Synthesis of 2,4-Diaminothieno- and Pyrrolo[2,3-*d*]pyrimidine-6-carboxylic Acid Derivatives

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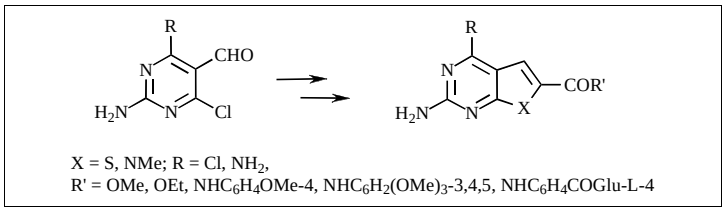

A series of new 2,4-diaminothieno[2,3-*d*]- and 2,4-diaminopyrrolo[2,3-*d*]pyrimidine derivatives were synthesised. Reaction of 2-amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) with ethyl mercaptoacetate, methyl *N*-methylglycinate or ethyl glycinate afforded ethyl (2-amino-4-chloro-5-formylpyrimidin-6-yl)thioacetate (**2a**), methyl *N*-(2-amino-4-chloro-5-formylpyrimidin-6-yl)-*N*-methylglycinate (**2b**) and ethyl *N*-(2-amino-4-chloro-5-formylpyrimidin-6-yl)glycinate (**2c**), respectively. Compounds **2a,b** by treatment with bases cyclised to the corresponding 2-amino-4-chlorothieno- and pyrrolo[2,3-*d*]pyrimidine-6-carboxylates (**3a,b**). Heating 2,4-diamino-6-chloropyrimidine-5-carbaldehyde (**5**) with ethyl mercaptoacetate or methyl *N*-methylglycinate gave 2,4-diaminothieno[2,3-*d*]- and 2,4-diaminopyrrolo[2,3-*d*]-pyrimidine-6-carboxylates (**6a,b**), whereas compound **5** with ethyl glycinate under the same reaction conditions afforded ethyl *N*-(2,4-diamino-5-formylpyrimidin-6-yl)glycinate (**7**). Treatment of 2,4-diaminothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**8a**) with 4-methoxy-, 3,4,5-trimethoxyanilines or ethyl *N*-(4-aminobenzoyl)-L-glutamate in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole furnished the corresponding *N*-arylamides **9-11**.

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Introduction.

Folate metabolism has long been recognized as an effective target for chemotherapy because of its crucial role in the biosynthesis of nucleic acid precursors [1,2].

