containing the iron chelator ethylenediaminebis(o-hydroxyphenylacetic acid) (EDDA; 250 μg/mL) was seeded with the bacterial strain at a concentration of 10^3/mL, poured into plates, and allowed to solidify.

Compounds were prepared as 10 mM stock solutions in water and were diluted in water to give appropriate test concentrations. Sterile Sensi-disks (BBL) containing 10 μL of the compounds were placed on the surface of the seeded agar and the plates were incubated at 37 °C for 18 h. Diameters of zones of stimulation were measured.

Liquid Growth Bioassay for Siderophore Activity. This assay was completed with *S. flexneri* SA240 (SA100 *iuc*D:Tn5), a siderophore biosynthesis deficient mutant and *E. coli* strains RW193, an *E. coli* K12 *ent*A, *fhu*A, and AN193, a *ent*A, *fhu*A negative mutant (deficient in ability to give the ferrichrome receptor). Overnight cultures of each strain were diluted 1:500 into Luria broth with 10 μg/mL of EDDA with or without 100 μg/mL of the test compound **2**. Growth was monitored by measuring turbidity by absorbance at 650 nm.

Liquid Growth Bioassay for Antimicrobial Activity. The preformed iron complex of each respective siderophore peptide or conjugate was added by filtration through an Acro-Disc 0.2-μm filter assembly to sterile Luria broth containing EDDA (either 0.1 or 1.0 mg/mL) to give solutions of 10 or 50 μM final concentration in each case. Immediately, 20 μL of a 26-h-old Luria broth culture of *E. coli* X580 was added. The culture flasks were then shaken at 37 °C at 300 rpm. Aliquots were removed every 2 h for culture turbidity measurements at 600 nm.

Minimum inhibitory concentration values (MIC) were determined by Eli Lilly and Co. using their standard cephalosporin broad screen assay.

Acknowledgment. We gratefully acknowledge support by the NIH (Grant G 25845). Mass spectra were kindly obtained by Dr. Bruce Plashko at Notre Dame, by Eli Lilly and Co., and by Finnigan MAT of Bremen, FRG. We also would like to thank Eli Lilly and Co. for determination of the MIC values. S.M.P. gratefully acknowledges support by the NIH (Grant Al16935).

Registry No. **1**, 124650-78-8; **2**, 124620-50-4; **3**, 131080-76-7; **4**, 131080-77-8; **5**, 875-74-1; **7**, 69489-40-3; **8**, 124620-57-1; **9**, 124620-58-2; **10**, 33125-05-2; **11**, 127526-64-1; **12**, 39249-27-9; **13**, 131080-78-9; **14**, 124620-56-0; **15**, 131080-79-0; **16**, 123932-46-7; **17**, 124620-59-3; **18**, 124620-60-6; **19**, 124620-61-7; **20**, 124620-62-8; **21**, 124620-63-9; **22**, 124620-64-0; **23**, 90849-37-9; **24**, 131080-80-3; **25**, 131080-81-4; **26**, 131080-82-5; **27**, 131080-83-6; Ph_3CCl, 76-83-5; *N*-hydroxysuccinimide, 6066-82-6.

Quinazoline Antifolates Inhibiting Thymidylate Synthase: 4-Thio-Substituted Analogues

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We report the synthesis of four new 4-thio-5,8-dideazafolic acid analogues and a 4-(methylthio) analogue structurally related to the thymidylate synthase (TS) inhibitor N^{10}-propargyl-5,8-dideazafolic acid. Three N^{10}-propargyl-4-thio-5,8-dideazafolic acid analogues had C^2 amino, hydrogen, and methyl substituents. A 4-thio and a 4-(methylthio) compound each with hydrogen at C^2 and ethyl at N^{10} were also synthesized. In general, the synthetic route involved thionation of the appropriate 4-oxoquinazoline; the sulfur thus introduced was then protected by methylation. Further protection with a pivaloyl group was required for the quinazoline bearing a 2-amino substituent. The protected quinazolines were treated with *N*-bromosuccinimide and the resulting 6-(bromomethyl) compounds were then coupled to the appropriate N-monoalkylated diethyl *N*-(4-aminobenzoyl)-L-glutamate in *N,N*-dimethylacetamide with calcium carbonate as base. The 4-thio-5,8-dideazafolic acids were obtained by removal of the methylthio group with sodium hydrosulfide, followed by deprotection of the carboxyl groups with cold dilute alkali. For the compound containing a pivaloyl protecting group, hot dilute alkali was used. To obtain the 5,8-dideazafolic acid containing a 4-(methylthio) substituent, the corresponding diester was treated with lithium hydroxide which selectively deprotected the carboxyl groups. The five compounds were tested as inhibitors of L1210 TS. It was found that replacement of the 4-oxygen of the quinazoline moiety by sulfur did not alter the TS inhibition. However, the introduction of a methylthio substituent at position 4 severely impaired TS inhibition. All 4-thio compounds were less cytotoxic to L1210 cells in culture than their 4-oxo counterparts.

It is well established that N^{10}-propargyl-5,8-dideazafolic acid[1] is a potent inhibitor of thymidylate synthase (TS)[2-4] and that its in vivo antitumor activity stems from this property alone.[5,6] In clinical trials it gave responses in patients with refractory ovarian, breast, liver, and lung cancer which indicated the potential of an antimetabolite acting cleanly upon TS.[7-11] However, the compound was nephrotoxic and this was thought to result from its poor aqueous solubility. Removal of the 2-amino group from N^{10}-propargyl-5,8-dideazafolic acid gave a much more soluble compound which was 8-fold worse a TS inhibitor yet 8.5-fold more cytotoxic against L1210 cells.[12] 2-

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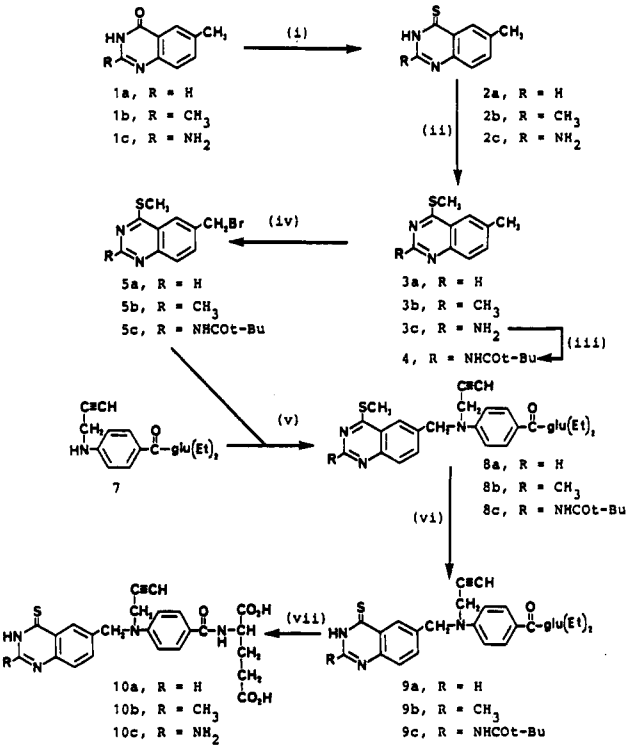
Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid was merely 2–3-fold worse a TS inhibitor than N^{10}-propargyl-5,8-dideazafolic acid yet 40-fold more cytotoxic against L1210 cells.[13-15] Neither desamino compound was nephrotoxic in mice.[16]

