–$H_2O$ | 41 | 159–161 | $C_{17}H_{30}N_2O_3S_2$ | C, H, N, S | 10 | 3 | 10 | 0 | >350 | 100[e] | 0 | 0 |
| 27 | 3,5-$(CH_3)_2$-1-Adm-$CH_2$ | A | MeOH–$Et_2O$ | 16 | 174–177 | $C_{15}H_{26}N_2O_3S_2$ | C, H, N, S | 44 | 15 | 27 | 0 | >600 | 600 | 7 | 0 |
| 28 | HO-$(CH_2)_2$ | A | EtOH, MeOH | 10 | 145–148 | $C_4H_{10}N_2O_4S_2$ | C, H, N, S | >600 | 300 | 93 | ++ | >1250 | 600 | 6 | 0 |
| | | | | | | | | | 150 | 87 | | | | | |
| 29 | HO-$(CH_2)_3$ | A | MeOH, $H_2O$ | 16 | 142–146 | $C_5H_{12}N_2O_4S_2$ | C, H, N, S | 250 | 120 | 0 | 0 | >1050 | 600 | 0 | 0 |
| 30 | HO-$(CH_2)_6$ | A | $i$-PrOH | 13 | 132–136 | $C_8H_{18}N_2O_4S_2$ | C, H, N, S | 175 | 80 | 0 | 0 | 900 | 600 | 0 | 0 |
| 31 | HO-$(CH_2)_2$-O-$(CH_2)_2$ | A | EtOH, 95% EtOH | 78 | 111–114 | $C_6H_{14}N_2O_5S_2$ | C, H, N, S | 350 | 150 | 0 | 0 | >900 | 450 | 0 | 0 |
| 32 | 4-$HOCH_2$-$cyclo$-$C_6H_{10}$-$CH_2$ | A | MeOH | 22 | 167–169 | $C_{10}H_{20}N_2O_4S_2$ | C, H, N, S | 150 | 50 | 0 | 0 | >1000 | 800 | 0 | 0 |
| 33 | $CH_3O$-$(CH_2)_3$ | A | $H_2O$ | 21 | 90–96 | $C_6H_{14}N_2O_4S_2\cdot H_2O$ | C, H, N, S | 150 | 50 | 0 | 0 | >900 | 600 | 0 | 0 |
| 34 | Tetrahydrofurfuryl | C | $Me_2CO$–$Et_2O$ | 13 | 130–135 | $C_7H_{14}N_2O_4S_2$ | C, H, N, S | 140 | 100 | 0 | 0 | | | | |
| 35 | 3-(2-Isobornyloxy)propyl | A | EtOH–$H_2O$ | 50 | 155–159 | $C_{15}H_{28}N_2O_4S_2$ | C, H, N, S | 12 | 6 | 0 | 0 | >350 | 300 | 0 | 0 |
| 36 | 3-(2-Bornyloxy)propyl | A | EtOH–$H_2O$ | 80 | 158 | $C_{15}H_{28}N_2O_4S_2$ | C, H, N, S | 19 | 12.5 | 60 | 0 | >600 | 600 | 0 | 0 |
| 37 | $cyclo$-$C_7H_{13}$-O-$(CH_2)_3$ | A | $H_2O$ | 21 | 127–132 | $C_{12}H_{24}N_2O_4S_2$ | C, H, N, S | | | | | | | | |
| 38 | 1-Adm-O-$(CH_2)_2$[h] | | | | | | | 75 | 30 | 80 | ++ | >600 | 400 | 33 | 0 |

| No. | R | Method | Purification solvents | Yield % | Mp °C | Formula | Analyses | LD₅₀ | Drug dose | Survival | Rating | LD₅₀ | Drug dose | Survival | Rating |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 39 | 1-Adm-O-(CH₂)₃[h] | | | | | | | 35 | 15 | 60 | + | >300 | 200 | 0 | 0 |
| 40 | 1-Adm-O-CH(CH₃)CH₂[h] | | | | | | | 60 | 15 | 87 | ++ | >300 | 300 | 7 | 0 |
| 41 | C₆H₅-O-(CH₂)₃ | A | EtOH | 78 | 145-147 | C₁₁H₁₆N₂O₄S₂ | C, H, N, S | 75 | 15 | 100 | ++ | 650 | 125 | 40 | + |
| 42 | C₆H₅-O-(CH₂)₄ | A | EtOH-H₂O | 55 | 154-157 | C₁₂H₁₈N₂O₄S₂ | C, H, N, S | 130 | 25 | 83 | ++ | | | | |
| 43 | C₆H₅CH₂-O-(CH₂)₂ | A | EtOH-H₂O | 16 | 140-142 | C₁₁H₁₆N₂O₄S₂ | C, H, N, S | 60 | 20 | 73 | + | 90 | 50 | 7 | 0 |
| 44 | C₆H₅CH₂-O-(CH₂)₃ | A | EtOH-H₂O | 70 | 144-146 | C₁₂H₁₈N₂O₄S₂ | C, H, N, S | 30 | 20 | 93 | + | 300 | 70 | 40 | + |
| 45 | p-CH₃O-C₆H₄-CH₂ | A | EtOH-Et₂O | 50 | 150-152 | C₁₀H₁₄N₂O₄S₂ | C, H, N, S | 87 | 32.5 | 80 | + | >600 | 100 | 0 | 0 |
| 46 | p-CH₃O-C₆H₄-(CH₂)₂ | A | H₂O | 35 | 153-156 | C₁₁H₁₆N₂O₄S₂ | C, H, N, S | 100 | 20 | 87 | ++ | 500 | 150 | 7 | 0 |
| 47 | 3,4-(CH₃O)₂-C₆H₃-(CH₂)₂ | A | i-PrOH, EtOH | 32 | 161-163 | C₁₂H₁₈N₂O₅S₂ | C, H, N, S | 250 | 50 | 60 | +++ | >900 | 600 | 0 | 0 |
| 48 | 3,4-(CH₃)₂-C₆H₃-CH₂ | A | EtOH-H₂O | 76 | 164 | C₁₁H₁₆N₂O₃S₂ | C, H, N | 130 | 38 | 60 | + | 500 | 100 | 0 | 0 |
| 49 | C₆H₅-S-(CH₂)₂ | A | EtOH-H₂O | 21 | 135-138 | C₁₀H₁₄N₂O₃S₃ | C, H, N, S | 60 | 40 | 10 | 0 | | | | |
| 50 | C₆H₅CH₂-S-(CH₂)₂ | A | EtOH-H₂O | 22 | 134-136 | C₁₁H₁₆N₂O₃S₃ | C, H, N, S | 95 | 20 | 67 | + | 110 | 80 | 40 | + |
| 51 | p-Cl-C₆H₄-S-(CH₂)₂ | A | EtOH-H₂O | 66 | 155-157 | C₁₀H₁₃ClN₂O₃S₃ | C, H, S; N[i] | 56 | 16 | 73 | ++ | 600 | 300 | 33 | 0 |
| 52 | p-CH₃-C₆H₄-S-(CH₂)₂ | A | EtOH-H₂O | 50 | 142-144 | C₁₁H₁₆N₂O₃S₃ | C, H, N, S | 53 | 30[e] / 15[e] | 93 / 51 | ++ | 350 | 80[e] | 0 | 0 |
| 53 | cyclo-C₆H₁₁-S-(CH₂)₂ | A | EtOH-H₂O | 33 | 142-145 | C₁₀H₂₀N₂O₃S₃ | C, H, N, S | 40 | 15 | 87 | + | 300 | 88 | 0 | 0 |
| 54 | (5-Methyl-2-thienyl)methyl | A | EtOH-H₂O | 10 | 125-127 | C₉H₁₄N₂O₃S₃ | H, N, S; C[j] | 32 | 18 | 80 | + | 155 | 80 | 27 | 0 |
| 55 | 2-(3-Indolyl)ethyl[k] | | | | | | | 180 | 28 / 14 | 93 / 67 | ++++ | 600 | 500 | 0 | 0 |
| 56 | 3-(3-Indolyl)propyl[k] | | | | | | | 38 | 12.5 | 0 | 0 | | | | |

