Synthesis of Classical and Nonclassical 2-Amino-4-oxo-6-benzylthieno-[2,3-*d*]pyrimidines as Potential Thymidylate Synthase Inhibitors

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A series of seven nonclassical 2-amino-4-oxo-6-substituted thieno[2,3-*d*]pyrimidines **2-8** and one classical *N*-[4-(2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-ylmethyl)benzoyl]-L-glutamic acid **9** (Table I) were designed as the first in a series of 6-substituted 6-5 fused ring analogs as potential thymidylate synthase (TS) inhibitors and as antitumor agents. The target compounds were synthesized *via* a Heck coupling of appropriately substituted iodobenzenes and allyl alcohol followed by cyclization using cyanoacetate and sulfur powder to afford substituted thiophenes. The resulting thiophenes were then cyclocondensed with chloroformamidine hydrochloride to afford 2-amino-4-oxo-6-substituted thieno[2,3-*d*]pyrimidines **2-8** and **26**. Hydrolysis of **26** followed by coupling with diethyl L-glutamate afforded **28**. The classical analog **9** was obtained by hydrolysis of **28**. None of the target compounds inhibited human recombinant thymidylate synthase at 23 μM except **9** for which the IC_{50} value was 100 μM.

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Thymidylate synthase (TS) catalyzes the reductive methylation of deoxyuridylate (dUMP) to deoxythymidylate (dTMP) utilizing 5,10-methylenetetrahydrofolate (5,10-CH_2-FH_4) as the source of the methyl group as well as the reductant [1]. This represents the sole *de novo* source of dTMP and hence inhibition of TS, in the absence of salvage, leads to "thymineless cell death" [2,3], and is an attractive target for the development of antitumor agents.

TS inhibitors that are clinically used as antitumor agent include 5-fluorouracil (5-FU), which is a mechanism-based inhibitor. Since different types of cancer either do not respond to 5-FU or develop resistance to it, there is a pressing need to design novel analogs as potential TS inhibitors and as antitumor agents [4]. Several TS inhibitors have entered clinical trails as antitumor agents, notable among them are CB3717 (PDDF) [5], ZD1694 (Tomudex) [6] and LY231514 (Alimta) (Figure 1) [7]. Alimta has been recently approved for treatment of lung cancer [8]. These compounds are "classical" antifolates, *i.e.* they contain a benzoyl-L-glutamate side chain, which makes them substrates for the enzyme folylpolyglutamate synthetase (FPGS). Although polyglutamylation of certain classical antifolates appears necessary for cytotoxicity, it has also been implicated as a possible cause of toxicity to host cells and in the development of resistance. In addition, classical antifolates require folate uptake systems to gain access to cells. Thus, lipophilic, nonclassical TS inhibitors that lack the benzoyl glutamate are not substrates for FPGS and do not require that folate uptake systems could be useful where resistance is due to decreased FPGS activity and/or inefficient uptake [9].

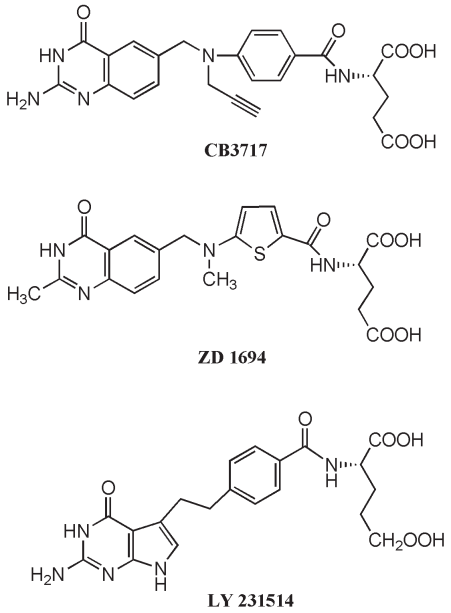


Figure 1

Nolatrexate dihydrochloride (Thymitaq^{TM}, AG337), a lipophilic TS inhibitor, was designed using X-ray crystallographic structures and molecular modeling, and is the first potent lipophilic TS inhibitor currently in clinical trials [9]. Molecular modeling of 6-5 ring-fused analogs such as pyrrolo[2,3-*d*]pyrimidines, furo[2,3-*d*]pyrimidines and thieno[2,3-*d*]pyrimidines superimposed on 6-6 ring-fused analogs such as AG337, indicate that the 5-substituents are closely positioned in both ring systems and the 6-substituent of the 6-5 system lies in between the 6- and 7-substituent of the 6-6 system (Figure 2) [10]. An example of potent TS inhibitors in the 6-6 system with a 5-substituent is AG337 [10], and an example of a 5-substituted 6-5 system is LY231514 [7]. The 6-6 fused systems that have been found to be potent inhibitors of TS are usually 6-substituted [4]. A recent report by Gangjee *et al.* [11] showed that a series of 5-substituted, nonclassical, 6-5 fused pyrrolo[2,3-*d*]pyrimidines **1** were inactive against TS and

