# 6-Substituted 1-Methylquinolinium-2-dithioacetic Acid Zwitterions: Antileukemia Activity

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**Abstract** □ Condensation of the anhydro bases derived from 6-substituted 1-methylquinaldinium iodides with carbon disulfide provided the 2-dithioacetic acid zwitterions. Since 1,6-dimethylquinolinium-2-dithioacetic acid zwitterion previously showed appreciable activity against P-388 lymphocytic leukemia in mice, a series of analogs with other 6-substituents was synthesized to assess the importance of substitution in this position. Both electron-donating and electron-attracting 6-substituents gave compounds with antileukemia activity. The 6-unsubstituted analog had only marginal activity.

**Keyphrases** □ Dithioacetic acids, quinolinium—zwitterions synthesized, antileukemic activity evaluated in mice □ Zwitterions—quinolinium-2-dithioacetic acids synthesized, antileukemic activity evaluated in mice □ Antileukemic activity—various quinolinium-2-dithioacetic acid zwitterions evaluated in mice □ Structure-activity relationships—quinolinium-2-dithioacetic acid zwitterions evaluated for antileukemic activity in mice

1,6-Dimethylquinolinium-2-dithioacetic acid zwitterion (IV, Scheme I, R = $CH_3$) showed appreciable activity against P-388 lymphocytic leukemia in mice (1). It was considered that the dithiocarboxylic anion was a necessary function for this activity; but since several other related structures having a dithiocarboxylic anion lacked antileukemic activity, the 6-substituent of the quinoline ring also was regarded as important to this activity.

A similar requirement for a 6-substituent in the quinoline ring was found necessary for antileukemic activity in a series of 6-nitro- and 6-aminobis(4-quinaldinyl)-$\alpha,\omega$-diaminoalkanes (2). Accordingly, a series of 6-substituted *N*-methylquinolinium-2-dithioacetic acid zwitterions was synthesized to assess the importance of the 6-substituent in antileukemic activity. Both electron-donating and electron-attracting groups were included.

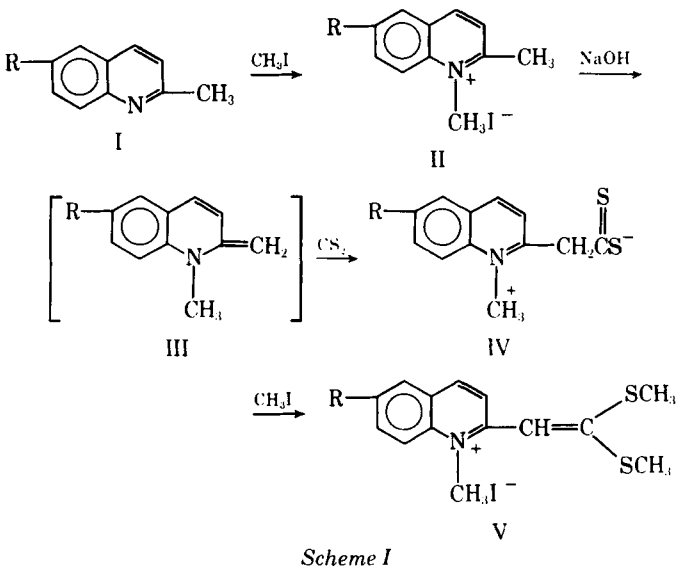
## DISCUSSION

**Chemistry**—Synthesis of the quinaldines (I, Scheme I), based on the method of Campbell and Schaffner (3), involved the condensation of the *para*-substituted anilines with crotonaldehyde, using ferric chloride as the oxidizing agent. The free bases rather than the aniline hydrochlorides could be used, and 85% crotonaldehyde gave satisfactory yields. Isolation of the quinaldines was accomplished by addition of toluene to the reaction mixtures, azeotropic distillation of water, and distillation of the resulting toluene solutions. 2,4-Dimethylquinoline was prepared from 3-penten-2-one.

