Synthesis, Chemical Reactivity, and Antitumor Evaluation of Congeners of Carmethizole Hydrochloride, an Experimental "Acylated Vinylogous Carbinolamine" Tumor Inhibitor[1]

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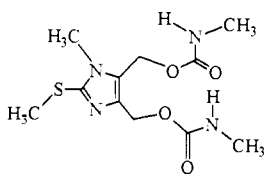
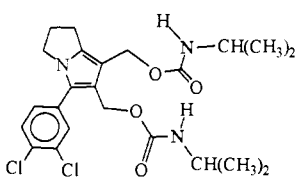
A series of analogues of 4,5-bis(((*N*-methylcarbamoyl)oxy)methyl)-1-methyl-2-(methylthio)-imidazole (1, carmethizole) were synthesized. The chemical reactivities of the analogues (as electrophiles) were evaluated and related to the antitumor activity (in vivo and in vitro). Changes in the alkylthio moiety had a significant effect upon the chemical reactivity. Electron-withdrawing groups on the sulfur decreased chemical reactivity and, in parallel, decreased antitumor activity. Carmethizole sulfoxide (11a) was unreactive as an electrophile and exhibited no antitumor activity either in vivo or in vitro; this led to the conclusion that carmethizole sulfoxide was not acting as a "carrier form" of carmethizole. The disulfides 17 and 18 were unreactive as electrophiles but did exhibit antitumor activity. The activity of 17 and 18 was attributed to the thiol 10 that would be generated upon cleavage of the disulfide bond.

Introduction

Carmethizole hydrochloride (1·HCl), a novel alkylating agent, is a new antitumor drug with relatively broad activity against murine leukemias, solid tumors, and advanced-staged human tumor xenografts in vivo.[2] The agent was produced in a congener development program to provide a compound with greater aqueous solubility and stability than isopropylpyrrolizine (IPP, 2).[3,4] Both compounds



1 (Carmethizole)



2 ("IPP")

possess the "acylated vinylogous carbinolamine" electrophilic functionalities that are believed to form interstrand DNA cross-links.[5-8] The proposed mechanism for alkylations by the "acylated vinylogous carbinolamines" involves a nucleophilic S_N1-type mechanism (AAL1) leading to the elimination of the carbamate leaving groups through an *O*-alkyl bond cleavage reaction (Figure 1). In the case of carmethizole this stepwise mechanism involves the reaction of two electrophilic centers that exhibit significantly different chemical reactivities. Theoretical, chemical and biochemical studies support this notion.[2,9-11] The difference in reactivity has been attributed to the greater ability of sulfur to participate in the stabilization of the transition state (or intermediate 3) involved in the expulsion of the C-5′ carbamate moiety compared to the C-4′ carbamate moiety. The initial *O*-alkyl fission step of this process results in the elimination of CO_2 and an amine and is most likely a concerted reaction, although a stepwise carbamate decomposition is a feasible process (where carbamic acid is initially formed). More detailed investigations of the role of the carbamate ester as esterase inhibitors in the pyrrolizine alkaloids[12] and in antitumor drugs[13,14] have been reported elsewhere. For the "acylated vinylogous carbinolamines", branched *N*-alkyl groups α to the carbamate nitrogen generally increase antitumor potency and decrease chemical reactivity (presumably because of decreased solvation of the more lipophilic leaving group).

The decomposition of carmethizole (1) in water involves consecutive hydrolysis of the two bis-carbamate moieties at C-5′ and C-4′, where hydrolysis of the C-5′ carbamate ($t_{1/2}$ = 2 h) was approximately ten times faster than the hydrolysis of the C-4′ carbamate ($t_{1/2}$ = 20 h).[9] When the reaction was run in ^{18}O water, analysis of the hydrolysis products by electron impact mass spectrometry showed that one ^{18}O atom was incorporated into the first degradation product.[9] This is consistent with a S_N1-type C-5′-*O*-alkyl bond fission mechanism rather than an addition–elimination mechanism of carbamate hydrolysis. The rate of hydrolysis was reduced in the presence of acid, consistent with the initial design hypothesis, because the protonated imidazole is less capable of stabilizing electron-deficient transition states than the unprotonated free base. Carmethizole (1) was hydrolyzed to the diol 8 in less than 24 h at 37 °C in 0.9% sodium chloride or phosphate buffer; the half-life was *ca.* 1 h at 37 °C in whole blood or plasma in vitro.[15]

A study of the metabolism of carmethizole revealed that the major product formed in vivo was the carmethizole diol 8.[15] The rapid plasma elimination of carmethizole appears to be due to chemical and metabolic processes since only 4.7% of the dose was eliminated unchanged in mouse urine (no analyses of fecal samples were made). The metabolic disposition of carmethizole has not been studied beyond the stability of the carbamate moieties; however, the C-2 methylthio group could undergo different metabolic transformations. An early series of lipophilic analogues was prepared (data not shown) in which either the N-1 or the S-2 methyl was replaced by an *n*-propyl or an *n*-hexyl group.[16] Replacement of the *S*-methyl group by either *n*-propyl or *n*-hexyl caused a reduction in activity in both the mammary carcinoma xenograft (MX-1) and the human LOX amelanotic melanoma assays. Replacement of the *N*-methyl group led to a decrease in the potency against MX-1 and LOX. The *N*-(*n*-propyl) and *N*-(*n*-hexyl) analogues were less active than carmethizole

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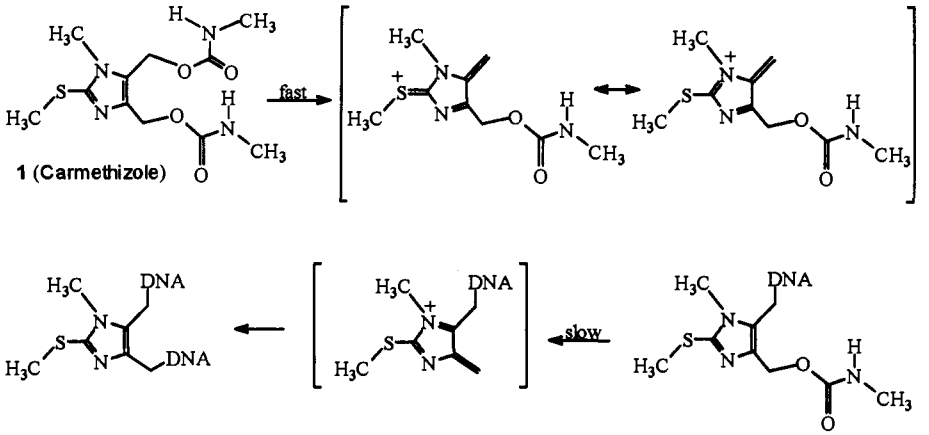


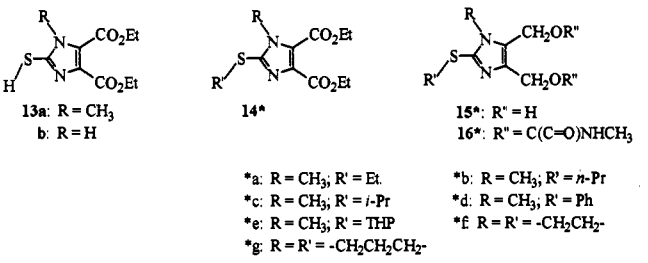
Figure 1. Proposed mechanism for the carmethizole DNA cross-linking reaction

hydrochloride against advanced staged LOX. The reduced activity of the larger C-2 (alkylthio) analogues of carmethizole does not appear to be due to increased lipophilicity because the more lipophilic N-1 alkyl analogues of carmethizole are quite active. The differences in the activities of the C-2-(alkylthio) carmethizole analogues could be due to differences in the metabolism of the *S*-alkyl substituents.