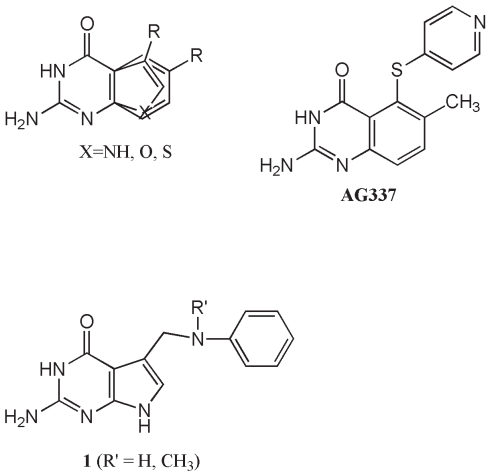
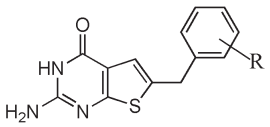


Figure 2

Table 1



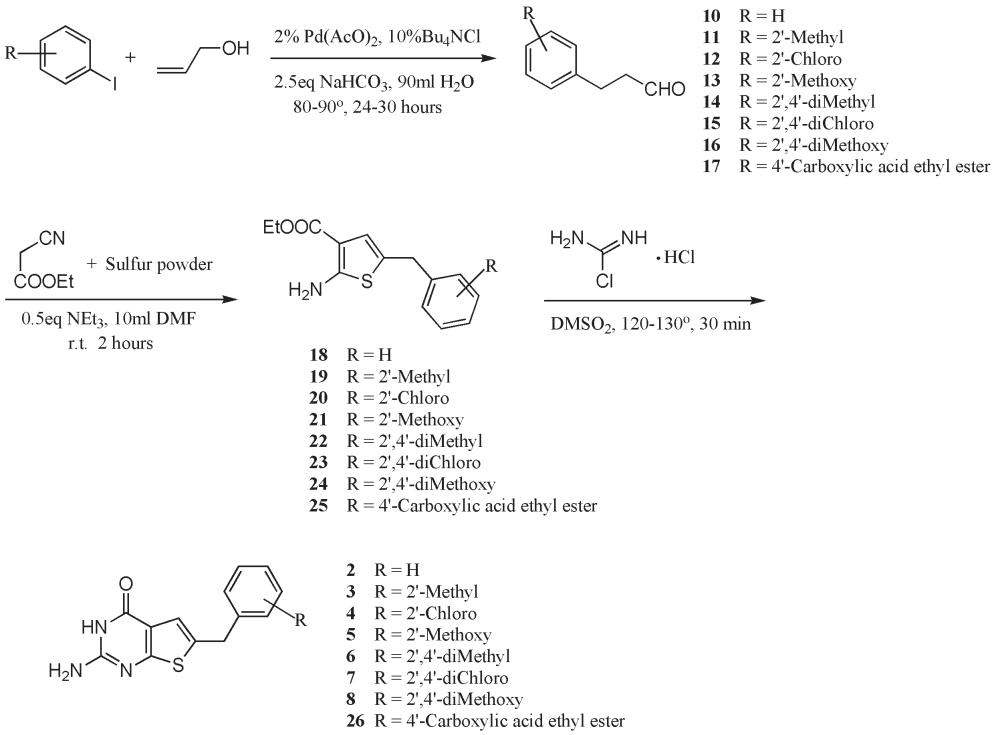
2 R = H
3 R = 2'-Methyl
4 R = 2'-Chloro
5 R = 2'-Methoxy
6 R = 2',4'-diMethyl
7 R = 2',4'-diChloro
8 R = 2',4'-diMethoxy
9 R = 4'-Carbonyl-L-glutamic acid

were also poor inhibitors of dihydrofolate reductase (DHFR) from rat liver and *Pneumocystis carinii*. It was thus of interest to synthesize 6-substituted thieno[2,3-*d*]-pyrimidines as potential inhibitors of TS. The sulfur atom of the thieno ring is larger than the nitrogen of a pyrrole and approximates a 6-6 ring system such as AG337. With these objectives in mind, we synthesized the nonclassical analogs **2-8** and the classical analog **9** (Table I).

The synthetic strategy of nonclassical analogs **2-8** and **26** is shown in Scheme I. The synthesis commenced from commercially available iodobenzenes and allyl alcohol. Heck arylation of allyl alcohol with appropriate iodobenzenes catalyzed by palladium (II) acetate in water for 24 to 30 hours at 80-90 °C afforded 3-phenylpropionaldehydes **10-17** in 70-85% yields [12]. The substituted thiophenes **18-25** were synthesized from the appropriate 3-phenylpropionaldehyde, cyanoacetate, triethylamine and sulfur powder in DMF, [13] in yields ranging from 30-40%. The thieno[2,3-*d*]pyrimidines **2-8** and **26** were obtained by chloroformamidine cyclization of the thiophenes **18-25** in dimethylsulfone in yields ranging from 50-60%.