As part of an extensive study of analogues of N^{10}-propargyl-5,8-dideazafolic acid, we report here on the effects of replacing oxygen by sulfur in position 4 of the quinazoline ring. The pK_a's of the lactam NH in the model heterocycles 3,4-dihydro-4-thioquinazoline[17] and 3,4-dihydro-4-oxoquinazoline[18] are 8.47 and 9.81, respectively. The thiolactam is thus more acidic and therefore a better potential hydrogen-bond donor. Thiocarbonyl sulfur is also less polar than carbonyl oxygen. It was possible that these changes would combine to give a better inhibitor. Therefore N^{10}-propargyl-4-thio-5,8-dideazafolic acid (**10c**), and its 2-desamino (**10a**) and 2-methyl (**10b**) analogues were synthesized and evaluated. 2-Desamino-10-ethyl-4-thio-5,8-dideazafolic acid (**13**) and a compound bearing a 4-(methylthio)quinazoline moiety (**14**) were also prepared and examined.

Chemistry

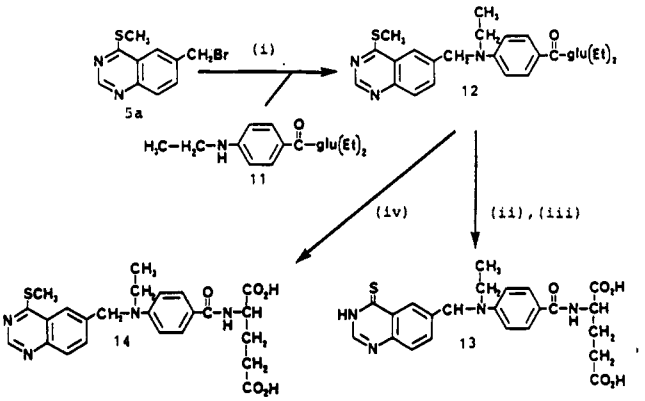
Many investigators have reported the preparation of 4-thiopteridines and 4-thioquinazolines. The most convenient way to obtain these compounds is by direct thionation of the corresponding 4-oxoquinazoline, for example with phosphorus pentasulfide in boiling xylene or pyridine.[19] Both 3,4-dihydro-4-thioquinazoline[19] and 3,4-dihydro-2-methyl-4-thioquinazoline[20] have been reported in the literature and have been resynthesized subsequently by many workers. The 6-methyl homologues have been synthesized by ring closure of 2-amino-5-methylbenzonitriles.[21] The yields were moderate, however,

Scheme I[a]



[a] Reagents: (i) P_2S_5, C_5H_5N; (ii) MeI, NaOH; (iii) *t*BuCOCl, CH_2Cl_2, Et_3N; (iv) *N*-bromosuccinimide, CCl_4, *hv*; (v) $CaCO_3$, Me_2NCHO; (vi) NaSH; (vii) NaOH.

Scheme II[a]



[a] Reagents: (i) DMA, $CaCO_3$; (ii) NaSH; (iii) NaOH; (iv) LiOH.

which led us to exemplify their preparation by thionation. Although 4-[*N*-[(2-amino-3,4-dihydro-4-thio-6-quinazolinyl)methyl]methylamino]benzoic acid and its N^{10}-hydrogen counterpart have been reported,[22] their syntheses or the synthesis of a possible precursor, 2-amino-3,4-dihydro-6-methyl-4-thioquinazoline, have not. 2-Amino-3,4-dihydro-4-thioquinazoline has been prepared in low yield via thionation of 2-(acetylamino)-3,4-dihydro-4-oxoquinazoline.[23] Attempts to prepare 2-amino-3,4-dihydro-4-thiopteridine by direct thionation of 2-amino-3,4-dihydro-4-oxopteridine were reported to be unsuccessful.[24] This was so despite the knowledge that

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direct thionation had earlier been reported for the synthesis of 4-thio-6,7-disubstituted-pteridines from the corresponding oxo compounds.[25] For us thionation worked well, even upon an unprotected aminoquinazoline.

Once introduced, the thiolactam sulfur has, on account of its nucleophilicity,[19] to be protected during further steps in the synthesis. Elliot et al. solved this problem, which occurred in their syntheses of N^{10}-methyl-4-thiofolic acid, by methylating the sulfur to give a thioether.[26,27] Displacement of the methylthio group by hydrosulfide anion later regenerated the 4-thio moiety. It is interesting that the sulfur atom first introduced is later deliberately substituted for by another. We decided to use this method in our quinazoline series. The targeted thiones (**10a–c**) (Scheme I) and **13** (along with its methylthio analogue, **14**) (Scheme II) were thus synthesized.

Quinazolines **1a**,[12] **1b**,[28] and **1c**[29] were treated with phosphorus pentasulfide to give thiones **2a–c** of which only **2a** could be purified. Methylation gave the pure thioethers **3a–c** and served to protect and to increase solubility for the subsequent bromination. Amine **3c** was additionally protected and solubilized by acylation to give pivalamide **4**. Reaction with *N*-bromosuccinimide in CCl_4 gave from **3a** pure bromomethyl compound **5a** and from **3b** and **4** a mixture of monobromo derivative **5b** or **5c** with unreacted starting material and dibrominated product of which one, dibromo compound **6**, was isolated from its mixture with **4** and **5c**. The proportion of **5b** and **5c** in their respective mixtures was determined by ^{1}H NMR spectroscopy by measurement of the integrals of signals for the appropriate hydrogens on the C-6 substituent. The bromo compounds **5a–c** were coupled with the appropriate amines, **7**[2] and **11**,[12] to give the diesters **8a–c** and **12** without cleavage of the methylthio group. The methylthio group of ester **12** was cleanly displaced with sodium hydrosulfide[24] and subsequent saponification could be carried out to give thione diacid **13** without the need to isolate the intermediate thione diester. When the N^{10}-substituent was propargyl (**8a–c**), byproducts were formed in the first step. Propargyl diesters **9a–c** were therefore isolated before hydrolysis to **10a–c**, elevated temperature being used in the case of **9c** for the additional removal of the pivaloyl group. Finally, when the methylthio diester **12** was treated with aqueous LiOH, the two ester groups were hydrolyzed selectively to give methylthio acid **14**. The stronger base NaOH additionally hydrolyzed the methylthio group, which is not surprising since this reagent was found to attack the related 4-(alkylthio)- and 4-(arylthio)-substituted pteridines.[24]

The five compounds **10a–c**, **13**, and **14** were tested as previously described for inhibition of partially purified L1210 TS[30,31] and for inhibition of the growth of L1210 cells in culture.[2] These results are collected in Table VI.

