evaporated to dryness under reduced pressure. The residue was recrystallized from a mixture of $CHCl_3$ and EtOH to provide 2.64 g (6 mmol) of the acetate. This was dissolved in 250 ml of hot dioxane and diluted with 250 ml of absolute EtOH. To the hot, stirred solution was added a small amount of W-2 Raney nickel followed by 4.0 g of 95% hydrazine added in portions over 20 min. The mixture was heated on the steam bath for 1 h and more Raney nickel was added to destroy the excess of hydrazine. The mixture was filtered through diatomaceous earth which was washed with hot EtOH. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was chromatographed on 100 g of silica gel (Mallinckrodt Silicar CC 7) with $CHCl_3$ to give as a first fraction 1.25 g of **5b** and as a second fraction 0.73 g of the title compound. This fraction was rechromatographed on 80 g of the silica gel with CH_2Cl_2 as the solvent and the first 1950 ml of eluent was discarded. The next 350 ml of eluent was evaporated to about 20 ml and cooled to 0° to give 0.46 g of crystalline product of mp 238–239° dec: ir 3460, 3380, 1650, 1630, 1590, 1490, 1240 cm^{-1}; NMR (Me_2SO-d_6) 12.54 (1 H, s, OH), 6.97 (1 H, d, J = 7 Hz, H_{3a}), 6.97 (1 H, s, H_{10}), 6.76 (1 H, t, H_2), 6.64 (1 H, s, H_5), 5.44 (1 H, t, H_1), 4.89–4.70 (3 H, m, H_{12c} and NH_2), 3.91 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3). Anal. ($C_{19}H_{15}NO_7$) H, N; C: calcd, 61.79; found, 62.34.

1,2,3a,12c-Tetrahydro-5-bromo-2,8-diacetoxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one (6). A solution of 456 mg (1 mmol) of the diacetoxy compound **3** in 10 ml of $CHCl_3$ was cooled and 3.0 ml of 0.5 M $NaHCO_3$ solution was added. The two-phase system was cooled to 5° and with stirring was treated dropwise over 2 h with a solution of 0.055 ml (1 mmol) of bromine in 5 ml of $CHCl_3$. The cooling bath was removed and, after the mixture had attained room temperature, stirring was continued for 3 h. The $CHCl_3$ layer was separated and washed with 4 × 25 ml portions of H_2O, then dried over $MgSO_4$, and evaporated to dryness under reduced pressure to give 546 mg of a solid. This was chromatographed on 100 g of silica gel (Mallinckrodt Silicar CC-4) using $CHCl_3$–MeOH (99:1) as the solvent. The first 368 ml of eluate was discarded and the next 42 ml gave the product which was recrystallized from isopropyl alcohol to afford 77 mg of pale yellow needles of mp 214–215°: ir 1760, 1660, 1490, 1210 cm^{-1}; NMR ($CDCl_3$) AB quartet centered at 7.04 (2 H, J = 9 Hz, H_9 and H_{10}), 6.64 (1 H, d, J = 6 Hz, H_{3a}), 6.52 (1 H, m, H_2), 4.58–4.10 (1 H, m, H_{12c}), 3.96, 3.93 (6 H, 2 s, OCH_3), 2.65–2.45 (5 H, m, H_1 and $OCOCH_3$), 1.65 (3 H, s, $OCOCH_3$). Anal. ($C_{23}H_{19}O_{10}Br$) C, H, Br.

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(1) (a) J. S. E. Holker and S. A. Kagal, *Chem. Commun.*, 1574 (1968). (b) In this paper, 1a will be referred to as both 5-methoxysterigmatocystin (5-MS) to preserve its relationship with previous publications on this compound and 3a,12c-dihydro-8-hydroxy-6,11-dimethoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one to facilitate naming of its derivatives.
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Synthesis and Biological Activity of 3,5-Dinitro-4- and -2-(1*H*-purin-6-ylthio)benzoates, Prodrugs of 6-Mercaptopurine

Robert Drawbaugh, Charlene Bouffard, and Michael Strauss*

Department of Chemistry, University of Vermont, Burlington, Vermont 05401.

