SYNTHESIS OF THIENO[2,3-d]PYRIMIDINE ANALOGUES OF THE POTENT ANTITUMOR AGENT *N*-{4-[2-(2-AMINO-4(3H)-OXO-7H-PYRROLO[2,3-d]PYRIMIDIN-5-YL)ETHYL]-BENZOYL}-L-GLUTAMIC ACID (LY231514)

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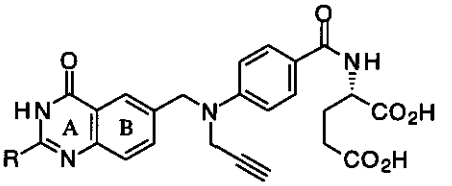
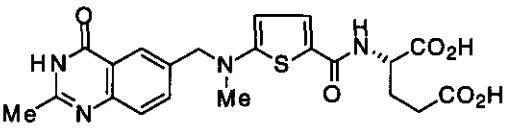
Abstract - Several thieno[2,3-d]pyrimidine analogues of the potent antitumor agent *N*-{4-[2-(2-amino-4(3H)-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl}-L-glutamic acid (LY231514, **7**) have been prepared by two different strategies. The first involved S-alkylation of the 6-mercaptopyrimidines (**13**) and (**14**) with the α-haloketone (**12**), followed by cyclization to the thieno-pyrimidines (**17**) and (**18**) respectively. The second involved synthesis of the 2-amino-3-carbomethoxy- and 2-amino-3-cyano-thiophenes (**19**) and (**20**) which were cyclized with acetonitrile to the corresponding thienopyrimidines (**21**) and (**22**). Compounds (**17**, **18**, **21**, and **22**) were hydrolyzed to the acids which were coupled with dimethyl L-glutamate, and the resulting monopeptide esters were saponified to the target analogs.

Inhibition of thymidylate synthase (TS), which mediates the methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to give 2'-deoxythymidine-5'-monophosphate (dTMP) and is thus essential for *de novo* DNA biosynthesis, has long been recognized as a prime objective for the development of an effective antitumor chemotherapeutic agent.[1] Several folate cofactor analogues which are TS inhibitors have emerged as clinical candidates. The earliest of these, the quinazoline antifolate CB3717 (**1**),[2] was withdrawn from clinical trials because of unexpected renal toxicity.[3] Newer TS inhibitors of continuing interest are second-

generation quinazoline antifolates with greater water solubility (and thus lower toxicity) than CB3717; e.g. the 2-desamino-,[4,5] 2-desamino-2-methyl- (ICI198583),[6-8] and 2-desamino-2-methyl thiophene (ZD1694,



1; R = NH_2
2; R = H
3; R = CH_3



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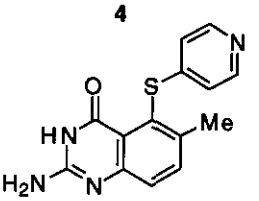

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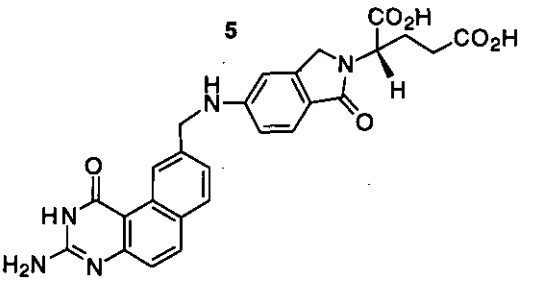

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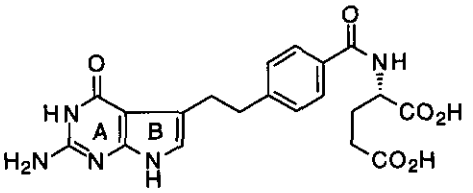
Tomudex)[9-12] analogues (**2**), (**3**) and (**4**), the non-classical quinazoline (**5**) (AG 337),[13] and the benzoquinazoline (**6**) (1843U89).[14]

A significant departure from the quinazoline family of TS inhibitors involved replacement of the ring-B fused benzene ring by a fused pyrrole ring, together with removal of the nitrogen atom from the bridge. The lead compound in this new series is *N*-{4-[2-(2-amino-4(3H)oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl}-L-glutamic acid (LY231514, **7**), which is currently in Phase II clinical trials.[15] A previous paper[16]

from our laboratories described the synthesis of B-ring modifications of LY231514 in which the pyrrole ring was replaced by a pyrazole ring. The present paper describes an additional B-ring modification of LY231514 in which the pyrrole moiety is replaced with a thiophene ring. Since thiophene is isosteric with benzene,



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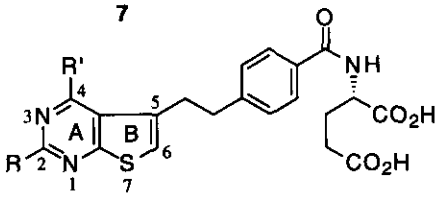


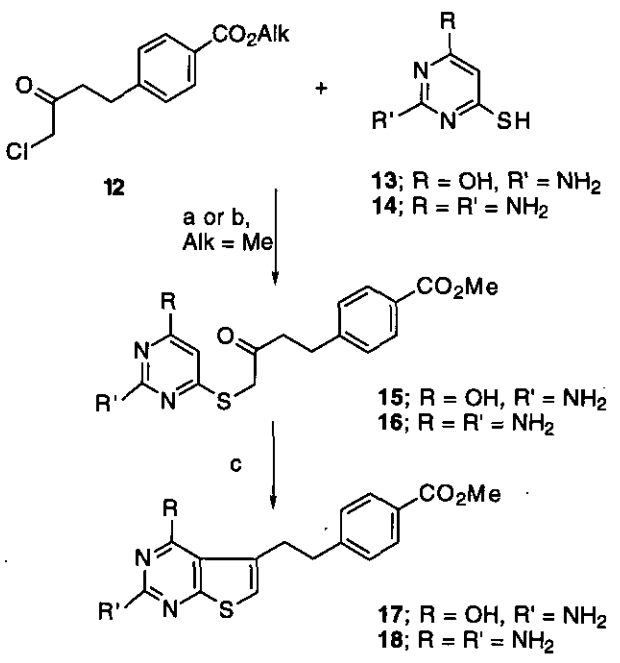
8; R = NH_2, R' = OH
9; R = R' = NH_2
10; R = Me, R' = OH
11; R = Me, R' = NH_2

compounds (**8**), (**9**), (**10**) and (**11**) may be regarded as analogues of the quinazoline series of TS inhibitors, but with presumably different aqueous solubility and transport properties. Preparation of these target molecules involved synthesis of the key intermediates (**17**), (**18**), (**21**), and (**22**) which were then converted to the corresponding target molecules using conventional chemistry.