The known redox biotransformations of sulindac, a nonsteroidal antiinflammatory agent, can be cited as precedent.[17,18] The only biotransformations of quantitative significance undergone by sulindac are changes in the oxidation state of the sulfinyl group (*i.e.* oxidation of the parent sulfoxide group to the sulfone or its reduction to the sulfide).[17-19] The formation of the sulfone metabolite is irreversible; however, reduction to the sulfide is readily reversible in five animal species including humans.[17] The reduced sulfide (active drug) is only marginally excreted as such and thus depends on reoxidation to the sulfoxide (parent compound) for its elimination. In addition to sulindac, a variety of other sulfide-containing drugs undergo a similar enzymatic biotransformation including dimethyl sulfoxide,[20] carbophenothion sulfoxide,[21] methionine sulfoxide,[22] mesoridazine, fenbendazole sulfoxide, and albendazole sulfoxide.[23-25] Sulfur oxidative *S*-dealkylation, analogous to *O*- and *N*-dealkylation mechanistically (*i.e.* involves a-carbon hydroxylation), has also been observed for various sulfur xenobiotics.[25] Examples of *S*-dealkylation in humans are limited due to competing metabolic processes, including *S*-oxidation. If the 2-(alkylthio)imidazoles can function as substrates in the above redox equilibrium, then this must be considered as one of the possible biotransformations of carmethizole. This metabolic transformation would lead to a decrease in electron density in the imidazole ring, by virtue of the electron-withdrawing character of the methyl sulfoxide and methyl sulfone, thereby making the compound less reactive (more stable) or inactive all together.

The C-2-(methylthio) group in carmethizole (1) potentially can undergo metabolic oxidation at either sulfur or carbon (Figure 2). Carbon oxidation will lead to *S*-dealkylation to give **10**, the thiol analogue of carmethizole. Oxidation at sulfur will produce the corresponding sulfoxide **11a** (R = CH_3) and, upon further oxidation, the sulfone **12**. The thiol **10** should be at least as reactive as carmethizole whereas the sulfoxide **11a** (R = CH_3) and the sulfone **12** should be much less reactive than carmethizole (1). The *n*-propylthio and the *n*-hexylthio analogues could be less active than the methylthio compound because of slower *S*-dealkylation (or, no *S*-dealkylation at all), or different rates of *S*-oxidation/reduction. The sulfoxide **11a** (or, less likely, the sulfone **12**) could actually be the "transport form" of the drug. The carbamate moieties in the sulfoxide **11a** should be a relatively unreactive (and chemically stable toward hydrolytic decomposition) because of the electron-withdrawing influence of the sulfoxide group (CH_3SO: $F = 0.52$; $R = 0.01$; $\sigma_m = 0.52$; $\sigma_p = 0.49$) relative to the sulfide (CH_3S: $F = 0.20$; $R = -0.18$; $\sigma_m = 0.15$; $\sigma_p = 0.00$).

This report describes the synthesis, antineoplastic evaluation (in vitro and in vivo in P388 murine leukemia and LOX human melanoma xenografts in nude athymic mice), and chemical reactivity [using the model nucleophile 4-(4-nitrobenzyl)pyridine (NBP) alkylation assay[26]] of a series of 2-(alkylthio)imidazole bis(carbamate) homologues, the corresponding sulfinyl derivatives, 2-(alkylthio) side chain restricted analogues, and novel disulfide analogues. The 2-alkylthio homologues **16a–c** probe potential steric effects of the alkylthio group at the C-2 position.



The imidazo[2,1-*b*]thiazole (**16f**) and imidazo[2,1-*b*]-thiazine (**16g**) restrict the conformation of the 2-(alkylthio) "side chain" and may also alter the chemical reactivity of the electrophilic centers through perturbation of the orbital overlap of the sulfur lone pair and imidazole p-electrons required for *O*-alkyl cleavage of the carbamate leaving groups. The sulfoxides **11a** and **11b** represent potential prodrugs that must be reduced to provide sufficient electron density in the heterocycle for reactivity and subsequent alkylation. The disulfides **17** and **18** are also potential prodrugs but would be activated by a different mechanism, disulfide reduction to yield the chemically reactive 2-mercaptoimidazole bis(carbamate) intermediate **10**. Enhanced levels of reducing agent have been associated with human tumor cell lines.[27] Furthermore, it is well documented that solid tumors, and particularly the necrotic tumor core, have inadequate vascularization and exist in oxygen-deficient, or hypoxic, states.[28]

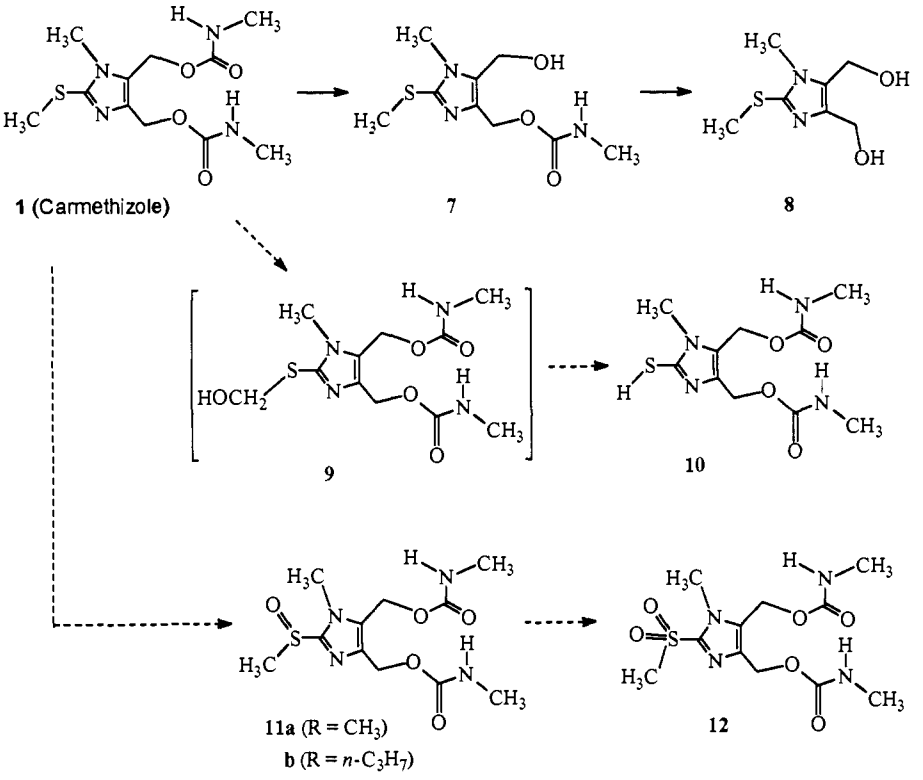
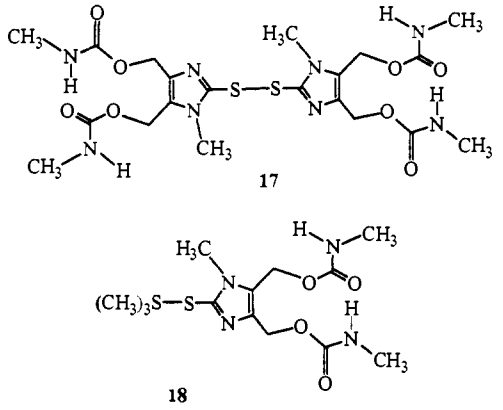


Figure 2. Metabolism of carmethizole.



Chemistry

The general synthetic approach previously described for carmethizole (1) was used to prepare the 1,2-disubstituted 4,5-bis[[(*N*-methylcarbamoyl)oxy]methyl]imidazoles.[2] The bis(carbamates) **16** were prepared from the corresponding diester **14** by reduction with lithium aluminum hydride (*ca.* 75–80% yields) followed by carbamoylation of the resulting diols **15** (*ca.* 90% yields using methyl isocyanate with a catalytic amount of di-*n*-butyltin diacetate). The 2-(alkylthio)imidazole homologues **14a-c** were prepared from diethyl 2-mercapto-1-methylimidazole-4,5-dicarboxylate [**13a**,obtained in four steps from sarcosine (48% overall yield)[2,29]] by treatment of the sodium hydride generated sulfur anion with the requisite iodoalkane. In this same manner **13b**, obtained from glycine ethyl ester hydrochloride (40% overall yield),[29] was treated with sodium hydride in anhydrous THF followed by either chloroethyl methanesulfonate or 3-bromopropyl methanesulfonate to afford the diesters of imidazo[2,1-*b*]thiazole (**14f**) and imidazo[2,1-*b*]thiazine (**14g**), respectively. The 2-(phenylthio) compound, **16d**, was prepared from **15** (R = CH_3; R′ = Ph; R″ = *t*$BuMe_2$Si) by deprotection of the bis(hydroxymethyl)silyl ether groups with fluoride ion followed by carbamoylation of the diol **15d**. Sulfide **15** (R = CH_3; R′ = Ph; R″ = *t*$BuMe_2$-Si) was obtained by ipso displacement of a C-2 methylsulfone.[30] The sulfoxides **11a** (R = CH_3) and **11b** (R = *n*-Pr) were prepared from the bis(carbamates) **1** and **16b**, respectively (*ca.* 85–90% yield), by *m*-chloroperbenzoic acid oxidation under biphasic reaction conditions (dichloromethane, 0.5 M aqueous sodium bicarbonate, 1:1).