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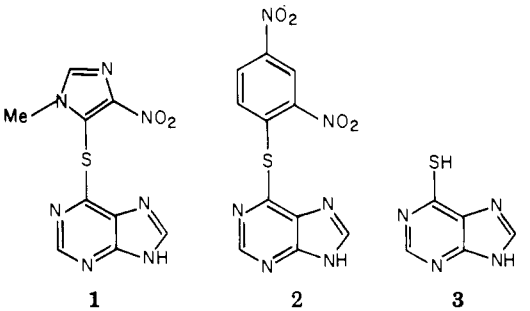
A series of prodrug modifications of 6-mercaptopurine (6-MP) containing dinitrobenzoate ester moieties with varying chain length has been prepared. These compounds were shown to be cytotoxic in several cell culture screens and also exhibited significant activity against L1210 lymphoid leukemia in vivo. The possibility exists that the transport and distribution of these compounds in vivo will be determined, in part, by increased lipophilic character, with a consequent selective localization in lymphatic and CNS tissue.

Lipid–water partition coefficients have been correlated with central nervous system (CNS) drug uptake in a study designed to assess the importance of lipophilicity in drugs designed for blood–brain barrier penetration and central nervous system effects.[1] A study of methotrexate diesters of increasing chain length and thus increasing lipophilicity has been reported.[2] The antitumor activity of these esters in vivo was the same as methotrexate in tests against both intracerebrally and ip inoculated L1210 lymphoid leukemia. This was because of rapid in vivo hydrolysis of the esters to parent drug.

We report here the preparation of prodrug derivatives of 6-mercaptopurine (6-MP) with increased lipophilicity. These were synthesized with the objective of favorably modifying transport and distribution of active purine in the lymphatic system and CNS. The desirability of having a clinically useful 6-MP derivative which readily reaches the CNS without the necessity for intrathecal injection is clear when the problems of treating CNS leukemias are considered.

6-MP has previously been modified with the objective of altering its transport, distribution, and metabolism. Two important modifications involve bonding of sulfur to nitroaromatic or nitroheteroaromatic rings, **1**[3] and **2**.[4] 6-MP is extensively metabolized in vivo, the main degradative pathways being oxidative. Both **1** and **2** were prepared with the hope that the protective group would



1 **2** **3**

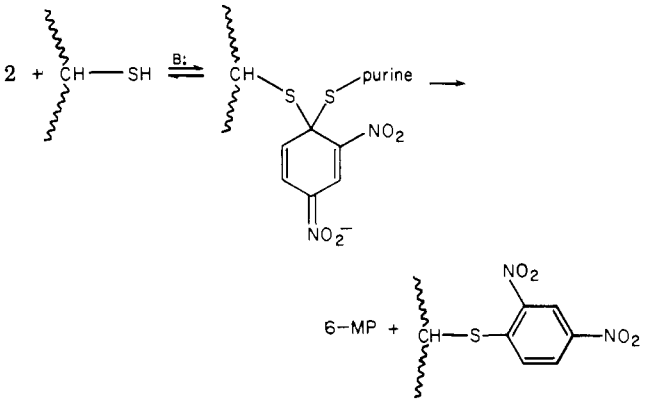
be able to shield the sulfur atom from oxidative attack.

Table I. Concentrations of the Various Esters 4 and 5 Necessary to Inhibit Growth in Cell Cultures and Optimum Dose and Increased Life Span (%) in L1210 in Vivo Screen

R	Ortho	Para	KB, human epidermoid carcinoma (in vitro)	LE, L1210 lymphoid leukemia (in vitro)	PS, P-388 lymphocytic leukemia (in vitro)	Increased life span (%) in L1210 in vivo screen	Optimum dose for highest ILS, mg/kg
Et	4a		0.16	2.4	0.49	26	37.5
Et		5a	0.15	1.7	0.72	30	40
Pr	4b		0.39	16	9.9	13	40
Pr		5b	0.59	0.87	1.2	65	62.5
i-Pr	4c		0.14	0.12	0.04	56	19
i-Pr		5c	0.17	1.3	0.37	57	25
Bu	4d		0.33	28	2.9	15	10
Bu		5d	0.15	10	1.9	50	60[a]
Hexadecyl	4e		1.2	1.9	0.53	41	125
Hexadecyl		5e	10	18	4.5	17	50
6-MP						42	80