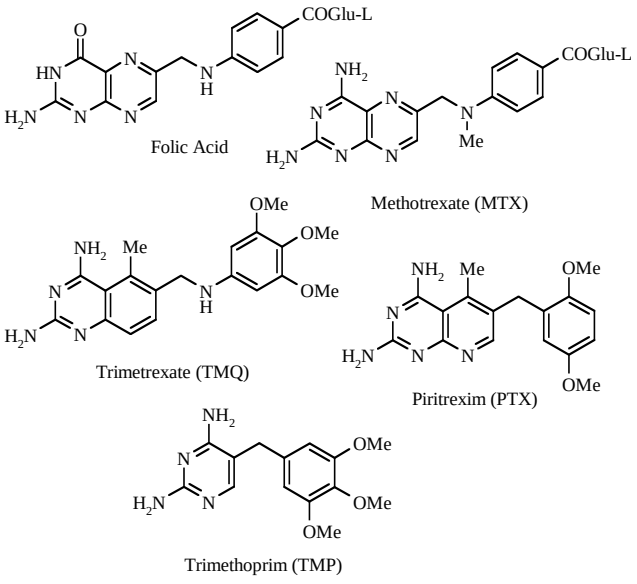


Figure 1

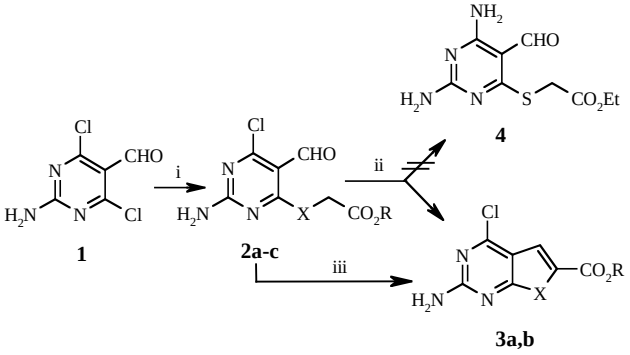
Inhibitors of folate-dependent enzymes in cancer, microbial, and protozoan cells provide compounds that have found clinical utility as antitumor, antimicrobial, and antiprotozoal agents [3]. As shown in Figure 1, folate analogues that inhibit dihydrofolate reductase usually contain 2,4-diamino substitution in their pyrimidine ring. Classical antifolate – methotrexate (MTX) is clinically used antitumor agent [2,4]. Trimetrexate (TMQ), piritrexim (PTX), and trimethoprim (TMP) are lipophilic antifolates and have been clinically used for the prophylaxis and treatment of *Pneumocystis carinii* and *Toxoplasma gondii* infections in patients with compromised immune system [5].

A vast number of groups that link heterocycle to the substituted benzene moiety of antifolates have been used that involve replacing the CH_2NH bridge in folic acid with methylene groups, heteroatoms, and $CH_2OC(=O)$ group [6-13]. However, a search of the literature indicates that there is a paucity of information of extending the bridge to an amide group. Recently we have described the synthesis of some potential classical and lipophilic antifolates - *N*-arylamides of 2-amino-4-oxothieno[2,3-*d*]pyrimidine-6-carboxylic acid [14]. In the present paper we present results on the synthesis of new derivatives of 2,4-diaminothieno- and pyrrolo[2,3-*d*]-pyrimidine-6-carboxylic acids.

Results and discussion.

For the synthesis 2-amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) [15] was used as starting material. Compound **1** easily reacted with ethyl mercaptoacetate, glycine and sarcosine (*N*-methylglycine) esters in the appropriate alcohols at room temperature in the presence of triethylamine to give the corresponding esters **2a-c** in 56-66% yields (Scheme 1).

Scheme 1



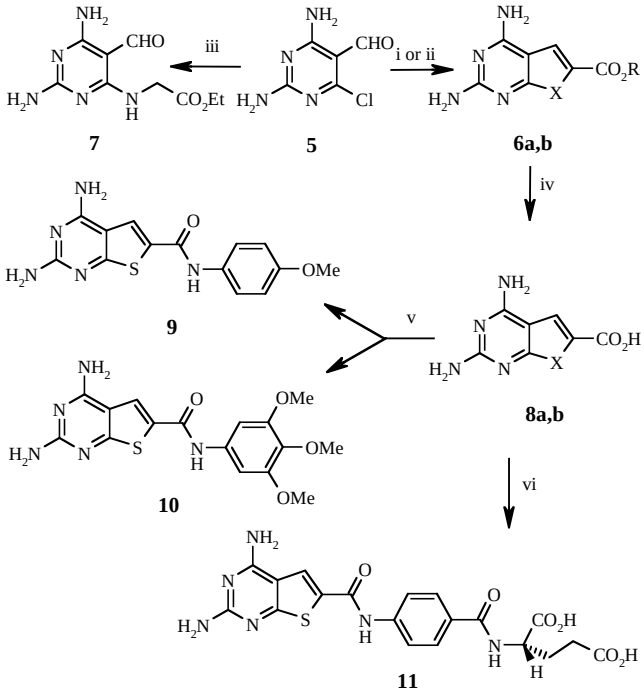
X = S, R = Et (**a**), X = N-Me, R = Me (**b**), X = NH, R = Et (**c**).

Reagents and conditions: (i) $HXCH_2CO_2R$, Et_3N, ROH, 1.5-3 h; (ii) NH_3/H_2O, Δ, 6 h (method A); (iii) Et_3N, ROH, Δ, 20-60 h (method B).