The synthetic strategy for the classical analog **9** is shown in Scheme II. The synthesis of **9** started with compound **26**

Scheme I

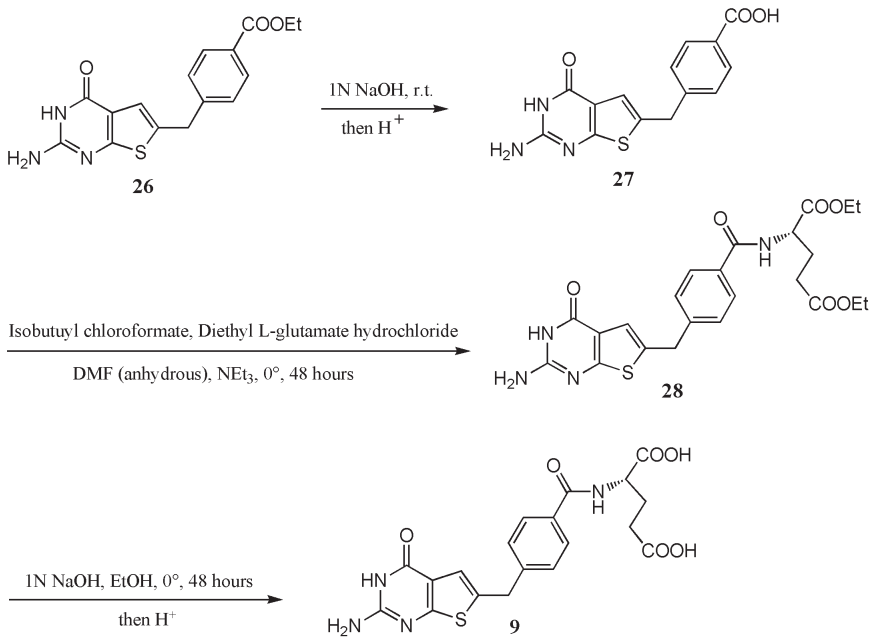


10 R = H
11 R = 2'-Methyl
12 R = 2'-Chloro
13 R = 2'-Methoxy
14 R = 2',4'-diMethyl
15 R = 2',4'-diChloro
16 R = 2',4'-diMethoxy
17 R = 4'-Carboxylic acid ethyl ester

18 R = H
19 R = 2'-Methyl
20 R = 2'-Chloro
21 R = 2'-Methoxy
22 R = 2',4'-diMethyl
23 R = 2',4'-diChloro
24 R = 2',4'-diMethoxy
25 R = 4'-Carboxylic acid ethyl ester

2 R = H
3 R = 2'-Methyl
4 R = 2'-Chloro
5 R = 2'-Methoxy
6 R = 2',4'-diMethyl
7 R = 2',4'-diChloro
8 R = 2',4'-diMethoxy
26 R = 4'-Carboxylic acid ethyl ester

Scheme II



which was obtained in Scheme I. Compound **26** was hydrolyzed with aqueous sodium hydroxide and then neutralized with aqueous hydrogen chloride to afford the acid **27**. Coupling of the acid **27** with diethyl L-glutamate using the mixed anhydride method [14] with isobutyl chloroformate and triethylamine, followed by a repeated cycle of activation (Scheme II), afforded, after chromatographic purification, the coupled product **28** in 51% yield. Hydrolysis of the diester **28** with aqueous sodium hydroxide at room temperature, followed by acidification gave the desired compound **9** in 93% yield.

Compounds **2**-**9** were evaluated as inhibitors of human recombinant thymidylate synthase. Accurate IC_{50} values could not be obtained for any of the target compounds except **9**. None of the target compounds inhibited human recombinant thymidylate synthase at 23 μM except **9** for which the IC_{50} value was 100 μM. This clearly indicated that 6-substituted one-carbon bridged classical and nonclassical 2-amino-4-oxo-6-benzylthieno[2,3-*d*]pyrimidine systems are not conducive to thymidylate synthase inhibitory activity.

EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator. Analytical samples were dried *in vacuo* (0.2 mmHg) in an Abderhalden drying apparatus over P_2O_5. Thin layer chromatography (TLC) was performed on silica gel plates with fluorescent indicator. Spots were visualized by UV light (254 and 365 nm). All analytical samples were homogeneous on TLC in at least two different solvent systems. Purification by column and flash chromatography was carried out using Merck silica gel 60 (200-400 mesh). The amount (weight) of silica gel for column chromatography was in the range of 50-100 times the amount (weight) of the crude compounds being separated. Columns were dry packed unless specified otherwise. Solvent systems are reported as volume percent mixture. Melting points were determined on a Mel-Temp II melting point apparatus with a digital thermometer and are uncorrected. ^{1}H nmr spectra were recorded on a Bruker WH-300 (300 MHz) nmr spectrometer. The chemical shift (δ) values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, bs = broad singlet, exch = protons exchangeable by addition of D_2O. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Elemental compositions were within 0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be removed despite 24 hours of drying *in vacuo* and were confirmed, where possible, by their presence in the ^{1}H nmr spectrum. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received except anhydrous solvents, which were freshly dried in the laboratory.