The first synthesis of 2,4-diamino-5-substituted thieno[2,3-d]pyrimidines, published by Roth in 1969,[17] involved the reaction of 2,4-diamino-6-mercaptopyrimidine with various α-haloketones to form intermediate pyrimidine sulfides which underwent cyclization in very low yield upon heating in diphenyl ether. This general strategy was adopted as our first approach (Scheme I). Thus, the key intermediate α-chloroketone (**12**) was prepared as previously described,[18a,b] and alternatively by a palladium-catalyzed Heck coupling approach from ethyl *p*-iodobenzoate and *t*-butyl acrylate (see Scheme II). Reaction of **12** (Alk = Me) with 2-amino-4-hydroxy-6-mercaptopyrimidine (**13**)[19] in DMF then gave pyrimidine sulfide (**15**) in 68% yield. Surprisingly, an attempt to prepare **16** by the reaction of 2,4-diamino-6-mercaptopyrimidine (**14**) with **12** was

unsuccessful under these reaction conditions. Compound (**16**) could, however, be prepared by generation of the anion of **14** with Et_3N and NaOAc followed by addition of the α-chloroketone (**12**) and heating the solution at 80 °C for 2 h. Both the sulfides (**15**) and (**16**) cyclized in the presence of *p*-toluenesulfonic acid to give the corresponding thieno[2,3-d]pyrimidines (**17**) and (**18**).

Scheme 1

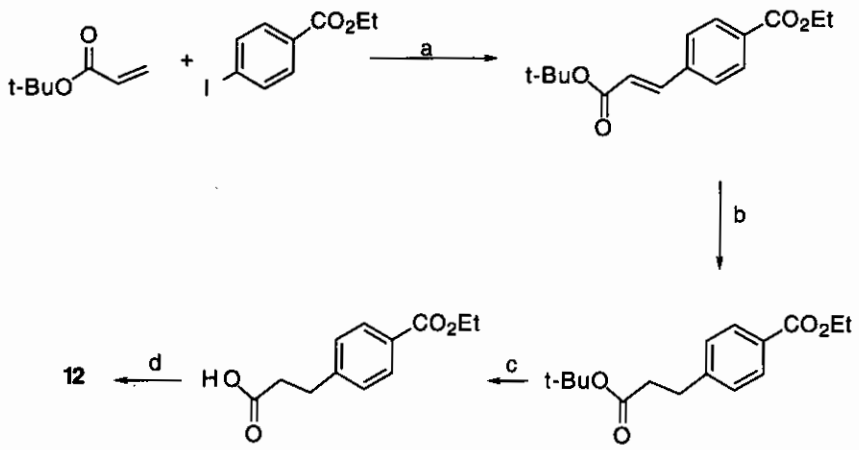


(a) DMF; (b) Et_3N, NaOAc, aq. MeOH;
(c) *p*-toluenesulfonic acid, ethylene glycol

Since the reaction of the α-chloroketone (**12**) with mercaptopyrimidines (**13**) and (**14**) was capricious, and the cyclization of **15** and **16** to **17** and **18** respectively required harsh reaction conditions, we also explored an alternate approach involving initial construction of the thiophene ring followed by annulation of the pyrimidine ring (Scheme III).[20]

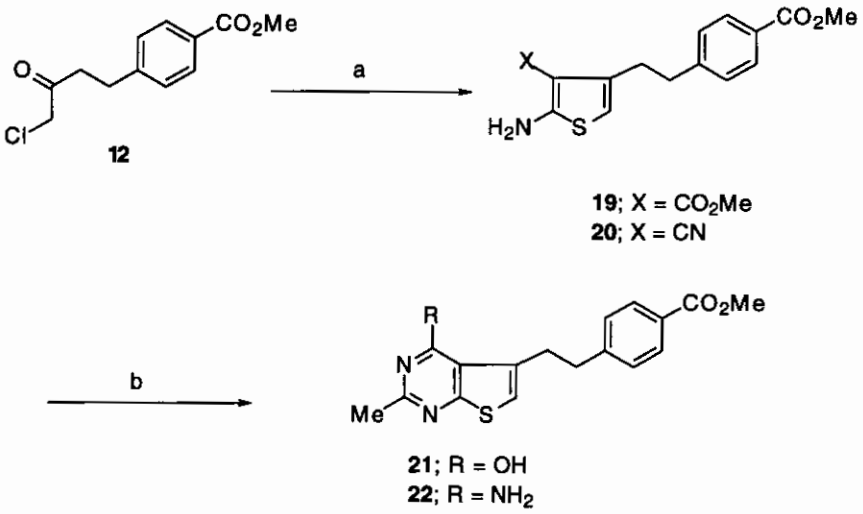
Thus, reaction of **12** with sodium hydrosulfide at 0 - 5 °C for 2 h, followed by addition of a mixture of methyl cyanoacetate and Et_3N in MeOH, gave the 2-amino-3-carbomethoxythiophene (**19**) in 65% yield.[21] All attempts to isolate the intermediate α-mercaptoketone from **12** were unsuccessful. In situ formation of

Scheme II



a, $Pd(OAc)_2$, MeCN, Et_3N; b, H_2, 10% Pd/C, 50 psi; c, $MeNO_2$, dry HCl; d, $SOCl_2$, then CH_2N_2; then HCl