Prolonged heating of esters **2a** in ethanol or **2b** in methanol in the presence of triethylamine afforded the thieno[2,3-*d*]- and pyrrolo[2,3-*d*]pyrimidines **3a,b**. However, compound **2c** remained unchanged by heating in ethanol or dimethylformamide using triethylamine or potassium carbonate as a base. It is also noteworthy, that reaction of **2a-c** with ammonia did not give the desired derivatives of 2,4-diaminopyrimidine **4**. For example, heating **2a** with an excess of ammonia furnished the cyclisation product **3a** in 53% yield. To overcome this problem 2,4-diamino-6-chloropyrimidine-5-carbaldehyde (**5**) [16] was employed for the construction of the 2,4-diaminothieno- and pyrrolo[2,3-*d*]pyrimidines. Refluxing compound **5** with ethyl mercaptoacetate in pyridine for 6 hours (Method A) or heating in dimethylformamide at 60°C in the presence of potassium carbonate and molecular sieves 4 Å (method B) resulted in the formation of thieno[2,3-*d*]pyrimidine **6a** in 68% and 72% yields, respectively (Scheme 2). Pyrrolo[2,3-*d*]pyrimidine **6b** in 59% yield was obtained after heating **5** with methyl *N*-methylglycinate in methanol for 36 hours in the presence of an excess of triethylamine. However reaction of **5** with glycine ester under the analogous conditions gave ethyl *N*-(2,4-diamino-5-formylpyrimdin-6-yl)glycinate (**7**). Formation of the corresponding pyrrolo[2,3-*d*]pyrimidine derivative was not observed. Cyclisation of **7** under different conditions, including heating with potassium carbonate or sodium hydride in dimethylformamide or with sodium ethoxide in ethanol, was also unsuccessful.

Esters **6a,b** appeared to be inert towards anilines. Similar inertness of the ester group was also observed in a series of ethyl 4-dialkylamino-2-methylthio- and 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylates [14,17]. Therefore, for the synthesis of *N*-arylamides we decided to use the corresponding acids. For this purpose esters **6a,b** were hydrolysed with sodium hydroxide in dilute alcohol to give the corresponding acids **8a** [18], **8b**. *N*-arylamides of 2,4-diaminothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**9**, **10**) were obtained by reaction of acid **8a** with the corresponding anilines in the presence of auxiliary reagents - dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt).

Scheme 2



X = S, R = Et (**a**), X = N-Me, R = Me (**b**)

Reagents and conditions: (i) $HXCH_2CO_2Et$, Et_3N, pyridine, Δ (method A); (ii) $HSCH_2CO_2Et$, K_2CO_3, MS 4Å, DMF, 60°C (method B); (iii) Et_3N, EtOH, 40 h, Δ; (iv) 1. NaOH, H_2O, EtOH, Δ, 1.5 h; 2. HCl; (v) aniline, DCC, HOBt, DMF, Ar, r.t., 24-60 h; (vi) 1. Diethyl N-(4-aminobenzoyl)-L-glutamate, DCC, HOBt, DMF, Ar, r.t., 1 week; 2. 1 M NaOH, r.t., 2 h; 3. aq. HCl till pH 4.

It is noteworthy that 2,4-diamino-7-methyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine-6-carboxylic acid (**8b**) did not react with anilines under these conditions. The reason of such behaviour can be its very poor solubility in various solvents. *N*-(4-{[(2,4-Diaminothieno[2,3-*d*]pyrimidin-6-yl)-carbonyl]amino}benzoyl)-L-glutamic acid (**11**) was prepared by the reaction of **8a** with diethyl *N*-(4-

aminobenzoyl)-L-glutamate by similar procedure applied for the synthesis of amides **9**, **10** and subsequent alkaline hydrolysis of the ester groups of glutamic acid moiety.

An investigation of the synthesised compounds **9-11** for possible inhibition of *P. carinii*, *T. gondii* and *Mycobacterium avium* DHFR is under progress and will be reported elsewhere.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. IR spectra were run on a Perkin-Elmer FT-IR spectrophotometer Spectrum BX II. ^{1}H and ^{13}C nmr spectra were recorded on a Varian INOVA spectrometer (300 MHz and 75 MHz respectively). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Mass spectrum was obtained on a mass spectrometer Kratos 115-30 (70 eV) by direct insertion probe. Elemental analyses were performed at the Elemental Analysis Laboratory of the Department of Organic Chemistry of Vilnius University. TLC was performed with silica gel plates Alugram Sil G/UV$_{254}$ (Macherey-Nagel).

Ethyl (2-Amino-4-chloro-5-formylpyrimidin-6-yl)thioacetate (**2a**).

To a stirred suspension of compound **1** [15] (0.38 g, 2.0 mmol) in ethanol (10 ml) triethylamine (0.22 g, 2.2 mmol) and ethyl mercaptoacetate (0.24 g, 2.0 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 hours. The precipitate was collected by filtration, washed with water (3 x 5 ml), methanol (1 ml), dried and recrystallised from 2-propanol to give 0.36 g (66%) of **2a**, mp 161-163°; ir (nujol): 3371, 3308 (NH_2), 1729, 1647 (CO) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.21 (t, *J* = 7.2 Hz, 3H, CH_3), 4.01 (s, 2H, SCH_2), 4.14 (q, *J* = 7.2 Hz, 2H, OCH_2), 7.96 (br. s, 1H, NH), 8.26 (br. s, 1H, NH), 10.10 (s, 1H, CHO); ^{13}C nmr (dimethylsulfoxide-d_6): δ 14.7, 32.4, 61.7, 113.7, 161.3, 165.3, 169.3, 173.8, 187.0.