General Procedure for the Synthesis of Compounds **10**-**17**.

To a 250 ml round-bottomed flask, fitted with a magnetic stir bar and a reflux condenser, were placed palladium diacetate (134.7 mg, 0.6 mmol), the appropriate substituted iodobenzenes (30 mmol), allyl alcohol (5.23 g, 90 mmol), sodium bicarbonate (6.3 g, 75 mmol), tetrabutylammonium chloride water (0.84 g, 3 mmol), and water (90 ml). The mixture was stirred vigorously at 80 °C for 24-30 hours under nitrogen. The reaction mixture was cooled and ethyl acetate (50 ml) was added to afford a solution. The ethyl acetate solution was washed with water (4 x 30 ml), dried over anhydrous sodium sulfate, and concentrated under

reduced pressure. The residue was loaded on column packed with silica gel and eluted with 5% ethyl acetate in hexanes and fractions containing the desired product (tlc) were pooled and evaporated to afford the products.

3-(2'-Methylphenyl)propionaldehyde (**11**).

This compound was obtained as a yellow liquid (3.18 g, 71.5%); R_f = 0.75 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 2.37 (3H, s, 2'-CH_3), 2.51-2.62 (2H, q, CH_2CH_2CHO), 2.69-2.80 (2H, t, CH_2CH_2CHO), 7.01-7.19 (4H, m, C_6H_4), 9.49 (1H, s, CHO).

3-(2'-Chlorophenyl)propionaldehyde (**12**).

This compound was obtained as a yellow liquid (3.5 g, 69%); R_f = 0.77 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 2.58-2.63 (2H, q, CH_2CH_2CHO), 2.82-2.87 (2H, t, CH_2CH_2CHO), 6.83-7.40 (4H, m, C_6H_4), 9.48 (1H, s, CHO).

3-(2'-Methoxyphenyl)propionaldehyde (**13**).

This compound was obtained as a yellow liquid (4.1 g, 83%); R_f = 0.70 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 2.54-2.59 (2H, q, CH_2CH_2CHO), 2.66-2.71 (2H, t, CH_2CH_2CHO), 3.81 (3H, s, 2'-OCH_3), 7.06-7.20 (4H, m, C_6H_4), 9.42 (1H, s, CHO).

3-(2',4'-Dimethylphenyl)propionaldehyde (**14**).

This compound was obtained as a yellow liquid (3.7 g, 76%); R_f = 0.71 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ2.30 (6H, s, CH_3); 2.67 (2H, t, CH_2CH_2CHO); 2.88 (2H, t, CH_2CH_2CHO); 6.90-7.10 (3H, m, C_6H_4); 9.72 (1H, s, CHO).

3-(2',4'-Dichlorophenyl)propionaldehyde (**15**).

This compound was obtained as a yellow liquid (3.9 g, 64%); R_f = 0.74 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 2.54-2.61 (2H, q, CH_2CH_2CHO), 2.81-2.86 (2H, t, CH_2CH_2CHO), 6.77-7.48 (3H, m, C_6H_4), 9.47 (1H, s, CHO).

3-(2',4'-Dimethoxyphenyl)propionaldehyde (**16**).

This compound was obtained as a yellow liquid (4.8 g, 82%); R_f = 0.71(hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 2.57 (2H, q, CH_2CH_2CHO), 2.59 (2H, t, CH_2CH_2CHO), 3.69 (3H, s, 2'-OCH_3), 3.71 (3H, s, 4'-OCH_3), 6.30-6.98 (3H, m, C_6H_4), 9.38 (1H, s, CHO).

4-(3'-Oxo-propyl)benzoic Acid Ethyl Ester (**17**).

This compound was obtained as a yellow liquid (5.2 g, 84%); R_f = 0.78 (hexanes: ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.28-1.29 (3H, t, $COOCH_2CH_3$), 2.70-2.72 (2H, t, CH_2CH_2CHO), 2.80-2.825 (2H, t, CH_2CH_2CHO), 4.25-4.29 (2H, t, $COOCH_2CH_3$), 6.84-7.85 (4H, m, C_6H_4), 9.43 (1H, s, CHO).

General Procedure for the Synthesis of Compounds **18-25**.