[a]A, RNHC(=NH₂⁺)CH₂Cl Cl⁻ + Na₂S₂O₃; B, RNHC(=NH₂⁺)CH₂ClBF₄⁻ + Na₂S₂O₃; C, RNHC(=NH₂⁺)CH₂Cl Cl⁻ + Tl₂S₂O₃. [b]The antiradiation data, generally, represent the lowest dose of drug for which a high rate of survival was obtained. For each test (see ref 10), usually 15 mice were treated with drug and irradiated either 15 or 30 min later. Compds were given ip or po in H₂O, physiologic saline, or as homogenized suspensions contg methylcellulose (0.2%) and Tween 80 (0.4%). The volumes injected did not exceed 2% of body wt. The radiation dose was 950 rads (30-50 rads/min) of γ radiation from a cobalt 60 source. Faster radiation dose rates (200 rads/min, 975 rads total) are noted in the table. [c]Ratings are based on the following ranges of protective indices (footnote § in the text): 0, 0-1; +, 2-5; ++, 6-10; +++, 11-14; ++++, 15-52. [d]Ref 2. [e]Fast dose rate, ca. 200 rads/min, 975 rads total. [f]Ref 4. [g]S: calcd, 20.76; found, 20.01; addn anal. H₂O. [h]Ref 6. [i]N, calcd, 8.22; found, 7.77. [j]C, calcd, 36.72; found, 37.17. [k]Ref 3.

Table II. N-Substituted-2-mercaptoacetamidine Hydrochlorides, RNHC(=NH₂⁺)CH₂SH Cl⁻ (II)

| No. | R | Method[a] | Purification solvents | Yield, % | Mp, °C dec | Formula | Analyses | Intraperitoneal data LD₅₀ ca. mg/kg | Drug dose, mg/kg | Survival, % | Rating | Peroral data LD₅₀ ca. mg/kg | Drug dose, mg/kg | Survival, % | Rating |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 57 | H[c] | | | | | | | 52 | 24 / 12 | 100 / 73 | ++ | 150 | 40 / 20 | 87 / 67 | +++ |
| 58 | cyclo-C₇H₁₃-CH₂ | D | H₂O[d] | 47 | 81-87 | C₁₀H₂₀N₂S·HCl·0.5H₂O | C, N, S, SH; H[e] | 18 | 7.5[g] | 90 | + | 35 | 15[g] | 40 | 0 |
| 59 | 1-Adm | E | MeOH-Et₂O, H₂O | 13 | 217-220 | C₁₂H₂₀N₂S·HCl·H₂O | C, N, S; H,[h] SH[f] | 40 | 20[g] / 10[g] | 93 / 47 | ++ | >75 | 40[g] | 60 | + |
| 60 | 2-Adm | E | i-PrOH-cyclohexane | 22 | 199-207 | C₁₂H₂₀N₂S·HCl | C, H, N, SH; S[i] | 17 | 10[g] | 70 | + | 35 | 20[g] | 90 | + |
| 61 | 1-Adm-CH₂[j] | | | | | | | 22 | 12 / 6 / 3 | 93 / 67 / 33 | ++ | 65 | 15 | 93 | ++ |
| 62 | 1-Adm-(CH₂)₃ | D | MeOH-Et₂O | 38 | 235-237 | C₁₅H₂₆N₂S·HCl·0.3H₂O | C, H, N, S; SH[f] | 25 | 5[g] | 93 | ++ | 350 | 50[g] | 67 | ++ |
| 63 | 3,5-(CH₃)₂-1-Adm-CH₂ | D | H₂O | 28 | 167 | C₁₅H₂₆N₂S·HCl | C, H, N, S; SH[f] | 10 | 5 / 2.5 | 64 / 33 | + | 175 | 50 / 25 | 93 / 80 | +++ |
| 64 | 3,4-(CH₃O)₂C₆H₃-(CH₂)₂ | D | MeCN-Et₂O | 48 | 155-157 | C₁₂H₁₈N₂O₂S·HCl | C, H, N, S | 125 | 50 | 63 | + | >500 | 200 | 0 | 0 |
| 65 | 3-Morpholinopropyl | D | EtOH-Et₂O | 38 | 170-175 | C₉H₁₉N₃OS·2HCl | C, H, N, S | 150 | 80 | 7 | 0 | | | | |
| 66 | 3-Quinuclidinyl | D | EtOH-i-PrOH | 44 | 217-219 | C₉H₁₇N₃S·2HCl | C, H, N, S | | | | | | | | |

[a]D, RNHC(=NH₂⁺)CH₂Cl Cl⁻ + (1) Na₃SPO₃, (2) HCl. E, RNHC(=NH₂⁺)CH₂BF₄⁻. [b]See Table I, footnotes b and c. [c]Ref 5. [d]Crude solid was washed with C₆H₆ and hexane. [e]H, calcd, 9.02; found, 8.52. [f]I₂ titrn of thiols in this series gave values within only 2% of calcd. [g]975 rads, 200 rads/min. [h]H, calcd, 8.31; found, 7.59. [i]S, calcd, 12.29; found, 11.78. [j]Ref 4.

Table III. 2,2'-Dithiobis[N-substituted acetamidine] Salts, [RNHC(=NH₂⁺)CH₂S]₂ 2X⁻ or X²⁻ (III)