Results and Discussion

In Table VI the IC_{50} values for the inhibition of L1210 TS by thiones **10a–c** and **13** can be compared with those of the corresponding 4-oxo analogues. For each pair of compounds under comparison the small difference seen between the IC_{50}'s are within the range of error of the determinations. Each 4-thio compound was thus equally inhibitory as its 4-oxo parent. Hence, replacing the 4-oxo group of a classical quinazoline antifolate with a 4-thio group has no effect upon TS inhibitory activity. This conclusion agrees broadly with that from the sole previous study of 4-thioquinazoline antifolates wherein the glutamate-lacking 4-[*N*-[(2-amino-3,4-dihydro-4-thio-6-quinazolinyl)methyl]methylamino]benzoic acid was found to be 3-fold more inhibitory to L1210 TS and equally inhibitory to *Lactobacillus casei* TS when compared to its 4-oxo analogue.[22] A comparison of the IC_{50} values of thiones **10a** and **13** which have, respectively, propargyl and ethyl at N^{10} indicates a 2-fold lesser inhibition by the ethyl compound. This is consistent with similar observations made in the related 2-amino,[32] 2-hydrogen,[12] 2-methyl,[28] and 2-methoxyquinazoline[33] series. 4-(Methylthio) analogue **14** was >500-fold less active as a TS inhibitor than its parent thione **13**. This greatly decreased activity is possibly due to an unfavorable steric interaction of the methylthio group with the enzyme. A more likely explanation is that since the molecule is locked in the thiolactim tautomer, the N^3 nitrogen cannot donate a hydrogen bond to the enzyme. This concept is in accord with the finding that the N^3-methyl substituted analogue of CB3717 is a poor inhibitor of TS.[34]

All four thiones **10a–c** and **13** were poor inhibitors of the growth of L1210 cells when compared to their 4-oxo counterparts; thioether **14** was also a poor inhibitor. In view of the potent activity of the thiones as TS inhibitors, their low activity against cells in culture seems due either to reduced transport into cells or to reduced metabolism by folylpolyglutamate synthetase.

Experimental Section

General procedures are as given in refs 12 and 32 except as follows. Elemental analyses were also obtained from C.H.N. Analysis, Alpha House, Countesthorpe Road, South Wigston, Leicester, England, and are correct within ±0.4% unless otherwise stated. NMR (200 MHz) spectra were determined on a Brucker AM 200 spectrometer. Mass spectra were determined with a VG 7070H spectrometer and VG 2235 data system, using the direct insertion method and an ionizing voltage of 70 eV. UV spectra were determined from solutions in 0.1 M NaOH on a Pye Unicam SP8-150 spectrophotometer.

3,4-Dihydro-6-methyl-4-thioquinazoline (2a). To a solution of **1a**[12] (1.602 g, 10 mmol) in anhydrous pyridine (20 mL) at 110 °C in the dark was added P_4S_{10} (5.90 g, 13.3 mmol) in portions over 2 min. The mixture was heated under reflux for 30 min, cooled, diluted with water (1.6 mL), stirred for 4 h, and then concentrated at 45 °C (0.1 mm) and the residue partitioned between EtOAc and aqueous NH_4Cl. A yellow-green insoluble solid (1.30 g) was recovered by filtration. The organic phase of the filtrate was separated, dried, and concentrated in vacuo to give a crystalline solid (0.31 g). The two solids were combined

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and recrystallized from ethanol to give the product as yellow crystals (1.48 g, 84%): mp >300 °C dec; mass spectrum, m/z 176 (M^+); NMR (250 MHz, Me_2SO-d_6) δ 2.49 (s, 3 H, CH_3), 7.64 (d, 1 H, J = 8.3 Hz, H^8), 7.74 (dd, 1 H, $J_{7,8}$ = 8.3 Hz, $J_{7,5}$ = 1.9 Hz, H^7), 8.13 (s, 1 H, H^2), 8.37 (d, 1 H, J = 1.9 Hz, H^5), 13.82 (br s, 1 H, NH). Anal. ($C_9H_8N_2S$) C, H, N; S: calcd, 18.19; found, 19.00.

2-Amino-3,4-dihydro-6-methyl-4-thioquinazoline (2c). A mixture of **1c**[29,34] (17.52 g, 0.1 mol) and P_4S_{10} (44.45 g, 0.1 mol) in dry pyridine (500 mL) was heated under reflux with stirring for 3 h. The mixture was cooled and H_2O (2 L) was added and the crude yellow product (19.76 g) was recovered by filtration. A small sample was dissolved in 1 M NaOH; filtration followed by acidification with AcOH gave partially purified **2c** as a bright yellow solid: mp 261–264 °C; NMR (250 MHz, Me_2SO-d_6) δ 2.35 (s, 3 H, CH_3), 6.56 (br s, 2 H, NH_2), 7.17 (d, 1 H, $J_{8,7}$ = 8.4 Hz, H^8), 7.48 (dd, 1 H, $J_{7,8}$ = 8.4 Hz, $J_{7,5}$ = 2.0 Hz, H^7), 8.15 (d, 1 H, J = 2.0 Hz, H^5), 12.53 (br s, 1 H, lactam NH). Anal. ($C_9H_9N_3S{\cdot}0.3H_2O$) C, H, N.

3,4-Dihydro-2,6-dimethyl-4-thioquinazoline (2b). Lactam **1b**[28] (4.36 g, 25 mmol) was converted into crude thione **2b** (5.22 g, >100%) by the procedure used to prepare **2c**.