Triethylamine (1.2 g, 0.01 mol) was added to a vigorously stirred mixture of ethylcyanoacetate (2.26 g, 0.02 mol), sulfur powder (0.64 g, 0.02 mol) and DMF (10 ml). The appropriately substituted phenylpropionaldehyde (0.02 mol) was added dropwise to this suspension, while maintaining the temperature at 50 °C. When the addition was complete, the reaction mixture was allowed to cool to room temperature and then stirred for 2 hours. To the mixture was added water and then ether was added to afford a solution. The ether solution was washed with water (4 x 30 ml), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford yellow oil. The residue was loaded on a column packed with silica gel and eluted with 5% ethyl acetate in hexanes and fractions containing the desired product (tlc) were pooled and evaporated to afford the products.

2-Amino-5-benzylthiophene-3-carboxylic Acid Ethyl Ester (**18**).

This compound was obtained as a red oil (1.6 g, 31%); R_f = 0.6 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.20-1.25 (3H, t, $COOCH_2CH_3$), 3.86 (2H, s, C_6H_5-CH_2), 4.10-4.17 (2H, q, $COOCH_2CH_3$), 6.56 (1H, s, 4-H), 7.13 (2H, s, NH_2, exch), 7.20-7.32 (5H, m, C_6H_5).

2-Amino-5-(2'-methylbenzyl)thiophene-3-carboxylic Acid Ethyl Ester (**19**).

This compound was obtained as a red oil (1.7 g, 31%); R_f = 0.6 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.30-1.34 (3H, t, $COOCH_2CH_3$), 2.33 (3H, s, C_6H_4-CH_3), 3.97 (2H, s, C_6H_4-CH_2), 4.28-4.35 (2H, q, $COOCH_2CH_3$), 5.85 (2H, s, NH_2, exch), 6.69 (1H, s, 4-H), 7.11-7.24 (4H, m, C_6H_4).

2-Amino-5-(2'-chlorobenzyl)thiophene-3-carboxylic Acid Ethyl Ester (**20**).

This compound was obtained as a red oil (3.6 g, 49%) under 1.25 equivalent of the general procedure; R_f = 0.65 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.19-1.24 (3H, t, $COOCH_2CH_3$), 3.96 (2H, s, C_6H_4-CH_2), 4.12-4.17 (2H, q, $COOCH_2CH_3$), 6.54 (1H, s, 4-H), 7.14 (2H, s, NH_2, exch), 7.27-7.45 (4H, m, C_6H_4).

2-Amino-5-(2'-methoxybenzyl)thiophene-3-carboxylic acid ethyl ester (**21**).

This compound was obtained as a red oil (3 g, 41%) under 1.25 equivalent of the general procedure; R_f = 0.55 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.20-1.24 (3H, t, $COOCH_2CH_3$), 3.79 (3H, s, C_6H_4-OCH_3), 4.04 (2H, s, C_6H_4-CH_2), 4.12-4.17 (2H, q, $COOCH_2CH_3$), 6.52 (1H, s, 4-H), 6.85-7.24 (4H, m, C_6H_4), 7.08 (2H, s, NH_2, exch).

2-Amino-5-(2',4'-dimethylbenzyl)thiophene-3-carboxylic Acid Ethyl ester (**22**).

This compound was obtained as a red oil (2.3 g, 40%); R_f = 0.6 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.19-1.23 (3H, t, $COOCH_2CH_3$), 2.19 (3H, s, C_6H_3-CH_3), 2.24 (3H, s, C_6H_3-CH_3), 3.79 (2H, s, C_6H_3-CH_2), 4.11-4.14 (2H, q, $COOCH_2CH_3$), 6.41 (1H, s, 4-H), 6.96-7.05 (3H, m, C_6H_3), 7.11 (2H, s, NH_2, exch).

2-Amino-5-(2',4'-dichlorobenzyl)thiophene-3-carboxylic acid ethyl ester (**23**).

This compound was obtained as a red oil (2.3 g, 35%); R_f = 0.6 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.18-1.23 (3H, t, $COOCH_2CH_3$), 3.995 (2H, s, C_6H_3-CH_2), 4.11-4.18 (2H, q, $COOCH_2CH_3$), 6.55 (1H, s, 4-H), 7.165 (2H, s, NH_2, exch), 7.33-7.59 (3H, m, C_6H_3).

2-Amino-5-(2',4'-dimethoxybenzyl)thiophene-3-carboxylic Acid Ethyl Ester (**24**).

This compound was obtained as a red oil (2.1 g, 33%); R_f = 0.64 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.19-1.23 (3H, t, $COOCH_2CH_3$), 3.70 (2H, s, C_6H_3-CH_2), 3.73 (3H, s, C_6H_3-OCH_3), 3.77 (3H, s, C_6H_3-OCH_3), 4.09-4.16 (2H, q,

COOCH_2CH$_3$), 6.43 (1H, s, 4-H), 6.46-6.53 (2H, d, 5', 6'-H), 7.03, 7.05 (1H, d, 3'-H), 7.05 (2H, s, NH_2, exch).