| No. | R | X | Method[a] | Recrystn solvents | Yield, % | Mp, °C | Formula | Analyses | LD₅₀ ca. mg/kg (IP) | Drug dose, mg/kg (IP) | Survival, % (IP) | Rating (IP) | LD₅₀ ca. mg/kg (PO) | Drug dose, mg/kg (PO) | Survival, % (PO) | Rating (PO) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 67 | H[c] | SO₄ | | | | | | | 60 | 25$^d$ / 12.5$^d$ / 6.3$^d$ | 100 / 100 / 27 | +++ | 125 | 50 / 25 / 13 / 6.3 | 87 / 67 / 40 / 20 | +++ |
| 68 | H | S₂O₃ | F | H₂O | 27 | 165–167 | C₄H₁₀N₄S₂·H₂S₂O₃ | C, H, N, S | | | | | | | | |
| 69 | cyclo-C₅H₉-CH₂ | Cl | G | n-BuOH–MeCN | 31 | 187–189 | C₁₆H₃₀N₄S₂·2HCl | C, H, N, S | 60 | 20$^d$ | 80 | ++ | 63 | 35$^d$ | 0 | 0 |
| 70 | cyclo-C₆H₁₁-(CH₂)₃ | Cl | G | EtOH–Et₂O | 26 | 218–219 | C₂₂H₄₂N₄S₂·2HCl | C, H, N, S | 5.8 | 1.75$^d$ | 40 | 0 | 70 | 30$^d$ | 53 | + |
| 71 | cyclo-C₇H₁₃ | Cl | G | EtOH–Et₂O | 42 | 230 | C₁₈H₃₄N₄S₂·2HCl·1.25H₂O | C, H, N, S, H₂O | 38 | 20$^d$ / 10$^d$ | 87 / 50 | + | 150 | 25$^d$ | 13 | 0 |
| 72 | cyclo-C₇H₁₃-CH₂ | Cl | G | H₂O, i-PrOH–MeCN–Et₂O | 8 | 210–212 | C₂₀H₃₈N₄S₂·2HCl$^e$ | C, H, N, S | 22 | 5 | 70 | ++ | 188 | 25 | 73 | +++ |
| 73 | cyclo-C₇H₁₃-(CH₂)₂ | Cl | G | EtOH–Et₂O | 48 | 228–231 | C₂₂H₄₂N₄S₂·2HCl$^e$ | C, H, N, S | 5 | 2.5$^d$ | 40 | 0 | 50 | 25$^d$ | 73 | + |
| 74 | cyclo-C₈H₁₅-CH₂ | Cl | G | n-BuOH–MeCN | 12 | 216–219 | C₂₂H₄₂N₄S₂·2HCl | C, H, N, S | 11 | 6$^d$ / 3$^d$ | 100 / 20 | + | >150 | 50$^d$ / 25$^d$ | 80 / 50 | ++ |
| 75 | 1-Adm-CH₂ | Cl | G / H | MeOH–H₂O | 93 / 80 | 245–250 | C₂₆H₄₂N₄S₂·2HCl·0.5H₂O | C, H, N, S | 100 | 50 | 7 | 0 | 325 | 50 / 25 | 80 / 53 | ++++ |
| 76 | 1-Adm-(CH₂)₃ | Cl | G | MeOH–Et₂O, EtOH | 4 | 228 | C₃₀H₅₀N₄S₂·2HCl·H₂O | C, H, N, S | 12 | 3.8 | 0 | 0 | >200 | 50 | 7 | 0 |
| 77 | 3,5-(CH₃)₂-1-Adm-CH₂ | Cl | G | MeOH–H₂O | 35 | 229–236 | C₃₀H₅₀N₄S₂·2HCl·H₂O | C, H, N, S | 5 | 3$^d$ | 40 | 0 | 250 | 35 / 25 | 60 / 53 | ++++ |
| 78 | 1-Adm-S-(CH₂)₂ | Cl | H | EtOH–H₂O | 50 | 162 | C₂₈H₄₆N₄S₄·2HCl·H₂O | C, H, N, S | 15 | 8$^d$ | 60 | + | 200 | 75$^d$ | 78 | + |
| 79 | p-tert-C₄H₉-S-C₆H₄-(CH₂)₂ | Cl | H | EtOH–H₂O | 32 | 180–183 | C₂₈H₄₂N₄S₄·2HCl | C, H, N, S | 23 | 8$^d$ | 67 | + | >300 | 150$^d$ | 47 | 0 |

[a]F, RNHC(=NH₂⁺)CH₂S₂O₃⁻ + H₂S (ref 9 using the procedure for H₂O-soluble Bunte salts); G, RNHC(=NH₂⁺)CH₂Cl Cl⁻ + (1) Na₃SPO₃. (2) HCl, (3) H₂O₂; H, RNHC(=NH₂⁺)CH₂S₂O₃⁻ + S=C(NH₂)₂.
[b]See Table I, footnotes b and c. [c]Ref 5. [d]975 rads. 230 rads/min. [e]Addnl anal. (N-ethylmaleimide, uv). SH, calcd. 0.0; found, 0.0.

Table IV. Sodium Hydrogen S-(Substituted amidino)methyl Phosphorothioates,[a] RNHC(=NH₂⁺)CH₂SPO₃²⁻ Na⁺ (IV)

| No. | R | Purificn solvents | Yield, % | Mp, °C dec | Formula | Analyses | LD₅₀ ca. mg/kg (IP) | Drug dose, mg/kg (IP) | Survival, % (IP) | Rating (IP) | LD₅₀ ca. mg/kg (PO) | Drug dose, mg/kg (PO) | Survival, % (PO) | Rating (PO) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 80 | H[c] | | | | | | 100 | 37.5 / 25 | 93 / 20 | + | 135 | 120 / 37.5 | 93 / 73 | ++ |
| 81 | cyclo-C₇H₁₃-CH₂ | d | 55 | 104–114 | C₁₀H₂₁N₂O₃PS·Na salt·0.75H₂O | C, H, N, S | 38 | 20$^e$ | 70 | + | 40 | 20$^e$ | 60 | + |
| 82 | cyclo-C₈H₁₅-CH₂ | d | 71 | 110–125 | C₁₁H₂₃N₂O₃PS·Na salt·1.25H₂O | C, N, S; H, Na$^f$ | 38 | 12.5$^e$ | 73 | + | 80 | 45$^e$ / 22.5$^e$ | 87 / 33 | + |
| 83 | 1-Adm-(CH₂)₂ | g | 66 | 108–111 | C₁₄H₂₅N₂O₃PS·Na salt·4H₂O | C, N, S, H₂O;[h] H[i] | | | | | | | | |
| 84 | 3,5-(CH₃)₂-1-Adm-CH₂ | j | 79 | 111 | C₁₅H₂₇N₂O₃PS·Na salt·3.5H₂O | C, H, N, S, H₂O[k] | 125 | 8$^e$ / 4$^e$ | 93 / 60 | ++++ | 425 | 100$^e$ / 50$^e$ | 100 / 30 | +++ |

[a]RNHC(=NH₂⁺)CH₂Cl Cl⁻ + Na₃SPO₃. [b]See Table 1, footnotes b and c. [c]Ref 5. [d]Crude solid from DMF-H₂O was washed with i-PrOH and Et₂O. [e]975 rads, ca. 200 rads/min. [f]H, calcd, 7.29; found, 6.81. Na, calcd, 6.78; found, 7.28. [g]Crude solid from DMF-H₂O was triturated with H₂O and dried. [h]Nmr in CD₃OD showed 11 exchangeable H's (4 H₂O). [i]H, calcd. 7.56; found, 8.04. [j]From H₂O-THF (reaction mixt), washed with MeCN. [k]Nmr in CD₃OD showed 10 exchangeable H's (3.5 H₂O).

Table V. N-Substituted-2-chloroacetamidine Hydrochlorides,[a] $RNHC(=NH_2^+)CH_2Cl\ Cl^-$ (V)

| R | Recrystn solvents | Yield, % | Mp, °C | Formula | Analyses |
|---|---|---|---|---|---|
| $H_2NCOCH_2$ | $MeOH-Me_2CO$ | 28 | 143–144 | $C_4H_8ClN_3O \cdot HCl$ | C, H, N |
| $HCl \cdot (CH_3)_2N(CH_2)_3$ | $EtOH-Et_2O$ | 92 | 118–120 | $C_7H_{16}ClN_3 \cdot 2HCl$ | C, H, N |
| $HCl \cdot (C_2H_5)_2N(CH_2)_3$ | EtOH | 100 | 182–186 | $C_9H_{20}ClN_3 \cdot 2HCl$ | C, H, N |
| $HCl \cdot$ 3-Piperidinopropyl | $EtOH-Et_2O$ | 67 | 162–165 | $C_{10}H_{20}ClN_3 \cdot 2HCl$ | C, H, N |
| $HCl \cdot$ 3-Morpholinopropyl | EtOH | 50 | 195–197 | $C_9H_{18}ClN_3O \cdot 2HCl$ | C, H, N |
| $cyclo$-$C_3H_5$ | $EtOH-Et_2O$ | 80 | 118–119 | $C_5H_9ClN_2 \cdot HCl$ | N |
| $cyclo$-$C_5H_9$ | $EtOH-Et_2O$ | 63 | 142–143 | $C_7H_{13}ClN_2 \cdot HCl$ | C, H, N |
| $cyclo$-$C_5H_9$-$CH_2$ | $i$-$PrOH-Et_2O$ | 15 | 124–127 | $C_8H_{15}ClN_2 \cdot HCl$ | C, H, N |
| $cyclo$-$C_6H_{11}$ | $i$-$PrOH-Et_2O$ | 80 | 181–182 | $C_8H_{15}ClN_2 \cdot HCl$ | C, H, N |
| $cyclo$-$C_6H_{11}$-$(CH_2)_3$ | $EtOH-Et_2O$ | 92 | 95–97 | $C_{11}H_{21}ClN_2 \cdot HCl$ | C, H, N |
| $cyclo$-$C_7H_{13}$-$CH_2$ | $i$-$PrOH-Et_2O$ | 41 | 129–131 | $C_{10}H_{19}ClN_2 \cdot HCl$ | C, H, N |
| $cyclo$-$C_7H_{13}$-$(CH_2)_2$ | $EtOH-Et_2O$ | 80 | 134–136 | $C_{11}H_{21}ClN_2 \cdot HCl$ | C, H, N |
| $3,5$-$(CH_3)_2$-$1$-$Adm$-$CH_2$ | b | 63 | 176–178 | $C_{15}H_{25}ClN_2 \cdot HCl$ | $H, N; C,^c Cl^c$ |