6-Trifluoromethylquinaldine, a new compound, was obtained in 21% yield. The NMR spectrum showed three C-methyl protons at $\delta$ 2.71 ppm, unsplit, and the aromatic protons at $\delta$ 7.2–8.2 ppm. Yields of the other substituted quinaldines ranged from 40 to 62%.

Preparation of the *N*-methylquinaldines first was attempted with dimethyl sulfate to provide higher yields and to avoid the discoloration associated with methiodides. Reaction of dimethyl sulfate with quinaldine gave an 80% yield of colorless material melting at 156–157° [lit. mp 152° (4) and 220° (5)]. Use of the methylsulfate of quinaldine gave only a 5% yield of the dithioacetic acid zwitterion, so the methiodides were employed.

The methiodides (II) were prepared by heating the quinaldines without solvent in a 25% excess of iodomethane (6). This method worked well for the 4-methyl, 6-methyl, 6-methoxy, and the 6-bromo compounds, but it gave no product with 6-chloroquinaldine. The 6-chloro methiodide was obtained by slow addition of iodomethane to a solution of the quinaldine in toluene at 100°. The only previous method reported for this compound was a sealed-tube reaction (7). The same procedure was used for the 6-trifluoromethyl methiodide, but the yield was only 13%.




*Scheme I*

A previous preparation of pyridinium- and quinoliniumdithioacetic acid zwitterions involved the condensation of *N*-methylpicolinium or *N*-methylquinaldinium iodide with carbon disulfide in a solution of sodium hydroxide in aqueous dioxane (1). Rosenhauer (8) reported the preparation of the 1-methylquinolinium-2-dithioacetic acid zwitterion (IV, R = H) by isolation of the intermediate methylene base (III) followed by condensation with carbon disulfide. Repetition of this method gave a green solid melting at 73–75° [lit. mp 195° (8) and 185–187° (1)], which decomposed slowly on standing in a desiccator. Gompper *et al.* (9) also found this preparation to be unstable at −60°. Therefore, the previous procedure (1) was employed, and some improvement in yields was realized by vigorous mechanical stirring, use of less sodium hydroxide, and prevention of overheating of the reaction mixtures. Yields of the dithioacetic acid zwitterions (IV) ranged from 27 to 68%. These compounds were too insoluble in the common solvents for NMR spectra to be taken.

Preparations of the bis(*S*-methyl) derivatives of dithio acids were reported (9, 10). With the 6-bromo-1-methylquinolinium-2-dithioacetic acid zwitterion, treatment with excess iodomethane gave a bis(*S*-methyl) derivative (V, R = Br) whose NMR spectrum showed five ring protons at $\delta$ 8.2–9.0 ppm, *N*-methyl protons at $\delta$ 4.35 ppm, *S*-methyl protons at $\delta$ 2.50 and 2.63 ppm, and a one-proton singlet at $\delta$ 6.65 ppm for the olefinic proton.

The dithio acid zwitterion of the 6-unsubstituted 1-methylquinaldinium iodide also was converted to the bis(*S*-methyl) ketene acetal and showed essentially the same NMR spectrum. The *N*-methyl protons appeared at $\delta$ 4.40 ppm, the *S*-methyl protons at $\delta$ 2.55 and 2.70 ppm, and the olefinic proton at $\delta$ 6.80 ppm. Gompper *et al.* (9) reported this compound, but the only physical constant listed was a decomposition point of 203°; in the present preparation, a melting point of 198–201° was obtained.

**Antileukemia Test Results**—Antileukemia testing was performed[1]

[1] At Lederle Laboratories; results were reported through the courtesy of Dr. Ralph G. Child.