Scheme III

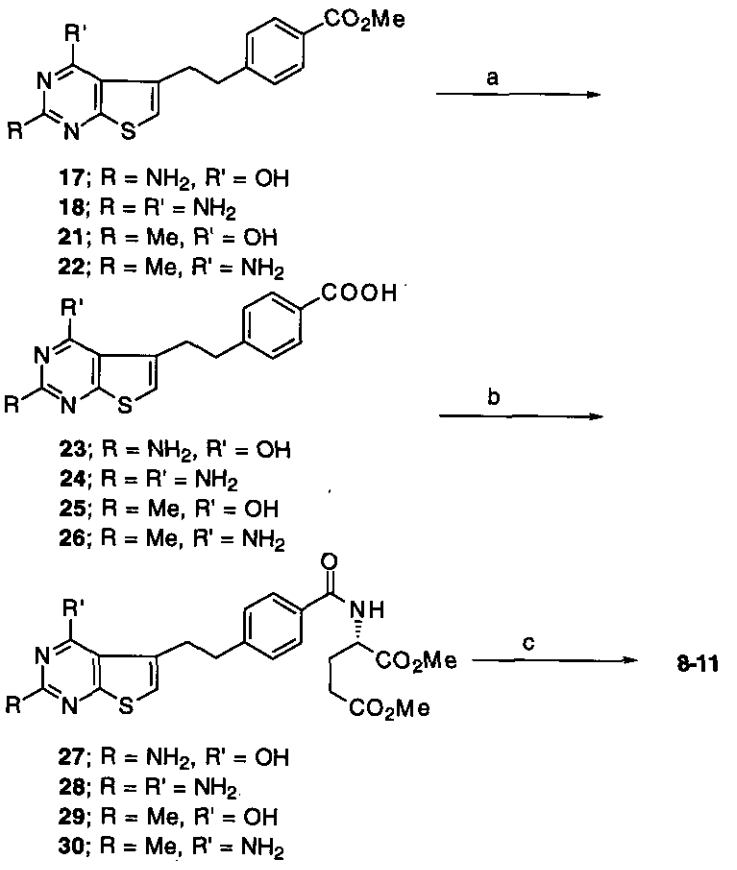


(a) i, NaSH, MeOH; ii, Et_3N, MeOH, NC-CH_2-X (X = CO_2Me or CN); (b) MeCN, dry HCl

the α-mercaptoketone from chloroketone (**12**) followed by treatment with malononitrile was also successful, and gave the corresponding 2-amino-3-cyanothiophene (**20**) in 85% yield. Both thiophenes (**19**) and (**20**) underwent cyclization with acetonitrile in dry HCl to give the cyclized thieno[2,3-d]pyrimidines (**21**) and (**22**) in reasonable yield.[22]

Saponification of the benzoate esters (**17**), (**18**), (**21**), and (**22**) (Scheme IV), followed by coupling of the resulting carboxylic acids (**23-26**) with dimethyl L-glutamate using 6-chloro-2,4-dimethoxy-1,3,5-triazine/N-methylmorpholine,[23] gave the intermediates (**27-30**). Final alkaline hydrolysis of **27-30** followed by acidification with acetic acid yielded the glutamic acid derivatives (**8-11**).

Scheme IV



(a) 1 *N* NaOH; (b) glutamate coupling; (c) 1 *N* NaOH.

Biological evaluation of compounds (**8**), (**10**), and (**11**) revealed that they were essentially inactive (IC_{50} > 20 mg/ml) as cell growth inhibitors.

EXPERIMENTAL SECTION

1-Chloro-4-(4-carboethoxyphenyl)-2-butanone (**12**, Alk = Et): A mixture of ethyl 4-iodobenzoate (5.00 g, 18.1 mmol), *t*-butyl acrylate (5.36 g, 41.8 mmol), $Pd(OAc)_2$ (0.5 g, 2.23 mmol), acetonitrile (50 ml) and Et_3N (30 ml) was heated at 80 °C for 15 h in a sealed tube. The reaction mixture was cooled and evaporated to dryness under reduced pressure, and the residue was dissolved in CH_2Cl_2 and filtered through a silica gel column using CH_2Cl_2 as eluent. Fractions containing the product were pooled and evaporated, the light yellow residual solid was triturated with a small amount of hexane, and the resulting white solid was recrystallized from hexane to give 4.35 g (91%) of ***t*-butyl 3-(4-carboethoxyphenyl)acrylate**, mp 68 °C. [^{1}HNmr ($CDCl_3$) δ 1.39 (t, *J* = 7.27 Hz, 3 H), 1.53 (s, 9 H), 4.37 (q, *J* = 6.9 Hz, 2 H), 6.44 (d, *J* = 16.11 Hz, 1 H), 7.55 (d, *J* = 7.32 Hz, 2 H), 7.60 (d, *J* = 15.42 Hz, 1 H), 8.03 (d, *J* = 8.12 Hz, 2 H); HRms calcd for $C_{16}H_{20}O_4$ 276.1361, found 276.1360. Anal. Calcd for $C_{16}H_{20}O_4$: C, 69.55; H, 7.30. Found: C, 69.58; H, 7.14].

To a solution of 5.0 g. of the above acrylate in 250 ml of MeOH was added 1.3 g of 10% Pd/C and the mixture was hydrogenated overnight at 50 psi of hydrogen. The mixture was then filtered through Celite, the filtrate evaporated to a smaller volume and then passed through silica gel, using CH_2Cl_2/MeOH (1:1, then 1:2). Fractions containing the product were pooled and evaporated to give crude *t*-butyl 3-(4-carboethoxyphenyl)propionate. A suspension of 1.6 g. of this material in 20 ml of nitromethane was cooled to O °C, and dry HCl gas was bubbled through the solution for 5 min. After stirring for 10 min at O °C and then at 30 min at room temperaure, the solution was filtered, washed with cyclohexane and dried to give 1.1 g (89%) of **3-(4-carboethoxyphenyl)propionic acid,** mp 109 °C (lit.,[18a] mp 108-110.5 °C). Subsequent conversion to (**12**, Alk = Et) was carried out as previously described for the corresponding methyl ester (**12**, Alk = Me)[18b]; yield 78%, mp 72 °C (lit.,[18a] mp 72-73 °C).

4-[2-Amino-4(3H)-oxopyrimidin-6-yl]thio-4-(4-carbomethoxyphenyl)-2-butanone (15). To a suspension of 2-amino-4-hydroxy-6-mercaptopyrimidine (**13**) (0.88 g, 6.1 mmol) in DMF (30 ml), the α-chloroketone (**12**)[18a,b] (Alk = Me, 1.63 g, 6.8 mmol) was added, the mixture was stirred at 55 °C for 1 h, and then cooled to room temperature and poured into ice-cold water (200 ml). The precipitated solid was filtered, washed with water and dried. Recrystallization of the crude material from MeOH gave **15** (1.41 g, 68%) as a white solid: mp 286-289 °C; ^{1}H nmr (DMSO-d_6) δ 2.84 (t, 2 H, J = 7.1 Hz), 2.94 (t, 2 H, J = 7.1 Hz), 3.80 (s, 3 H), 3.95 (s, 2 H), 5.35 (s, 1 H), 6.61 (s, 2 H), 7.31 (d, 2 H, J = 8.1 Hz), 7.82 (d, 2 H, J = 8.1 Hz), 10.63 (br s, 1 H); EIms, *m/z* (relative intensity) 347 (3), 329 (45), 180 (100); HRms calcd for $C_{16}H_{17}N_3O_4S$ 347.0939, found 347.0926.