[a]Only purified intermediates are shown. [b]Triturated with cold $H_2O$ and several portions of $C_6H_6$. [c]C, calcd, 59.02; found, C, 58.44. Cl, calcd, 23.24; found, 23.65.

Table VI. N-Substituted Ethyl 2-Chloroacetimidate Salts,[a]
$$ClCH_2\overset{\displaystyle OEt}{\underset{}{C}}=N^+\overset{\displaystyle R}{\underset{\displaystyle R'}{\diagdown}}\ BF_4^-\ (VI)$$

| R | R' | Yield,[b] % | Mp, °C | Half-life,[c] min | Formula | Analyses |
|---|---|---|---|---|---|---|
| $(CH_3)_3C$ | H | 64 | 58–63 | 40 | $C_8H_{16}ClNO \cdot HBF_4$ | C, H, Cl, N |
| $C_6H_5$ | H | 49 | 103–106 |  | $C_{10}H_{12}ClNO \cdot HBF_4$ | C, H, N |
| $cyclo$-$C_6H_{11}$ | H | 15 | 126–129 | 200 | $C_{10}H_{18}ClNO \cdot HBF_4$ | C, H, Cl, N |
| $(CH_2)_6$ |  | 50 | 72–73 | 440 | $C_{10}H_{19}ClNOX(X = BF_4)$ | C, H, Cl, N |
| $CH_3(CH_2)_5CH(CH_3)$ | H | 45 | 54–56 | 200 | $C_{12}H_{24}ClNO \cdot HBF_4$ | $C, H, N; Cl^d$ |
| 1-Adm | H | 63 | 142–144 | 56 | $C_{14}H_{22}ClNO \cdot HBF_4$ | C, H, N |
| 1-Adm | $CH_3$ | 71 | 135–138 | 1.3 | $C_{15}H_{25}ClNOX(X = BF_4)$ | C, H, N |
| 1-Adm-$CH(CH_3)$ | H | 38 | 125–128 | 240 | $C_{16}H_{26}ClNO \cdot HBF_4$ | C, H, N |

[a]Only purified intermediates are shown. [b]Recrystd from EtOAc. [c]Stability studies were done in MeOH at room temp. A decrease in uv absorption at 225 nm was used to follow the decompn. The decompd material had practically no absorption at this wavelength. Initial concns varied from 0.02 to 0.4%. [d]Cl, calcd, 11.02; found, Cl, 11.55.

min were observed for the other imidates included in the stability study. The adamantylmethyl derivative of VI ($R_1 =$ 1-adamantyl; $R_2 = CH_3$) was considerably more stable in MeCN, a solvent which could be used for amidine formation by employing $NH_4OAc$. Even under these conditions, however, the imidate with a half-life of 1.3 min in MeOH again gave N-methyl-1-adamantanamine. Also, correlation between half-life values of 2-chloroacetimidates and successful displacement of ethoxide with $NH_3$ cannot be made for VI, when $R_1 =$ 1-admCH($CH_3$) and $R_2 =$ H; an attempted conversion to V gave crude solid product with no ir amidine absorption. This was unexpected because the 2-chloroacetimidate in this case had a half-life in MeOH of 240 min. No satisfactory explanation can be advanced at present for this phenomenon.
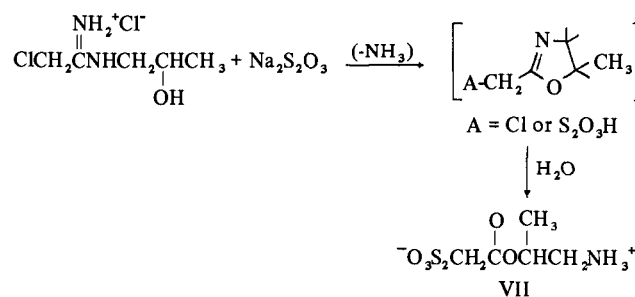
Reaction of V with $S_2O_3^{2-}$ invariably furnished crystalline Bunte salts (I). Considerable difficulty was experienced in isolating amidinium phosphorothioates (IV) from reaction of V with $O_3PS^{3-}$, but immediate acid hydrolysis of IV afforded the target 2-mercaptoacetamidines (II).

Published syntheses of the disulfides (III) of 2-mercaptoacetamidines involve mild $H_2O_2$ oxidation of the thiol (II). A more convenient route to III was developed by using Swan's procedure[8] to allow the Bunte salts (I) to react with thiourea to give III. Sulfur was an undesirable side product from this reaction, but it could be selectively extracted from III with $CS_2$ or $C_6H_6$. Klayman's[9] use of $H_2S$ to prepare disulfides from Bunte salts was employed successfully in the amidine series also. The advantage of these two syntheses of III is that the very accessible Bunte salts are used as precursors.

Attempts to functionalize the side chain of the aliphatic substituent R met with some success by incorporating alcoholic, amino, ether, and thioether groups in relatively

close proximity to the amidine site. Although 2-aminoethanol and several homologs were allowed to react normally in the Pinner synthesis and subsequent conversion to I [$R = (CH_2)_nOH$], use of 1-amino-2-propanol afforded an amino ester (VII) rather than a hydroxy amidine (Scheme

Scheme II

$$\begin{array}{c} NH_2^+Cl^- \\ \parallel \\ ClCH_2CNHCH_2CHCH_3 \\ | \\ OH \end{array} + Na_2S_2O_3 \xrightarrow{(-NH_3)} \left[ \begin{array}{c} N \\ \diagup \diagdown \\ A\text{-}CH_2 \diagdown_O \diagup CH_3 \end{array} \right]$$

$A = Cl\ or\ S_2O_3H$

$\downarrow H_2O$

$$\begin{array}{cc} O & CH_3 \\ \parallel & | \\ ^-O_3S_2CH_2COCHCH_2NH_3^+ \end{array}$$

VII

II). The 2-chloroacetamidine may have formed normally, but on addition of $Na_2S_2O_3$ the amidine base was liberated, thereby facilitating cyclization and loss of $NH_3$ to give the labile oxazoline intermediate. The $Cl^-$ displacement with $S_2O_3^{2-}$ could have occurred either before or after cyclization.