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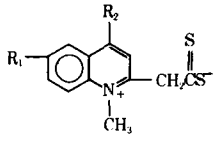
**Table I—Antileukemia Activities in Mice**



| | | | Median Survival Time, T/C × 100 at | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 300 | 200 | 100 | 50 | 12.5 | 3.25 |
| $R_1$ | $R_2$ | Test | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg | mg/kg |
| H | H | 1 | — | 130 | — | 110 | 100 | 100 |
| | | 2 | 130 | 120 | 120 | — | — | — |
| H | $CH_3$ | 1 | — | 110 | — | 110 | 90 | 115 |
| | | 2 | 110 | 110 | — | — | — | — |
| $CH_3$ | H | 1 | — | 140 | — | 120 | 120 | 110 |
| Cl | H | 1 | — | 130 | — | 130 | 115 | 110 |
| | | 2 | 145 | 140 | 130 | 115 | — | — |
| Br | H | 1 | — | 135 | — | 120 | 105 | 115 |
| | | 2 | 145 | 120 | 120 | — | — | — |
| $CH_3O$ | H | 1 | — | 135 | — | 125 | 110 | 115 |
| | | 2 | 150 | 140 | 130 | 120 | — | — |
| $CF_3$ | H | 1 | — | 105 | — | 105 | 100 | 105 |
| | | 2 | 100 | 100 | — | — | — | — |

using P-388 lymphocytic leukemia essentially in accordance with the protocol of the National Cancer Institute (11). $BDF_1$ (C57BL/6 × DBA/2)$F_1$ hybrid mice, 18–22 g, were inoculated intraperitoneally with $10^6$ tumor cells. Test compounds were administered intraperitoneally on Days 1, 5, and 9 following tumor inoculation. Control mice received saline or 0.2% Noble agar[2] in saline. Six mice were used in each test group, and 12–18 mice were in each placebo control group. Animals were observed daily for deaths, and the median survival time was calculated. No weight loss was found at the highest dose used.

Of the seven compounds tested, five showed positive activity (a T/C × 100 ratio of 125 or better) (Table I). Although the 6-unsubstituted compound gave marginal activity, somewhat better results were shown by the 6-substituted derivatives. Little difference in activity appeared between electron-attracting and electron-donating substituents, although the strongly electronegative trifluoro derivative was inactive. It may be concluded that 6-substituents contribute to the antileukemic activity of these compounds; but similarly to the series of 6-nitro- and 6-amino-bis(4-quinaldinyl)-α,ω-diaminoalkanes (2), the relative electronegativities of the substituents seem unimportant.

## EXPERIMENTAL[3]

**2,4-Dimethylquinoline**—This compound was prepared in 38% yield by the method of Campbell and Schaffner (3), bp 81–82° (0.8 torr) [lit. (3) bp 103–105° (2–3 torr)].

**6-Methoxyquinaldine**—This compound was prepared in 40% yield by the method of Campbell *et al.* (12), bp 95–96° (0.5 torr) [lit. (12) bp 145–146° (8 torr)].

**6-Chloroquinaldine**—To a stirred solution of 100 g (0.783 mole) of 4-chloroquinoline and 562 ml of 95% ethanol were added 77.5 g (0.781 mole) of 37% hydrochloric acid, 337 g of ferric chloride hexahydrate, and 12.5 g of zinc chloride. The reaction mixture was heated to 60°, and 51.5 g (0.625 mole) of 85% crotonaldehyde was added during 2.75 hr. The mixture was refluxed for 2 hr, and ethanol was distilled until the boiling temperature reached 100°.

After the resulting mixture had cooled, 100 ml of water and 500 ml of toluene were added. Free quinaldine was liberated by addition of 180 ml of 50% sodium hydroxide with stirring, the temperature was maintained below 65°. With a Dean–Stark trap, 200 ml of water was removed azeotropically, 100 ml of toluene was added, and 60 ml more of water was removed. The resulting liquid was decanted through coarse filter paper, and solvent was removed by rotary evaporation at 60° (40 torr).

The residue was extracted with 200 ml of toluene, filtered, and evaporated with the first extract. The resulting dark oil was distilled, with stirring, through a plug of stainless steel wool at 123–145° (9.5 torr) and solidified. The yellow solid was recrystallized twice from hexane at 5° and once from a mixture of 150 ml of methanol and 80 ml of water to give 52.28 g (47%) of white spars, mp 95–98° [lit. (13) mp 91°].