4-[2,4-Diaminopyrimidin-6-yl]thio-4-(4-carbomethoxyphenyl)-2-butanone (16). To a mixture of 2,4-diamino-6-mercaptopyrimidine (**14**) hemisulfate (1.91 g, 10 mmol) and NaOAc (2.46 g, 30 mmol) in 50% aqueous MeOH (50 ml) was added Et_3N (1.01 g, 10 mmol), and the mixture was heated to 80 °C for 10 min. To the resulting mixture, a solution of α-chloroketone (**12**, Alk = Me) (3.03 g, 12.6 mmol) in MeOH (30 ml) was added and heating was continued for additional 2 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The solid product was filtered, washed with water and chromatographed on silica gel eluting with 3% MeOH/CH_2Cl_2. Fractions containing the product were combined and evaporated to give **16** (2.73 g, 79%) as a light yellow solid: mp 140-142 °C; ^{1}H nmr (DMSO-d_6) δ 2.82 (t, 2 H, J = 6.9 Hz), 2.97 (t, 2 H, J = 6.9 Hz), 3.79 (s, 3 H), 3.92 (s, 2 H), 5.53 (s, 1 H), 5.93 (s, 2 H, exchangeable with D_2O), 6.19 (s, 2 H, exchangeable with D_2O), 7.32 (d, 2 H, J = 8.1 Hz), 7.84 (d, 2 H, J = 8.1 Hz); EIms, *m/z* (relative intensity) 346 (5), 328 (30), 179 (74), 155 (100); HRms calcd for $C_{16}H_{17}N_4O_3S$ 346.1099, found 346.1099. Anal. Calcd for $C_{16}H_{17}N_4O_3S$: C, 55.48; H, 5.24; N, 16.17; S, 9.26. Found: C, 55.61; H, 5.10; N, 15.92; S, 9.00.

Methyl 4-[2-(2-Amino-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoate (17). A mixture of **15** (1.04 g, 3 mmol) and *p*-toluenesulfonic acid (100 mg) in ethylene glycol (10 ml) was heated at 190 °C for 1 h. The dark red solution was cooled to room temperature, poured into ice-cold water (100 ml) and extracted with EtOAc (4 x 200 ml). The combined extracts were dried ($MgSO_4$) and concentrated in

vacuo. The crude product was chromatographed on silica gel eluting with 70% EtOAc/hexanes. Fractions containing the product were combined and evaporated to give **17** (0.73 g, 74%) as a light yellow solid: mp 290-293 °C (decomp.); ^{1}H nmr (DMSO-*d6*) δ 2.89-3.02 (m, 4 H), 3.79 (s, 3 H), 6.48 (s, 1 H), 6.50 (s, 2 H), 7.32 (d, 2 H, *J* = 8.1 Hz), 7.84 (d, 2 H, *J* = 8.1 Hz); EIms, *m/z* (relative intensity) 329 (42), 180 (100); HRms calcd for $C_{16}H_{15}N_3O_3S$ 329.0834, found 329.0831.

Methyl 4-[2-(2,4-Diaminothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoate (18). Compound (**16**) (843 mg, 2.43 mmol) was cyclized in the presence of *p*-toluenesulfonic acid (100 mg) as described above, and the crude product was recrystallized from MeOH to give **18** (443 mg, 55%) as a light brown solid: mp 202-204 °C (decomp.); ^{1}H nmr (DMSO-*d6*) δ 3.06 (t, 2 H, *J* = 6.9 Hz), 3.12 (t, 2 H, *J* = 6.9 Hz), 6.27 (brs, 2 H, exchangeable with D_2O), 6.51 (s, 1 H), 6.69 (s, 2 H, exchangeable with D_2O), 7.35 (d, 2 H, *J* = 8.1 Hz), 7.83 (d, 2 H, *J* = 8.1 Hz); EIms, *m/z* (relative intensity) 328 (35), 179 (100); HRms calcd for $C_{16}H_{16}N_4O_2S$ 328.0994, found 328.0993.

Methyl 4-{2-[2-Amino-3-carbomethoxythiophen-4-yl]ethyl}benzoate (19). A solution of NaSH (1.50 g, 26.7 mmol) in MeOH (100 ml) was prepared and cooled to -10 °C. To this, a solution of the α-chloroketone (**12**, Alk = Me) (2.40 g, 10 mmol) in MeOH (40 ml) was added, and the resulting reaction mixture was stirred at -10 °C for 2 h. A mixture of methyl cyanoacetate (1.01 g, 10 mmol) and Et_3N (5 ml) in MeOH (10 ml) was added and stirring was continued for an additional 3 h at -5 - 5 °C. The reaction mixture was poured onto water (400 ml) and extracted with ether (3 x 500 ml). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo. The crude product was chromatographed on silica gel eluting with 17% EtOAc/hexanes. Fractions containing the product were combined and evaporated to give **19** (2.08 g, 65%) as a white solid. A small sample of **19** was recrystallized from EtOAc-hexanes: mp 148-150 °C; ^{1}H nmr ($CDCl_3$) δ 2.88-3.02 (m, 4 H), 3.85 (s, 3 H), 3.90 (s, 3 H), 5.79 (s, 1 H), 6.10 (br s, 2 H, exchangeable with D_2O), 7.24 (d, 2 H, *J* = 8.1 Hz), 7.95 (d, 2 H, *J* = 8.1 Hz); HRms calcd for $C_{16}H_{17}NO_4S$ 319.0878, found 319.0884. Anal. Calcd for $C_{16}H_{17}NO_4S$: C, 60.18; H, 5.37; N, 4.39; S, 10.04. Found: C, 60.39; H, 5.51; N, 4.39; S, 10.01.