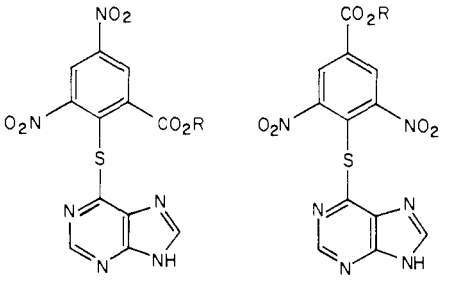
[a] Optimum dose not yet reached. This is the maximum dose tested.

Enhanced chemotherapeutic activity was expected, especially if the masking group is eventually cleaved. Liberation of free 6-MP is expected to be facile for **1** and **2** because of the ease with which structurally related compounds undergo nucleophilic aromatic substitution,[5,6] especially with sulfur nucleophiles. Such reactions have also been shown to occur in vivo.[3,7]



The imidazole derivative **1** has found greater use as an immunosuppressant than as an antineoplastic drug. Although **2** has been studied clinically, it does not appear to have significant advantages over 6-MP.[4]

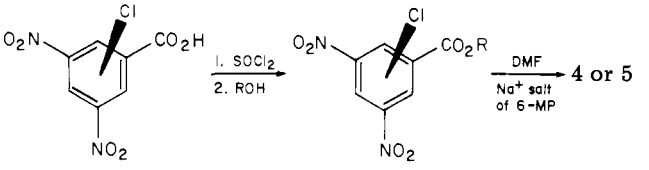
To enhance the lipophilicity of masked 6-MP analogues and make nucleophilic displacement and liberation of free 6-MP more facile, we have prepared a series of esters, **4** and **5**, analogues of **2** with carboalkoxy functionality containing R groups of different chain lengths ortho and para to sulfur. Both the increased number of electron-withdrawing groups and the increased steric compression about the benzene carbon bonded to sulfur are expected to facilitate the in vivo liberation of free 6-MP in **4** relative to **2**. The reactivity of **5** relative to **2** is more difficult to assess because *p*-nitro functionality is very effective in promoting nucleophilic substitution.[6] We have found that several of the esters **4a–e** and **5a–e** are at least as active as 6-MP in screens of mice infected with L1210 lymphoid leukemia. The partition coefficients of a series of related alkyl chain derivatives provide evidence that **4** and **5** probably are more lipophilic than 6-MP.[8] Our own determinations of partition coefficients for 6-MP, **4**, and **5**, confirm this (see Experimental Section). The wide variation in activity between various isomers of **4**, **5**, and 6-MP shows that either the transport, distribution, and/or metabolism of these antineoplastic compounds (before they liberate free purine) are quite different from 6-MP itself or that they need not be converted to free 6-MP to be active.



4a, R = Et
b, R = Pr
c, R = *i*-Pr
d, R = Bu
e, R = cetyl

5a, R = Et
b, R = Pr
c, R = *i*-Pr
d, R = Bu
e, R = cetyl

All of the compounds **4a–e** and **5a–e** were prepared by esterification of the commercially available 2- or 4-chlorodinitrobenzoic acids, followed by displacement of chloride with the sodium salt of 6-MP. All of the compounds except one analyzed within 0.35% for C, H, and N, and all were characterized by ^{1}H NMR (see Experimental Section).