Anal. Calcd. for $C_9H_{10}ClN_3O_3S$: C, 39.21; H, 3.66; N, 15.24. Found: C, 39.49; H, 3.61; N, 15.36.

Methyl *N*-(2-Amino-4-chloro-5-formylpyrimidin-6-yl)-*N*-methylglycinate (**2b**).

This compound was prepared analogously to **2a** from compound **1** (1.0 g, 5.2 mmol). The reaction time 3 h. Yield 0.76 g (56%), mp 160-163°C (dec) (from 2-propanol); ir (nujol): 3362, 3331, (NH_2), 1756, 1746 (CO) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 2.99 (s, 3H, NCH_3), 3.69 (s, 3H, OCH_3), 4.26 (s, 2H, NCH_2), 7.47 (br.s, 1H, NH), 7.63 (br.s, 1H, NH), 9.94 (s, 1H, CHO); ^{13}C nmr (dimethylsulfoxide-d_6): δ 41.5, 52.5, 53.5, 104.0, 161.4, 164.0, 166.4, 170.2, 184.7.

Anal. Calcd. for $C_9H_{11}ClN_4O_3$: C, 41.79; H, 4.29; N, 21.66. Found: C, 42.07; H, 4.25; N, 21.70.

Ethyl *N*-(2-Amino-4-chloro-5-formylpyrimidin-6-yl)glycinate (**2c**).

To a mixture of compound **1** (1.0 g, 5.2 mmol), hydrochloride of ethyl glycinate (0.88 g, 6.3 mmol) and ethanol (10 ml) triethylamine (1.16 g, 11.5 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h. Solid was collected by filtration and washed with water. Filtrate was concentrated to 1/3 of the initial volume and poured into water. The precipitate was collected by filtration, combined with the earlier obtained and recrystallised from 2-propanol to give 0.88 g (65%) of **2c**, mp 141.5-144.5° (dec.); ir (Nujol): 3413, 3348, 3274, 3241 (NH_2, NH); 1732, 1663 (CO) cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.27 (t, *J* = 7.8 Hz, 3H, CH_3), 4.09-4.36 (m, 4H, NCH_2, OCH_2), 5.82 (s, 2H, NH_2), 9.51 (s, 1H, NH); 10.09 (s, 1H, CHO). ^{13}C nmr (deuteriochloroform): δ 14.8, 42.7, 61.4, 101.8, 162.7, 162.9, 165.9, 170.2, 188.1.

Anal. Calcd. for $C_9H_{11}ClN_4O_3$: C, 41.79; H, 4.29; N, 21.66. Found: C, 42.02; H, 4.26; N, 21.53.

Ethyl 2-Amino-4-chlorothieno[2,3-*d*]pyrimidine-6-carboxylate (**3a**).

Method A.

A suspension of compound **2a** (0.28 g, 1.0 mmol) in aqueous ammonia (25%, 5 ml) was refluxed for 6 hours. The reaction mixture was cooled to room temperature, the precipitate was collected by filtration, well washed with water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.14 g (53 %) of **3a**, mp 249-252°; ir (nujol): 3397, 3326 (NH_2), 1694 (CO) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.34 (t, *J* = 7.2 Hz, 3H, CH_3), 4.34 (q, *J* = 7.2 Hz, 2H, OCH_2), 7.71 (br.s, 2H, NH_2), 7.91 (s, 1H, C5-H); ^{13}C nmr (dimethylsulfoxide-d_6): δ 14.8, 62.4, 120.4, 125.9, 127.0, 157.2, 161.9, 162.0, 172.8.

Anal. Calcd. for $C_9H_8ClN_3O_2S$: C, 41.95; H, 3.13; N, 16.31. Found: C, 42.39; H, 3.19; N, 16.60.

Method B.