Methyl 4-[2-(2-Amino-3-cyanothiophen-4-yl)ethyl]benzoate (20). A solution of NaSH (1.50 g, 26.7 mmol) in MeOH (100 ml) was prepared and cooled to -10 °C. To this, a solution of the α-chloroketone (**12**, Alk = Me) (2.4 g, 10 mmol) in MeOH (30 ml) was added and the resulting reaction mixture was stirred at -10 °C for 2.5 h. A mixture of malononitrile (0.66 g, 10 mmol) and Et_3N (1.01 g, 10 mmol) in MeOH (20 ml) was added and stirring was continued for an additional 2 h at -5 - 0 °C. The reaction mixture was poured onto water (100 ml) and extracted with ether (4 x 300 ml). The combined extracts were dried ($MgSO_4$) and concentrated in vacuo. The crude product was chromatographed on silica gel eluting with 35% EtOAc/hexanes. Fractions containing the product were combined and evaporated to give **20** (2.42 g, 85%) as a light yellow solid. A small sample of **20** was recrystallized from CH_2Cl_2: mp 138-140 °C; ^1H nmr ($CDCl_3$) δ 2.84 (t, 2 H, *J* = 7.5 Hz), 2.96 (t, 2 H, *J* =7.5 Hz), 3.90 (s, 3 H), 4.60-5.00 (br s, 2 H), 5.88 (s, 1 H), 7.24 (d, 2 H, *J* = 8.1 Hz), 7.95 (d, 2 H, *J* = 8.1 Hz); EIms *m/z* (relative intensity) 286 (M^+, 13), 254 (29), 228 (48), 197 (37); HRms calcd for $C_{15}H_{14}N_2O_2S$ 286.0776, found 286.0766. Anal. Calcd for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78; S, 11.20. Found: C, 62.70; H, 5.01; N, 9.79; S, 11.31.

Methyl 4-[2-(2-Methyl-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoate (21). A stream of dry HCl was bubbled through a suspension of **19** (850 mg, 2.67 mmol) in MeCN (50 ml) for 8 h. The reaction mixture was stirred at 25 °C for an additional 72 h. The excess MeCN was removed in vacuo and the solid was dissolved in water (20 ml). The pH of the solution was adjusted to 9 with conc. NH_4OH and the precipitated solid was filtered, washed with water and dried. The crude product was purified by silica gel chromatography eluting with 60% EtOAc/hexanes. Fractions containing the product were combined and evaporated to give **21** (350 mg, 40%) as a light brown solid. A small sample of **21** was recrystallized from MeOH-MeCN: mp 250-252 °C; ^1H nmr (DMSO-*d6*) δ 2.31 (s, 3 H), 2.95 (t, 2 H, *J* = 7.3 Hz), 3.14 (t, 2 H, *J* = 7.3 Hz), 3.79 (s, 3 H), 6.98 (s, 1 H), 7.34 (d, 2 H, *J* = 8.1 Hz), 7.84 (d, 2 H, *J* = 8.1 Hz), 12.30 (s, 1 H, exchangeable with D_2O); EIms *m/z* (relative intensity) 328 (M^+, 100), 296 (30), 268 (24); HRms calcd for $C_{17}H_{16}N_2O_3S$ 328.0881, found 328.0876. Anal. Calcd for $C_{17}H_{16}N_2O_3S$: C, 62.18; H, 4.91; N, 8.53; S, 9.76. Found: C, 62.47; H, 5.01; N, 8.30; S, 9.88.

Methyl 4-[2-(4-Amino-2-methylthieno[2,3-d]pyrimidin-5-yl)ethyl]benzoate (22). Compound (**20**) (286 mg, 1 mmol) was reacted with MeCN (15 ml) in the presence of dry HCl as described above (but for 4 h; silica gel chromatography eluting with 60% EtOAc/hexanes) to give **22** (280 mg, 86%) as a light yellow solid. A small sample of **22** was recrystallized from MeOH-MeCN: mp 216-218 °C; ^{1}H nmr (DMSO-*d6*) δ 2.36 (s, 3 H), 2.98 (t, 2 H, *J* = 7.6 Hz), 3.21 (t, 2 H, *J* = 7.6 Hz), 3.79 (s, 3.H), 6.92 (br, s, 2 H, exchangeable with D_2O), 7.34 (d, 2 H, *J* = 8.2 Hz), 7.83 (d, 2 H, *J* = 8.2 Hz); EIms *m/z* (relative intensity) 327 (M^+, 31), 178 (100); HRms calcd for $C_{17}H_{17}N_3O_2S$ 327.1041, found 327.1040. Anal. Calcd for $C_{17}H_{17}N_3O_2S$: C, 62.37; H, 5.23; N, 12.83; S, 9.79. Found: C, 62.39; H, 5.33; N, 13.09; S, 9.96.

4-[2-(2-Amino-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoic Acid (23). To a suspension of compound (**17**) (330 mg, 1 mmol) in MeOH (5 ml) was added 1 *N* NaOH (4 ml), and the mixture was allowed to stir at room temperature for 12 h. It was then acidified with $CH_3CO_2H_{(gl)}$ and the solid was filtered, washed with water and dried to give **23** (300 mg, 95%) as a light yellow solid: mp > 320 °C; ^{1}H nmr (DMSO-*d6*) δ 2.92-3.08 (m, 4 H), 6.48 (br s, 2 H), 6.51 (s, 1 H), 7.30 (d, 2 H, *J* = 8.0 Hz), 7.81 (d, 2 H, *J* = 8.0 Hz), 10.81 (s, 1 H), 12.75 (br s, 1 H); HRms calcd for $C_{15}H_{13}N_3O_3S$ 315.0677, found 315.0665.

4-[2-(2,4-Diaminothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoic Acid (24). Compound (**18**) (360 mg, 1 mmol) was saponified in 80% MeOH (25 ml) and 1 *N* NaOH (3 ml) as described above (but at reflux for 4 h) to give **24** (340 mg, 99%) as a white solid: mp > 300 °C; ^{1}H nmr (DMSO-*d6*) δ 2.95 (t, 2 H, *J* = 6.6 Hz), 3.11 (t, 2 H, *J* = 6.6 Hz), 6.01 (s, 2 H, exchangeable with D_2O), 6.43 (s, 2 H, exchangeable with D_2O), 6.47 (s, 1 H), 7.33 (d, 2 H, *J* = 8.0 Hz), 7.81 (d, 2 H, *J* = 8.0 Hz); FABHRms calcd for $C_{15}H_{15}N_4O_2S$ (MH^+) *m/z* 315.0916, found 315.0907.

4-[2-(2-Methyl-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoic Acid (25). Compound (**21**) (340 mg, 1.04 mmol) was saponified in 1 *N* NaOH (4 ml) and MeOH (20 ml) as described above (but at reflux for 90 h) to give **25** (207 mg, 66%) as a white solid: mp 280-283 °C (decomp.); ^{1}H nmr (DMSO-*d6*) δ 2.31 (s, 3 H), 2.93 (t, 2 H, *J* = 7.5 Hz), 3.12 (t, 2 H, *J* = 7.5 Hz), 6.98 (s, 1 H), 7.30 (d, 2 H, *J* = 8.1 Hz),

7.80 (d, 2 H, J = 8.1 Hz), 12.30 (s, 1 H, exchangeable with D_2O); EIms *m/z* (relative intensity) 314 (M^+, 38), 179 (100), 138 (26); HRms calcd for $C_{16}H_{14}N_2O_3S$ 314. 0725, found 314.0727. Anal. Calcd for $C_{16}H_{14}N_2O_3S \cdot 0.5\ H_2O$: C, 59.43; H, 4.68; N, 8.66; S, 9.92. Found: C, 59.76; H, 4.38; N, 8.02; S, 9.92.

