contains ca. 22% (by VPC) of a presumed centrally N-monoalkylated isomer (with a slightly lower boiling point) plus dialkylated material, $C_8H_{21}N_3O_2$. Fractional distillation of 200 g of Aldrich material in a spinning brush column rated at 50 theoretical plates was continued until distillation ceased (at 4.5 mm pressure, with an oil bath at 228 °C). The last cut (cut 6) amounted to 36.6 g (18%) of the desired product: bp 160.5 °C (4.5 mm), lit.[28] 153–155 °C (3.5–3.8 mm); n^{25}_D 1.4960, lit. 1.4974; VPC in a 15.4-cm column packed with 3% OV 225 (a cyanopropylsilicone) supported on 80/100 Supelcoport[29] run at 150 °C with N_2 flow at 45 mL/min showed two peaks, with retention times of 5.4 and 6.8 min and peak areas of 2 and 98%, respectively. Anal. ($C_6H_{17}N_3O$) C, H, N. Cut 2 was mostly isomeric: yield 6.4 g; bp 156 °C (4.0 mm); $n^{24.7}$ 1.4962; VPC showed the 5.4- and 6.8-min peaks in a 68:32 ratio; NMR ($CDCl_3$), in contrast to cut 6, showed the complexity expected from a mixture. Anal. Calcd for $C_6H_{17}N_3O$: C, 49.0; H, 11.6; N, 28.6. Found: C, 47.9; H, 12.1; N, 27.9. Cut 8 amounted to 18.4 g: bp 184 °C (0.1 mm); n^{25} 1.5047. Anal. ($C_8H_{21}N_3O_2$) C, H, N.

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Synthesis and Biological Evaluation of Tetramisole Analogues as Inhibitors of Alkaline Phosphatase of the 6-Thiopurine-Resistant Tumor Sarcoma 180/TG[1]

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Tetramisole and its analogues are potent inhibitors of alkaline phosphatase, including isoenzymes of Sarcoma 180/TG which appear to be involved in the mechanism of resistance of this neoplastic cell line to the 6-thiopurines. To determine the requirement for the thiazole ring system of tetramisole for inhibitory potency, 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]oxazole, 2,3-dihydro-6-phenylimidazo[2,1-*b*]oxazole, and 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*a*]imidazole were synthesized and tested for inhibitory activity against alkaline phosphatase isolated from Sarcoma 180/TG. The results indicate that 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]oxazole caused 50% inhibition at 0.21 mM, while the other synthesized compounds were inactive at a concentration of 1 mM; in contrast, tetramisole required only 0.045 mM for 50% inhibition of alkaline phosphatase activity. The findings support the concept that the thiazole ring system of the tetramisole structure is required for maximum inhibitory potency of this series against alkaline phosphatase.

Investigations by this laboratory[2-4] and by others[5] of the mechanisms by which neoplastic cells acquire resistance to the growth-inhibitory action of the 6-thiopurines (i.e., 6-mercaptopurine and 6-thioguanine) have shown that the acquired insensitivity to these drugs by the murine neoplasm Sarcoma 180/TG and by acute leukemia cells of