A suspension of compound **2a** (0.30 g, 1.1 mmol) in ethanol (10 ml) and triethylamine (0.11 g, 1.1 mmol) was refluxed for 20 hours. The reaction mixture was cooled to room temperature, the precipitate was collected by filtration, washed with water (3 x 5 ml), methanol (1 ml), dried and recrystallised from a mixture of dimethylformamide and water to give 0.20 g (71%) of **3a**, whose properties were identical to those of the product obtained in Method A.

Methyl 2-Amino-4-chloro-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (**3b**).

A mixture of compound **2b** (0.52 g, 2.0 mmol), methanol (15 ml), and triethylamine (0.40 g; 4.0 mmol) was refluxed for 60 hours. After cooling to room temperature the precipitate was collected by filtration, washed with water and recrystallised from 1,4-dioxane to give 0.27 g (56%) of compound **3b**, mp > 200° (dec.); ir (nujol): 3417, 3307 (NH_2), 1726 (CO) cm^{-1}; ^{1}H nmr (dimethylsulfoxide-d_6): δ 3.83 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 7.07 (s, 1H, C5-H), 7.17 (br. s, 2H, NH_2); ^{13}C nmr (dimethylsulfoxide-d_6): δ 31.1, 52.6, 107.8, 108.2, 125.9, 154.9, 155.6, 161.4, 161.6.

Anal. Calcd. for $C_9H_9ClN_4O_2$: C, 44.92; H, 3.77; N, 23.28. Found: C, 45.17; H, 3.92; N, 23.45.

Ethyl 2,4-Diaminothieno[2,3-*d*]pyrimidine-6-carboxylate (**6a**).

Method A.

A suspension of compound **5** [16] (1.38 g, 8.00 mmol), ethyl mercaptoacetate (0.96 g, 8.0 mmol) and triethylamine (1.62 g, 16.0 mmol) in pyridine (20 ml) was refluxed for 6 hours. After

cooling to room temperature the reaction mixture was poured into water (*ca*. 200 ml), the precipitate was collected by filtration, well washed with water, methanol (5 ml), dried and recrystallised from 1,4-dioxane to give 1.29 g (68 %) of **6a**, mp 249-252°; ir (nujol): 3395, 3333, 3226, 3150 (NH_2), 1691 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.30 (t, *J* = 7.2 Hz, 3H, CH_3), 4.28 (q, *J* = 7.2 Hz, 2H, CH_2), 6.51 (br. s, 2H, NH_2), 7.35 (br. s, 2H, NH_2), 8.21 (s, 1H, C5-H). ^{13}C nmr (dimethylsulfoxide-d_6): δ 15.0, 61.4, 109.8, 119.6, 128.0, 160.7, 162.9, 163.2, 172.7.

Anal. Calcd. for $C_9H_{10}N_4O_2S$: C, 45.37; H, 4.23; N, 23.51. Found: C, 45.58; H, 4.03; N, 23.62.

Method B.

To a solution of compound **5** (0.20 g, 1.16 mmol) in dry dimethylformamide (5 ml) in sequence as following molecular sieves 4 Å (0.2 g), ethyl mercaptoacetate (0.15 g, 1.3 mmol) and anhydrous potassium carbonate (0.80 g, 5.8 mmol) were added. The reaction mixture was stirred at 60° for 2 hours, cooled to room temperature and poured into water (*ca*. 50 ml). The precipitate was collected by filtration, washed with water (3 x 3 ml), methanol (1 ml), dried and recrystallised from 1,4-dioxane to give 0.20 g (72 %) of **6a**, whose properties were identical to those of the product obtained in Method A.

Methyl 2,4-Diamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate (**6b**).

To a mixture of compound **5** (1.0 g, 5.8 mmol), hydrochloride of methyl *N*-methylglycinate (0.98 g, 7.0 mmol) and methanol (15 ml) triethylamine (1.30 g, 12.8 mmol) was added dropwise. The reaction mixture was refluxed for 18 hours. Then an additional amount of triethylamine (1.17 g, 11.6 mmol) was added and the reaction mixture was refluxed for additional 18 hours. After cooling to room temperature the precipitate was collected by filtration. Filtrate was concentrated to 1/3 of the initial volume and poured into water. The precipitate was collected by filtration, combined with that earlier obtained, washed with water and recrystallised from methanol to give 0.76 g (59%) of **6b**, mp > 260° (dec.); ir (nujol): 3421, 3324, 3117 (NH_2), 1712 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 3.75 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 6.05 (s, 2H, NH_2), 6.99 (s, 2H, NH_2), 7.32 (s, 1H, C5-H); ^{13}C nmr (dimethylsulfoxide-d_6): δ 31.4, 51.8, 96.8, 110.5, 120.5, 155.7, 156.0, 162.1, 162.9.