**Biological Evaluation.** Antiradiation effects were determined in mice using established[10] test methods. Although a radioprotective agent effective parenterally may have usefulness, a primary objective of the antiradiation program at Walter Reed Army Institute of Research has been to develop a drug which can be given orally. Target compds were tested using both ip and po administration. Ratings given in Tables I–IV are based on protective index§ values

§Protective index = (protection factor) X ($LD_{50}$/min effective dose); doses are in mg/kg and the protection factor is 1.4 for 40% survival, 1.5 for 50% survival, etc.

which incorporate both therapeutic index and dose–response factors. The ratings do not reflect the high potency in both the ip or po tests of compds included in this study. 2-Aminoethanethiol (MEA) can be considered the standard for comparison. At 150 mg/kg ip of MEA 87% survival of mice in the 30 day test can be obtained. Its ip $LD_{50}$ is *ca.* 250 mg/kg and it is rated ++ in the ip test. The po $LD_{50}$ for MEA is *ca.* 625 mg/kg. At 300 mg/kg 73% survival can be obtained in the po test giving MEA a rating of ++. Slightly better results can be obtained with MEA as well as with most active compds, by using faster radiation dose rates, although for MEA differences are not large enough to change the assigned ratings.

Amidine Bunte salts (I) (Table I) generally, because of synthetic practicality and the often established biological advantage of this S-covering function in other series,[11–13] were prepared and tested first. The parent thiols (II) (Table II), disulfides (III) (Table III), and/or phosphorothioates (IV) (Table IV) were then synthesized and tested in selected cases. The Bunte salts as a group were highly effective in the ip test. The unsubstituted *S*-(amidino)methyl hydrogen thiosulfate (1) was one of the most active compds, giving 100% survival at 50 mg/kg ip and 55% at 25 mg/kg po. Preirradiation administration of the 2-(cyclohexyl)ethyl derivative (8) resulted in 100% survival at 10 mg/kg ip. With the cyclooctylmethyl derivative (15) at 5 mg/kg ip and the 1-adamantylmethyl compd (23) at 10 mg/kg ip 93% survival, and with the 2-admantyl derivative (22) 100% survival at 10 mg/kg ip were obtained. The 2-bornyl (19) and *endo-2*-norbornyl (16) compds were effective in obtaining 87–93% survivals using doses of 15–30 mg/kg ip. The adamantyl ethers (38–40) also were very effective ip. Aryloxyalkyl (41–42) and aralkyl (45–46) derivatives offered excellent radioprotection at doses ranging from 15 to 30 mg/kg ip. Related thioethers (49–50) were less striking in their effectiveness, although the *p*-chlorophenyl (51) and *p*-tolyl (52) analogs compared favorably. Survival of 87% is associated with the nonaromatic cyclohexyl thioether (53) at 15 mg/kg ip. Use of one of the few heterocyclic derivatives, the 2-(3-indolyl)ethyl compd (55), gave 93 and 67% survival at 28 and 14 mg/kg ip, respectively; the lower dose was only *ca.* 7.7% of the acute ip $LD_{50}$ dose.

Hydroxyalkyl amidine Bunte salts were not effective except for the 2-hydroxyethyl compd (28) which is associated with 87% survival at 150 mg/kg ip. The good radioprotection provided by this one compd and the complete lack of activity with close analogs were unexpected.

Amidine Bunte salts (I) given perorally, though not promising candidates for additional biological tests, afforded moderate antiradiation activity. Administration of the 2-cyclohexylethyl derivative (8) po gave 93% survival at 50 mg/kg, and the 2-bornyl compd (19) 87% survival at the same dose. In the case of 19, however, the effective dose was only 0.25 the po $LD_{50}$ dose.

The parent thiols (II) (Table II) were found to be unusually potent radioprotectors, either parenterally or orally. The unsubstituted analog (57) was active in this series also, giving 100% survival at 24 mg/kg ip and 87% at 40 mg/kg po. At 20 mg/kg po (0.14 po $LD_{50}$), 57 afforded 67% survival. At least 90% survival rates in the ip test were observed with the cycloheptylmethyl (58), 1-adamantyl (59), 1-adamantylmethyl (61), and 3-(1-adamantyl)propyl (62) derivatives of 2-mercaptoacetamidine (II). Radioprotection was obtained with ip doses as low at 2.5 mg/kg for 63, 3 mg/kg for 61, and 5 mg/kg for 62.

Perorally, the 2-mercaptoacetamidines are very potent

antiradiation agents. Survival rates of 90–93% were obtained in the po test using the 1-adamantyl derivative (59) at the remarkably low dose of 15 mg/kg, the 2-adamantyl derivative (60) at 20 mg/kg, and the 3,5-dimethyl-1-adamantyl derivative (63) at 50 mg/kg. 63 at 25 mg/kg po, a dose equivalent to *ca.* 14% the po $LD_{50}$, afforded 80% survival of the mice.

Disulfides as a class previously have not been favorably considered[13] over the parent thiols or corresponding Bunte salts, phosphorothioates, and thiazolidines. Disulfides (III) (Table III) of II, however, are extremely potent antiradiation agents, and, as in the case of the thiols (II), some compds could be given either ip or po. Again, the N-unsubstituted compd (67) is an important member of the series, resulting in 100% survival at two ip dose levels (25 and 12.5 mg/kg) and 87% survival at 50 mg/kg po. Significant protection (40%) was obtained perorally at 13 mg/kg, or just slightly more than 10% the po $LD_{50}$. Survival of 100% was obtained using only 6 mg/kg ip of the cyclooctylmethyl derivative (74). Other examples provided protection at corresponding low ip doses (Table III). The 1-adamantylmethyl (75) and 3,5-dimethyl-1-adamantylmethyl (77) compds are particularly significant because at least 50% survival rates were obtained in the po test at only 7.6 and 10%, respectively, of the corresponding po $LD_{50}$ doses. At 50 mg/kg po for 75 and 35 mg/kg po for 77, 80 and 60% survival, respectively, was obtained. The poor activity of 75 and 77 in the ip test showed again that a potent radioprotector may not be effective parenterally.

Phosphorothioates, useful derivatives in other series,[14] gave rise to promising compds in the amidine series also. Again, highly potent compds were obtained (Table IV). Administration of the N-unsubstituted analog (80) at 37.5 mg/kg ip or 120 mg/kg po resulted in 93% survival; 73% survival was obtained at 37.5 mg/kg po. The cyclooctyl derivative (81) afforded good protection in both test systems. With the 3,5-dimethyl-1-adamantyl compd (84) 100% survival was obtained at 100 mg/kg ip, and 93 and 60% survival given ip at only 8 and 4 mg/kg, respectively. The 4-mg dose represents a safety factor of over 30 to 1 (3.2% ip $LD_{50}$, acute). The phosphorothioate 84 in mice is one of the most potent and safe antiradiation agents known.

A comparison of the parent thiol with the protected thiols used in this work is illustrated in Figure 1. 2-Aminoethanethiol and its disulfide, thiosulfate, and phosphorothioate are included as reference compounds in Figure 1. Five different N substituents are shown. Generally thiols and disulfides were more active given by mouth than parenterally, whereas the Bunte salts and phosphorothioates can be considered more effective by injection. Of course, there are exceptions to these generalizations. The thiol bearing the *N*-cycloheptylmethyl substituent was essentially inactive po and moderately active ip. The best overall activity either ip or po and regardless of S-covering function was obtained with the N-unsubstituted compounds (I–IV, R = H). On the other hand, activity from some analogs was associated with only certain S-covering groups. For example, the 3,5-dimethyl-1-adamantyl analog as a phosphorothioate was the most potent compd tested ip and po, but the corresponding Bunte salt was inactive both ip and po and the disulfide, although excellent for po use, was essentially inactive ip.