The symmetrical and unsymmetrical disulfide imidazole bis(carbamates), **17** and **18**, respectively, were prepared from **16e**. The bis(carbamate) **16e** was prepared from **13a**: treatment with dihydropyran under pyridinium *p*-toluenesulfonate catalysis[31] afforded 2-[(tetrahydro-2*H*-pyranyl)thio] **14e** in excellent yield (95%). Other acid catalysts such as boron trifluoride etherate,[32] *p*-toluenesulfonic acid,[33] or amberlite resins[34] gave inferior yields of **14e** (<55%). Compound **16e** was subjected to halogen promoted in situ THP deprotection[35] to generate the reactive sulfinyl iodide that without isolation afforded the symmetrical disulfide[36] **17** in 70% yield. Several attempts were made to isolate the 2-mercaptoimidazole bis(carbamate) **10**, but the compound was too reactive and underwent rapid decomposition. The THP moiety in **16e** could not be cleaved with either pyridinium *p*-toluenesulfonate or *p*-toluenesulfonic acid in chloroform at 0°C (or at 45 °C) but was cleaved in methanol. The difficulty was that **10** was unstable under the reaction conditions and underwent rapid decomposition (other conditions such as aqueous silver nitrate, or aqueous hydrochloric acid also led to decomposition). Interestingly, various 2-(tetrahydropyranylthio)imidazole derivatives bearing derivatized 4,5-bis(hydroxymethyl) substituents did not undergo thioether deprotection under conditions that worked well when the 4- and 5-substituents were at the dicarboxylate stage (*e.g.*, triphenylphosphine–carbon tetrabromide or amberlite resins). The unsymmetrical disulfide **18** was

Table I. In Vivo Activity of Carmethizole Analogues against ip Implanted LOX Amelanotic Melanoma in Nude Mice[a,b]

compd	dose (mg/kg)	% ILS[c] (survivors)[d]	KE[e]	compd	dose (mg/kg)	% ILS[c] (survivors)[d]	KE[e]
1[f]	200	−24 (2/10)	toxic	16c[g]	188	45	−0.2
	100	143 (9/10)	−4.0 (−7.2)		94	28	+0.6
	50	117 (1/10)	−2.9 −5.9)		47	24	+0.7
	25	34 (1/10)	+0.3 (−5.9)		23.5	13	+1.3
11a	182[g]	2	+1.8	16e[g]	212	8	+1.3
	91[f]	−8	+2.0		106	15	+1.0
	45.5[f]	−8	+2.0		53	15	+1.0
	22.75[f]	−7	+2.0		26.5	11	+1.2
11b[g]	198	21	+0.8	17	328[g]	−69	toxic
	99	1	+1.6		164[g]	−5	+1.8
	49.5	15	+1.0		82[f]	−5	+1.8
	24.75	−2	+1.7		41[f]	3	+1.5
16a	174[g]	95 (5/10)	−2.5 (−6.4)	18[g]	215	−67	toxic
	87[f]	85 (3/10)	−2.1 (−6.2)		107.5	−37	toxic
	43.5[f]	52 (1/10)	−0.5 (−5.9)		53.7	−7	+1.8
	21.75[f]	44 (1/10)	−0.2 (−5.9)		26.8	−5	+1.8
16b[g]	188	76	−1.6				
	94	22	+0.6				
	47	24	+0.7				
	23.5	13	+1.3				

[a] Determined under the auspices of the National Cancer Institute. [b] Ascitic fluid containing *ca.* 1.7×10^6 cells was implanted into CD_2F_1 female mice (Simonsen Laboratories). The drugs were given by intraperitoneal injection on days 1, 5, and 9 beginning 24 h after tumor implantation, and all treatment was according to exact body weight. Fresh solutions (or suspensions) of the test compounds were prepared daily. [c] % ILS refers to the increase in life span of the dying mice (based on the median day of death; 60 day survivors not used in this calculation) compared to control animals. [d] Refers to 60-day survivors per total number of animals in the initial test group. [e] KE refers to tumor cell kill and is the net log change in viable tumor cell population at the end of the treatment period as compared to the start of the treatment period (e.g. a −6.0 log change means that there was a 99.9999% reduction in tumor burden at the end of the treatment period). Figures in parentheses are based on the percent survivors, assuming that the number of cells surviving treatment in each animal obeys Poisson distribution and that those animals still alive at 60 days have no tumor cells that survive treatment. [f] The drug was given as a solution in a saline solution plus Tween 80. [g] The drug was given as a suspension in a saline solution plus Tween 80.

Table II. In Vivo Activity of Carmethizole Analogues against ip Implanted P388 Leukemia in Mice[a,b]

compd	dose (mg/kg)	% ILS[c] (survivors)[d]	KE[e]	compd	dose (mg/kg)	% ILS[c] (survivors)[d]	KE[e]
1[f]	100	−34 (0/6)	toxic	16c[g]	94	+59	−1.5
	50	+17 (0/6)	toxic		47	+27	+0.9
	25	+96	−4.1		23.5	+25	+1.0
	12.5	+59	−1.5		11.8	+31	+0.6
11a[f]	91	+17	+1.3	16e[g]	106	+10	+1.4
	45.5	+27	+0.9		53	+16	+1.4
	22.8	+10	+1.4		26.5	+9	+1.4
	11.4	+10	+1.4		13.3	+1	+1.6
11b[f]	99	+27	+0.9	17[f]	164	+26	+0.9
	49.5	+27	+0.9		82	+17	+1.3
	24.8	+25	+1.0		41	+20	+1.3
	12.4	+10	+1.4		20.5	+17	+1.3
16a[f]	87	+99	−4.3	18[g]	108	−52	toxic
	43.5	+64	−1.9		54	−34	toxic
	21.8	+56	−1.3		27	+1	+1.6
	10.9	+47	−0.6		13.5	+11	+1.4
16b	94[g]	+84	−3.3				
	47[g]	+59	−1.5				
	23.5[f]	+40	−0.1				
	11.8[f]	+40	−0.1				

[a] See footnote *a* in legend for Table I. [b] Ascitic fluid containing *ca.* 5.2×10^6 cells was inoculated into Portage-CD_2F_1 female mice. The drugs were given by intraperitoneal injection on days 1–5 beginning 24 h after tumor inoculation and all treatment was determined by average body weight. Fresh solutions (or suspensions) of the test compounds were prepared daily. [d] Refers to 24-day survivors per total number of animals in the initial test group. [c,e–g] See the corresponding footnotes in Table I.

obtained by the sulfenyl thiocyanate method.[37,38] Thus, treatment of **16e** with zinc chloride and thiocyanogen (prepared in situ from lead thiocyanate and bromine in chloroform[38–40]), followed by treatment of the thiol **10** with *tert*-butyl mercaptan, afforded the unsymmetrical disulfide **18** in 60% yield.

Results and Discussion.

Biological Activity Studies. The imidazole bis(carbamates) were evaluated for antineoplastic activity by either in vivo or in vitro methods where carmethizole hydrochloride (1·HCl) or the free base **1** served as a comparative control. The in vivo data from intraperitoneally implanted LOX amelanotic melanoma and P388 lymphocytic leukemia assays in mice are given in Tables I and II, respectively. These data show that no analogue was more active than carmethizole hydrochloride (1·HCl) which showed a 143% ILS at its most effective dose (100 mg/kg) for ip LOX and a 96% ILS for P388 assay at its most effective dose (25 mg/kg). Other more advanced dosing schedules and pharmacokinetic studies for **1** have been recently reported.[41] 2-(Ethylthio)imidazole **16a**, at its highest dose (174 mg/kg) in the ip LOX assay, was less effective (95% ILS) compared to carmethizole; however, in the P388 model **16a** was as active as the parent compound (95% ILS at 87 mg/kg).

