Antiradiation Compounds XXII. Methyl 3-Amino-2-phenyldithiopropenoates and 1,1-Bis(methylthio)-3-amino-2-phenyl-1-propenes

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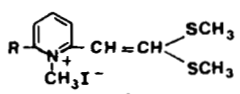
Abstract □ The title compounds were prepared in the attempt to provide methylthio and bis(methylthio) analogues of the radioprotective pyridinium- and quinolinium-2-dithioacetic acid derivatives in which the methylthio function is attached to an amino group through an aliphatic chain. The methyl 3-amino-2-phenyldithiopropenoates were obtained by the reaction of amines with 4-phenyl-3-methylthio-1,2-dithiolium iodide, and the 1,1-bis(methylthio)-3-amino-2-phenyl-1-propenes were obtained by methylation and reduction of the dithiopropenoates. The methyl dithiopropenoates with aliphatic substituents on the nitrogen gave only fair or poor radiation protection in mice, and one example of the reduced bis(methylthio) derivatives tested was inactive. The precursor 1,2-dithiole-3-thione and its methiodide, predicted to be radiation protective, were found inactive in this test.

A series of bis(methylthio)vinyl derivatives (ketene dithioacetals) of *N*-methylquinolinium and *N*-methylpyridinium iodides (1) has shown appreciable radiation-protective activities against lethal doses of ionizing radiation in mice.[1] Bis(methylthio)vinyl compounds, in which this function is attached to an aliphatic amino group, have not been evaluated for this activity. Compounds of this type (2) should be obtainable from the reaction of primary and secondary amines with 1,2-dithiole-3-thiones (3),[2] followed by methylation of the resulting methyl dithio ester (4), as shown in Scheme I. Preparation and testing of these compounds should indicate the relative importance of the bis(alkylthio)vinyl function and the quaternized aromatic ring in conferring radioprotective properties to 1. The mechanism by which compounds of type 1 exert radiation protection to animals is unknown, although the possibility exists that they may complex copper ion and act as mimics of superoxide dismutase.[3,4] Another possible mechanism lies in the fact that aryl-1,2-dithiole-3-thiones are known to raise glutathione levels in cells.[5] It is a possibility that since the 1,2-dithiole-3-thiones react rapidly with amines to give 4, the products of the amine reaction (4) may be responsible for raising glutathione levels. In this event, compounds of type 2 or 4 could be radioprotective; increased glutathione levels have been shown to provide protection in mice against hepatotoxic agents and have been postulated to be of value in radiation protection.[5]

Accordingly, a number of methylthio derivatives of types 2 and 4 were prepared. In order to obtain stable derivatives of type 2, a reduction step was necessary. Examples of each type were screened for radiation-protective properties in mice. The products obtained are listed in Table I.



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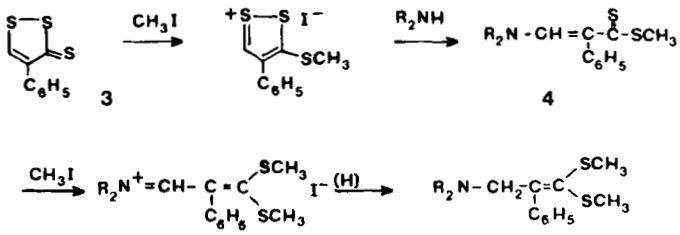


3 4 2

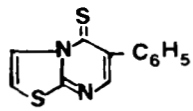
Scheme I

Results and Discussion

Chemistry—Treatment of the methyl iodide salt of 4-phenyl-1,2-dithiole-3-thione (3) with a secondary or tertiary amine produces the methyl ester of a 3-amino-2-phenyldithiopropenoic acid.[2] Preparation of the methylamino and dimethylamino products proceeded with good yields and rapid reactions at room temperature. Reaction with morpholine and *N*-aminomorpholine also gave moderately good yields, but required longer reaction times. Reaction with ammonia gave an unstable product. LeCoustemer and Mollier[2] reported that tertiary amino products, as from dimethylamine, exist as the E isomer, while secondary amino products prefer the Z configuration. Comparison of the ^{1}H NMR spectrum of the methylamino product (**4a**) with that of **4b** indicated the presence of both isomers, the spectrum of the minor component resembling more closely that of the E form of **4b**.