6-Methyl-4-(methylthio)quinazoline (3a). A mixture of **2a** (4.44 g, 25.2 mmol), EtOH (50 mL), 1 M NaOH (50 mL), and MeI (1.76 mL, 28.3 mmol) was stirred at 25 °C for 30 min, then diluted with brine (100 mL), acidified with 10% aqueous citric acid, and extracted with EtOAc (200 mL). The extract was washed with water, dried, and concentrated to give an oil which crystallized on standing. Column chromatography on silica gel (Merck, Art. 7734) eluting with CH_2Cl_2–EtOAc (7:3) gave crystalline **2b** which was recrystallized from MeOH containing a trace of H_2O to give white needles (4.40 g, 92%): mp 96–98 °C; mass spectrum, m/z 190 (M^+); NMR (250 MHz, $CDCl_3$) δ 2.54 (s, 3 H, C-Me), 2.70 (s, 3 H, SMe), 7.65 (dd, 1 H, $J_{7,8}$ = 8.7 Hz, $J_{7,5}$ = 1.9 Hz, H^7), 7.81 (signal hidden, 1 H, H^5), 7.84 (d, 1 H, J = 8.7 Hz, H^8), 8.94 (s, 1 H, H^2). Anal. ($C_{10}H_{10}N_2S$) C, H, N, S.

2,6-Dimethyl-4-(methylthio)quinazoline (3b). To a stirred solution of crude **2b** (5.22 g, ca. 0.025 mol) in 1 M NaOH (25 mL, 0.025 mol) was added H_2O (25 mL) and EtOH (50 mL), followed by MeI (3.89 mL, 62.5 mmol). After 30 min the brown mixture was extracted with CH_2Cl_2 (250 mL) and the dried organic layer was concentrated to give a red, viscous oil (3.65 g) which was redissolved in CH_2Cl_2 and applied to a silica column (Merck, Art. 9385, 100 g) which was eluted with CH_2Cl_2–EtOAc (4:1) to give **3b** as a pale yellow solid (2.57 g, 50%): mp 81–82 °C; mass spectrum, m/z 204 (M^+); NMR (90 MHz, $CDCl_3$) δ 2.51 (s, 3 H, C^6-CH_3), 2.69 (s, 3 H, SCH_3), 2.79 (s, 3 H, C^2-CH_3), 7.56 (dd, 1 H, $J_{7,8}$ = 9.1 Hz, $J_{7,5}$ = 2.5 Hz, H^7), 7.65 (d, 1 H, 9.1 Hz, H^8), 7.78 (d, 1 H, 2.5 Hz, H^5). Anal. ($C_{11}H_{12}N_2S$) C, H, N, S.

2-Amino-6-methyl-4-(methylthio)quinazoline (3c). To a stirred, ice-cooled solution of crude **2c** (19.76 g, ca. 0.1 mol) in 1 M NaOH (200 mL, 0.2 mol) was added MeI (15.57 mL, 0.25 mol) in one portion. After a period of 30 min, the mixture was extracted with CH_2Cl_2 (3 × 200 mL). Concentration of the dried ($MgSO_4$) extracts yielded an orange, viscous oil (17.23 g). It was dissolved in $CHCl_3$ (60 mL) and applied to a column of silica gel (Merck, Art. 7734, 360 g). Elution with EtOAc–$CHCl_3$ (4:1) gave first a minor product, 6-methyl-2,4-bis(methylthio)quinazoline, as a bright yellow oil (0.317 g, 1.3%). Recrystallization from hexane gave yellow crystals: mp 89–93.5 °C (lit.[35] mp 104–105 °C); mass spectrum, m/z 236 (M^+); NMR (250 MHz, $CDCl_3$) δ 2.50 (s, 3 H, CH_3), 2.67 (s, 3 H, SCH_3), 2.68 (s, 3 H, SCH_3), 7.57 (dd, 1 H, $J_{7,5}$ = 1.8 Hz, $J_{7,8}$ = 8.6 Hz, H^7), 7.67 (d, 1 H, J = 8.6 Hz, H^8), 7.73 (d, 1 H, J = 1.8 Hz, H^5). Anal. ($C_{11}H_{12}N_2S_2$) C, H, N, S.

Eluted next was **3c**, as a yellow solid (9.41 g, 46%) which was recrystallized from toluene: mp 158.5 °C; IR ν 3490 and 3290 cm^{-1} (NH); mass spectrum, m/z 205 (M^+); NMR (250 MHz, $CDCl_3$) δ 2.45 (s, 3 H, CH_3), 2.63 (s, 3 H, SCH_3), 5.06 (br s, 2 H, NH_2), 7.43 (d, 1 H, J = 8.6 Hz, H^8), 7.49 (dd, 1 H, $J_{7,5}$ = 1.8 Hz, $J_{7,8}$ = 8.6 Hz, H^7), 7.68 (d, 1 H, $J_{5,7}$ = 1.8 Hz, H^5). Anal. ($C_{10}H_{11}N_3S$) C, H, N, S.

6-Methyl-2-(pivaloylamino)-4-(methylthio)quinazoline (4). To a solution of **3c** (5.13 g, 25 mmol) and Et_3N (5.06 g, 50 mmol) in dry CH_2Cl_2 (250 mL) was added pivaloyl chloride (6.029 g, 50 mmol) with stirring. The solution was heated under reflux for 18 h, cooled, washed with H_2O (2 × 400 mL), dried, and concentrated to give a solid residue which was recrystallized from toluene (50 mL) to yield **4** as pale yellow crystals (6.10 g, 84%). Recrystallization from EtOH gave white crystals: mp 197–199 °C; IR ν 3300 (NH), 1690 cm^{-1} (C=O); mass spectrum, m/z 289 (M^+); NMR (250 MHz, $CDCl_3$) δ 1.38 (s, 9 H, tBu), 2.50 (s, 3 H, CH_3), 2.74 (s, 3 H, SCH_3), 7.59 (dd, 1 H, $J_{7,5}$ = 1.7 Hz, $J_{7,8}$ = 8.7 Hz, H^7), 7.75 (d, 1 H, J = 8.7 Hz, H^8), 7.77 (d, 1 H, J = 1.7 Hz, H^5), 8.10 (br s, 1 H, NH). Anal. ($C_{15}H_{19}N_3OS$) C, H, N, S.