**6-Bromoquinaldine**—This compound was prepared from 4-bromoaniline in the same manner as the chloro analog, giving a 45% yield of colorless spars, mp 103–104.5° [lit. (13) mp 96–97°].

**6-Trifluoromethylquinaldine**—This compound was prepared from 4-aminobenzotrifluoride in the same manner as the chloro analog. The crude oil distilled at 50–68° (0.35–0.5 torr). It was freed from solvent by rotary evaporation, dissolved in 50 ml of toluene, and reevaporated. It was then refluxed with 100 ml of toluene and 4.3 g of succinic anhydride for 1 hr, cooled to 20°, and stirred for 0.5 hr with 100 ml of 5% sodium hydroxide. The mixture was heated to 30° to dissolve any solid, and the organic phase was washed with 30 ml of warm water and evaporated.

The resulting oil was crystallized from 50 ml of hexane at −20° and washed with 25 ml of hexane at −20° to give 11.37 g (21%) of colorless plates, mp 62.5–64.5°; NMR (carbon tetrachloride): δ 2.71 (s, 3H, 2-$CH_3$), 7.20 (d, 1H, 4-H, $J$ = 8 Hz), and 7.5–8.2 (m, 4H, aromatic H) ppm; IR (carbon tetrachloride): 1607, 1573, 1508, 1485 (quinoline ring), 1330, 1170, and 1140 ($CF_3$) $cm^{-1}$.

*Anal.*—Calc. for $C_{11}H_8F_3N$: C, 62.56; H, 3.82; N, 6.63. Found: C, 61.99; H, 3.37; N, 6.81.

The compound was lachrymatory and caused eyelid inflammation.

**1,2-Dimethyl-6-methoxyquinolinium Iodide**—A solution of 17.3 g (0.1 mole) of 6-methoxyquinaldine and 17.7 g (0.125 mole) of iodomethane was heated to 98° for 23 hr. The resulting solid was refluxed with 150 ml of 95% ethanol for 2 hr, the solvent was decanted, and the extraction was repeated twice. The yellow needles were washed with acetone and dried, giving 25.5 g (81% yield), mp 235–238° dec. [lit. (14) mp 238° dec.].

*Anal.*—Calc. for $C_{12}H_{14}INO$: I, 40.33. Found: I, 40.27.

**1,2-Dimethyl-6-chloroquinolinium Iodide**—A solution of 13.23 g (0.0745 mole) of 6-chloroquinaldine and 100 ml of toluene was heated to 100°. Iodomethane (14.2 g, 0.1 mole) was added dropwise in two 7-hr periods on successive days while the temperature was maintained at 100° overnight both during and after the addition. The cooled mixture was filtered, washed with 100 ml of acetone, dried, and recrystallized from 100 ml of 95% ethanol, giving 9.53 g (40% yield) of yellow platelets, mp 223–224° [lit. (7) mp 211–212°].

**1,2-Dimethyl-6-bromoquinolinium Iodide**—This compound was prepared by the method used for the 6-methoxy derivative, except that 95% ethanol was used for recrystallization. A 75% yield of brown spars was obtained, mp 233–236° dec. [lit. (15) mp 237° dec.].

*Anal.*—Calc. for $C_{11}H_{11}BrIN$: I, 34.86. Found: I, 34.52.

**1,2-Dimethyl-6-(trifluoromethyl)quinolinium Iodide**—This compound was prepared by the method used for the 6-chloro analog, except that heating at 100° was continued for 4 days after the addition of iodomethane and the product was not recrystallized. A 13% yield of yellow prisms was obtained, mp 241–242° dec.; IR (KBr): 1320, 1175, and 1130 ($CF_3$) $cm^{-1}$.