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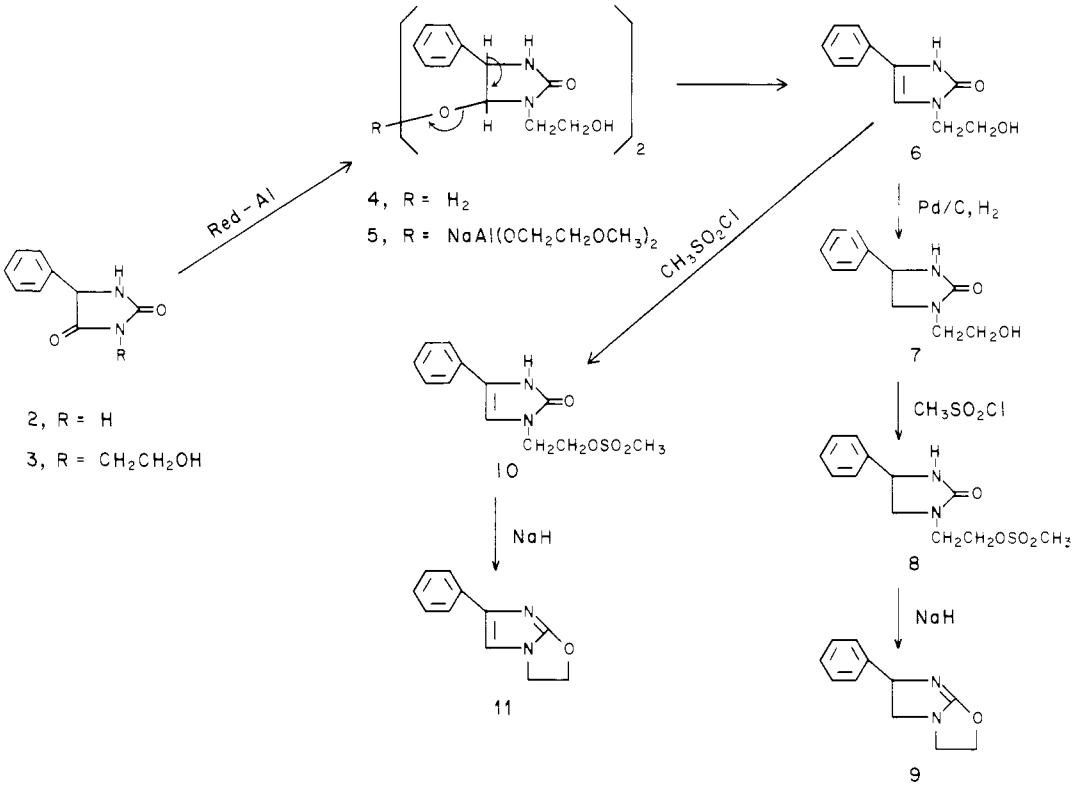
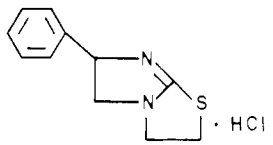
Scheme I



man is at least partially due to an increase in the activity of particulate-bound alkaline phosphatase. This change leads to an increase in the rate of degradation of the active tumor-inhibitory nucleotide form(s) of the 6-thiopurines and loss of this material from neoplastic cells.[6,7] An effective inhibitor of alkaline phosphatase activity would appear to be capable of restoring sensitivity to the 6-thiopurines in neoplasms attaining insensitivity by this mechanism and thereby may have clinical utility.

The anthelmintic tetramisole (2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]thiazole hydrochloride, **1**) and certain



1

derivatives thereof have been reported to be relatively potent inhibitors of alkaline phosphatase(s) of most mammalian tissues,[8-10] including the alkaline phosphatases of Sarcoma 180/TG.[11,12] In an effort to develop a more efficacious inhibitor of this catalyst, the present study has investigated the importance of the thiazole ring system by the synthesis and characterization of three analogues of tetramisole: 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]-oxazole (**9**), 2,3-dihydro-6-phenylimidazo[2,1-*b*]oxazole (**11**), and 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*a*]imidazole (**15**). The potency of these three agents as inhibitors of alkaline phosphatase of Sarcoma 180/TG was determined and the findings demonstrated the essentiality of the thiazole ring system of tetramisole for inhibitory activity.