2-Amino-5-(4'-ethoxycarbonyl-benzyl)thiophene-3-carboxylic Acid Ethyl ester (**25**).

This compound was obtained as a red oil (2.2 g, 33%); R_f = 0.65 (hexanes:ethyl acetate = 3:1); ^{1}H nmr (DMSO-d_6): δ 1.19-1.23 (3H, t, COOCH$_2$CH_3), 1.30 (3H, t, COOCH$_2$CH_3), 4.00 (2H, s, C_6H_4-CH_2), 4.12-4.16 (2H, q, COOCH_2CH$_3$), 4.28-4.30 (2H, q, COOCH_2CH$_3$), 6.60 (1H, s, 4-H), 7.15 (2H, s, NH_2, exch), 7.36-7.95 (4H, m, C_6H_4).

General Procedure for the Synthesis of Compounds **2-8** and **26**.

A solution of hydrogen chloride in diethyl ether (100 ml, 0.1 mol) was cooled in an ice-bath. To this vigorously stirred solution was added a solution of cyanamide (1.8 g, 0.05 mol) in diethyl ether (34.5 ml) over a 15 minute period. The reaction mixture was then allowed to stir for 15 minutes. A white, solid precipitate of chloroformamidine hydrochloride that formed was collected by filtration and washed with diethyl ether. An intimate mixture of appropriate thiophenes and chloroformamidine hydrochloride (1:1.5) in dimethylsulfone (4 g) was heated at 120-130° C for 30 minutes. The mixture was cooled to room temperature, 10 ml of water was added and ammonium hydroxide was used to neutralize the suspension. The greenish brown solid obtained was collected by filtration, washed with water and dried. The crude product was dissolved in DMF and silica gel was added followed by evaporation of the DMF to afford a dry silica gel plug. The plug was then loaded onto a silica gel column and eluted with 3% methanol in chloroform. The fractions containing the desired product (tlc) were pooled and the solvent evaporated to afford the product.

2-Amino-4-oxo-6-benzylthieno[2,3-*d*]pyrimidine (**2**).

Compound **2** (0.54 g, 34%) was obtained from **18** (1.6 g, 6 mmol) and chloroformamidine hydrochloride (1.0 g, 9 mmol). R_f = 0.63, (chloroform: methanol = 7:1); mp 306-309 °C; ^{1}H nmr (DMSO-d_6): δ 4.03 (2H, s, C_6H_5-CH_2), 6.48 (2H, s, 2-NH_2, exch), 6.83 (1H, s, 5-H), 7.22-7.34 (5H, m, C_6H_5); 10.83 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33; S, 12.46. Found: C, 60.75; H, 4.38; N, 16.41; S, 12.56.

2-Amino-4-oxo-6-(2'-methylbenzyl)thieno[2,3-*d*]pyrimidine (**3**).

Compound **3** (1.6 g, 65%) was obtained from **19** (2.57 g, 9 mmol) and chloroformamidine hydrochloride (1.7 g, 14 mmol). R_f = 0.60, (chloroform:methanol = 7:1); mp 295-297 °C; ^{1}H nmr (DMSO-d_6): δ 2.25 (3H, s, C_6H_4-CH_3), 4.03 (2H, s, C_6H_4-CH_2), 6.47 (2H, s, 2-NH_2, exch), 6.69 (1H, s, 5-H), 7.165 (4H, m, C_6H_4); 10.82 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{14}H_{13}N_3OS \cdot 0.6H_2O$: C, 59.60; H, 5.07; N, 14.89; S, 11.36. Found: C, 59.35; H, 4.74; N, 14.68; S, 11.13.

2-Amino-4-oxo-6-(2'-chlorobenzyl)thieno[2,3-*d*]pyrimidine (**4**).

Compound **4** (0.85 g, 24%) was obtained from **20** (3.6 g, 12 mmol) and chloroformamidine hydrochloride (2.1 g, 18 mmol). R_f = 0.73, (chloroform:methanol = 7:1); mp 290-294 °C; ^{1}H nmr (DMSO-d_6): δ 4.15 (2H, s, C_6H_4-CH_2), 6.50 (2H, s, 2-NH_2, exch), 6.78 (1H, s, 5-H), 7.31-7.44 (4H, dd, C_6H_4); 10.85 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{13}H_{10}N_3OSCl$: C, 53.52; H, 3.45; N, 14.40; S, 10.99; Cl, 12.15. Found: C, 53.26; H, 3.54; N, 14.26; S, 10.87; Cl, 12.43.

2-Amino-4-oxo-6-(2'-methoxybenzyl)thieno[2,3-*d*]pyrimidine (**5**).