The 2-(*n*-propylthio) derivative **16b** and 2-(2-propylthio) derivative **16c** were less active in both in vivo assays. In the ip LOX assay, **16c** exhibited marginal activity, resulting in tumor cell population stasis during the treatment period.

Note that none of the 2-alkylthio homologues (16b–d) were tested at toxic levels. The THP derivative 16e, an intermediate in the synthesis of the oxidized disulfide compounds 17 and 18, was inactive. This observation is significant in that the 2-(pyranylthio) substituent carries all the necessary structural and electronic components responsible for complete modulation of antitumor activity and in this way represents an interesting lead compound for further development. The sulfinyl analogues 11a and 11b were inactive in the LOX amelanotic melanoma at doses up to *ca.* 200 mg/kg and were marginally active against P388 lymphocytic leukemia assays. Neither sulfoxide was toxic at doses up to *ca.* 200 mg/kg. These data indicate that under the nonhypoxic assay conditions, sulfoxide bioreduction does not appear to play a significant role for these compounds, or that sulfoxide reduction occurs at a slower rate compared to the rate of hydrolysis of the carbamate leaving groups. It should be noted that this "sulfoxide trigger" strategy could function as a prodrug form of vinylogous carbinolamine-like alkylating agents in hypoxic tumor cells which are known to have a greater reduction potential than noncancerous cells.[42a] In this regard, we have earlier suggested in vivo activation of similar antitumor agents via sulfone reduction.[42b]

The effect of hypoxia on the elimination of omeprazole, a potent inhibitor of gastric excretion,[43] was studied in the isolated perfused rat liver.[44] Although the sulfone and sulfoxide derivatives do not appear as major metabolites under aerobic conditions, the alterations in the omeprazole metabolite profiles during hypoxia showed the inhibition of oxidative metabolism and potentiation of reductive metabolism. A similar study[45] with misonidazole, a nitro-substituted imidazole radiation sensitizing agent, demonstrated that misonidazole metabolism was also changed under anaerobic conditions leading to an accelerated clearance of the drug by metabolic pathways not observed under aerobic conditions. In the light of these studies, sulfoxide compounds 11a and 11b will be evaluated for antitumor activity under hypoxic conditions. Further sulfoxide prodrug designs would need to consider the redox potential of the sulfoxide moiety such that selective reduction to the "active" sulfide compound occurs in the tumor cell. The reduced sulfide compound must also show alkylating ability.

The symmetrical 17 and unsymmetrical 18 disulfides were cytotoxic in vitro but, in vivo, were found to be toxic at higher doses. These data would suggest that disulfide reduction does occur and that the cytotoxicity and toxicity is mediated through the putative thiol 10 (because the (carbamoyloxy)methyl groups in the latentiated disulfides are chemically unreactive). Thiol-containing-drug action and toxicity can be influenced by factors which influence the metabolic formation and degradation of disulfides including NADPH levels, the glutathione:oxidized glutathione ratio, and the redox status of the cell.[46] The toxicity of 10 could be due to the free thiol or, more likely, to the reactivity of the (carbamoyloxy)methyl groups.

The in vitro cytotoxicity data against a number of organ specific human solid tumor cell lines is given in Table III. This in vitro primary screen assays for cellular growth using a sulforhodamine colorimetric assay[47] (see the Experimental Section). Sulfide metabolism by sulfoxidases is known to be mediated by nonmicrosomal flavin dependent oxidases in mammalian tissues.[23,48] Sulfide oxidation of the test compounds by microsomal or cytosolic

Table III. In Vitro Antitumor Activity of Selected Target Compounds

	IC_{50} (μM)[a]				
compd	L1210	A549	HT-29	MFC7	PSN-1
1	4.3	218	162	347	110
1[b]			108		
16b	18	190	108	147	115
16d	>100	>100	>100	>100	>100
16f	11	190	108	147	118
16g	>100	>1000	>1000	>1000	>650

[a] The cell lines tested were as follows: L1210, murine lymphocytic leukemia; A549, human lung carcinoma (NSCLC); HT-29, human colon adenocarcinoma; MCF7, human breast adenocarcinoma; PSN-1, human pancreatic carcinoma. Test compounds were dissolved in RPMI-1640 plus HEPES at a maximum soluble concentration. Test compound exposure time was 48 h for L1210 and 72 h for the remaining cell lines. [d] Test compounds were dissolved in 25% DMSO.

enzymes in the tumor cell or in the cell culture serum cannot be excluded; however, this possibility is much less likely than that expected in vivo in murine tumor models.

These data show that as the length of the 2-(alkylthio)-imidazole side chain increases, antitumor potency decreases. The 2-(*n*-propylthio)imidazole **16b** is less active than 1 as measured by IC_{50} values in the indicated cell lines. A striking difference in cytotoxicity was observed for the bicyclic heterocycles **16f** and **16g**. The imidazo-[2,1-*b*]thiazole **16f** was only slightly less potent than carmethizole (1) free base, while the imidazo[2,1-*b*]thiazine **16g** was found to be 5–10 times less potent. Optimized structures calculated using MNDO showed the 5–5 fused bicyclic heterocycle **16f** to be very nearly planar, whereas the 5–6 fused bicyclic homologue, **16g**, was not. The torsion angle N8–C8a–S1–C2 was calculated (MNDO) to be 166° in **16g**. The major consequence of the conformational constraint is the modification of the electronic interactions between the C-2 sulfur atom and imidazole π-electron system.[49] The MNDO optimized structure for the putative cationic intermediate (*cf.* Figure1) for the displacement of the C-6′ carbamate has a torsion angle N8–C8a–S1–C2 of 139° (more planar than the ground-state structure of **16g** but not as planar as **16f**). The effects of this change are also apparent in the chemical properties of the compounds. For example, the relative basicity of the azine ring nitrogen increases with the ability of the sulfur to stabilize the protonated imidazole ring: thus, the imidazothiazine diol **15g** (pK_a = 5.58) is more basic than the imidazothiazole diol **15f** (pK_a = 5.05) and the carmethizole diol 8 (pK_a = 4.97). The reactivities of the bis(carbamates) are similarly influenced (see below) and the increased reactivity of **16g**, relative to **16f**, presumably is associated with an increase in decomposition under the assay conditions.

Chemical Reactivity Studies. The rates at which the analogues alkylate the model biological nucleophile, 4-(4-nitrobenzyl)pyridine (NBP) were determined by using a modification of published methods,[26] and the rate constants (K'_{NBP}) are given inTable IV. The NBP assay provides a measure of relative reactivity of a structurally similar group of alkylating agents, but it is not able to discriminate whether an analogue is reacting as a bifunctional or monofunctional electrophile.

The K'_{NBP} rates for 2-(ethylthio)imidazole **16a** and carmethizole (1) were comparable, but the rates decreased as the 2-(alkylthio)imidazole side chain was homologated to *n*-propyl (**16b**) and branching (*i*-propyl, **16c**). The heterobicyclic analogues **16f** and **16g** exhibited marked

Table IV. Reactivity of Imidazole Bis(carbamate) Derivatives toward the Model Nucleophile 4-(4-Nitrobenzyl)pyridine[a]

compd	concn (mM/mL)	$K'_{NBP} \times 10^{-2}$ (relative to 1)	r^2
1	1.0	2.45	0.991
1	0.1	0.152	0.992
11a	1.0	NR[b]	
11b	1.0	NR[b]	
16a	1.0	2.52 (upr1.03×)	0.991
16b	1.0	2.00 (↓1.28×)	0.998
16c	1.0	1.06 (↓2.31×)	0.998
16d	1.0	0.072 (↓34.0×)	0.986
16e	1.0	0.011 (↓223×)	0.985
16f	1.0	1.02 (↓2.40×)	0.992
16g	1.0	15.87 (↑6.48×)	0.994
17	1.0	0.05 (↓49.0×)	0.996
18	1.0	*ca. 0.05*[c]	

[a] The K' value represents the pseudo-first-order rate constant for alkylation of NBP (Experimental Section).[26] The r^2 value is the square of the correlation coefficient for the linear (regression analysis. [b] Compound failed to react with NBP after 1 h under the conditions described for 1. [c] Compound 18 NBP alkylation product did not completely extract into the EtOAc layer and prohibited quantitative measurement of the extent of alkylation (qualitatively the rate was comparable to that of 17).

differences in reactivity. Compound **16f** was *ca.* 15-fold less reactive than **16g**.[49] The 2-(phenylthio)imidazole analogue **16d** was much less reactive than carmethizole (1) because of the electron-withdrawing influence of the phenyl moiety. The electron-withdrawing influence [and the steric effect (*cf.*, **16b** *vs* **16c**)] of the THP moiety in the 2-(pyranylthio)imidazole **16e** reduced the electrophilic reactivity still further. The sulfoxides **11a** and **11b** and the disulfides **17** and **18** exhibited negligible NBP alkylation reactivity. The absence of significant electrophilic reactivity for these oxidized-sulfur prodrugs was expected on the basis of the electron-withdrawing properties of the C-2 substituent.