Based only on screening data of a large group of very potent antiradiation agents, sodium hydrogen *S*-{*N*-[(3,5-dimethyl-1-adamantyl)methyl]amidino}methyl phosphorothioate (84) stands out as the agent of choice for ip administration, being effective at 4 mg/kg ip (3.2% ip $LD_{50}$). Given
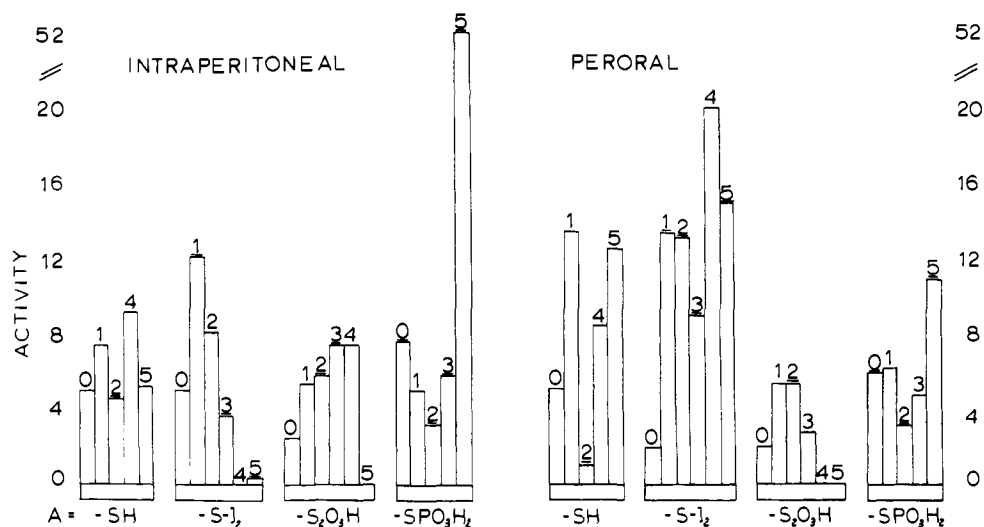
Figure 1. Effect of sulfur-covering group on antiradiation activity (expressed as protective index values, footnote § in text) of substituted amidines, RNHC(=NH)CH$_2$A (as salt). 1, R = H; 2, R = *cyclo*-C$_7$H$_{13}$-CH$_2$; 3, R = *cyclo*-C$_8$H$_{15}$-CH$_2$; 4, R = 1-Adm-CH$_2$; 5, R = 3,5-(CH$_3$)$_2$-1-Adm-CH$_2$. 0, reference compds, H$_2$NCH$_2$CH$_2$A. Underlined numerals indicate a radiation dose rate of *ca.* 200 rads/min; 30–50 rads/min was used for the other compds.

orally, the group of amidines described in this study, particularly as thiols, disulfides, and phosphorothioates, are among the most potent antiradiation agents known. 2,2'-Dithiobis{N-[(1-adamantyl)methyl]acetamidine} dihydrochloride (75) may be the best compd for oral administration, giving 80% survival at 50 mg/kg (17% po LD$_{50}$) and 53% at 25 mg/kg po.

The antiradiation effectiveness of mercaptoacetamidine derivatives included in this study is noteworthy because (1) candidates potentially useful for both ip and po administration have been found, (2) 90–100% survival rates in the 30-day test were obtained for selected compds regardless of the route of administration, (3) minimum effective doses were well below toxic levels in both the ip and po tests, and (4) several compds were extremely potent, being effective at doses below 10 mg/kg ip and below 50 mg/kg po. Further evaluation of these new compounds is indicated.

## Experimental Section[#]

**Starting Materials.** Unless otherwise specified, the starting amines for the amidine syntheses were either purchased commercially or made by literature methods. The free bases or hydrochloride salts were used. The synthesis of some intermediates is indicated: 3-(cycloheptyloxy)propylamine[15] by Raney Co hydrogenation of the nitrile; cyclopentanemethylamine,[16] bp 128°, by LAH redn of cyclopentanecarboxamide; cycloheptaneethylamine by a modification of the reported[17] method; 3-[(bornyl- and isobornyl)oxy]propylamines by an adaptation of recently published methods;[18,19] cyclooctanemethylamine, bp 90–94° (13 mm) [lit.[20] bp 95–104° (10 mm)], by hydrazine cleavage of N-(cyclooctylmethyl)phthalimide, mp 62–65°; α-methyl-1-adamantanemethylamine;[21] 1-adamantaneethylamine,[22] bp 67° (0.2 mm), by LAH redn of 1-adamantaneacetonitrile;[23] 1-adamantanepropylamine, bp 70–77° (0.2 mm) and glc 93%, by LAH redn of crude 1-adamantanepropionitrile[23] ] mp 36–38°, bp 98–100° (0.4 mm)] [the LAH redn resulted also in 24% of 3,3'-di-1-adamantylpropylamine, bp 193–195° (0.1 mm) and glc 98%, *Anal.* (C$_{26}$H$_{43}$N) C, H, N]; 1-adamantanepentylamine[24] [1-adamantanebutyric acid[23] + LAH-THF → 83% of 1-adamantanebutanol, bp 93–96° (0.05 mm), → crude 1-adamantane-

butyl tosylate → crude 1-adamantanebutyronitrile, bp 112–116° (0.2 mm), + LAH → 45% of 1-adamantanepentylamine, bp 99–102° (0.1 mm) and glc 95% (lit.[24] bp 171–172°, 11 mm)]; crude 3,5-dimethyl-1-adamantanemethylamine, bp 71–74° (0.1 mm) by LAH redn of 3,5-dimethyladamantane-1-carboxamide.[25]

**1-(2-Bromoethyl)adamantane.** Several attempts (some involved elaborate precaution) to scale up the published method[23] using AlBr$_3$ and ethylene failed to give reasonable yields. A mixt of 46.8 g (0.26 mole) of 1-adamantaneethanol and 300 ml of 40% by wt of dry HBr in AcOH was heated in a glass pressure bottle for 7 hr on a steam bath. The cooled mixt was poured over ice; the solid product was filtered off and dried to give 60 g (90%) of 1-(2-bromoethyl)-adamantane, mp 54–57° (without recrystn), bp 85–87° (0.2 mm) (lit.[23] mp 68–69° from MeOH).

**N-Substituted-2-chloroacetamides.** N-Butyl-2-chloro-N-methylacetamide had bp 68–75° (0.4 mm), glc 97% [*Anal.* (C$_7$H$_{14}$ClNO) C, H, N]; 1-(2-chloroacetyl)hexahydro-1H-azepine,[26] N-1-adamantyl-2-chloroacetamide,[27] crude N-2-adamantyl-2-chloroacetamide, mp 114–117° (C$_6$H$_6$), N-[1-(1-adamantyl)ethyl]-2-chloroacetamide, mp 103–106° [*Anal.* (C$_{14}$H$_{22}$ClNO) C, H, N]; 2-chloro-N-cyclohexylacetamide;[28] N-methyl-2-chloroacetanilide.[29]

**N-Substituted-S-(amidinomethyl) Hydrogen Thiosulfates (I). Method A. From 2-Chloroacetamides** *via* N-Substituted-ethyl 2-Chloroacetimidate Fluoroborates (VI) or N,N-Disubstituted(2-chloro-1-ethoxyethylidene)ammonium Tetrafluoroborates (VI). The general procedure is given by this example. A soln of freshly prepd Et$_3$O$^+$BF$_4^-$ (40 g, 0.21 mole) in CH$_2$Cl$_2$ (250 ml) was added to the amide 1-(2-chloroacetyl)hexahydro-1H-azepine (36.6 g, 0.21 mole) in CH$_2$Cl$_2$ (300 ml). After 18 hr of stirring at 25°, solvents were removed under vacuum. Addn of dry Et$_2$O to the residue caused crystn of the salt, ethyl (2-chloro-1-ethoxyethylidene)hexahydro-1H-azepin-1-ium tetrafluoroborate, whose characteristics are listed in Table VI.