*Anal.*—Calc. for $C_{12}H_{11}F_3IN$: C, 40.81; H, 3.14; N, 3.97. Found: C, 40.92; H, 3.08; N, 4.00.

**6-Methoxy-1-methylquinolinium-2-dithioacetic Acid Zwitterion**—To a solution of 6-methoxy-1-methylquinolinium iodide (12.61 g, 0.04 mole), water (20 ml), dioxane (40 ml), and carbon disulfide (20 ml, 0.233 mole) was added, at 25°, 30 ml of 50% sodium hydroxide at such a rate that the temperature did not exceed 29°. After 6 hr of stirring and addition of 50 ml of water, the mixture was filtered, washed with 400 ml of water, and dried (25°, 0.6 torr) to give 2.82 g (27%) of red-brown solid, mp 181° dec.; IR (KBr): 1315 (C=S) $cm^{-1}$.

*Anal.*—Calc. for $C_{13}H_{13}NOS_2 \cdot 2H_2O$: C, 52.14; H, 5.68; N, 4.68; S, 21.42. Found: C, 52.33; H, 4.90; N, 4.49; S, 20.84.

**6-Chloro-1-methylquinolinium-2-dithioacetic Acid Zwitterion**—This compound was prepared in the same manner as the 6-methoxy-1-methylquinolinium-2-dithioacetic acid zwitterion. An 80% yield of orange-brown solid was obtained, mp 300° dec.; IR (KBr): 1285 (C=S) and 885 (C=S) $cm^{-1}$.

*Anal.*—Calc. for $C_{12}H_{10}ClNS_2$: C, 53.82; H, 3.78; N, 5.23. Found: C, 54.02; H, 3.60; N, 5.10.

**6-Bromo-1-methylquinolinium-2-dithioacetic Acid Zwitterion**—The same procedure was used as for the 6-methoxy analog. A 55% yield of green-brown powder was obtained, mp 210–212° dec.; IR (KBr): 1305 (C=S) and 930 (C=S) $cm^{-1}$.

*Anal.*—Calc. for $C_{12}H_{10}BrNS_2 \cdot H_2O$: C, 43.64; H, 3.33; N, 4.24. Found: C, 43.96; H, 3.52; N, 3.86.

**6-Trifluoromethyl-1-methylquinolinium-2-dithioacetic Acid**

[2] Difco.

[3] Melting points were determined in capillaries with a Mel-Temp block and are uncorrected. IR absorption spectra were obtained with a Perkin-Elmer model 457A grating spectrophotometer and were corrected against polystyrene bands. NMR spectra were measured with a Varian T60 spectrometer with tetramethylsilane as internal standard. Elemental analyses were done by F. B. Strauss, Oxford, England. TLC was carried out using silica gel, and products were detected by exposure to iodine vapor. Organic reagents were supplied by Aldrich Chemical Co. or Eastman Organic Chemicals.

**Zwitterion**—This compound was prepared in the same manner as the 6-methoxy analog. A 68% yield of red-brown solid was obtained, mp 185° dec.

*Anal.*—Calc. for $C_{13}H_{10}F_3NS_2$: C, 51.82; H, 3.32; N, 4.65. Found: C, 52.16; H, 3.49; N, 4.35.

**6-Bromo-1-methyl-2-bis(2-methylthio)vinylquinolinium Iodide**—A mixture of 6-bromo-1-methylquinolinium-2-dithioacetic acid zwitterion (6.81 g, 0.0208 mole), iodomethane (10 ml), and dimethylformamide (40 ml) was allowed to stand at room temperature, with occasional shaking, for 17 hr. The dark-brown solid was filtered, washed with acetone, and dried at 23° (0.2 torr) for 6 days, giving 4.81 g (49% yield), mp 194–197° dec.; NMR (dimethyl sulfoxide-$d_6$): $\delta$ 2.50 (s, 3H, $SCH_3$), 2.63 (s, 3H, $SCH_3$), 4.35 (s, 3H, $NCH_3$), 6.65 (s, 1H, vinyl H), and 8.2–9.0 (m, 5H, ring H) ppm.