Compound **5** (1.7 g, 59%) was obtained from **21** (2.9 g, 10 mmol) and chloroformamidine hydrochloride (1.7 g, 15 mmol). R_f = 0.83, (chloroform:methanol = 7:1); mp 291-293 °C; ^{1}H nmr (DMSO-d_6): δ 3.79 (3H, s, C_6H_4-OCH_3), 3.95 (2H, s, C_6H_4-CH_2), 6.44 (2H, s, 2-NH_2, exch), 6.74 (1H, s, 5-H), 6.88-7.20 (4H, m, C_6H_4); 10.81 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{14}H_{13}N_3O_2S \cdot 0.2H_2O$: C, 57.80; H, 4.64; N, 14.44; S, 11.02. Found: C, 57.75; H, 4.58; N, 14.44; S, 11.07.

2-Amino-4-oxo-6-(2',4'-dimethylbenzyl)thieno[2,3-*d*]pyrimidine (**6**).

Compound **6** (1.4 g, 61%) was obtained from **22** (2.3 g, 8 mmol) and chloroformamidine hydrochloride (1.38 g, 12 mmol). R_f = 0.7, (chloroform:methanol = 7:1); mp 297-300 °C; ^{1}H nmr (DMSO-d_6): δ 2.19 (3H, s, C_6H_3-CH_3), 2.24 (3H, s, C_6H_3-CH_3), 3.97 (2H, s, C_6H_3-CH_2), 6.49 (2H, s, 2-NH_2, exch), 6.67 (1H, s, 5-H), 6.94-7.09 (3H, dd, C_6H_3); 10.84 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{15}H_{15}N_3OS \cdot 0.2H_2O$: C, 62.35; H, 5.37; N, 14.54; S, 11.10. Found: C, 62.26; H, 5.34; N, 14.60; S, 11.16.

2-Amino-4-oxo-6-(2',4'-dichlorobenzyl)thieno[2,3-*d*]pyrimidine (**7**).

Compound **7** (1.1 g, 56%) was obtained from **23** (2.0 g, 6 mmol) and chloroformamidine hydrochloride (1.0 g, 9 mmol). R_f = 0.7, (chloroform:methanol = 7:1); mp 298-301 °C; ^{1}H nmr (DMSO-d_6): δ 4.14 (2H, s, C_6H_3-CH_2), 6.52 (2H, s, 2-NH_2, exch), 6.80 (1H, s, 5-H), 7.43-7.63 (3H, m, C_6H_3); 10.87 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{13}H_9N_3OSCl_2$: C, 47.60; H, 2.83; N, 12.81; S, 9.78; Cl, 21.62. Found: C, 47.41; H, 2.99; N, 12.78; S, 9.63; Cl, 21.31.

2-Amino-4-oxo-6-(2',4'-dimethoxybenzyl)thieno[2,3-*d*]pyrimidine (**8**).

Compound **8** (1.2 g, 63%) was obtained from **24** (1.9 g, 6 mmol) and chloroformamidine hydrochloride (1.0 g, 9 mmol). R_f = 0.72, (chloroform:methanol = 7:1); mp 262-265° C; ^{1}H nmr (DMSO-d_6): δ 3.74 (3H, s, C_6H_3-OCH_3), 3.78 (3H, s, C_6H_3-OCH_3), 3.88 (2H, s, C_6H_3-CH_2), 6.43 (2H, s, 2-NH_2, exch), 6.48 (1H, s, 5-H), 6.56-7.10 (3H, m, C_6H_3); 10.79 (1H, s, 3-NH, exch).

Anal. Calcd. for $C_{15}H_{15}N_3O_3S$: C, 56.77; H, 4.76; N, 13.24; S, 10.10. Found: C, 56.62; H, 4.87; N, 13.26; S, 10.16.

4-(2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-ylmethyl)benzoic Acid Ethyl Ester (**26**).

Compound **26** (1.0 g, 51%) was obtained from **25** (2.0 g, 6 mmol) and chloroformamidine hydrochloride (1.0 g, 9 mmol). R_f = 0.6, (chloroform:methanol = 7:1); mp 193-198 °C; ^{1}H nmr (DMSO-d_6): δ 1.15-1.17 (3H, t, COOCH$_2$CH_3), 4.12 (2H, s, C_6H_4-CH_2), 4.25-4.32 (2H, q, COOCH_2CH$_3$), 6.51 (2H, s, 2-NH_2, exch), 6.88 (1H, s, 5-H), 7.24-7.91 (4H, dd, C_6H_4); 10.54 (1H, s, 3-NH, exch).

4-(2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-ylmethyl)benzoic Acid (**27**).