6-(Bromomethyl)-4-(methylthio)quinazoline (5a). To a solution of **3a** (0.952 g, 5.0 mmol) in hot CCl_4 (30 mL) was added *N*-bromosuccinimide (0.904 g, 5.08 mmol) and the resulting mixture, under reflux, was irradiated with two 500-W tunsten lamps. After 30 min the mixture was cooled and diluted with CH_2Cl_2, and succinimide was removed by filtration. The filtrate was concentrated in vacuo to give a brown solid. Column chromatography on silica gel (Merck, Art. 15111) eluting with CH_2Cl_2–EtOAc (9:1) gave **5a** (0.539 g, 40%) as a white, crystalline solid which decomposed on heating with no defined melting point: mass spectrum, m/z 268, 270 (M^+); NMR (250 MHz, $CDCl_3$) δ 2.72 (s, 3 H, CH_3), 4.65 (s, 2 H, CH_2), 7.86 (dd, 1 H, $J_{7,8}$ = 8.7 Hz, $J_{7,5}$ = 1.9 Hz, H^7), 7.94 (d, 1 H, J = 8.7 Hz, H^8), 8.05 (d, 1 H, J = 1.9 Hz, H^5), 8.99 (s, 1 H, H^2). Anal. ($C_{10}H_9BrN_2S$) C, H, N, S; Br: calcd, 29.69; found, 30.37.

6-(Bromomethyl)-2-methyl-4-(methylthio)quinazoline (5b). A mixture of (methylthio)quinazoline **3b** (1.02 g, 5 mmol), *N*-bromosuccinimide (0.98 g, 5.5 mmol), dibenzoyl peroxide (10 mg), and CCl_4 (20 mL) was stirred in the presence of a light source (2 × 500 W) for 18 h. The filtered solution was concentrated to give a yellow, crystalline solid (1.60 g) containing 66% **5b** (by ^1H NMR spectroscopy), which was not purified further.

6-(Bromomethyl)-2-(pivaloylamino)-4-(methylthio)-quinazoline (5c). A mixture of **4** (4.63 g, 16 mmol) and *N*-bromosuccinimide (3.13 g, 17.6 mmol) in CCl_4 (160 mL) was heated under reflux with stirring in the presence of a strong (2 × 500 W) light source. After 30 min $CHCl_3$ (100 mL) was added to the cooled solution which was filtered and concentrated to give an orange oil (9.83 g) which partially crystallized. A solution in cyclohexane–CH_2Cl_2–EtOAc (5:4.5:0.5, 20 mL) was applied to a column (300 g) of silica gel (Merck, Art. 15111) which was eluted with the same solvent mixture. Pure 6-(dibromomethyl)-2-(pivaloylamino)-4-(methylthio)quinazoline (**6**, 0.42 g) eluted first: mp 205–209 °C; IR ν 3250 (NH), 1690 cm^{-1} (C=O); mass spectrum, m/z 447 (M^+); NMR (250 MHz, $CDCl_3$) δ 1.39 (s, 9 H, tBu), 2.77 (s, 3 H, SCH_3), 6.77 (s, 1 H, CH), 7.87 (d, 1 H, J = 8.8 Hz, H^8), 8.01 (d, 1 H, J = 2.0 Hz, H^5), 8.08 (dd, 1 H, $J_{7,8}$ = 8.8 Hz, $J_{7,5}$ = 2.0 Hz, H^7), 8.15 (br s, 1 H, NH). Anal. ($C_{15}H_{17}Br_2N_3OS$) C, H, N, S, Br: calcd, 35.74; found, 35.12. Subsequently eluted was a mixture of **6**, **5c**, and **4** (5.94 g) containing 51% of the desired product **5c** by ^1H NMR spectroscopy.

Diethyl *N*-[4-[*N*-[[2-(Pivaloylamino)-4-(methylthio)-6-quinazolinyl]methyl]prop-2-ynylamino]benzoyl]-L-glutamate (8c). A mixture of **7**[2] (1.44 g, 4 mmol), the product containing 51% **5c** (2.95 g, 3.96 mmol), and $CaCO_3$ (dried at 100 °C, 0.8 g, 8.0 mmol) was stirred in *N*,*N*-dimethylacetamide (dried over 3-Å molecular sieves, 12 mL) at 50 °C for 48 h in the dark. The pale orange slurry was filtered through Celite and concentrated at 0.1 mmHg to give an oil. It was dissolved in EtOAc–CH_2Cl_2 (1:9, 30 mL) and subjected to preparative HPLC on silica gel (Merck, Art. 15111, 250 g), with the same solvent mixture as eluant to yield **8c** (2.35 g, 91.7%) as a pale yellow amorphous solid after drying at 70 °C over P_2O_5 at 0.1 mm for 6 h: mp 185.5–187.5 °C; mass spectrum, m/z 205 ($C_{10}H_{11}N_3S^+$), 190 ($C_9H_8N_3S^+$); NMR (250 MHz, Me_2SO-d_6) δ 1.15 (t, 3 H, J = 7.1 Hz, ester CH_3), 1.18 (t, 3 H, J = 7.1 Hz, ester CH_3), 1.26 (s, 9 H, tBu), 2.05 (m, 2 H, Glu CH_2^β), 2.41 (m, 2 H, Glu CH_2^γ), 2.69 (s, 3 H, SCH_3), 3.26 (m, 1 H, propargyl CH), 4.03 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.09 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.39 (br s, 2 H, propargyl CH_2), 4.39 (m, 1 H, Glu CH^α), 4.87 (s, 2 H, CH_2^9), 6.86 (d, 2 H, J = 8.9 Hz, $H^{3'}$, $H^{5'}$), 7.74 (d, 1 H, J = 8.5 Hz, H^8), 7.75 (d, 2 H, J = 8.9 Hz, $H^{2'}$, $H^{6'}$), 7.82 (dd, 1 H, $J_{7,8}$ = 8.5 Hz, $J_{7,5}$ = 1.6 Hz, H^7), 7.92 (d, 1 H, J = 1.6 Hz, H^5), 8.38 (d, 1 H, J = 7.4 Hz, Glu NH), 9.97 (br

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s, 1 H, pivalamide NH). Anal. ($C_{34}H_{41}N_5O_6S$) C, H, N, S.

By a similar procedure the methylthio diesters **8a**, **8b**, and **12** were prepared (Table I). ^{1}H NMR data for these products are collected in Table IV.