Conclusions

This work was undertaken to further develop structure–activity relationships at the C-2 position of carmethizole hydrochloride (1·HCl), while attempting to provide additional clues to the metabolic fate of this cytotoxic agent. Earlier we reported electron-donating substituents at the C-2 position gave active compounds.[2] Any change in electron-donating ability of the C-2 sulfur atom of 1 must be considered in terms of how it modulates electrophilic reactivity, drug stability, and lipophilicity. We observed a decrease in antitumor activity with decreased chemical reactivity for **16b–e**, **11a**, and **11b**. Compounds **16c** and **16f** exhibited similar antitumor activity and chemical reactivity. We also observed decreased activity with the chemically more reactive **16g**.

The predominant effect of varying the 2-(alkylthio)-imidazole chain length on chemical reactivity may be attributed to a combination of steric and resonance effects. It is generally considered that molecular orbital coplanarity is a prerequisite for effective participation of sulfur p_π-orbitals in conjugations.[50] Length and width components of the steric parameter[51] of the C-2 substituent may alter its own free rotation with respect to the imidazole ring and in this way disrupt the sulfur p_π/ imidazole π-electron overlap required for electron-donating resonance effect. Similar effects have been observed in the change in basicity and nucleophilicity of 4-alkoxy-3,5-dialkylpyridines where the higher alkoxy homologues are less basic and weaker nucleophiles. This effect is most prominent when comparing **16f** and **16g** where the resonance donating ability of **16f** is decreased by the restricted 5–5 fused imidazo-[2,1-*b*]thiazole ring system leading to a decreased rate of alkylation in the NBP assay.[49] The sulfoxide compounds **11a** and **11b** were not active in vitro, but more significantly, **11a** and **11b** were inactive in vivo when evaluated using either ip LOX or P388. This provides indirect evidence for the absence of a role of carmethizole sulfoxide as a "carrier form" of carmethizole. The absence of cytotoxicity of the sulfoxides and sulfones could be viewed positively because these compounds might be involved in the metabolism and excretion of the thioethers. Nevertheless, sulfoxides as prodrugs in this series of compounds warrants further investigation, particularly if the redox potential of the sulfoxide can be modified. The disulfide **17** and **18** proved to be very toxic in vivo but were relatively unreactive as electrophiles; these data support the concept that disulfide reduction is a prerequisite for activation of these drugs. These compounds along with related analogues are currently under further investigation.

Experimental Section

Melting points (uncorrected) were determined in an open capillary with a Thomas-Hoover Unimelt apparatus. IR spectra were determined as KBr pellets (unless noted otherwise) with a Matteson FT-IR interferometer. ^{1}H NMR spectra were determined at 90 MHz with a EM 390 spectrometer in $CDCl_3$ solution (unless noted otherwise) with TMS as internal standard. Microanalyses were performed by Atlantic Microlab, Atlanta, GA. Silica gel for flash column chromatography (230–400-mesh ASTM) was obtained from EM Science.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-(methylsulfinyl)imidazole (11a). A solution of 1[2] (3.0 g, 9.08 mmol) in dichloromethane (50 mL) was treated with 0.5 M aqueous sodium bicarbonate (7.0 equiv, 63.56 mmol, 5.34 g). The mixture was cooled on an ice bath, and then *m*-chloroperbenzoic acid (1.1 equiv, 10 mmol, 2.16 g of 80% wt/wt) was added portionwise over 15 min and allowed to come to room temperature over 3 h. The clear solution was extracted with dichloromethane (150 mL) and then NaCl (45 g) was added to the aqueous phase followed by dichloromethane extraction (4 × 50 mL). The pooled organic layers were dried ($MgSO_4$), concentrated, and purified by flash column chromatography (dichloromethane–MeOH, 9:1) to give a clear oil that was triturated with hexane to yield 11a (85%) as a white crystalline solid: mp 127-130 °C; ^{1}H NMR δ 2.72 (d, J = 2.5 Hz, 3 H), 2.81 (d, J = 2.5 Hz, 3 H), 3.14 (s, 3 H), 3.90 (s, 3 H), 4.90 (br m, 2 H), 5.16 (s, 2 H), 5.24 (s, 2 H); IR 3319, 3083, 3005, 2915, 2890, 1702, 1545, 1469, 1130, 1040 cm^{-1}. Anal. ($C_{11}H_{18}N_4O_5S$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-(propylsulfinyl)imidazole (11b). The procedure used for the preparation of 11a was modified to use **16b** to give **11b** as a fine white powder (90%): mp 98–101 °C; ^{1}H NMR δ 1.11 (t, J = 7.5 Hz, 3 H), 1.88 (m, 2 H), 2.75 (d, J = 2.0 Hz, 3 H), 2.81 (d, J = 2.0 Hz, 3 H), 3.41 (t, J = 7.5 Hz, 2 H), 3.91 (s, 3 H), 4.92 (br, 2 H), 5.14 (s, 2 H); IR 3326, 2966, 1726, 1698, 1559, 1261, 1142 cm^{-1}. Anal. ($C_{13}H_{22}N_4O_5S$) C, N, H, S.

General Procedure for the *S*-Alkylation of Diethyl 2-Mercapto-1-methylimidazole-4,5-dicarboxylate (13a). Sodium hydride (1.75 equiv, 1.63 g, 68 mmol) was added batchwise to stirred suspension of diethyl 2-mercapto-1-methylimidazole-4,5-dicarboxylate[2] (13a) (10.0 g, 38.7 mmol) in dry THF (250 mL) while under argon and at 5°C to give an olive green suspension that was allowed to stir for an additional hour. The electrophile (1.5 equiv) was then added dropwise, or slowly by syringe, and the reaction mixture was allowed to stir until complete disappearance of starting material (determined by TLC; usually between 6 and 24 h) while approaching ambient or reflux temperatures. The resulting tan suspensions were either quenched with absolute ethanol or directly concentrated to a brown residue. This residue was partitioned between EtOAc–0.5 M HCl (1:1, 300 mL) and the aqueous layer extracted with

EtOAc (2 × 150 mL). The pooled organic layers were dried ($MgSO_4$), concentrated at reduced pressure, and purified by flash chromatography (50–70% hexanes–EtOAc). In some cases the crude oil was used directly in the next step (lithium aluminum hydride reduction).

Diethyl 2-(Ethylthio)-1-methylimidazole-4,5-dicarboxylate (14a). Iodoethane was used as an electrophile on a 11.61-mmol scale to afford **14a** as a yellow oil (2.31 g, 73%): ^{1}H NMR δ 1.38 (t, J = 7.5 Hz, 9 H), 3.25 (q, J = 7.5 Hz, 2 H), 3.77 (s, 3 H), 4.39 (q, J = 7.5 Hz, 4 H); IR (neat) 2980, 1718, 1713, 1542, 1460, 1370, 1344, 1325, 1271, 1100 cm^{-1}. Anal. ($C_{12}H_{18}N_2O_4S$) C, H, N, S.