The results of cell culture screening studies with **4a–e** and **5a–e** on three different cell cultures are summarized in Table I. They are expressed as the ED_{50} for inhibition of growth to 50% of control. Most of these compounds exhibit considerable cytotoxicity, with wide variations in activity for each compound between different cell cultures and for a single cell culture as the structures are varied. It is interesting to note, however, that in the L1210 cell culture screen the three most cytotoxic compounds, *p*-propyl, *o*-isopropyl, and *p*-isopropyl esters **5b**, **4c**, and **5c**, appear quite effective in producing an increase in life span in the in vivo L1210 screen (vide infra). In addition, **4c** appears to be the most cytotoxic compound for all three cell cultures, especially P-388 lymphocytic leukemia.

The results of in vivo screening with L1210 lymphoid leukemia are also summarized in Table I, expressed as percentage increase in life span relative to untreated control. The optimum dose for most of these compounds

Table II. Dose-Response Data for 6-MP Control (L1210 in Vivo Screen)

Dose, mg/kg	% ILS Group 1	Group 2	Group 3	Group 4	% ILS (optimum dose av)
20	6	30	11	4	
40	9	50	20	12	
80	14	68	47	41	42
160	0	7	0	23	

is also recorded. It should be noted that in this screen **4c**, **4e**, and **5b–d** are about as effective as 6-MP.

The statistical significance of the data in Table I (and the dose–response curves in Figure 1) is apparent when the data obtained from four different experiments with a 6-MP control are considered (Table II). There is a substantial variation from one group to another, but the optimum dose (of those tested) is the same in each case. This variation should be kept in mind when comparing the % ILS values for the esters **4** and **5** (Table III).

Presentation of the dose–response curves for these data is also informative and some of these are shown in Figure 1. It is interesting to note the very high dosage level achieved with **4e**. This is expected since the molecular weight, 682, is significantly higher for the same number of equivalents of active purine than the molecular weight of 6-MP, 152. At a level of 125 mg/kg the increased life span resulting from **4e** is 41%. Since the large lipophilic side chain in **4e** is a major portion of its structure, it is to be expected that if rapid hydrolysis does not occur, the transport and distribution of **4e** in vivo may well be determined in large part by the properties of this moiety.

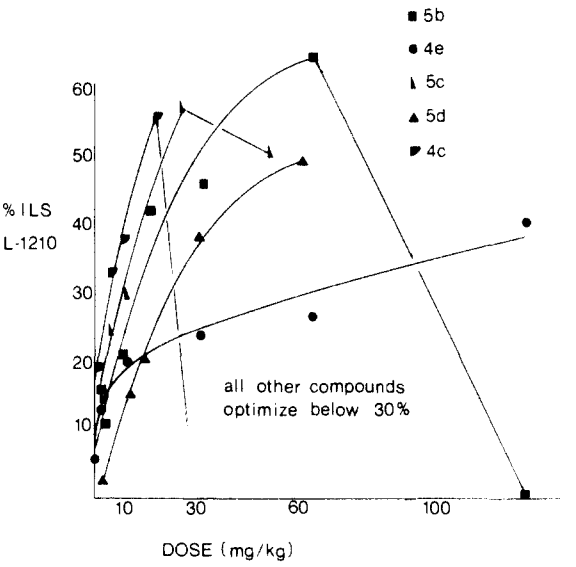


Figure 1. Dose-response curves for several of the esters 4 and 5.

Continued screening of **4e** and **5b** is continuing at the National Cancer Institute and at the Department of Noeplastic Diseases, Mount Sinai School of Medicine.

Experimental Section

All melting points were determined by the capillary method using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Analyses were performed by Robertson Laboratory, Florham Park, N.J., and Galbraith Laboratories, Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.35% of the