Diethyl *N*-[4-[*N*-[[3,4-Dihydro-2-(pivaloylamino)-4-thio-6-quinazolinyl]methyl]prop-2-ynylamino]benzoyl]-L-glutamate (9c). To a solution of **8c** (1.36 g, 21 mmol) in dry EtOH (80 mL) was added powdered NaSH (dried P_2O_5/50 °C/0.1 mm, 1.36 g). The mixture was stirred under N_2 at 70 °C for 3 h then filtered and the filtrate concentrated to give a yellow, amorphous solid. This was dissolved in CH_2Cl_2 (30 mL) and then filtered through Celite. The filtrate (40 mL) was applied to a column of silica gel (Merck, Art. 7734, 200 g) which was eluted with CH_2Cl_2–EtOAc (10:1) to yield **9c** (1.17 g, 85.5%) as a bright yellow gum: NMR (250 MHz, Me_2SO-d_6) δ 1.16 (t, 3 H, J = 7.1 Hz, CH_3), 1.18 (t, 3 H, J = 7.1 Hz, CH_3), 1.27 (s, 9 H, *t*Bu), 2.04 (m, 2 H, Glu CH_2^β), 2.42 (m, 2 H, Glu CH_2^γ), 3.25 (s, 1 H, propargyl CH), 4.04 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.09 (q, 2 H, J = 7.1 Hz, ester CH_2), 4.36 (s, 2 H, propargyl CH_2), 4.36 (m, 1 H, Glu CH^α), 4.82 (s, 2 H, CH_2^9), 6.87 (d, 2 H, J = 8.8 Hz, $H^{3'}$, $H^{5'}$), 7.54 (d, 1 H, J = 8.4 Hz, H^8), 7.75 (d, 2 H, J = 8.8 Hz, $H^{2'}$, $H^{6'}$), 7.75 (dd, 1 H, $J_{7,8}$ = 8.4 Hz, $J_{7,5}$ = 2.0 Hz, H^7), 8.39 (d, 1 H, J = 7.3 Hz, Glu NH), 8.43 (d, 1 H, J = 2.0 Hz, H^5), 11.29 (s, 1 H, pivalamide NH), 13.75 (s, 1 H, lactam NH). Anal. ($C_{33}H_{39}N_5O_6S$·$1H_2O$) C, H, N, S.

By a similar procedure the thione diesters **9a** and **9b** were prepared (Table II). ^{1}H NMR data for these products are collected in Table V.

***N*-[4-[*N*-[(2-Amino-3,4-dihydro-4-thio-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamic Acid (10c).** A mixture of **9c** (0.135 g, 0.213 mmol), H_2O (10 mL), EtOH (10 mL), and 1 M NaOH (1.37 mL, 1.37 mmol) was stirred at 50 °C for 94 h in the dark. The solution was filtered through Celite and acidified to pH 3.0 to precipitate **10c** as a bright yellow powder (0.088 g, 78%): mp 180–190 °C dec; NMR (250 MHz, Me_2SO-d_6) δ 1.98 (m, 2 H, Glu CH_2^β), 2.23 (m, 2 H, Glu CH_2^γ), 3.21 (s, 1 H, propargyl CH), 4.29 (s, 2 H, propargyl CH_2), 4.34 (m, 1 H, Glu CH^α), 4.69 (s, 2 H, CH_2^9), 6.62 (br s, 2 H, NH_2), 6.68 (d, 2 H, J = 8.8 Hz, $H^{3'}$, $H^{5'}$), 7.22 (d, 1 H, J = 8.5 Hz, H^8), 7.55 (dd, 1 H, $J_{7,8}$ = 8.5 Hz, $J_{7,5}$ = 1.7 Hz, H^7), 7.74 (d, 2 H, J = 8.8 Hz, $H^{2'}$, $H^{6'}$), 8.25 (d, 1 H, J = 7.5 Hz, amide NH), 8.30 (d, 1 H, J = 1.7 Hz, H^5), 12.38 (br s, 2 H, COOH's), 12.58 (br s, 1 H, lactam NH); UV nm (ε) λ max 225 (43 500), 247.5 (29 800), 298 (29 800), 363 (8900); λ min 242.5 (28 700), 259.5 (14 400), 336 (5900). Anal. ($C_{24}H_{23}N_5O_5S$·$2H_2O$) C, H, N, S.

By a similar procedure, but done at ambient temperature, the thio diacids **10a** and **10b** (the latter washed by centrifugation, 3 × 100 mL) were prepared (Table III). ^{1}H NMR data for these products are collected in Table V; UV for **10a** nm (ε) λ max 219.6 (44 690), 299.5 (24 623), 351 (10 050); λ min 252.8 (9916), 333.5 (8475)°, UV for **10b** nm (ε) λ max 220.6 (47 866), 300.2 (25 091), 351.8 (10 152); λ min 258.9 (10 366), 334.2 (8567).

***N*-[4-[*N*-[(3,4-Dihydro-4-thio-6-quinazolinyl)methyl]-ethylamino]benzoyl]-L-glutamic Acid (13).** A solution of **12** (0.150 g, 0.278 mmol) in EtOH (6 mL) was treated with a freshly prepared aqueous MeOH solution of NaSH[36] (5.25% w/v, 2 mL, 1.87 mmol) and the mixture stirred under N_2 at 85 °C for 5 h, it becoming homogeneous after 20 min. The alcohols were evaporated in vacuo, the concentrate was diluted with H_2O (10 mL), and the resulting solution was acidified to pH 3.5 with 10% aqueous citric acid. The yellow precipitate was recovered by filtration and dissolved in EtOH (2.0 mL) and the solution treated with 1 M NaOH (1.2 mL, 1.2 mmol) at 25 °C for 18 h. It was clarified by filtration and acidified to pH 3.5 with 10% aqueous citric acid. The resulting precipitate was separated by centrifugation (2500*g*/15 min) and washed twice by cycles of resuspension (H_2O, 40 mL), centrifugation, and decantation. The resulting solid was dried over P_2O_5 in vacuo to give **13** (0.112 g, 86%) as an amorphous, yellow powder: mp 155–190 °C dec; NMR (250 MHz, CD_3OD) δ 1.27 (t, 3 H, J = 7.0 Hz, ethyl CH_3), 2.18 (m, 2 H, Glu CH_2^β), 2.45 (t, 2 H, J = 7.3 Hz, Glu CH_2^γ), 3.64 (q, 2 H, J = 7.0 Hz, ethyl CH_2), 4.58 (m, 1 H, Glu CH^α), 4.77 (s, 2 H, CH_2^9), 6.74 (d, 2 H, J = 9.0 Hz, $H^{3'}$, $H^{5'}$), 7.63–7.73 (m, 4 H, $H^{2'}$, $H^{6'}$, H^7, H^8),

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Table I. Preparation of Methylthio Antifolate Diesters 8a, 8b, and 12

compd	amine	mmol	quinazoline	mmol	$CaCO_3$, mmol	DMF, mL	reaction temp, °C	reaction time, h	chromatography	property of product	M+	% yield	formula	anal.
8a	**7**[a]	1.0	**5a**	1.0	2.0	1.5	50	18	CH_2Cl_2–EtOAc 3:1, then 2:1	glass/gum	548	57.0	$C_{28}H_{32}N_4O_6S$·$1H_2O$	C,H,N,S
8b	**7**[a]	2.5	**5b**	3.3[b]	5.0	7.5	50	18	CH_2Cl_2–EtOAc 2:1	glass/gum	562	70.7	$C_{30}H_{34}N_4O_6S$	C,H,N,S
12	**11**[c]	0.836	**5a**	0.836	0.84	5[d]	25	66	0–40% EtOAc in CH_2Cl_2	glass		69.5	$C_{28}H_{34}N_4O_6S$	C,H,N

[a] Reference 2. [b] Crude starting material, purity determined by NMR. [c] Reference 12. [d] DMA.