Diethyl 1-Methyl-2-(propylthio)imidazole-4,5-dicarboxylate (14b). Iodopropane (7.3 g, 4.19 mL, 43.17 mmol) was used as an electrophile on a 35.97 mmol scale. The excess sodium hydride was quenched with 95% EtOH, the pH adjusted with 0.5 M HCl to 9 (litmus), and the product was purified by flash column chromatography (hexane–EtOAc, 1:1) to give **14b** as a yellow oil (6.5 g, 70%): ^{1}H NMR δ 0.92 (t, J = 7.5 Hz, 3 H), 1.22 (m, 6 H), 1.60 (m, 2 H), 3.19 (t, J = 7.5 Hz, 2 H), 3.70 (s, 3 H), 4.30 (m, 4 H); IR (neat) 2987, 1739, 1372, 1231, 1206, 1045 cm^{-1}. Anal. ($C_{13}H_{20}N_2O_4S$) C, H, N, S.

Diethyl 1-Methyl-2-(2-propylthio)imidazole-4,5-dicarboxylate (14c). 2-Iodopropane was used on a 11.61-mmol scale to afford **14c** as a yellow oil (2.75 g, 79%): ^{1}H NMR δ 1.32 (t, J = 6.0 Hz, 6 H), 1.37 (d, J = 7.5 Hz, 6 H), 3.78 (s, 3 H), 3.90 (t, J = 6.0 Hz, 1 H), 4.38 (m, 4 H); IR (neat) 2979, 1718, 1541, 1459, 1369, 1271, 1200, 1136 cm^{-1}. Anal. ($C_{13}H_{20}N_2O_4S$) C, H, N, S.

Diethyl 1-methyl-2-(phenylthio)imidazole-4,5-dicarboxylate (14d) was prepared by published methodology.[30]

Diethyl 1-Methyl-2-[(tetrahydro-2*H*-pyranyl)thio]imidazole-4,5-dicarboxylate (14e). Pyridinium *p*-toluenesulfonate (1.25 g, 5 mmol) was added at room temperature to a stirred solution of **13a** (12.9 g, 50 mmol) and dihydropyran (6.31 g, 75 mmol) in dichloromethane (120 mL). The reaction mixture stirred at 40–45 °C for 24 h and then cooled to room temperature, the dichloromethane was evaporated, and the crude product was purified by silica gel flash column chromatography (dichloromethane–MeOH, 19:1) to afford pure **14e** as white solid (16.2 g, 95%): mp 112–114 °C; ^{1}H NMR δ 1.36 (t, J = 6 Hz, 6 H), 1.51–2.36 (m, 6 H), 3.86 (s, 3 H), 3.98–4.22 (m, 2 H), 4.36 (q, J = 6 Hz, 4 H), 5.91–6.16 (m, 1 H); IR 2946, 2861, 1745, 1721, 1587 cm^{-1}. Anal. ($C_{15}H_{22}N_2O_5S$) C, H, N, S.

Diethyl 2,3-Dihydroimidazo[2,1-*b*]thiazole-5,6-dicarboxylate (14f). Sodium hydride (0.984 g, 41.0 mmol) was added batchwise over 15 min to a stirred suspension of diethyl 2-mercaptoimidazole-4,5-dicarboxylate[2] (**13b**) (4.0 g, 16.4 mmol) in dry THF (60 mL) under argon at 5 °C. The mixture was stirred for 1 h, chloroethyl methanesulfonate (7.7 g, 5.95 mL, 32.8 mmol) was added dropwise, and the mixture was allowed to come to room temperature over 20 h. The reaction mixture was worked up in a similar manner as described above and purified by crystallization from THF–isopropyl ether to yield **14f** as fine white crystals (3.9 g, 58%): mp 104–106 °C; ^{1}H NMR δ 1.41 (t, J = 6.0 Hz, 6 H), 3.88 (t, J = 6.0 Hz, 2 H), 4.22 (m, 6 H); IR 3451, 2966, 1735, 1700, 1552, 1448, 1193 cm^{-1}. Anal. ($C_{11}H_{14}N_2O_4S$) C, H, N, S.

3-Bromopropyl Methanesulfonate. 3-Bromopropanol (10 g, 71.94 mmol) was added dropwise to a stirred solution of methylsulfonyl chloride (1.25 equiv, 10.3 g, 90 mmol) and triethylamine (1.25 equiv, 9.11 g, 90.0 mmol) in anhydrous dichloromethane (150 mL) at 5 °C. The reaction mixture was stirred at 5 °C for 1 h, brought to room temperature over 2 h, and subsequently washed with water (30 mL) and brine (2 × 30 mL), dried ($MgSO_4$), concentrated at reduced pressure to an orange oil, and purified by fractional distillation (11 mmHg), collecting fractions boiling at 145–150 °C to give as a clear oil (11.5 g, 75%): ^{1}H NMR δ 2.28 (m, 2 H), 3.04 (s, 3 H), 3.53 (t, 2 H), 4.38 (t, 2 H). Anal. ($C_4H_9BrO_3S$) C, H, S.

Diethyl 2*H*,3,4-Dihydroimidazo[2,1-*b*]thiazine-5,6-dicarboxylate (14g). The procedure used for the preparation of **14f** was modified to use 3-bromopropyl methanesulfonate (6.67 g, 30.71 mmol). Flash chromatography (hexane–EtOAc, 2:3) of the resultant oil gave a clear oil that was crystallized by trituration in isopropyl ether to yield **14g** as fine white crystals (3.76 g, 57%): mp 68–69 °C; ^{1}H NMR δ 1.38 (t, J = 7.0 Hz, 6 H), 2.33 (m, 2 H), 3.15 (m, 2 H), 4.18–4.54 (m, 6 H); IR 2989, 2917, 1746, 1711, 1545, 1199 cm^{-1}. Anal. ($C_{12}H_{16}N_2O_4S$) C, H, N, S.

4,5-Bis(hydroxymethyl)-2-(ethylthio)-1-methylimidazole (15a). A solution of diester **14a** (1.46 g, 5.10 mmol) in freshly distilled dichloromethane (50 mL) was added dropwise over 15 min to a stirred suspension of lithium aluminum hydride ($LiAlH_4$) (3 equiv, 0.581 g, 5.3 mmol) in dry THF or diethyl ether (50 mL) while under argon and at 2 °C. The reaction mixture was brought to room temperature over 3–12 h, and the excess hydride was destroyed at 5 °C by the slow sequential addition of water (1 mL), 15% sodium hydroxide solution (1 mL), and water (3 mL) for each gram of $LiAlH_4$ used. Methanol (20–1500 mL) was added to the white precipitate of inorganic salts followed by filtration and concentration in vacuo gave a clear oil that was purified either by crystallization (hot acetone–isopropyl ether) or flash column chromatography (dichloromethane–MeOH, 9:1) to give colorless crystals **15a** (0.867 g, 87%): mp 117–119 °C; ^{1}H NMR ($CDCl_3$/MeOH-d_4) δ 1.28 (t, J = 7.5 Hz, 3 H), 3.00 (q, J = 7.5 Hz, 2 H), 3.71 (s, 3 H), 4.33–4.77 (br s, 2 H), 4.55 (s, 2 H), 4.62 (s, 2 H); IR 3350 st, br, 2965, 1467, 1408, 1029, 1001 cm^{-1}. Anal. ($C_8H_{14}N_2O_2S$) C, H, N, S.

4,5-Bis(hydroxymethyl)-1-methyl-2-(propylthio)imidazole (15b). Compound **15b** was prepared with the method described for **15a** and was purified by flash chromatography (dichloromethane–MeOH, 9:1) to give a white solid (60%): mp 51–54 °C; ^{1}H NMR (MeOH-d_4) δ 0.93 (t, J = 6.0 Hz, 3 H), 1.57 (m, 2 H), 2.92 (t, J = 6.0 Hz, 2 H), 3.66 (s, 3 H), 4.48 (s, 2 H), 4.60 (s, 2 H), 4.79 (br, 2 H); IR (KBr) 3354 st, br, 1704, 1462, 1026, 1008 cm^{-1}. Anal. ($C_9H_{16}N_2O_2S$) C, H, N, S.

4,5-Bis(hydroxymethyl)-1-methyl-2-(2-propylthio)imidazole (15c). The procedure used for the preparation of **15a** was modified to use **12c** (1.70 g, 5.66 mmol), affording **15c** (1.08 g, 88%): mp 111–113 °C; ^{1}H NMR δ 1.29 (d, J = 7.0 Hz, 6 H), 3.38 (t, J = 7.0 Hz, 1 H), 3.58 (s, 3 H), 4.39 (d, 2 H), 4.78 (t, 1 H), 5.00 (t, J = 7.0 Hz, 1 H); IR 3297 br, 2969, 2840 br, 1459,1442, 1406, 1367, 1027, 1011 cm^{-1}. Anal. ($C_9H_{16}N_2O_2S$) C, H, N, S.