Chemistry. 2,3,5,6-Tetrahydro-6-phenylimidazo-[2,1-*b*]oxazole (**9**) and 2,3-dihydro-6-phenylimidazo[2,1-*b*]oxazole (**11**) were prepared as described in Scheme I. Treatment of 5-phenylhydantoin (**2**) with chloroethanol gave 3-(2-hydroxyethyl)-5-phenylhydantoin (**3**).[13] Employing a reported procedure for reduction of hydantoins with $LiAlH_4$,[14,15] no selective reduction of the amide carbonyl of **3** was obtained. However, when excess Red-Al®[16] in THF was employed, **3** was selectively reduced to give **6**. This result differed from the findings of Marquez et al.[17] that 3-(2-hydroxyethyl)-5,5-diphenylhydantoin was reduced by refluxing with excess Red-Al® in THF to give 1-(2-hydroxyethyl)-4,4-diphenyl-2-imidazolidinone. The presence of the acidic labile 5-hydrogen in **3** and its participation during the process of reduction to convert the intermediate **4** or its metal complex **5** to the corresponding 2-imidazolone **6** can account for the difference in the findings. Hydrogenation of **6** with Pd/C as the catalyst provided the desired product **7**. The conversion of **7** to **8** was easily accomplished by treatment with methanesulfonyl chloride in dry pyridine at room temperature. When the mesylate **8** was reacted with NaH in dimethoxyethane (DME), only O-alkylation occurred to give 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*]oxazole (**9**). No N-3 alkylation was detected in the reaction mixture. This result was in agreement with earlier reports with similar systems.[17,18] Treatment of **6** with methanesulfonyl chloride afforded **10** in good yield, and by employing the same conditions used to cyclize **8** the mesylate **10** was converted to 2,3-dihydro-6-phenylimidazo[2,1-*b*]oxazole (**11**) in reasonable yield.

2-(Methylmercapto)-4-phenyl-2-imidazoline (**12**) was employed as the starting material to prepare 2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*a*]imidazole (**15**), as shown in Scheme II. Compound **12** can be obtained synthetically from either α-aminoacetophenone hydrochloride or α-aminophenylacetonitrile.[19] Displacement of the methylmercapto group of **12** by refluxing this material with aminoethanol in $CHCl_3$ led to 2-[(β-hydroxyethyl)-amino]-4-phenyl-2-imidazoline (**13**).[20] Reaction of **13** with excess $SOCl_2$ gave the corresponding chloro derivative **14**, which was cyclized by refluxing with ethanolic KOH to provide the final product **15**.

Scheme II

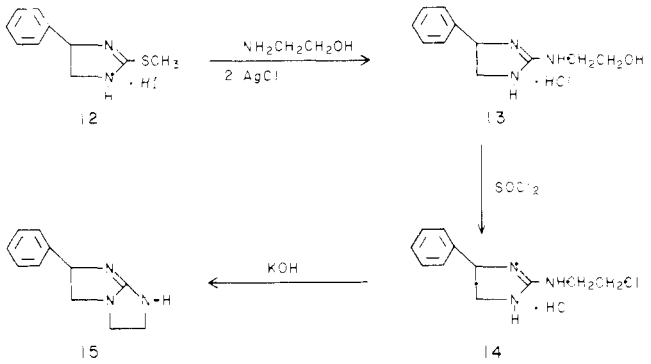
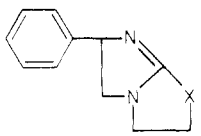


Table I. Inhibitory Activity of Tetramisole Analogues against Alkaline Phosphatase of Sarcoma 180/TG



no.	X	I_{50}, mM[a]
1	S[b]	0.045
9	O	0.21
15	N	c
11[d]		c

[a] I_{50} is the concentration of drug required to reduce enzyme activity by 50%. [b] Tetramisole (hydrochloride); product of Aldrich Chemical Co. [c] Noninhibitory at concentrations up to 1 mM. [d] See Scheme I for structure.