Reaction of the methiodide of 3 with aqueous ammonia gave a significant amount of starting dithiolethione 3, unlike the reactions with primary or secondary amines. This was attributed to removal of the S-methyl group to form methylamine hydroiodide, which was also isolated.

Reaction of the methiodide of 3 with several heterocyclic amines at room temperature or slightly above gave the expected 3-amino-2-phenyldithiopropenoic methyl esters in good yield. Generally, a mixture of cis and trans isomers was found from the NMR singlets at either δ 2.66 or 2.56, attributed to the S—CH_3 protons. Reaction with 2-aminothiazole, however, gave a yellow, crystalline compound which showed no S—CH_3 protons in the NMR spectrum, and only eight protons, all in the aromatic region. Elemental analysis confirmed that cyclization had taken place to give 4*H*-3-phenyl-4-thiothiazolo-[2,3-*b*]pyrimidine (5).



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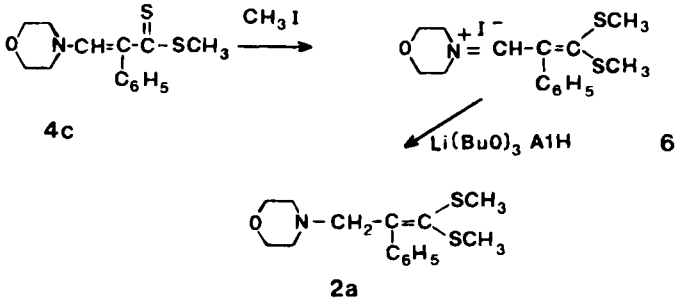
To convert the amino adducts 4 to the bis(thiomethyl)vinyl derivatives, 4c was treated with methyl iodide to give the easily hydrolyzable salt 6. Suitable reducing conditions were found using lithium tri(*tert*-butoxy)-aluminum hydride in a mixture of methylene chloride and tetrahydrofuran. Approximately 50% of the reduced derivative (**2a**) was obtained (Scheme II). Some morpholine and an aldehyde were also formed, possibly as a result of hydrolysis of unreacted 6 during the workup. The pyrrolidino derivative **4e** was also reduced to the bis(methyl) derivative (**2b**) by this procedure. The pyrimidinyl compound **4g** was reduced with sodium borohydride in dimethylformamide without prior conversion



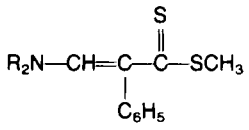
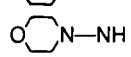
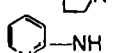
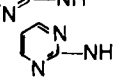
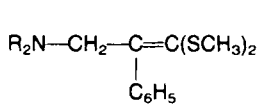
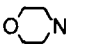
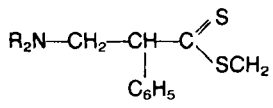
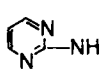
Scheme II

to the bis(methylthio) derivative and gave the reduced thio ester 7. Only one methylthio group appeared in the ^{1}H NMR spectrum, at 2.57 ppm.

Radiation-Protective Activities—Testing results for radiation protection were supplied by the Walter Reed Army Institute of Research. Tests were performed by whole-body γ-irradiation of mice, using 1000 rads, generally 30 min after administration of compound, which was given intraperitoneally in 10–20% ethanol or Klucel. The testing results are shown in Table II.

The methyl 3-amino-2-phenyldithiopropenoates derived from methylamine and dimethylamine provided fair (25–45%) and poor (<25%) protection, respectively, at dose levels of 150–600 mg/kg. The morpholino derivatives of both the dithiopropenoate ester and the reduced bis(methylthio) compound gave no protection at dose levels of 300–1200 mg/kg, but the *N*-aminomorpholine derivative of the dithiopropenoate ester gave poor protection. The methylthio morpholino derivative of the 1-methylquinolinium-2-dithioacetic acid,[1] however, provided 60% protection to mice at the exceptionally low dose of 9.38 mg/kg, although the corresponding pyridinium derivative was inactive. The increase in activity realized by inclusion of morpholine in a series of antileukemic methylthio heterocyclic amino derivatives of the 1-methylquinolinium-2-dithioacetic acids[6] was not observed in the radiation-protective properties of the morpholine-containing derivatives of the dithiopropenoate esters.