4,5-Bis(hydroxymethyl)-1-methyl-2-(phenylthio)imidazole (15d). Tetrabutylammonium fluoride (4.0 equiv, 2.08 mmol) was added to a solution of **15** (R = CH_3; R′ = Ph; R″ = *t*BuMe$_2$Si)[30] (250 mg, 0.52 mmol) in anhydrous THF (10 mL) at 5 °C under argon. The yellow solution was stirred for 1 h, concentrated in vacuo, and purified by flash chromatography (dichloromethane–MeOH, 47:3) to give **15d** as a fine white solid (130 mg, 100%): mp 109–110 °C; ^{1}H NMR δ 3.68 (s, 3 H), 4.53 (s, 2 H), 4.60 (s, 2 H), 7.20 (m, 5 H); IR 3299, 3072, 3040, 2782, 1458, 1022, 1004 cm^{-1}. Anal. ($C_{12}H_{14}N_2O_2S$) C, H, N, S.

4,5-Bis(hydroxymethyl)-1-methyl-2-[(tetrahydro-2*H*-pyranyl)thio]imidazole (15e). The procedure used for the preparation of **15a** was modified to use **14e**. The crude product was purified by flash silica gel column and crystallized (dichloromethane–hexane, 1:1) to afford **15e** as a white solid (7.5 g, 70%): mp 125–128 °C; ^{1}H NMR δ 1.51–2.17 (m, 6 H); 3.66 (s, 3 H), 3.69–3.93 (br exchanges with D_2O), 4.01–4.42 (m, 2 H), 4.51 (unresolved d, 2 H), 4.63 (unresolved d, 2 H), 6.11–6.39 (m, 1 H); IR 3412, 3291, 2934, 1449, 1381 cm^{-1}. Anal. ($C_{11}H_{18}N_2O_3S$) C, H, N, S.

2,3-Dihydro-5,6-bis(hydroxymethyl)imidazo[2,1-*b*]thiazole (15f). The procedure used for the preparation of **15a** was modified to use **14f** (6.0 g, 22.2 mmol) in dichloromethane (125 mL), $LiAlH_4$ (2.53 g, 66.6 mmol) in dry THF (200 mL). Purification by flash chromatography (dichloromethane–MeOH, 19:1) gave **15f** as white crystals (3.46 g, 84%): mp 165–167 °C; ^{1}H NMR ($CDCl_3$/MeOH-d_4) δ 3.87 (t, J = 6.0 Hz, 2 H), 4.21 (t, J = 6.0 Hz, 2 H), 4.45 (s, 2 H), 4.56 (s, 2 H), 4.72 (s, 2 H), IR 3298 br, 3069 br, 1485, 1462, 1024, 1005 cm^{-1}. Anal. ($C_7H_{10}N_2O_2S$) C, H, N, S.

2*H*-3,4-Dihydro-6,7-bis(hydroxymethyl)imidazo[2,1-*b*]thiazine (15g). The procedure used for the preparation of **12a** was modified to use **14g** (14.28 g, 0.05 mol) and $LiAlH_4$ (5.72 g, 0.152 mol) in dry THF (200 mL), and after flash chromatography (dichloromethane–MeOH, 19:1) gave **15g** as colorless crystals (5.60 g, 56%): mp 183–184 °C; ^{1}H NMR δ 2.38 (m, 2 H), 3.14 (t, J = 6.0 Hz, 2 H), 4.10 (t, J = 6.0 Hz, 2 H), 4.41 (s, 2 H), 4.44 (s, 2 H), 4.54 (s, 2 H); IR 3269, 3059 br, 1428, 1019, 926 cm^{-1}. Anal. ($C_8H_{12}N_2O_2S$) C, H, N, S.

4,5-Bis[[(*N*-methy)carbamoyl)oxy]methyl]-2-(ethylthio)-1-methylimidazole (16a). Methyl isocyanate (4.0 equiv, 80 mmol) was added dropwise to an argon-purged stirred suspension or solution of diol (20.0 mmol) in dry dichloromethane (200 mL), followed by catalytic amount of dibutyltin diacetate at room temperature. For large-scale reactions, additions should be made at 5 °C. The reaction mixture was allowed to stir until thin-layer chromatography (dichloromethane–MeOH, 19:1 or 9:1) showed complete disappearance of starting material (usually between 4 and 12 h), and then it was concentrated at reduced pressure to a white residue that was again dissolved in dichloromethane and concentrated. The white residual solid was purified either by flash chromatography (dichloromethane–MeOH, 19:1) or crystallization from hot acetone–isopropyl ether to give **16a–g** as clear to white fluffy crystals (85-95%). In this manner **15a** (1.40 g, 6.92 mmol) as a solution in dichloromethane was reacted for 6 h to give **16a** as white crystals (1.90 g, 88%): mp 87–89 °C; ^{1}H NMR δ 1.35 (t, J = 8.0 Hz, 3 H), 2.75 (m, 6 H), 3.80 (q, J = 8.0 Hz, 2 H), 3.55 (s, 3 H), 5.00 (br s, 2 H), 5.07 (s, 2 H), 5.17 (s, 2 H); IR ($CHCl_3$) 3462, 3019, 1719, 1521, 1517, 1237, 1221 cm^{-1}. Anal. ($C_{12}H_{20}N_4O_4S$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-(propylthio)imidazole (16b). The procedure used for the preparation of **16a** was modified to use **15b** (5.5 g, 21.3 mmol) in dichloromethane (200 mL) to give a white suspension which became clear 1.0 h after addition of methyl isocyanate (total reaction time was 3 h). The mixture was concentrated, and the residue was crystallized to give **16b** (6.63 g, 94%): mp 94–97 °C; ^{1}H NMR δ 1.0 (t, J = 7.5 Hz, 3 H), 1.65 (m, 2 H), 2.71 (d, J = 2.0 Hz, 3 H), 2.80 (d, J = 2.0 Hz, 3 H), 3.08 (t, J = 7.5 Hz, 2 H), 3.58 (s, 3 H), 4.95 (br, 2 H), 5.08 (s, 2 H), 5.15 (s, 2H); IR 3340, 3249, 2963, 1726, 1700, 1542, 1267, 1234, 1109 cm^{-1}. Anal. ($C_{13}H_{22}N_4O_4S$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-(2-propylthio)imidazole (16c). A mixture of **15c** (1.50 g, 6.94 mmol) in dichloromethane (150 mL) gave a white suspension that became clear 1.0 h after the addition of methyl isocyanate (total reaction time 7 h). Purification by flash column gave **16c** as a white solid (2.17 g, 93%): mp 101–102 °C; ^{1}H NMR δ 1.30 (d, J = 6.0 Hz, 6 H), 2.75 (m, 6 H), 3.57 (m, 1 H), 3.60 (s, 3 H), 5.00 (br s, 2 H), 5.07 (s, 2 H), 5.17 (s, 2 H); IR 3462, 3024, 2971, 2964, 2958, 1720, 1518, 1458, 1238, 1217, 1132 cm^{-1}. Anal. ($C_{13}H_{22}N_4O_4S$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-(phenylthio)imidazole (16d). The procedure used for the preparation of **16a** was modified to use diol **15d** (150 mg, 0.60 mmol) in dichloromethane to give a white suspension that went clear 0.5 h after addition of methyl isocyanate and total reaction time 3 h. Purification by flash column gave **16d** as a white solid (185 mg, 85%): mp 113–115 °C; ^{1}H NMR δ 2.73 (d, 3 H), 2.80 (d, 3 H), 3.60 (s, 3 H), 5.15 (s, 2 H), 5.21 (s, 2 H), 5.30 (s, 2 H), 7.23 (m, 5 H); IR ($CHCl_3$) 3464, 1720, 1520, 1517, 1458, 1252, 1237, 1132 cm^{-1}. Anal. ($C_{16}H_{20}N_4O_4S$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-[(tetrahydro-2*H*-pyranyl)thio]imidazole (16e). The procedure used for the preparation of **16a** was modified to use **15e**. The product was crystallized from benzene–hexane to afford **16e** (7.9 g, 95%) as a white solid: mp 151–152 °C; ^{1}H NMR δ 1.45–2.14 (m, 6 H), 2.82 (d, J = 6 Hz, 6 H), 3.61 (s, 3 H), 3.98–4.26 (m, 2 H), 4.81–5.00 (br, 2 H), 5.02-5.13 (2 H), 5.33 (2 H), 6.12–6.37 (m, 1 H); IR 3345, 2944, 2859, 1710, 1534 cm^{-1}. Anal. ($C_{15}H_{24}N_4O_5S$) C, H, N, S.