Biological Results and Discussion. Compounds **9**, **11**, and **15** were compared to tetramisole as inhibitors of a particulate-bound alkaline phosphatase isolated from Sarcoma 180/TG ascites tumor cells.[21] The results shown in Table I are expressed as the concentration of inhibitors required to produce 50% inhibition of enzyme activity; the findings presented are representative data from three replicate experiments, each yielding essentially identical results. Tetramisole was the most potent of the compounds tested, requiring a concentration of 0.045 mM for 50% inhibition of enzyme activity. Analogue **9** was active as an inhibitor of alkaline phosphatase, but required five times more material to cause a 50% decrease in enzyme activity. Compounds **11** and **15** were inactive at concentrations up to 1 mM, and only 15% inhibition was obtained with **15** at a level of 5 mM. These findings support the previous observation[12] that the thiazole ring system of the tetramisole structure is at least partially responsible for the enzyme-inhibitory potency of this class of agents. The results, together with our previous data,[11,12] suggest that a bicyclic ring system consisting of both thiazoline and imidazoline rings to which a 6-phenyl substituent is attached are critical portions of the molecule for the expression of the maximum inhibitory potential of this series against alkaline phosphatase.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by the Baron Consulting Co., Orange, CT. NMR spectra were obtained with a Varian T-60A NMR spectrometer; tetramethylsilane was used as an internal standard in $CDCl_3$ and as an external reference in dimethyl-d_6 sulfoxide. The prepared compounds were homogeneous when analyzed by micro thin-layer chromatography on silica gel, and NMR spectra and elemental analyses were consistent with the reported chemical structures.

3-(2-Hydroxyethyl)-5-phenylhydantoin (3). Alkylation of the sodium salt of 5-phenylhydantoin with chloroethanol using the procedure of Schlögl et al.[13] gave **3** (66%): mp 96 °C (lit.[13] 93–95 °C); NMR (Me_2SO-d_6) δ 3.38 (m, 4, CH_2CH_2), 4.76 (m, 1, OH), 5.18 (s, 1, H_5), 7.36 (s, 5, phenyl), 8.60 (s, 1, NH). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

1-(2-Hydroxyethyl)-4-phenyl-2-imidazolone (6). A mixture of Red-Al®[16] (19 mL, 70 mmol) and freshly distilled THF (30 mL) was added dropwise to a solution of **3** (2.2 g, 10 mmol) in THF (30 mL) under N_2 at room temperature. The reaction mixture was stirred for another 3 h, and the excess hydride was destroyed by the careful addition of 20% HCl in an ice–acetone bath until neutral conditions were obtained. The reaction mixture was filtered through Celite and the organic layer was collected. $CHCl_3$ (100 mL) was added to the organic layer, and this mixture was washed twice with H_2O and dried ($MgSO_4$). After evaporation of the solvent, the residual semisolid was triturated with ether. Recrystallization from EtOH afforded pure **6** (0.9 g, 45%): mp 195–197 °C; NMR (Me_2SO-d_6) δ 3.60 (s, 4, CH_2CH_2), 4.90 (m, 1, OH), 6.98 (s, 1, H_5), 7.10–7.56 (m, 5, phenyl), 10.69 (s, 1, NH). Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

1-(2-Hydroxyethyl)-4-phenyl-2-imidazolidinone (7). A solution of **6** (1.02 g, 5 mmol) in CH_3OH (80 mL) and AcOH (2 mL) was hydrogenated in a Parr pressure apparatus at 50 psi using 10% Pd/C (150 mg) at room temperature for 4–5 h. After removal of the catalyst by filtration and evaporation of the solvent, the residue was triturated with ether to give pure **7** (0.91 g, 88%): mp 84–86 °C; NMR (Me_2SO-d_6) δ 3.23 (m, 3, CH_2O + 1 H_5), 3.66 (t, 2, CH_2N), 3.96 (t, 2, 1 H_5 + OH, OH signal D_2O exchangeable), 4.79 (t, 1, H_4), 6.90 (s, 1, NH), 7.40 (s, 5, phenyl). Anal. ($C_{11}H_{14}N_2O_2$) C, H, N.