Table I—Physical Properties of Methyl 3-Amino-2-phenyldithiopropenoates and 1,1-Bis(methylthio)-3-amino-2-phenyl-1-propenes

No.	R_2N	Melting Point, °C	Yield, %	Formula[a]	Analyses
					
4a	CH_3NH	134–136	83	$C_{11}H_{13}NS_2$	C,H,N
4b	$(CH_3)_2N$	149–151	49	$C_{12}H_{13}NS_2$	C,H,N
4c		120–126	65	$C_{14}H_{17}NOS_2$	C,H,N
4d		132–135	44	$C_{14}H_{18}N_2OS_2$	C,H,N
4e		145–149	82	$C_{14}H_{17}NS_2$	C,H,N
4f		170–175	91	$C_{15}H_{14}N_2S_2$	C,H,N
4g		134–139	60	$C_{14}H_{13}N_3S_2$	C,H,N
					
2a		oil	49	$C_{15}H_{21}NOS_2$	C,H,N
2b		oil	52	$C_{15}H_{21}NS_2$	C,H,N
					
7		105–107	42	$C_{14}H_{15}N_3S_2$	C,H,N

[a] Satisfactory analytical data (±0.4% for C,H,N) were found for all compounds listed.

The comparatively high dose levels at which the 3-amino-2-phenyldithiopropenoate esters showed activity (150–600 mg/kg) is in contrast to the exceptionally low dose levels (1–20 mg/kg) at which the 1-methylquinolinium- and 1-methylpyridinium-2-dithioacetic acid derivatives gave protection.[1] The only bis(methylthio) derivative of the present series which was tested for protective activity provided no protection, which indicates as well that the low activity of the amino dithiopropenoate esters is most likely not due to the same mechanism as that responsible for the activity of the pyridinium and quinolinium dithioacetic acid derivatives.

The possibility that the amino dithiopropenoate esters might provide protection by raising glutathione levels in cells (in analogy to this activity of the dithiolethiones) appears unlikely in view of the lack of radiation-protection activity of the two 1,2-dithiole-3-thione precursors tested. A more likely manner in which the 3-amino-2-phenyldithiopropenoate esters provide radiation protection is through hydrolysis to the dithio acid which can undergo H atom transfer to radiation-caused free radicals of important cellular constituents, a mechanism proposed for the protective action of the aminoalkanethiols.[7] Another potential mechanism is the complexation of copper(II) ion by the dithio ester and the resulting action of the copper complex as a superoxide dismutase mimic.[8]

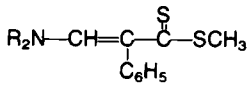
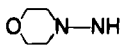
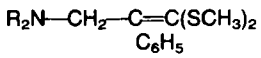
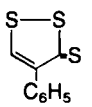
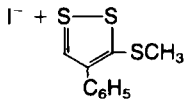
Experimental Section

Melting points were determined in capillaries with a Mel-Temp block and are uncorrected. The ^1H NMR spectra were obtained with a Varian T-60 spectrometer, using tetramethylsilane as internal standard. The IR spectra were obtained with a Perkin-Elmer model 457A spectrophotometer using KBr pellets. Elemental analyses were done by Multi Chem Laboratories, Lowell, MA, and are within ±0.4% of theoretical values (Table III). Thin-layer chromatography was carried out using silica gel plates with fluorescent indicator. Organic reagents were supplied by Aldrich Chemical Co. or Eastman Organic Chemicals.

4-Phenyl-1,2-dithiole-3-thione—The method of Fields[9] was followed, giving a 48% yield of red-brown solid, mp 121–122.5 °C (lit[9] 122 °C).

4-Phenyl-3-methylthio-1,2-dithiolium Iodide—The method of Fields[9] was used, giving a 97% yield of brown powder, mp 177–178 °C dec (lit[9] 194 °C dec).

Table II—Radiation-Protective Properties in Mice[a]

R_2N	LD_{50}, mg/kg, 10 d[b]	Route	Dose, mg/kg	Survival, %[c]
				
CH_3NH	>600	ip	600	10
			300	40
			150	20
$(CH_3)_2N$	>1200	ip	1200	0 (3 tox)
			600	20
			300	0
	>1200	ip	1200	0
			600	0
			300	0
	>600	ip	600	0
			300	10
			150	0
				
	>1200	ip	1200	0
			600	0
			300	0
				
	>300	ip	300	0
			150	0
			75	0
				
	~100	ip	75	0 (1 tox)
	~100		37.5	0 (1 tox)
			18.75	0

[a] Groups of 10 mice were exposed to 1000 rad of γ radiation 30 min after dosing. [b] Toxic doses were determined in 10–20% ethanol by ip administration in mice; animals were observed for 10 d. [c] Percent survival was determined over a 30-d period; deaths due to toxicity of drug (shown in parentheses) appeared before 5 d.