*Anal.*—Calc. for $C_{14}H_{15}BrINS_2$: C, 35.91; H, 3.23; N, 2.99; S, 13.70. Found: C, 35.55; H, 3.08; N, 2.94; S, 13.12.

**1-Methyl-2-bis(2-methylthio)vinylquinolinium Iodide**—The same procedure was used as the for 6-bromo analog. The resulting dark-yellow solid was washed with ether, recrystallized from water, and dried at 25° (1.5 torr), giving a 15.5% yield of yellow crystals, mp 198–201° dec. [lit. (9) mp 203° dec.]; NMR (dimethyl sulfoxide-$d_6$): $\delta$ 2.55 (s, 3H, $SCH_3$), 2.70 (s, 3H, $SCH_3$), 4.40 (s, 3H, $NCH_3$), 6.80 (s, 1H, vinyl H), and 8.3–9.0 (m, 6H, ring H) ppm.

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# Differential Pulse Polarographic Analysis of Thyroid Hormone: Determination of Iodine, Thyroxine, and Liothyronine


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Received June 30, 1978, from the *Food and Drug Administration, Department of Health, Education, and Welfare, Brooklyn, NY 11232.* Accepted for publication August 24, 1978.



**Abstract** □ A differential pulse polarographic method for the analysis of thyroid and thyroid tablets for total iodine, thyroxine, and liothyronine is described. The procedure for iodine, which is also applicable to individual tablet assay, consists of ashing the sample, converting iodide to iodate, and analyzing by differential pulse polarography. The procedure for thyroxine and liothyronine involves hydrolysis of the sample with barium hydroxide and isolation and separation of the iodoamino acids using ion exchangers, followed by differential pulse polarographic determination in a supporting electrolyte composed of 0.5 *N* $Na_2CO_3$ in 20% 2-propanol containing 1% tetrabutylammonium bromide. The differential pulse polarographic results for iodine agree with values obtained using the USP XIX procedure, and the quantities of thyroxine and liothyronine found agree with literature values.



**Keyphrases** □ Thyroid—differential pulse polarographic analysis of iodine, thyroxine, and liothyronine in hormone and tablets □ Polarography, differential pulse—analysis of iodine, thyroxine, and liothyronine in thyroid hormone and tablets □ Iodine—differential pulse polarographic analysis in thyroid hormone and tablets □ Thyroxine—differential pulse polarographic analysis in thyroid hormone and tablets □ Liothyronine—differential pulse polarographic analysis in thyroid hormone and tablets


The thyroid gland produces various iodoamino acids that are derivatives of thyronine and tyrosine including thyroxine, liothyronine, 3,3′,5′-triiodothyronine, 3,5-diiodothyronine, diiodotyrosine, and monoiodotyrosine (1, 2). The major biological activity is present only in thyroxine and liothyronine. 3,3′,5′-Triiodothyronine and 3,5-diiodothyronine have little or no biological activity and are present in small amounts; diiodotyrosine and monoiodotyrosine have no biological activity.

## BACKGROUND

The USP XIX method for the estimation of thyroid potency involves the determination of total iodine. The compendial assay requires ashing the sample with potassium carbonate, which releases iodide ion. The chemical transformations consist of oxidation of iodide to iodate, reduction to iodine, and, finally, titration with sodium thiosulfate.

This titrimetric procedure is not applicable to individual tablet analysis because it lacks sufficient sensitivity. For this reason, a procedure that requires a Schöniger flask oxygen combustion treatment followed by the chemical transformations is used. However, the determination of total iodine is a nonspecific test for thyroid potency since iodine is available from biologically inactive compounds as well as the biologically active hormones. This assay is used because total iodine is relatively simple to determine accurately.

A better indication of thyroid potency would be the determination of the biologically active hormones, thyroxine and liothyronine. The development of a simple and reliable method for their determination has been a challenge. The approach has been to hydrolyze the sample by




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