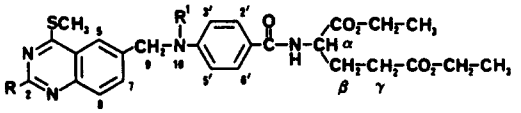
Table II. Preparation of Thio Antifolate Diesters **9a** and **9b**

compd	starting material	mmol	NaSH, g	EtOH, mL	temp, °C	time, h	chromatography	% yield	property of product	mp, °C	formula	anal.
9a	**8a**	0.353	0.179	15	70	5	filter through SiO_2,EtOAc	48.2	glass	176–179	$C_{28}H_{30}N_4O_5S$	C,H,N,S
9b	**8b**	1.0	0.5	40	70	5	CH_2Cl_2–EtOAc 2:1 then 1:1	47.8	glass	196–201	$C_{29}H_{32}N_4O_5S$	C,H,N,S

Table III. Preparation of Thio Antifolate Diacids **10a** and **10b**

compd	starting material	mmol	NaOH (aq), molar equiv	reaction time, h	drying temp, °C	% yield	mp, °C	formula	anal.
10a	**9a**	1.87	5.0	4	20	17	175–178	$C_{24}H_{22}N_4O_5S\cdot 1H_2O$	C,H,N,S
10b	**9b**	0.337	3.37	5	70	98	150–152	$C_{25}H_{24}N_4O_5S\cdot 1H_2O$	C,H,N,S

Table IV. NMR Spectral Data of Methylthio Antifolate Esters **8a**, **8b**, and **12**

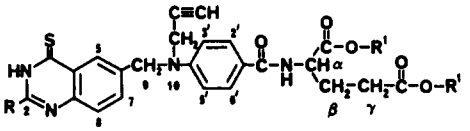


8a: R = H, $R^1 = CH_2C{\equiv}CH$
8b: R = CH_3, $R^1 = CH_2C{\equiv}CH$
12: R = H, $R^1 = CH_2CH_3$

compd	ester CH_3	Glu CH_2^β	N^{10} CH/CH_3	Glu CH_2^γ	SCH_3	ester CH_2	N^{10} CH_2	Glu CH^α	CH_2^9	amide NH	$H^{3',5'}$	$H^{2',6'}$	H^7	H^8	H^5	C^2 CH_3/H
8a[a]	1.21, 1.30	2.29	2.32	2.43	2.69	4.10, 4.22	4.19	4.78	4.82	6.85	6.88	7.75	7.78	7.94	7.97	8.98
8b[b]	1.16, 1.18	2.03	3.23	2.41	2.68	4.04, 4.10	4.38	4.38	4.89	8.33	6.85	7.74	7.84	7.84	7.95	2.63
12a[a]	1.29, 1.29	2.21	1.20	2.45	2.68	4.09, 4.21	3.60	4.80	4.75	6.78	6.70	7.69	7.70	7.92	7.88	8.97

[a] 250 MHz, $CDCl_3$. [b] 200 MHz, Me_2SO-d_6.

Table V. ^1H NMR Spectral Data of Thio Antifolate Esters and Acids **9a**, **9b**, **10a**, and **10b**[a]



9a: R = H, $R^1 = CH_2CH_3$
9b: R = CH_3, $R^1 = CH_2CH_3$
10a: R = H, R^1 = H
10b: R = CH_3, R^1 = H

compd	ester CH_3	Glu CH_2^β	prop CH	Glu CH_2^γ	ester CH_2	prop CH_2	Glu CH^α	CH_2^9	amide NH	$H^{3',5'}$	$H^{2',6'}$	H^7	H^8	H^5	lactam NH	C^2 CH_3/H	CO_2H
9a[b]	1.15, 1.17	2.02	3.25	2.41	4.04, 4.07	4.35	4.78	4.84	8.37	6.85	7.74	7.80	7.7	8.51	13.85	8.14	
9b[c]	1.16, 1.18	2.04	3.18	2.41	4.04, 4.09	4.33	4.39	4.82	8.33	6.85	7.73	7.73	7.58	8.47	13.67	2.45	
10a[b]		1.97	3.22	2.32		4.35	4.35	4.84	8.23	6.86	7.74	7.79	7.70	8.51	13.80	8.14	12.40
10b[c]		2.01	3.18	2.33		4.33	4.37	4.82	8.21	6.86	7.75	7.75	7.59	8.48	13.68	2.45	12.27

[a] Spectra determined in Me_2SO-d_6. [b] 250 MHz. [c] 200 MHz.

Table VI. Biological Properties of Antifolates[a]

compd	IC_{50} L1210 TS,[b,c] μM	IC_{50} L1210 cells in culture,[c] μM
10a	0.11 (0.16)	7.4 (0.4)
10b	0.051 (0.061)	8.0 (0.085)
10c	0.019 (0.015)	18.0 (3.4)
13	0.26 (0.29)	31.0 (2.7)
14	117	15.0

[a] Figures in parentheses are for those of the corresponding 4-oxo analogue. [b] The (±)-5,10-CH_2FH_4 concentration was 200 μM. [c] IC_{50} values were derived from data obtained from at least five inhibitor concentrations, each in duplicate.

8.01 (s, 1 H, H^2), 8.57 (d, 1 H, J = 1.6 Hz, H^5); UV nm (ε) λ_{max} 350 sh (12 600), 310 (28 300), 219 (45 000); λ_{min} 262 (7800). Anal. ($C_{23}H_{24}N_4O_5S\cdot 1H_2O$) C, H, N, S.