1-(2-Hydroxyethyl)-4-phenyl-2-imidazolidinone Methanesulfonate (8). Methanesulfonyl chloride (0.5 mL, 6 mmol) was added to a solution of **7** (1.03 g, 5 mmol) in pyridine (5 mL) in an ice bath, and the reaction mixture was stirred for 4 h. Water (50 mL) was added and the precipitate was collected by filtration. Two crystallizations from CH_3OH gave **8** (1.02 g, 72%): mp 155–156 °C; NMR (Me_2SO-d_6) δ 3.04 (s, 3, CH_3), 3.31 (m, 3, CH_2N + 1 H_5), 3.81 (t, 1, H_5), 4.26 (t, 2, CH_2SO_2), 4.66 (t, 1, H_4), 7.00 (br, 1, NH), 7.26 (s, 5, phenyl). Anal. ($C_{12}H_{16}N_2O_4S$) C, H, N.

2,3,5,6-Tetrahydro-6-phenylimidazo[2,1-*b*]oxazole (9). A 57% mineral oil suspension (70 mg) of NaH (30 mg, 1.2 mmol) was washed with dimethoxyethane (DME) freshly distilled from $LiAlH_4$ and added to a stirred cold mixture of **8** (284 mg, 1 mmol) in dry DME under N_2. The reaction mixture was stirred for 2 h at 0 °C and for another 3 h at room temperature under N_2. Insoluble material was removed by filtration, and the solvent was removed by evaporation to give a solid, which was extracted with $CHCl_3$. Evaporation of the solvent provided **9** as a white solid (103 mg, 55%): mp 132–133 °C; NMR ($CDCl_3$) δ 3.20 (m, 3, CH_2N + 1 H_5), 3.70 (t, 1, H_5), 4.75 (dd, 2, CH_2O), 5.17 (t, 1, H_6), 7.3 (s, 5, phenyl). Anal. ($C_{11}H_{12}N_2O$) C, H, N.

1-(2-Hydroxyethyl)-4-phenyl-2-imidazolone Methanesulfonate (10). Using the procedure employed for the preparation of **8**, compound **6** (0.6 g, 3 mmol) was treated with methanesulfonyl chloride (0.3 mL, 3.6 mmol) in pyridine. The resulting precipitate was crystallized from CH_3OH to give **10** (0.55 g, 65%): mp 131–132 °C; NMR (Me_2SO-d_6) δ 3.12 (s, 3, CH_3), 3.88 (t, 2, CH_2N), 4.40 (t, 2, CH_2SO_2), 6.97 (br, 1, NH), 7.17–7.47 (m, 5, phenyl). Anal. ($C_{12}H_{14}N_2O_4S$) C, H, N.

2,3-Dihydro-6-phenylimidazo[2,1-*b*]oxazole (11). To compound **10** (282 mg, 1 mmol) in DME (30 mL) was added NaH (36 mg, 1.5 mmol) under N_2, and the reaction mixture was stirred at 0 °C for 2 h and then at room temperature overnight. After removal of the insoluble solid and evaporation of the solvent, the residue was applied to a column of silica gel which was eluted with a mixture of CH_3OH–$CHCl_3$ (1:49, v/v). The appropriate fractions were collected and evaporated to give pure **11** (93 mg, 50%): mp 136–138 °C; NMR ($CDCl_3$) δ 4.10 (dd, 2, CH_2N), 4.94 (dd, 2, CH_2O), 6.84 (s, 1, H_5), 7.23 and 7.67 [(m, 3), (m, 2), phenyl]. Anal. ($C_{11}H_{10}N_2O$·H_2O) C, H, N.

4-Phenyl-2-(methylmercapto)imidazoline Hydroiodide (12). Compound **12** was prepared by the procedure of Matier et al.[19] using either α-aminoacetophenone hydrochloride or α-aminophenylacetonitrile hydrochloride as the starting material: overall yield 60 and 75%, respectively; mp 136–137.5 °C (lit.[19] 135.5–137.5 °C); NMR (Me_2SO-d_6) δ 2.71 (s, 3, SCH_3), 3.70 (t, 1,