Anal. Calcd. for $C_9H_{11}N_5O_2$: C, 48.87; H, 5.01; N, 31.66. Found: C, 49.01; H, 4.99; N, 31.96.

Ethyl *N*-(2,4-Diamino-5-formylpyrimidin-6-yl)glycinate (**7**).

To a mixture of compound **5** (1.0 g, 5.8 mmol), hydrochloride of ethyl glycinate (0.98 g, 7 mmol) in ethanol (15 ml) triethylamine (1.3 g; 12.8 mmol) was added dropwise. The reaction mixture was refluxed while stirring for 40 hours. After cooling to room temperature the precipitate was collected by filtration, washed with water and recrystallised from methanol to give 0.60 g (43%) of **7**, mp > 200° (dec.); ir (nujol): 3418, 3319, 3143 (NH_2, NH), 1731, 1691 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.22 (t, *J* = 7.0 Hz, 3H, CH_3), 4.13 (q, *J* = 7.0 Hz, 2H, OCH2), 4.18 (d, *J* = 6.3 Hz, 2H, NCH_2), 6.56 (br. s, 2H, NH_2), 6.97 (br. s, 2H, NH_2), 9.44 (br. s, 1H, NH), 9.80 (s, 1H, CHO); ^{13}C nmr (dimethylsulfoxide-d_6): δ 19.6, 47.1, 65.9, 96.0, 168.7, 169.2, 171.7, 175.7, 189.0.

Anal. Calcd. for $C_9H_{13}N_5O_3$: C, 45.19; H, 5.48; N, 29.27. Found: C, 45.50; H, 5.21; N, 29.43.

2,4-Diamino-7-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid (**8b**).

A mixture of compound **6b** (1.0 g, 4.5 mmol), methanol (20 ml), water (10 ml) and sodium hydroxide (0,44 g, 11 mmol) was refluxed while stirring for 1 hour. Then the reaction mixture was concentrated under reduced pressure to 1/3 of the initial volume and acidified with dilute hydrochloric acid to pH 2. The precipitate was collected by filtration, washed with water and recrystallised from dimethylsulfoxide to give 0.88 g (94%) of **8b**, mp >300° (dec.); ir (nujol): 3421 cm^{-1}, 3318 cm^{-1}, 3202 cm^{-1}, 3130 cm^{-1} (NH_2, OH); 1683 cm^{-1} (CO); ^1H-nmr (deuterated trifluoroacetic acid): δ 3,73 (3H, s, NCH_3); 7,62 (1H, s, C5-H).

Anal. Calcd. for $C_8H_9N_5O_2$: C, 46.38; H, 4.38. Found: C, 46.63; H, 4.41.

N-(4-Methoxyphenyl)amide of 2,4-Diaminothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**9**).

To a stirred suspension of compound **8a** [18] (0.68 g, 3.2 mmol) in dry dimethylformamide (30 ml) *N,N'*-dicyclohexylcarbodiimide (1.0 g, 4.8 mmol), 1*H*-1,2,3-benzotriazol-1-ol (0.65 g, 4.8 mmol) and 4-methoxyaniline (0.6 g, 4.8 mmol) were added. The flask was capped with rubber septum, flushed with argon gas and stirred at room temperature for 60 hours. Water (0.5 ml) was added and the mixture was stirred for an additional 1 hour. The precipitate was filtered off, washed with dimethylformamide (1 ml) and the filtrate was poured into water (*ca*. 300 ml). The precipitate was collected by filtration, recrystallised from a mixture of dimethylformamide and water, and dried in a drying oven at *ca*. 105 °C to give 0.76 g (75 %) of **9**, mp > 300° (dec.); ir (nujol): 3461, 3313, 3186 ($2NH_2$, NH), 1624 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 3.77 (s, 3H, C4'-OCH_3), 6.38 (br. s, 2H, NH_2), 6.94 (d, *J* = 9.2 Hz, 2H, C3',5'-H), 7.18 (br. s, 2H, NH_2), 7.64 (d, *J* = 9.2 Hz, C2',C6'-H), 8.05 (s, 1H, C5-H), 10.11 (br. s, 1H, CONH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 55.9, 109.7, 114.5, 122.4, 124.0, 126.6, 132.8, 156.2, 160.6, 161.2, 162.7, 171.5; ms (m/z, % I): 315 (M^+, 7), 200 (48), 156 (16), 154 (36), 150 (100), 134 (15), 123 (18), 122 (31), 119 (58), 105 (26), 92 (36), 91 (68).