***N*-[4-[*N*-[[4-(methylthio)-6-quinazolinyl]methyl]ethylamino]benzoyl]-L-glutamic Acid (14).** A solution of **12** (0.257 g, 0.477 mmol) in EtOH (3 mL) was treated with 1 M LiOH (1.4 mL, 1.4 mmol). The resulting solution was stirred at 25 °C for 5 h, clarified, and acidified with 10% aqueous citric acid. The precipitate was collected by centrifugation (2500*g*/15 min.) and washed by five cycles of resuspension (H_2O, 30 mL), centrifugation, and decantation, then dried over P_2O_5 in vacuo to give **14** (0.210 g, 90%) as a pale yellow powder: mp 124–134 °C; NMR (250 MHz, CD_3OD) δ 1.29 (t, 3 H, J = 7.0 Hz, ethyl CH_3), 2.18 (m, 2 H, Glu CH_2^β), 2.45 (t, 2 H, J = 7.5 Hz, Glu CH_2^γ), 2.67 (s, 3 H, SCH_3), 3.66 (q, 2 H, J = 7.0 Hz, ethyl CH_2), 4.60 (m, 1 H, Glu CH^α), 4.85 (s, 2 H, CH_2^9), 6.76 (d, 2 H, J = 9.0 Hz, $H^{3'}$, $H^{5'}$), 7.72 (d, 2 H, J = 9.0 Hz, $H^{2'}$, $H^{6'}$), 7.83–7.94 (m, 3 H, H^5, H^7, H^8), 8.88 (s, 1 H, H^2). Anal. ($C_{24}H_{26}N_4O_5S\cdot 0.25H_2O$) C, H, N, S.

Acknowledgment. This work was supported by grants from the Cancer Research Campaign and Medical Research Council. We thank Mr. M. H. Baker for determining the mass spectra and Mrs. J. Hawkes and Mrs. F. Gallway who kindly provided NMR spectra under the auspices of the University of London Intercollegiate Research Service. We also thank Ms. L. Nevill, who expertly

typed the manuscript, and Drs. L. R. Hughes, M. Jarman, and R. G. Moran for helpful discussions.

Registry No. **1a**, 19181-53-4; **1b**, 18731-19-6; **1c**, 50440-82-9; **2a**, 13116-91-1; **2b**, 13116-92-2; **2c**, 131066-72-3; **3** (R = SMe), 91088-93-6; **3a**, 131066-74-5; **3b**, 131066-75-6; **3c**, 131066-73-4; **4**, 131066-76-7; **5a**, 131066-77-8; **5b**, 131066-78-9; **5c**, 131066-79-0; **6**, 131066-80-3; **7**, 76858-72-5; **8a**, 131078-76-7; **8b**, 131078-77-8; **8c**, 131066-81-4; **9a**, 131066-82-5; **9b**, 131066-83-6; **9c**, 131066-84-7; **10a**, 131066-85-8; **10b**, 131066-86-9; **10c**, 131066-87-0; **11**, 70280-71-6; **12**, 131078-75-6; **13**, 131066-88-1; **14**, 131066-89-2; TS, 9031-61-2.

Synthesis and Antitumor Activity of Structural Analogues of the Epipodophyllotoxins

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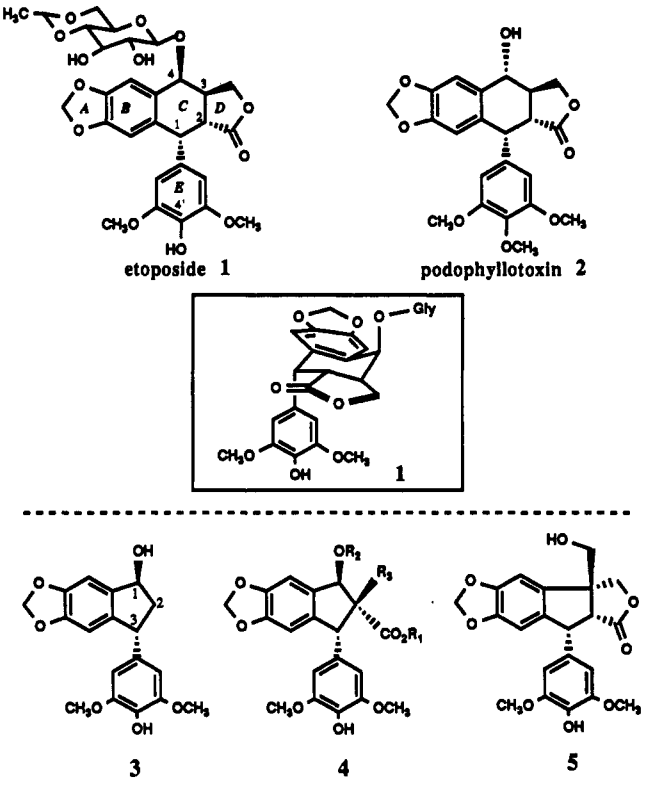
Several ring-contracted analogues of the antitumor agent etoposide have been prepared. The synthesis of the simple indanyl system **3** is described along with two bicyclic systems of general structure **4** prepared through a stereoselective allylation of the keto-ester **6**. A cis-fused lactone analogue **5**, which is isomeric with the etoposide aglycone, has been synthesized via a dialkylation of the indene-2-carboxylate anion. Regiochemical and stereochemical results of these alkylations are described. The cytotoxicity of these derivatives toward several tumor cell lines is described and generally follows the structure–activity relationships known for the agent podophyllotoxin (**2**).

Introduction

The antitumor agent etoposide **1** is a semisynthetic compound prepared in several steps from the naturally occurring lignan podophyllotoxin (**2**)[1a] (Scheme I). While both these molecules exhibit antitumor activity, podophyllotoxin has been shown to enact its effects via inhibition of cellular microtubule assembly mechanisms.[1b] In contrast to this activity, the potent antitumor activity of etoposide is believed to be due to inhibition of the enzyme topoisomerase-II and not due to any direct interaction with endogenous DNA.[2a] Although etoposide has seen much use in the clinic, problems such as myelosuppression, drug resistance, and poor bioavailability[2b] have encouraged further modifications. Recently, several amino analogues,[3a] ring C aromatic analogues,[3b] ring E desoxy analogues,[3c] and even a phosphorylated prodrug[3d] have been prepared and utilized in order to circumvent these problems.

At present, almost all reported antitumor agents in the podophyllotoxin series have been derived from the naturally occurring parent or incorporate its carbon skeleton and thus, the type and variation of analogues has been limited by the structure and stability of this parent. Besides some minor modifications on the two aromatic rings, little work has been done to affect changes in the gross structural skeleton.[4] In order to have greater freedom for modification of these molecules and access to new structural congeners, we felt a total synthetic effort was required. Therefore, we report here the synthesis of several sets of racemic analogues of **1** and **2** having modified ring systems, along with their in vitro antitumor activities against the A549, HT-29, and P388-D1 tumor cell lines.

Scheme I



Chemistry

Initially, we were interested in delineating which structural elements of etoposide were *minimally* required in order to produce the maximum desired activity. Upon investigation, the most striking structural aspect of etoposide is the trans diaxial placement[4] of the C4 glycoside and the C1 aryl substituents as shown in Scheme I. This

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