Methyl 3-Methylamino-2-phenylpropenedithiocarboxylate (4a)—To a suspension of 4-phenyl-3-methylthio-1,2-dithiolium iodide (1.0 g, 2.84 mmol) in 30 mL of benzene was added 1 mL of a 40% aqueous solution of methylamine. The reaction was stirred vigorously for 15 min at room temperature, the aqueous phase was separated, and the benzene solution was dried (Na_2SO_4). Removal of solvent on a rotary evaporator gave a dark yellow solid which was purified by chromatography on alumina to give 0.49 g (83%) of orange crystals, mp 128–132 °C; IR (KBr): ν 1620 (C=C), 1250 (C=S), 930 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.5 (s, 3H, SCH_3), 3.0–3.2 (q, 3H, NCH_3), 6.7–7.0 (d, 1H, CH=C), and 7.3 (s, 5H, arom) ppm.

Anal.—($C_{11}H_{13}NS_2$) C,H,N.

Methyl 3-(*N*-Morpholinylamino)-2-phenylpropenedithiocarboxylate (4d)—To a suspension of 1.0 g (2.84 mmol) of 4-phenyl-3-methylthio-1,2-dithiolium iodide in 20 mL of benzene was added 0.4 mL (2.87 mmol) of triethylamine and 0.3 g (2.94 mmol) of *N*-aminomorpholine. The mixture was stirred for 45 min at room temperature and filtered, and the residue was washed with benzene. Evaporation of the combined filtrates gave 1.02 g of an orange solid which was chromatographed on silica and recrystallized from benzene:hexane, giving 0.37 g (44%) of brown crystals, mp 132–135 °C; IR (KBr): ν 1605 (C=C), 1225 (C=S), 900 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.45 (s, 3H, SCH_3), 2.8–3.1 (m, 4H, $N-(CH_2)_2$), 3.55–3.80 (m, 4H, $O(CH_2)_2$), 7.03–7.33 (m, 6H, CH=C, arom), and 13.05 (s, 1H, NH, J = 11 Hz) ppm.

Anal.—($C_{14}H_{18}N_2OS_2$) C,H,N.

Methyl 3-(2-Pyrimidinylamino)-2-phenylpropenedithiocarboxylate (4g)—To a stirred suspension of 4-phenyl-3-methylthio-1,2-dithiolium iodide (7.04 g, 20 mmol) in dimethylformamide (70 mL) was added 2-aminopyrimidine (3.80 g, 40 mmol) in dimethylformamide (15 mL) in a dropwise manner over a period of 10 min. The mixture was warmed at 40–50 °C for 2 h and then cooled to room temperature and filtered. The filtrate was diluted with ice-cold water (200 mL), extracted with ethyl acetate (4 × 100 mL), washed with water (5 × 50 mL), and dried overnight (Na_2SO_4). Solvent was removed and a red-orange solid (4.74 g) was obtained. Crystallization from methylene chloride:heptane gave a mixture of cis and trans isomers as red-orange needles, mp 136–139 °C; IR (KBr): ν 1600 (C=C, 1200 (C=S), 950 (C=S) cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.56 and 2.66 (2s, 3H, SCH_3), 6.7–6.9 (m, 1H, CH=C), 7.0–7.4 (br s, 5 H, arom), and 7.8–9.3 (m, 3H; pyr) ppm.

Anal.—($C_{14}H_{13}N_3S_2$) C,H,N.

1,1-Bis(methylthio)-3-(1-morpholinylidene)-2-phenyl-1-propene Iodide (6)—A solution of 1.00 g (1.35 mmol) of **4c**, 25 mL of toluene, and 1.1 mL (17.7 mmol) of methyl iodide was stirred at room temperature for 20 h. The resulting solid was filtered, washed with toluene, and dried under reduced pressure to give 1.27 g of orange powder, mp 130–147 °C. It was recrystallized by dissolving in 3 mL of hot acetonitrile, adding 9 mL of ethyl acetate in a dropwise manner, and cooling. The red crystals were washed with ethyl acetate and dried, giving 1.10 g (81.5%), mp 145–150 °C; IR(KBr): ν 1580 (C=N^+) cm $^{-1}$; ^1H NMR ($CDCl_3$): δ 2.27 (br s, 3H, SCH_3), 2.82 (br s, 3H, SCH_3), 3.20–3.50 (d, 4H, $(CH_2)_2N$), 4.10–4.33 (d, 4H, $(CH_2)_2O$), 7.27–7.67 (m, 5H, arom), and 9.47 (s, 1H, CH=N) ppm.