Table III. Characterization of the Adducts 4 and 5

Compd	Yield, %	Mp,[a] °C	Ir,[b] cm^{-1}	^{1}H NMR[c]	Uv,[d] nm	Formula	Analyses[e]
4a	80	184	1740, 1350, 1000	1.02 (t, 3), 4.10 (q, 2), 8.52 (s, 1), 8.59 (s, 1), 8.70 (d, 1), 8.92 (d, 1)	214, 275, 350 (b)	$C_{14}H_{10}N_6O_6S$	C, H, N
4b	71	163	1735, 1340, 1000	0.78 (t, 3), 1.40 (m, 2), 4.00 (t, 2), 8.54 (s, 1), 8.61 (s, 1), 8.82 (d, 1), 9.14 (d, 1)	217, 275, 358 (b)	$C_{15}H_{12}N_6O_6S$	C, H, N
4c	65	175	1730, 1350, 1000	1.00 (s, 3), 1.10 (s, 3), 4.88 (m, 1), 8.51 (s, 1), 8.58 (s, 1), 8.77 (d, 1), 9.04 (d, 1)	215, 278, 360 (b)	$C_{15}H_{12}N_6O_6S$	C, H, N
4d	74	166	1730, 1345, 990	0.76 (t, 3), 1.10 (m, 4), 4.04 (t, 2), 8.48 (s, 1), 8.55 (s, 1), 8.78 (d, 1), 9.02 (d, 1)	210, 276, 342 (b)	$C_{16}H_{14}N_6O_6S$	C, H, N
4e	79	90	1725, 1345, 1010	1.20 (s, 31), 4.05 (t, 2), 8.54 (s, 1), 8.61 (s, 1), 8.87 (d, 1), 9.15 (d, 1)	210, 276, 350 (b)	$C_{28}H_{38}N_6O_6S$	C, H
5a	74	174	1740, 1350, 995	1.4 (t, 3), 4.45 (q, 2), 8.55 (s, 1), 8.62 (s, 1), 8.87 (s, 2)	213, 276, 335 (b)	$C_{14}H_{10}N_6O_6S$	C, H, N
5b	85	170	1725, 1275, 950	1.00 (t, 3), 1.78 (m, 2), 4.35 (t, 2), 8.52 (s, 1), 8.68 (s, 1), 8.96 (s, 2)	212, 278, 348 (b)	$C_{15}H_{12}N_6O_6S$	C, H, N
5c	77	174	1730, 1345, 995	1.32 (s, 3), 1.42 (s, 3), 5.20 (m, 1), 8.50 (s, 1), 8.58 (s, 1), 8.77 (s, 2)	217, 276, 345 (b)	$C_{15}H_{12}N_6O_6S$	C, H, N
5d	72	165	1730, 1350, 995	0.98 (t, 3), 1.35 (m, 2), 1.70 (m, 2), 4.40 (t, 2), 8.52 (s, 1), 8.61 (s, 1), 8.82 (s, 2)	213, 275, 342 (b)	$C_{16}H_{14}N_6O_6S$	C, H, N
5e	48	136	1740, 1350, 1010	0.85 (t, 3), 1.20 (s, 28), 4.40 (t, 2), 8.51 (s, 1), 8.59 (s, 1), 8.80 (s, 2)	210, 275, 335 (b)	$C_{28}H_{38}N_6O_6S$	C, H, N

[a] All melting points are approximate as decomposition occurs with gas evolution. [b] KBr pellet. [c] In Me_2SO-d_6 with Me_4Si as internal standard. [d] In MeOH. [e] Analysis for each symbol shown was within ±0.35%.

theoretical values. Ir spectra were determined on a Perkin-Elmer 237B grating spectrophotometer; ^{1}H NMR spectra were determined on JEOL MH-100 or C-60 NMR spectrometers; uv spectra were determined using a Perkin-Elmer 402 ultraviolet–visible spectrophotometer.

Materials. 2- and 4-chloro-3,5-dinitrobenzoic acid were obtained from Aldrich Chemical Co., Inc. 6-MP was obtained as the monohydrate from Aldrich Chemical Co., Inc., and from the National Cancer Institute.

Esters. All esters used were prepared from the chlorodinitrobenzoic acid chlorides. The acid chlorides were prepared from the acid by treatment under reflux with thionyl chloride. The appropriate acid chloride was then treated with a slight excess of the alcohol and warmed for several hours. Cooling the reaction mixture afforded solid ester which was recrystallized from methanol to yield crystalline products having sharp melting points and appropriate ^{1}H NMR spectra.