4-[2-(4-Amino-2-methylthieno[2,3-d]pyrimidin-5-yl)ethyl]benzoic Acid (26). Compound **(22)** (654 mg, 2 mmol) was saponified in 1 *N* NaOH (4 ml) and MeOH (10 ml) as described above (but at reflux for 3 h) to give **26** (560 mg, 89%) as a white solid: mp > 300 °C (decomp.); ^{1}H nmr (DMSO-*d6*, 300 Hz) δ 2.36 (s, 3 H), 2.96 (t, 2 H, J = 7.5 Hz), 3.21 (t, 2 H, J = 7.5 Hz), 3.79 (s, 3.H), 6.88 (s, 2 H, exchangeable with D_2O), 6.93 (s, 1 H), 7.29 (d, 2 H, J = 7.8 Hz), 7.80 (d, 2 H, J = 7.8 Hz); EIms *m/z* (relative intensity) 313 (80, M^+), 178 (100), 137 (40); HRms calcd for $C_{16}H_{15}N_3O_2S$ 313.0885, found 313.0892. Anal. Calcd for $C_{16}H_{15}N_3O_2S \cdot 0.5\ H_2O$: C, 59.61; H, 5.00; N, 13,03. Found: C, 59.31; H, 4.69; N, 12.87.

Dimethyl *N*-{4-[2-(2-Amino-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (27). To a suspension of **23** (236 mg, 0.75 mmol) in DMF (15 ml) at 25 °C was added *N*-methylmorpholine (NMM, 0.098 ml, 0.89 mmol) followed by 6-chloro-2,4-dimethoxy-1,3,5-triazine (168 mg, 0.90 mmol) and the resulting solution was stirred at 25 °C for 1 h. NMM (0.125 ml, 1.14 mmol) was added to the solution followed by dimethyl L-glutamate hydrochloride (191 mg, 0.90 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo (0.5 mm Hg) and the residue was taken up in CH_2Cl_2 (50 ml). The CH_2Cl_2 layer was washed with 5% $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel eluting with 10% MeOH/CH_2Cl_2. Fractions containing the product were combined and evaporated to give a gummy residue which was triturated with ether-hexanes. The solid product was filtered and dried to give **27** (250 mg, 72%) as a white solid: mp 212-214 °C; ^{1}H nmr (DMSO-*d6*) δ 1.95-2.12 (m, 2 H), 2.42 (t, 2 H, J = 6.4 Hz), 2.88-3.04 (m, 4 H), 3.54 (s, 3 H), 3.60 (s, 3 H), 4.38-4.45 (m, 1 H), 6.48 (br s, 2 H), 6.51 (s, 1 H), 7.30 (d, 2 H, J =7.0 Hz), 7.77 (d, 2 H, J = 7.0 Hz), 8.64 (d, 1 H, J = 7.4 Hz), 10.80 (s, 1 H).

Dimethyl *N*-{4-[2-(2,4-Diaminothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (28). The acid (**24**) (236 mg, 0.75 mmol) was coupled with dimethyl L-glutamate (169 mg, 0.8 mmol) as described above (but for 3 h; silica gel chromatography, eluting with 70% EtOAc/hexanes) to give **28** (280 mg, 78%) as a white solid: mp 130-132 °C; ^{1}H nmr (DMSO-*d6*) δ 2.02-2.17 (m, 2 H), 2.37 (t, 2 H, *J* = 7.6 Hz), 2.98-3.11 (m, 4 H), 3.54 (s, 3 H), 3.63 (s, 3 H), 4.55 (m, 1 H), 5.15 (s, 2 H), 5.77 (s, 2 H), 6.27 (s, 1 H), 7.14 (d, 2 H, *J* =7.9 Hz), 7.72 (d, 2 H, *J* = 7.9 Hz), 8.07 (d, 1 H, *J* = 7.6 Hz); EIms (relative intensity) *m/z* 471 (30, 439 (10), 297 (13), 268 (24), 179 (100); HRms calcd for $C_{22}H_{25}N_5O_5S$ 471.1576; found, 471.1581.

Dimethyl *N*-{4-[2-(2-Methyl-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (29). The acid (**25**) (157 mg, 0.5 mmol) was coupled with dimethyl L-glutamate (120 mg, 0.55 mmol) as described above (but for 12 h; silica gel chromatography, eluting with 2% MeOH/CH_2Cl_2) to give **29** (190 mg, 81%) as a white solid: mp 174-176 °C; ^{1}H nmr ($CDCl_3$) δ 2.11-2.47 (m, 4 H), 2.55 (s, 3 H), 3.03 (t, 2 H, *J* = 7.5 Hz), 3.25 (t, 2 H, *J* = 7.5 Hz), 3.64 (s, 3 H), 3.77 (s, 3 H), 4.81 (m, 1 H), 6.69 (s, 1 H), 7.17 (d, 1 H, *J* = 7.4 Hz), 7.26 (d, 2 H, *J* = 7.8 Hz), 7.73 (d, 2 H, *J* = 7.8 Hz), 12.56 (s, 1 H); EIms *m/z* (relative intensity) 471 (M^+, 16), 439 (17), 311 (8), 297 (58), 268 (100), 179 (64); HRms calcd for $C_{23}H_{25}N_3O_6S$ 471.1464, found 471.1473.