Table III—Analyses

Compound	Theoretical, %			Found, %		
	C	H	N	C	H	N
4a	59.15	5.86	6.27	59.21	5.98	5.91
4c	60.18	6.13	5.01	59.99	6.06	4.94
4d	57.11	6.16	9.51	57.23	6.19	9.41
4e	63.83	6.51	5.32	64.14	6.57	5.34
4f	62.90	4.93	9.78	62.97	5.06	9.65
4g	58.51	4.56	14.62	58.61	4.60	14.42
5	58.99	3.30	11.47	58.99	3.48	11.43
2a	60.98	7.16	4.74	60.76	7.12	4.73
2b	64.47	7.57	5.01	64.39	7.61	5.49
7	58.10	5.22	14.52	58.08	5.39	14.28

1-Bis(methylthio)-3-(1-morpholinyl)-2-phenyl-1-propene (2a)—To a stirred solution of 1.00 g (2.37 mmol) of 1,1 bis(methylthio)-3-(1-morpholinylidene)-2-phenyl-1-propene in 20 mL of methylene chloride was added a solution of 0.61 g (2.40 mmol) of lithium tri(*t*-butoxy)aluminum hydride in 24 mL of dry tetrahydrofuran over a 20-min period. Evaporation of the solvents after 18 h of stirring at room temperature gave an orange paste. This was taken up in 50 mL of 1 M HCl and extracted with three 20-mL portions of ethyl acetate. The aqueous phase was made alkaline with 50% KOH solution and extracted with two 20-mL portions of ethyl acetate. The extracts were dried (Na_2SO_4), filtered, and evaporated to give 0.44 g of a clear yellow oil. Chromatography on silica afforded 0.34 g (49%) of product as yellow oil and 0.08 g of an aldehyde. IR(neat): ν 1590 (C=C), 1110 (CH_2—O) cm $^{-1}$; ^1H NMR ($CDCl_3$); δ 2.18 (s, 3H, SCH_3), 2.22–2.33 (m, 7H, SCH_3 + $N(CH_2)_2$), 3.40–3.67 (m, 6H, $O(CH_2)_2$ + NCH_2), and 7.25 (s, 5H, arom) ppm.

Anal.—($C_{15}H_{21}NOS_2$) C,H,N.

***4H*-3-Phenyl-4-thiothiazolo[2,3-*b*]pyrimidine (5)**—To a stirred suspension of 4-phenyl-3-methylthio-1,2-dithiolium iodide (5.25 g, 0.15 mol) in 100 mL of dimethylformamide (DMF) was added 2-aminothiazole (4.0 g, 0.04 mol) in 15 mL of DMF over a 5–10-min period. The mixture was stirred at room temperature overnight and was filtered. The filtrate was diluted with water (200 mL) and extracted with ethyl acetate (5 × 50 mL). The extract was washed with water (3 × 50 mL) and dried (Na_2SO_4). Solvent was removed in a rotary evaporator, and the resulting solid was crystallized from methylene chloride:heptane giving 2.1 g of yellow crystals, mp 152–154 °C; ^1H NMR ($CDCl_3$): δ 7.10–7.69 (m, 6H, arom), 8.0 (s, 1H, arom), and 9.0 (d, 1H, J = 5 Hz, arom) ppm.

Anal.—($C_{12}H_8N_2S_2$) C,H,N.

Test for Radiation Protection—Compounds were administered to groups of 10 mice at each dose level 30 min prior to whole-body irradiation with 1000 rads from a ^{60}Co source. Female C57 B16 mice, 18–20 g, were used. Compounds were generally injected intraperitoneally in water or 10–20% aqueous ethanol; in some cases Klucel was also required. Animals surviving beyond 30 d were considered protected.[10]

References and Notes

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10. Tests for radiation protection were provided by the Walter Reed Army Institute of Research.

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