To a solution of **26** in ethanol (10 ml) was added aqueous 1 *N* NaOH (1ml) and the reaction mixture stirred at room temperature for 18 hours and carefully acidified to pH 4 with drop wise

addition of 3 *N* HCl. The resulting suspension was left at 0 °C for 2 hours at which time the residue was collected by filtration, washed with water (10 ml), acetone, and ethyl ether and dried over P_2O_5/vacuum at 78° C to afford the free acid **27** (0.8 g, 87%) as a light yellow solid. mp 296-300° C. ^{1}H nmr (DMSO-d_6): δ 4.11 (2H, s, C_6H_4-CH_2); 6.51 (2H, s, NH_2, exch); 6.88 (1H, s, 5-H); 7.36-7.90 (4H, m, C_6H_4); 10.79 (1H, s, NH, exch).

Diethyl 2-[4-(2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-ylmethyl)benzoyl]-L-glutamate (**28**).

To a suspension of the acid **27** (0.302 g, 1 mmol) in anhydrous DMF (10 ml) under nitrogen was added triethylamine (0.418 ml, 3 mmol) and the suspension heated to 80 °C to form a solution. The solution was cooled to 0 °C and isobutyl chloroformate (0.26 ml, 2 mmol) was added, followed, after 15 mintues, with diethyl L-glutamate hydrochloride (0.36 g, 1.5 mmol) and immediately followed by triethylamine (0.418 ml, 3 mmol). The reaction mixture was warmed slowly to room temperature and stirred for 12 hours. At this time the reaction mixture was cooled to 0 °C and the activation steps described above were repeated using triethylamine (0.21 ml, 1.5 mmol), followed by isobutyl chloroformate (0.13 ml, 1 mmol). After stirring for 15 mintues at 0 °C diethyl L-glutamate hydrochloride (0.18 g, 0.76 mmol) was added followed immediately by triethylamine (0.21 ml, 1.5 mmol). The reaction mixture was stirred for another 24 hours at room temperature and to this solution was added silica gel (1.2 g) and the suspension evaporated to dryness under reduced pressure. The silica gel plug obtained was loaded on a dry silica gel column which was rapidly eluted with chloroform and then stepwise with 1-5% methanol in chloroform. The fractions showing a single spot (tlc) were pooled and evaporated to afford compound **28** (0.25 g, 51.4%) as an off-white solid. R_f = 0.50 (chloroform: methanol = 7:1). mp 186-187.5 °C. ^{1}H nmr (DMSO-d_6): δ 1.03-1.21 (m, 6 H, $COOCH_2CH_3$); 1.95-2.12 (m, 2H, Gluβ-CH_2); 2.41 (t, 2H, Gluγ-CH_2, ***J*** = 7.2 Hz); 4.06 (m, 4 H, $COOCH_2CH_3$); 4.14 (s, 2H, C_6H_4-CH_2); 4.35-4.43 (m, 1 H, Gluα-CH), 6.49 (s, 2 H, 2-NH_2, exch); 6.88 (s, 1H, 5-H); 7.36,7.39-7.81,7.84 (dd, 4H, C_6H_4, J = 8.1 Hz), 8.67-8.69 (d, 1 H, CONH, J = 7.5 Hz, exch), 10.84 (s, 1 H, 3-NH, exch).

Anal. Calcd. For $C_{23}H_{26}N_4O_6S$: C, 56.78; H, 5.39; N, 11.52; S, 6.59. Found: C, 56.89; H, 5.40; N, 11.59; S, 6.55.

N-[4-(2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-ylmethyl)benzoyl]-L-glutamic Acid (**9**).

To a solution of **28** (0.25 g, 0.5 mmol) in ethanol (15 ml) was added 1 *N* NaOH (1 ml) and the solution stirred at room temperature for 24 hours. The ethanol was evaporated under reduced pressure, the residue was dissolved in water (15 ml), and the solution was stirred for a further 24 hours. The solution was then cooled in an ice-bath and carefully acidified to pH 4.0 with the drop wise addition of 3 *N* HCl. This suspension was left at 0-5 °C for 24 hours and then filtered. The residue was washed well with water and dried over P_2O_5/vacuum to afford **9** (0.21 g, 92.8%) as a white solid. mp 214-217 °C. ^{1}H nmr (DMSO-d_6): δ1.94-2.06 (m, 2H, Gluβ-CH_2); 2.32-2.49 (t, 2H, Gluγ-CH_2, *J* = 7.2 Hz); 4.11 (s, 2H, $C_6H_4CH_2$); 4.38 (m, 1 H, Gluα-CH), 6.50 (s, 2 H, 2-NH_2, exch); 6.87 (s, 1H, 5-H); 7.35,7.38-7.81,7.84 (dd, 4H, C_6H_4, J = 8.1 Hz), 8.568-8.593 (d, 1 H, CONH, J = 7.5 Hz, exch), 10.86 (s, 1 H, 3-NH, exch).

Anal. Calcd. For $C_{19}H_{18}N_4O_6S\cdot 0.2H_2O$: C, 52.58; H, 4.27; N, 12.91; S, 7.39. Found: C, 52.21; H, 4.47; N, 12.74; S, 7.33.

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