Dimethyl *N*-{4-[2-(4-Amino-2-methylthieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (30). The acid (**26**) (313 mg, 1 mmol) was coupled with dimethyl L-glutamate (241 mg, 1.14 mmol) as described above (but for 4 h; silica gel chromatography eluting with 3% MeOH/CH_2Cl_2) to give **30** (360 mg, 77%) as a white solid: mp 124-126 °C; ^{1}H nmr ($CDCl_3$) δ 2.07-2.50 (m, 4 H), 2.52 (s, 3 H), 3.06-3.13 (AA'BB', 4 H), 3.62 (s, 3 H), 3.74 (s, 3 H), 4.76 (m, 1 H), 5.72 (s, 2 H), 6.71 (s, 1 H), 7.16 (d, 2 H, *J* = 8.1 Hz), 7.21 (d, 1 H, *J* = 7.8 Hz), 7.70 (d, 2 H, *J* = 8.1 Hz); EIms *m/z* (relative intensity) 470 (M^+, 12), 438 (22), 296 (20), 267 (38), 178 (100); HRms calcd for $C_{23}H_{26}N_4O_5S$ 470.1624, found 470.1621. Anal. Calcd for $C_{23}H_{26}N_4O_5S$: C, 58.71; H, 5.57; N, 11.91; S, 6.81. Found: C, 58.93; H, 5.51; N, 11.88; S, 6.64.

***N*-{4-[2-(2-Amino-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (8).** A solution of **27** (100 mg, 0.21 mmol) in 1 *N* NaOH (1 ml) was allowed to stir at room temperature for 24 h. The reaction mixture was acidified with gl. AcOH and the solid product was filtered, washed with water, and dried to give **8** (85 mg, 90%) as a white solid: mp 292-295 °C (decomp.); ^{1}H nmr (DMSO-*d6*) δ 1.91-2.07 (m, 2 H), 2.31 (t, 2 H, *J* = 7.2 Hz), 2.90-3.03 (m, 4 H), 4.35 (m, 1 H), 6.49 (br s, 2 H), 6.51 (s, 1 H), 7.27 (d, 2 H, *J* = 8.0 Hz), 7.76 (d, 2 H, *J* = 8.0 Hz), 8.47 (d, 1 H, *J* = 7.5 Hz), 10.82 (s, 1 H), 12.43 (br s, 2 H). Anal. Calcd for $C_{20}H_{20}N_4O_6S$: C, 54.05; H, 4.54; N, 12.61; S, 7.21. Found: C, 54.24; H, 4.51; N, 12.40; S, 7.31.

***N*-{4-[2-(2,4-Diaminothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (9).** Compound (**28**) (157 mg, 0.33 mmol) was saponified in 0.5 *N* NaOH (2 ml) as described above to give **9** (119 mg, 81%) as a white solid: mp 208-210 °C (decomp.); ^{1}H nmr (DMSO-*d6*) δ 1.87-2.10 (m, 2 H), 2.31 (t, 2 H, *J* = 7.0 Hz), 2.95 (t, 2 H, *J* = 6.8 Hz), 3.11 (t, 2 H, *J* = 6.8 Hz), 4.36 (m, 1 H), 5.99 (s, 2 H), 6.42 (s, 2 H), 6.47 (s, 1 H), 7.30 (d, 2 H, *J* = 7.8 Hz), 7.78 (d, 2 H, *J* = 7.8 Hz), 8.48 (d, 1 H, *J* = 7.4 Hz), 11.50-13.50 (br s, 2 H); FABHRms calcd for $C_{20}H_{22}N_5O_5S$ (MH^+) 444.1342, found: 444.1331. Anal. Calcd. for $C_{20}H_{21}N_5O_5S$: C, 54.17; H, 4.77; N, 15.79; S, 7.23. Found: C, 54.01; H, 4.59; N, 15.53; S, 7.12.

***N*-{4-[2-(2-Methyl-4(3H)-oxothieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (10).** Compound (**29**) (100 mg, 0.21 mmol) was saponified in 0.5 *N* NaOH (1.5 ml) as described above to give **10** (72 mg, 77%) as a white solid: mp 220-222 °C; ^{1}H nmr (DMSO-*d6*) δ 1.86-2.10 (m, 2 H), 2.25-2.37 (m 2 H), 2.31 (s, 3 H), 2.93 (t, 2 H, *J* = 7.5 Hz), 3.12 (t, 2 H, *J* = 7.5 Hz), 4.33 (m, 1 H), 6.97 (s, 1 H), 7.27 (d, 2 H, *J* = 8.0 Hz), 7.76 (d, 2 H, *J* = 8.0 Hz), 8.47 (d, 1 H, *J* = 7.5 Hz, exchangeable with D_2O), 12.30 (s, 1 H, exchangeable with D_2O); FABHRms calcd for $C_{21}H_{22}N_3O_6S$ (MH^+) 444.1229, found 444.1232. Anal. Calcd for $C_{21}H_{21}N_3O_6S \cdot H_2O$: C, 54.66; H, 5.02; N, 9.11; S, 6.95. Found: C, 54.39; H, 4.93; N, 9.04; S, 6.95.

***N*-{4-[2-(4-Amino-2-methylthieno[2,3-d]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic Acid (11).** Compound (**30**) (235 mg, 0.5 mmol) was saponified in 1 *N* NaOH (1.5 ml) as described above to give **11** (146 mg, 66%) as a white solid: mp 150-152 °C; ^{1}H nmr (*DMSO-d6*) δ 1.89-2.05 (m, 2 H), 2.31 (t, 2 H, *J* = 7.4 Hz), 2.36 (s, 3 H), 2.96 (t, 2 H, *J* = 7.3 Hz), 3.23 (t, 2 H, *J* = 7.3 Hz), 4.31-4.37 (m, 1 H), 6.92 (s, 3 H, 2.H exchangeable with D_2O), 7.33 (d, 2 H, *J* = 8.1 Hz), 7.76 (d, 2 H, *J* = 8.1 Hz), 8.49 (d, 1 H, *J* = 7.6 Hz, exchangeable with D_2O),12.10-12.80 (br s, 2 H, exchangeable with D_2O); FABHRms calcd for $C_{21}H_{23}N_4O_5S$ (MH^+): 443.1389; found: 443.1391. Anal. Calcd for $C_{21}H_{22}N_4O_5S$: C, 57.00; H, 5.01; N, 12.67; S, 7.23. Found: C, 56.88; H, 4.89; N, 12.44; S, 7.01.