Purine Derivatives. All of the purine adducts were prepared by the following procedure with the exception of the high molecular weight hexadecyl compounds. A sample of the sodium salt of 6-MP was dissolved in a small portion of dry, freshly distilled dimethylformamide. To the resulting solution was added a slight molar excess (10–20%) of the appropriate chloro ester. The reaction mixture immediately turned light orange and proceeded to lighten in color to a bright yellow solution upon stirring at room temperature for approximately 18 h. The cloudy mixture was cooled for several minutes and filtered through a fine-fritted filter to remove most of the NaCl. The filtrate was then evaporated under partial vacuum at 50° to yield a thick dark-orange oil. The oil was treated with a few milliliters of methanol and a large portion of Et_2O. The resulting yellow powder was filtered and washed with several portions of water, followed by several portion of Et_2O. The product was dried under vacuum at 62°. All compounds were characterized by melting point, ir, ^{1}H NMR, uv, and analysis.

For the hexadecyl compounds, after evaporation yielded the oil, it was treated with a few milliliters of methanol and a large portion of water. After stirring, the resulting mixture was filtered and the powder washed with a few small portions of ether and larger portions of water. These compounds were dried and characterized in the same manner as the others.

Partition Coefficients. Partition coefficients for the compounds **4a–e** and **5a–e** were determined in an octanol–water system, each phase being saturated with the other solvent. Solutions of the compounds to be partitioned were prepared from freshly distilled octanol. In partitioning these samples gentle inversion was carried out at room temperature (25 ± 5°) for 15 min. Longer mixing times generally gave no significantly different results. The volume ratio of the two solvents and the concentration of the sample were chosen such that the uv absorption of the partitioned sample fell in the range of 0.2–1.0 absorbance units using a 1-cm cell. The samples were taken from the octanol layer of the system after adequate separation had been accomplished and were centrifuged for 15–30 min. These were then used to record the uv spectrum from which the partitioned concentrations could be determined. The partition coefficients were calculated as $P = C_{octanol}/C_{water}$. P values were determined for each compound in duplicate using various concentrations of sample and at least two different solvent volume ratios.

Log P values for the methyl, ethyl, propyl, isopropyl, and butyl ester fell in the range from 1.2 to 1.6. The value of 1.9 for the cetyl derivative was significantly greater as expected. All of the esters were found to be considerably more lipophilic than 6-MP. The determined log P for the parent drug was 0.8 (lit.[8] 1.0).

Biological Testing. The curve for each of the compounds **4** and **5** (Figure 1) represents the results from two tests. The treatment schedule involved one ip dose per day for 5 days beginning with the first day of infection. The compounds were administered as suspensions in water–Tween mixtures.

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Some 10-Substituted 5-Ethyl-10-dialkylaminoalkyl-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepines as Potential Antiarrhythmic Agents

Charles R. Ellefson* and John W. Cusic

Department of Chemical Research, Searle Laboratories, Division of G. D. Searle & Company, Chicago, Illinois 60680.
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A series of 10-dialkylaminoalkyl-10-(cyano-, carboxamido-, aminomethyl-, and acyl-)-5-ethyl-10,11-dihydro-5*H*-dibenz[*b*,*f*]azepines was prepared by alkylation and further reaction of 10-cyano-10,11-dihydro-5-ethyl-5*H*-dibenz[*b*,*f*]azepine (**5**). Several of these compounds showed good activity in three assays for antiarrhythmic potential.

In the course of some alterations of the 5*H*-dibenz[*b*,*f*]azepine ring system, we prepared 10-cyano-10,11-dihydro-5-ethyl-5*H*-dibenz[*b*,*f*]azepine (**5**). The activated proton at the 10 position thus made 10-alkylated products readily accessible. This report describes the preparation and the antiarrhythmic activity of some 10-dialkylaminoalkyl-10-(cyano-, carboxamido-, aminomethyl-, and acyl-)-10,11-dihydro-5-ethyl-5*H*-dibenz[*b*,*f*]azepines. The 10-carboxamido compounds may be seen as analogues of the potent antiarrhythmic agent disopyramide[1] (Norpace),