Conversion of the preceding tetrafluoroborate salt (15.3 g, 0.053 mole) to the amidine fluoroborate was effected by dissolving it in EtOH (100 ml) and adding 100 ml of 1.2 N NH$_3$ in EtOH. After 3 hr of stirring at 0°, the mixt was permitted to come to 25°. Salts were filtered off and the filtrate was concd under vacuum. The residue of 1-(2-chloroacetimidoyl)hexahydro-1H-azepine fluoroborate (15.6 g) was used immediately for subsequent conversion.

Reaction of the crude acetamidine fluoroborate (15.6 g) with Na$_2$S$_2$O$_3$·5H$_2$O (13.0 g, 0.053 mole) in MeOH (200 ml) at the reflux yielded, after being chilled, the amidinium Bunte salt, S-[(hexahydro-1H-azpin-1-yl)formidoyl]methyl hydrogen thiosulfate (12.8 g, 96%), which crystd from MeOH, mp 173–175°. *Anal.* (C$_8$H$_{16}$N$_2$O$_3$S$_2$) C, H, N, S.

Another example using a secondary amine involves N-methylbutylamine as its 2-chloroacetyl deriv. Conversion with Et$_3$O$^+$BF$_4^-$, then NH$_3$ in EtOH, followed by Na$_2$S$_2$O$_3$·5H$_2$O produced S-(N-butyl-N-methylamidino)methyl hydrogen thiosulfate (17%), mp 148–150° (from H$_2$O). *Anal.* (C$_7$H$_{16}$N$_2$S$_2$O$_3$) C, H, N, S.

[#]All melting and boiling points are uncorrected. Melting points were determined using a Mel-Temp or Thomas-Hoover apparatus. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions are within ±0.4% of the theoretical values. Nmr and/or ir spectra were used in making structural assignments of new compounds.

Reaction of 1-Adamantyl(2-chloro-1-ethoxyethylidene)methyl-ammonium Tetrafluoroborate with NH₃.** The 2-chloroacet-imidate salt [1-admN⁺(CH₃)=C(OEt)CH₂ClBF₄⁻] (25 g, 0.07 mole) from *N*-1-adamantyl-*N*-methyl-2-chloroacetamide treated as under method A with ethanolic NH₃ gave solid product whose ir spectrum showed only a trace of amidine absorption. An Et₂O extract of the solid was washed with H₂O, dried (MgSO₄), and treated with dry HCl-*i*-PrOH to give 3.0 g of amine salt, mp 244–246°. The free base was liberated to give solid which was identical with authentic *N*-methyl-1-adamantanamine.

A mixture of 2.0 g (0.0057 mole) of 1-adamantyl(2-chloro-1-ethoxyethylidene)methylammonium tetrafluoroborate and 0.5 g of NH₄OAc in 40 ml of MeCN was stirred at 25° for 55 min. Most of the solid NH₄OAc had disappeared after 30 min. The solvent was removed, and the residue was washed with Et₂O and recrystd from EtOAc to give 0.7 g (50%) of *N*-methyl-1-adamantanamine fluoroborate (ir and nmr spectra), mp 152–156°. This was an unexpected result because a uv study (Table VI) indicated this 2-chloroacet-imidate salt to be relatively stable in MeCN at 25°. Furthermore, ethyl 2-chloro-*N*-cyclohexylacetimidate (Table VI) was converted in 23% yield to the Bunte salt (6) *via* reaction with NH₄OAc in MeCN at 25° for 1.5 hr and followed by Na₂S₂O₃.

All other examples of I prepd by this general method are compiled in Table I.

Method B. From *N*-Substituted-2-chloroacetamidine Hydrochlorides. This general procedure using chloroacetonitrile and a primary aliphatic amine has been described previously.⁵ Certain precautions are indicated to maximize yields: chloroacetonitrile should be freshly distd; equiv molar quantities of commercial or freshly prepd NaOMe and nitrile are used as a 0.1 *M* soln; addn of amine, followed by dry HCl in suitable alcohol, can be substituted for amine hydrochloride; both steps of the reaction sequence can 'e followed to completion with pmr;³ when the soln becomes intensely colored, it is imperative not to prolong the reaction time, *i.e.*, isolate crystalline salts or use crude 2-chloroacetamidines immediately; Na₂S₂O₃·5H₂O can be used in either MeOH or H₂O; solid Bunte salts were obtained by chilling the reaction mixts, concentrating the mixts to near dryness, or triturating the oily products with Et₂O and *i*-PrOH.

*S*-[1-(2-Hydroxyethyl)-2-imidazolin-2-yl]methyl Hydrogen Thiosulfate. 2-[(2-Aminoethyl)amino]ethanol (11.4 g, 0.11 mole) on being subjected to the conditions of method B gave 11.4 g (43%) of the imidazoline formed by cyclization³ of the aminoethyl amidine: mp 127–128°, ir (KBr) 1580 and 1610 cm⁻¹ (C=N). *Anal.* (C₆H₁₂N₂O₄S₂) C, H, N, S.



$$HO(CH_2)_2N \underset{}{\overset{}{\bigcirc}} CH_2S_2O_3H$$

Method C. *S*-(Cyclopentylamidino)methyl Hydrogen Thiosulfate (4). A soln of 2-chloro-*N*-cyclopentylacetamidine hydrochloride obtained as a gum from 0.057 mole of chloroacetonitrile in H₂O (50 ml) and Tl₂S₂O₃ (31.8 g, 0.057 mole) was mixed and heated at 96° for 1.25 hr. The cooled (25°) reaction mixt was filtered to remove TlCl. The aqueous filtrate was then evapd under vacuum to yield a pale cream solid (4) (5 g, 77%), mp 161–167°.

*N*-Substituted-2-mercaptoacetamidine hydrochlorides (II) were prepd by HCl hydrolysis of IV as described previously,⁵ except that 30 ml of 25–45% aqueous THF (peroxide-free) was used in the displacement with SPO₃³⁻ for each 0.01 mole of the slightly H₂O-soluble 2-chloroacetamidines. 2-Chloroacetamidine precursors to 58 and 60 were prepd in H₂O. Thiol percentages obtained by I₂ titration usually were checked using the *N*-ethylmaleimide uv assay.³⁰

2,2'-Dithiobis[*N*-substituted acetamidine] Hydrochlorides (III). The thiols were prepd as described for II, but without isolation, using H₂O as the reaction solvent for 69 and 71–74, 25% aqueous THF (27 ml/0.1 mole) for 70, and 45% aqueous THF (14 ml/0.1 mole) for 76. Following HCl hydrolysis, the mixt was removed from heat and treated with *ca.* 1 molar equivalent of 30% H₂O₂. Alternatively, insoluble thiols were dissolved in hot MeOH before reaction with H₂O₂. The pptd (from chilled or concd mixt) disulfides (III) were purified as indicated in Table III.

2,2'-Dithiobisacetamidine Sulfate. A soln of *S*-(amidinomethyl) hydrogen thiosulfate (17.0 g, 0.1 mole), thiourea (7.6 g, 0.1 mole), and 1 *N* H₂SO₄ (250 ml) was heated on a steam bath for 2.5 hr. Pptd S was separated and the filtrate was concd to half vol. EtOH was added to the cooled soln until it became cloudy, effecting

crystn of the disulfide sulfate. Recrystn from H₂O–EtOH gave 3.9 g (28%), mp 212–215° dec (lit.⁵ mp 225–227° dec).