2,3-Dihydro-5,6-bis[[(*N*-methylcarbamoyl)oxy]methyl]-imidazo[2,1-*b*]thiazole (16f) was prepared from **15f** (2.7 g, 14.5 mmol) in dichloromethane (150 mL) to give a white suspension that went clear 1.0 h after addition of methyl isocyanate (total reaction time 7 h). Purification by flash column gave **16f** as a white solid (3.26 g, 75%): mp 134–135 °C; ^{1}H NMR (MeOH-d_4) δ 2.70 (m, 6 H), 3.90 (t, J = 7.0 Hz, 2 H), 4.25 (t, J = 7.0 Hz, 2 H), 4.78 (br, 2 H), 4.95 (s, 2 H), 5.12 (s, 2 H); IR 3310, 3242, 3073, 1703, 1566, 1293, 1154, 991 cm^{-1}. Anal. ($C_{11}H_{18}N_4O_4S$) C, H, N, S.

2*H*-3,4-Dihydro-6,7-bis[[(*N*-methylcarbamoyl)oxy]methyl]imidazo[2,1-*b*]thiazine (16g). A solution of **15g** (0.4 g, 2.0 mmol) in dichloromethane (6 mL) was treated (*via* syringe) with methyl isocyanate (0.47 mL, 8.0 mmol) to give a white suspension that became clear 1.0 h after addition of methyl isocyanate (total reaction time 4 h). The product was purified by crystallization to give **16g** (625 mg, 99%): mp 153.5–154.5 °C; ^{1}H NMR ($CDCl_3$/MeOH-d_4) δ 2.35 (m, 2 H), 2.67 (m, 6 H), 3.16 (m, J = 6.0 Hz, 2 H), 4.09 (t, J = 6.0 Hz, 2 H), 4.78 (s, 2 H), 4.97 (s, 2 H), 5.14 (s, 2 H); IR 3363, 3227, 2953, 1718, 1692, 1534, 1257, 1119 cm^{-1}. Anal. ($C_{12}H_{18}N_4O_4S$) C, H, N, S.

Bis[4,5-bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-imidazoyl] Disulfide (17).[35] Iodine (1.55 g, 6 mmol) was added to cold stirring solution of **16e** (2.16 g, 5.8 mmol) in ethanol (15 mL) while under argon. The reaction mixture was stirred at room temperature for 3 h. The ethanol was evaporated, the residue was filtered through a short silica gel column, and the solvent was evaporated. The yellow residue was twice purified by silica gel column chromatography (dichloromethane–MeOH, 9:1) to afford **17** as a yellow solid (1.23 g, 70%): mp 92-95 °C dec; ^{1}H NMR δ 2.79 and 2.81 (2 d, J = 6 Hz, 12 H), 3.63 (s, 6 H), 5.05–5.16 (4 H), 5.18–5.28 (4 H), 5.33–5.68 (br, 4 H); IR (KBr) 3345, 1700, 1540, 1458, 1261 cm^{-1}; mass spectrum (FAB), m/e 575 (parent +H), 289, 217, 157, 140. Anal. ($C_{20}H_{30}N_8O_8S_2$) C, H, N, S.

4,5-Bis[[(*N*-methylcarbamoyl)oxy]methyl]-1-methyl-2-imidazoyl *tert*-Butyl Disulfide (18).[35] A cold (0 °C), stirred solution of thiocyanogen (13 mmol) in chloroform (60 mL) [prepared from $Pb(SCN)_2$ and bromine in chloroform][40] was treated with zinc chloride (1.77 g, 13 mmol), and after 10 min, a solution of **16e** (4.05 g, 10.8 mmol) in chloroform (30 mL) was added to the mixture. After 20 min, 2-methyl-2-propanethiol (1.48 g, 16.5 mmol) in chloroform (10 mL) was added dropwise at 0 °C, and the reaction mixture was allowed to stir for 0.5 h at 0 °C and then at room temperature for 3 h. The reaction mixture was filtered through a small silica gel column, and the solvent was evaporated. The crude product was purified by silica gel flash column chromatography (MeOH–EtOAc, 1:19) to afford a gum that was dried azeotropically with benzene (2 × 30 mL) to give **18** (2.45 g, 60%) as a yellow powder: mp 128–130 °C; ^{1}H NMR δ 1.3 (s, 9 H), 2.65 (d, J = 6 Hz, 3 H), 2.72 (d, J = 6 Hz, 3 H), 3.69 (s, 3 H), 4.68–4.95 (br, 2 H), 5.02 (s, 2 H), 5.18 (s, 2 H); IR 3405, 3232, 3057, 1714, 1541 cm^{-1}. Anal. ($C_{14}H_{24}N_4O_4S_2$) C, H, N, S.

Determination of in Vitro Antiproliferative Activity.[23] A121, A549, HT-29, and MCF7 cells were propagated in RPMI 1640 medium containing 5% fetal calf serum, 5% NuSerum IV, and 20 mM HEPES. Exponentially growing cells were seeded into 96-well microtiter plates and incubated overnight to allow the cells to adhere. A 5 log range (10 concentrations) of each test compound was then added to the cells. Final cell densities were determined after 72 h (3–4 cell doublings) using a sulforhodamine B (SRB) assay.[47] L1210 cells were maintained as suspension cultures in RPMI 1640 medium containing 10% NuSerum IV, and 20 mM HEPES. Cells were seeded in 24-well plates and a 5 log range (5 concentrations) of each test compound was added to the cells. After a 48-h incubation period (4 cell doublings), cell numbers were determined using a Coulter Counter Model Zf. The median-effect dose was calculated from the concentration-effect curve using nonlinear regression analysis.

Determination of Relative Rates of Alkylation Using 4-(4-Nitrobenzyl)pyridine (NBP) Assay. These were determined by a modification of the standard NBP colorimetric assay.[26] Water (2.0 mL), 0.025 M sodium acetate buffer (10 mL) adjusted to pH 6 with glacial acetic acid, acetone (0.89 mL), 5% wt/wt NBP in acetone (0.4 mL), and test compound prepared at 40 mM in DMSO, or DMSO blank (0.11 mL), were sequentially added to 15-mL glass centrifuge tubes. The tubes were capped, mixed vigorously, and placed in a water bath maintained at 37 °C (±0.1). Individual tubes (including one "blank" each time) were removed from the water bath at various time intervals (*i.e.*, 5, 10, 20, 30, and 40 min) depending on the reactivity of the alkylating agent. Reactions were terminated by placing the tube on ice for 2 min, followed by addition of EtOAc (5.0 mL), acetone (2.0 mL), and 0.25 N NaOH (1.5 mL). The samples were mixed vigorously and centrifuged for 1 min to separate the phases. The clear blue EtOAc layer was transferred to a cuvette and quantified spectrophotometrically at 540 nm using the solvent as a blank. The slope of the line obtained by the least-squares analysis of the absorbance plotted against time gave the comparative

alkylating activity (K'_{NBP}). Since the K'_{NBP} values are determined from the initial rates in the presence of excess NBP, they are proportional to (but not the same as) the pseudo-first-order rate constants for NBP alkylation.

Determination of the pK_a Values for Diols 15f, 15g, and 8. The pK_a values were determined using the method reported previously.[2] A sample (ca. 0.125 g) of the diol of interest was dissolved in an (0.10 N) HCl solution, and then the solution was titrated with 0.20 N NaOH. The pH of the solution was monitored with a standard pH meter and recorded after each NaOH addition. The pK_a of the diol was determined by a plot of the pH vs volume of 0.20 N NaOH. The pK_a of imidazole was used as a control constant by using the above procedure; imidazole had a pK_a of 6.89 (lit.[52] pK_a = 6.95).

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