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REFERENCES

1. (a) P. V. Danenberg, *Biochim. Biophys. Acta*, 1977, **473**, 73. (b) K. T. Douglas, *Med. Res. Rev.*, 1987, **7**, 441.
2. T. R. Jones, A. H. Calvert, A. L. Jackman, S. J. Brown, M. Jones, and K. R. Harrap, *Eur. J. Cancer*, 1981, **17**, 11.
3. For leading references, see (a) G. M. F. Bisset, K. Pawelczak, A. L. Jackman, A. H. Calvert, and L. R. Hughes, *J. Med. Chem.*, 1992, **35**, 859. (b) E. M. Berman and L. M. Werbel, *J. Med. Chem.*, 1991, **34**, 479. (c) D. R. Newell, Z. H. Siddik, A. H. Calvert, A. L. Jackman, D. L. Alison, K. G. McGhee, and K. R. Harrap, *Proc. Am. Assoc. Cancer Res.*, 1982, **23**, 181. (d) D. R. Newell, D. L. Alison, A. H. Calvert, K. R. Harrap, M. Jarman, T. R. Jones, M. Manteuffel-Cymborowska, and B. O'Connor, *Cancer Treat. Rep.*, 1986, **70**, 971. (e) D. L. Alison, D. R. Newell, C. Sessa, S. J. Harland, L. I. I. Hart, K. R. Harrap, and A. H. Calvert, *Cancer Chemother. Pharmacol.*, 1985, **14**, 265.
4. A. L. Jackman, G. A. Taylor, B. M. O'Connor, J. A. Bishop, R. G. Moran, and A. H. Calvert, *Cancer Res.*, 1990, **50**, 5212.

5. A. L. Jackman, D. R. Newell, G. A. Taylor, B. O'Connor, L. R. Hughes, and A. H. Calvert, *Proc. Am. Assoc. Cancer Res.*, 1987, **28**, 271.

6. L. R. Hughes, P. R. Marsham, J. Oldfield, T. R. Jones, B. M. O'Connor, J. A. M. Bishop, A. H. Calvert, and A. L. Jackman, *Proc. Am. Assoc. Cancer Res.*, 1988, **29**, 286.

7. T. R. Jones, J. T. Thornton, A. Flinn, A. L. Jackman, D. R. Newell, and A. H. Calvert, *J. Med. Chem.*, 1989, **32**, 847.

8. L. R. Hughes, A. L. Jackman, J. Oldfield, R. C. Smith, K. D. Burrows, P. R. Marsham, J. A. M. Bishop, T. R. Jones, B. M. O'Connor, and A. H. Calvert, *J. Med. Chem.*, 1990, **33**, 3060.

9. P. R. Marsham, L. R. Hughes, A. L. Jackman, A. J. Hayter, J. Oldfield, J. M. Wardleworth, J. A. M. Bishop, B. M. O'Connor, and A. H. Calvert, *J. Med. Chem.*, 1991, **34**, 1594.

10. T. C. Stephens, J. A. Calvete, D. Janes, S. E. Waterman, B. E. Valcaccia, L. R. Hughes, and A. H. Calvert, *Proc. Am. Assoc. Cancer Res.*, 1990, **31**, 342.

11. D. I. Jodrell, D. R. Newell, J. A. Calvete, T. C. Stephens, and A. H. Calvert, *Proc. Am. Assoc. Cancer Res.*, 1990, **31**, 341.

12. A. L. Jackman, G. A. Taylor, W. Gibson, R. Kimbell, M. Brown, A. H. Calvert, I. R. Judson, and L. R. Hughes, *Cancer Res.*, 1991, **51**, 5579.

13. S. E. Webber, T. M. Bleckman, J. Attard, J. G. Deal, V. Kathardekar, K. M. Welsh, S. Webber, C. A. Janson, D. A. Matthews, W. W. Smith, S. T. Freer, S. R. Jordan, R. J. Bacquet, E. F. Howland, C. L. J. Booth, R. W. Ward, S. M. Hermann, J. White, C. A. Morse, J. A. Hilliard, and C. A. Bartlett, *J. Med. Chem.*, 1993, **36**, 733.

14. (a) W. Pendergast, S. Dickerson, I. Dev, R. Ferone, D. Duch, and G. Smith, *Proc. Am. Assoc. Cancer Res.*, 1992, **33**, A47. (b) D. S. Duch, S. Banks, I. K. Dev, S. H. Dickerson, R. Ferone, L. S. Heath, J. Humphreys, V. Knick, W. Pendergast, S. Singer, G. K. Smith, K. Waters, and H. R. Wilson, *Cancer Res.*, 1993, **53**, 810.

15. E. C. Taylor, D. Kuhnt, C. Shih, S. M. Rinzel, G. B. Grindey, J. Barredo, M. Jannatipour, and R. G. Moran, *J. Med. Chem.*, 1992, **35**, 4450.

16. E. C. Taylor and H. H. Patel, *Tetrahedron*, 1992, 48, 8089.

17. B. Roth, *J. Med. Chem.*, 1969, **12**, 227.

18. (a) J. I. DeGraw, P. Tsakotellis, R. L. Kisliuk, and Y. Gaumont, *J. Heterocycl. Chem.*, 1971, **8**, 105. (b) E. C. Taylor, W. B. Young, R. Chaudhari, and H. H. Patel, *Heterocycles*, 1993, **36**, 1897.

19. D. Isbecque, R. Promel, R. C. Quinaux, and R. H. Martin, *Helv. Chim. Acta*, 1959, **42**, 1347.

20. This general approach to the thieno[2,3-d]pyrimidine ring system, albeit primarily *via* utilization of o-aminonitriles generated by the Gewald process [K. Gewald, E. Schinke, and H. Bottcher, *Chem. Ber.*, 1966, **99**, 94], has been exploited previously; see, for example, (a) A. Rosowsky, M. Chaykovsky, K. K. N. Chen, M. Lin, and E. J. Modest, *J. Med. Chem.*, 1973, **16**, 185. (b) M. Chaykovsky, M. Lin, A. Rosowsky, and E. J. Modest, *J. Med. Chem.*, 1973, **16**, 188. (c) A. Rosowsky, K. K. N. Chen, and M. Lin, *J. Med. Chem.*, 1973, **16**, 191. (d) A. Rosowsky, C. E. Mota, J. E. Wright, J. H. Freisheim, J. J. Heusner, J. J. McCormack, and S. F. Queener, *J. Med. Chem.*, 1993, **36**, 3103. (e) E. F. Elslager, P. Jacob, and L. M. Werbel, *J. Hetercycl. Chem.* 1972, **9**, 775.

21. Mitsui Toatsu Chemicals, Inc. *Jpn. Kokai Tokkyo Koho JP* 59,204,186, 1984 (Chem. Abstr., 1985, **102**, 113281k).

22. K. G. Dave, C. J. Shishoo, M. G. Devani, R. Kalyanaram, S. Ananthan, G. V. Ullas, and V. S. Bhadti, *J. Heterocycl. Chem.* 1980, **17**, 1497.

23. Z. J. Kaminski, *Tetrahedron Lett.*, 1985, **26**, 2901.

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