1 H_5), 4.40 (t, 1, 1 H_5), 5.49 (t, 1, H_4), 7.41 (s, 5, phenyl), 9.31 (br s, 1, NH).

2-[(β-Hydroxyethyl)amino]-4-phenyl-2-imidazoline Hydrochloride (13). A solution of **12** (6.4 g, 20 mmol) and aminoethanol (2 mL, 24 mmol) in freshly distilled $CHCl_3$ (40 mL) was refluxed for 4 h. The evolved methyl mercaptan was absorbed in a 20% NaOH solution. The reaction mixture was allowed to remain at room temperature overnight and was then evaporated in vacuo to a syrup. The syrup was dissolved in H_2O (100 mL) and then stirred with AgCl (2.86 g, 20 mmol) overnight. The yellow precipitate of AgI which formed was removed by filtration, and evaporation of the solvent afforded the desired product **13** as a syrup: yield 3.3 g (70%); NMR (Me_2SO-d_6) δ 3.44 (m, 5, ethylene and 1 H_5), 4.03 (t, 1, 1 H_5), 5.14 (t, 1, H_4), 5.84 (br, 1, OH), 7.38 (s, 5, phenyl). The picrate salt melted at 130 °C, and NMR showed δ 7.35:8.68 = 5:2, indicative of a 1:1 ratio for the picrate complex. Anal. ($C_{17}H_{18}N_6O_8$) C, H, N.

2-[(β-Chloroethyl)amino]-4-phenyl-2-imidazoline Hydrochloride (14). A mixture of **13** (5.2 g, 22 mmol) and excess $SOCl_2$ (8 mL) was warmed at 50–60 °C for 6 h. After the excess $SOCl_2$ was removed by distillation, EtOH was added and the solution was evaporated to remove the remaining $SOCl_2$. The residue in EtOH was decolorized by passage through charcoal and then evaporated to a semisolid. The crude product was purified by silica gel column chromatography using a mixture of CH_3OH–$CHCl_3$ (1:9, v/v) as eluent to give pure **14**: yield 3 g (52%); NMR (Me_2SO-d_6) δ 3.48 (m, 5, ethylene and 1 H_5), 4.04 (t, 1, 1 H_5), 5.10 (t, 1, H_4), 7.30 (s, 5, phenyl). Its picrate complex melted at 145–146 °C. Anal. ($C_{17}H_{17}N_6O_7Cl$) C, H, N.

2,3,5,6-Tetrahydro-6-phenyl-1*H*-imidazo[1,2-*a*]imidazole (15). An ethanolic solution of KOH (15 mL, 6 mmol) was added to a solution of **14** (1.3 g, 5 mmol) in absolute EtOH (10 mL) at a rate which maintained a slight excess of alkali during the course of the reaction. The mixture was then refluxed for 5 h. The precipitated KCl was removed by filtration and the filtrate was evaporated to dryness. The residue was extracted with acetone and the crude product was crystallized from acetone to give **15**: yield 0.5 g (57%); mp 168 °C dec; NMR (Me_2SO-d_6) δ 3.20 (m, 3, 2 H_3 + 1 H_5), 3.51 (t, 2, H_4), 3.76 (t, 1, 1 H_5), 4.76 (t, 1, H_6), 7.26 (s, 5, phenyl). Anal. ($C_{11}H_{13}N_3$) C, H, N.

Evaluation of Inhibitory Potency against Alkaline Phosphatase. Alkaline phosphatase (enzyme A) from the murine neoplasm Sarcoma 180/TG was partially purified by a previously reported procedure[21] and assayed as described earlier.[11] Inhibitor stock solutions were made at a concentration of 10^{-2} M in 25–100% Me_2SO and varying amounts of this solution were added to the reaction mixture. The concentration of Me_2SO was less than 5% in the final assay mixture, and appropriate controls containing Me_2SO were included in each assay. The enzyme partially purified up to the step of ethanol fractionation[21] (specific activity 500 units/mg of protein) was used. Enzyme activity was measured by determination of the initial rate of hydrolysis of *p*-nitrophenyl phosphate spectrophotometrically at 410 nm using a Beckman kinetic recording spectrophotometer.

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(1) This work was supported in part by U.S. Public Health Service Research Grants CA-02817 and CA-16359 and a grant from the Bristol-Myers Co.
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