When less soluble disulfides were formed, S coprecipitated with the product. Extn of the solid with several small vols of either boiling CS₂ or C₆H₆ was effective in separating the S.

Sodium Hydrogen *S*-[(Substituted amidino]methyl Phosphoro-thioates (IV) were synthesized as described previously⁵ from *N*-substituted-2-chloroacetamidine hydrochlorides or fluoroborates and Na₃SPO₃, except that either 30–35 ml of 30% aqueous THF (peroxide-free) or 15–20 ml of 40% aqueous DMF was used for each 0.01 mole of the slightly H₂O-soluble 2-chloroacetamidines.

Mercaptoacetic Acid, 2-Amino-1-methylethyl Ester, *S*-Thio-sulfate Ester [HO₃S₂CH₂CO₂CH(CH₃)CH₂NH₂]. A cold (5°) soln of methyl 2-chloroacetimidate (from 0.2 mole of chloroacetonitrile) in 100 ml of abs MeOH was treated in one portion with 15.0 g (0.2 mole) of 1-amino-2-propanol dissolved in 40.6 ml of *i*-PrOH, 4.9 *N* in dry HCl. The mixt was stirred in the cold for 5 hr, the pH was adjusted to *ca.* 3 with 1 *N* NaOH, and then 49.6 g (0.2 mole) of Na₂S₂O₃·5H₂O in 50 ml of H₂O was added. The mixt was heated under reflux for 1 hr and evaporated to dryness. The residue in hot *i*-PrOH was filtered; the filtrate was chilled to give 20.7 g (59%) of product, mp 151–157°. Four recrystns from MeOH gave material having mp 174–178° dec; ir (KBr) 1728 cm⁻¹ (C=O); nmr (DMSO-*d₆*) δ 7.75 (m, 3, removed with D₂O, H₃N⁺), 4.78–5.34 (m, 1, OCH), 3.73 (s, 2, SCH₂), 2.92–3.28 (m, 2, CH₂N), and 1.24 ppm (d, 3, *J* = 7 Hz, CH₃); p$K_a$' = 8.0 and mol w t 226 (titration in 50% DMF, single inflection). *Anal.* (C₅H₁₁NO₅S₂) Calcd: C, 26.23; H, 4.84; N, 6.12; O, 34.93; S, 28.00; H₂O, 0.00. Found: C, 26.22; H, 4.95; N, 6.04; O, 35.19; S, 28.00; H₂O, 0.00.

Reaction of 2-aminoethanol under identical conditions gave crude solid whose ir spectrum showed amidine absorption and no ester at all; 28 was isolated and characterized.

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# *Notes*

## Pyridine Isosteres of the β-Adrenergic Antagonists, 2-(p-Nitrophenyl)-1-isopropylamino-2-ethanol and 3-(p-Nitrophenoxy)-1-isopropylamino-2-propanol†
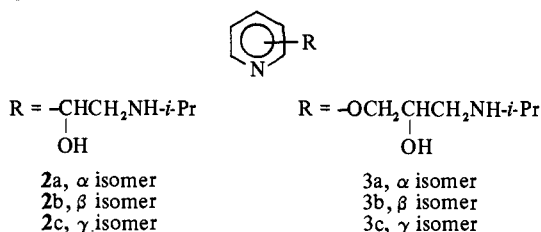
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Structure–activity relationships (SAR) of cardiac β-adrenergic agonists and antagonists are of great current interest and are becoming clearer with newer understanding of the mode of action of adrenergic drugs.[1] Yet the structural criteria for agonism and antagonism are still elusive. As part of a general SAR program, the nitrobenzene ring of known β-antagonists[2] was replaced with the isosteric pyridine ring to ascertain whether these compounds would be bioisosteric as is often the case with this transformation.[3]
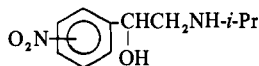


1a, ortho isomer
1b, meta isomer
1c, para isomer (INPEA)

The pyridine compounds selected were the following types



R = -CHCH₂NH-*i*-Pr
         |
        OH

2a, α isomer
2b, β isomer
2c, γ isomer

R = -OCH₂CHCH₂NH-*i*-Pr
                 |
                OH

3a, α isomer
3b, β isomer
3c, γ isomer

as these are the side chains known to confer β-adrenergic receptor antagonism to many aromatic ring systems.[4]

**Chemistry.** The syntheses of the three isomeric pyridyl isopropylaminoethanols from the known ω-bromo ketones followed the sequence shown in Scheme I. This is essentially the method of Friz[5] who prepared the 4-pyridyl isomer. The intermediate bromohydrins are unstable in alkaline solution forming deep colored products, presumably from condensation reactions. Decomposition was prevented by rapid conversion to the HCl salts. The alternate sequence involving reaction of the bromo ketone with isopropylamine, followed by NaBH₄ reduction, was not as satisfactory. The properties of the amino alcohols prepared are listed in Table I.

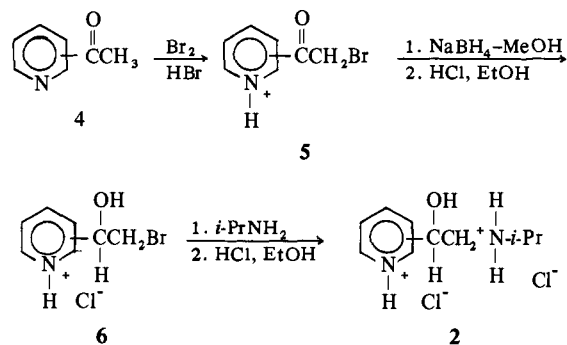The synthetic approaches to the 2,3- and 4-pyridyl ana-

**Scheme I**



**Table I**



| Compd | % yield (base) | Recrystn solvent (2HCl) | Mp, °C dec (2HCl) | Formula |
|-------|------|------|------|------|
| 2a | 37 | *i*-PrOH | 153–156 | C₁₀H₁₈N₂OCl₂[a] |
| 2b | 46 | EtOH | 162–164 | |
| 2c | 73 | EtOH–Et₂O | 182–183[b] | |

*[a] Anal.* C, H; H: calcd, 7.16; found, 7.63.  *[b] Lit.[5] 186° dec.*

logs of the 3-(nitrophenoxy)-1-isopropylamino-2-propanols were quite different and it was possible to prepare only the 4 isomer. From the reaction of 3-pyridol with epichlorohydrin, followed by excess *i*-PrNH₂, the expected pyridyl ether was not isolated but 1,3-diisopropylamino-2-propanol (see Experimental Section) and some unreacted 3-pyridol were. Recently Howe, *et al.*,[6] reported that reaction of the sodium salt of 3-pyridol with 1-chloro-3-(*N*-benzyl-*N*-isopropylamino)-2-propanol gave the betaine (7).
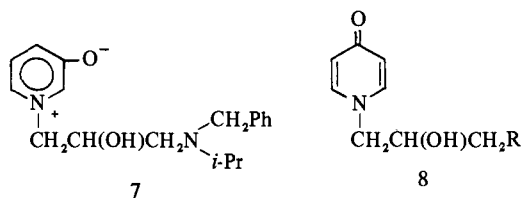


A similar reaction sequence involving 4-hydroxypyridine and epichlorohydrin followed by isopropylamine led only to N-substitution, giving 4-pyridone compounds of general structure 8. Reaction of 4-hydroxypyridine with epichlorohydrin and Ag₂CO₃ in acetone also gave only ketone products in accord with the reported reaction of alkyl halides with the silver salt of 4-pyridol.[7] A successful synthesis of the 4-pyridyl analog is outlined in Scheme II.

For the addition of HOBr to the known 4-allyloxy ether,[8]