Anal. Calcd. for $C_{14}H_{13}N_5O_2S$: C, 53.32; H, 4.15; N, 22.21. Found: C, 53.10; H, 4.35; N, 22.42.

N-(3,4,5-Trimethoxyphenyl)amide of 2,4-Diaminothieno[2,3-*d*]-pyrimidine-6-carboxylic acid (**10**).

This compound was prepared analogously to **9** from compound **8a** (0.30 g, 1.4 mmol). The reaction time 24 hours. Yield 0.26 g (49%), mp > 300° (dec.) (from a mixture of dimethylformamide and water); ir (nujol): 3454, 3324, 3181 (NH_2, NH), 1628 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 3.66 (s, 3H, C4'-OCH_3), 3.78 (s, 6H, C3',5'-OCH_3), 6.40 (br. s, 2H, NH_2), 7.17 (s, 2H, C2',6'-H) 7.20 (br. s, 2H, NH_2,), 8.06 (s, 1H, C5-H), 10.14 (br. s, 1H, CONH); ^{13}C nmr (dimethylsulfoxide-d_6): δ 56.4, 60.8, 98.4, 109.7, 124.2, 126.5, 134.2, 135.9, 153.3, 160.6, 161.4, 162.7, 171.6.

Anal. Calcd. for $C_{16}H_{17}N_5O_4S$: C, 51.19; H, 4.56; N, 18.66. Found: C, 51.22; H, 4.68; N, 18.91.

N-(4-{[(2,4-diaminothieno[2,3-*d*]pyrimidin-6-yl)carbonyl]amino}-benzoyl)-L-glutamic acid (**11**).

To a stirred suspension of compound **8a** (0.285 g, 1.36 mmol) in dry dimethylformamide (12 ml) *N,N'*-dicyclohexylcarbodiimide (0.56 g, 2.7 mmol), 1*H*-1,2,3-benzotriazol-1-ol (0.27 g,

2.0 mmol) and *N*-(4-aminobenzoyl)-L-glutamic acid diethyl ester (0.48 g, 1.5 mmol) were added. The flask was capped with rubber septum, flushed with argon gas and stirred at room temperature for 1 week. Water (0.5 ml) was added, the precipitate was filtered off after 1 hour, washed with dimethylformamide (1 ml); the filtrate was poured into water (*ca*. 200 ml) and left for several hours. The resulted precipitate was collected by filtration and suspended in 1 *M* sodium hydroxide solution (*ca*. 7 ml). The mixture was stirred at room temperature for 2 hours and the solids filtered off. The filtrate was roughly acidified with concentrated hydrochloric acid to pH 7 and then adjusted to pH 4 with 5% hydrochloric acid. The precipitate was collected by filtration, washed with water (3 x 3 ml), recrystallised from a mixture of dimethylformamide and water, and dried in drying oven at *ca*. 105 °C to give 0.232 g (37 %) of **11**, mp > 300° (dec.); ir (nujol): 3430, 3346 (NH_2, NH, CO_2H), 1653, 1645 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): δ 1.90-2.19 (m, 2H, CH_2), 2.38 (t, *J* = 7.5 Hz, 2H, CH_2), 4.37-4.46 (m, 1H, CH), 6.50 (br. s, 2H, NH_2) 7.28 (br. s, 2H, NH_2,), 7.82 (d, *J* = 8.7 Hz, 2H, C2',6'-H), 7.90 (d, *J* = 8.7 Hz, 2H, C3',5'-H), 8.13 (s, 1H, C5-H), 8.53 (d, *J* = 7.5 Hz, 1H, CONH), 10.49 (br. s, 1H, CONH), 12.43 (br. s, 2H, CO_2H); ^{13}C nmr (dimethylsulfoxide-d_6): δ 26.6, 31.1, 52.6, 109.8, 119.7, 125.0, 126.1, 129.0, 129.2, 142.6, 154.0, 160.6, 161.8, 162.5, 166.7, 174.2, 174.6.

Anal. Calcd. for $C_{19}H_{18}N_6O_6S$: C, 49.78; H, 3.96; N, 18.33. Found: C, 49.65; H, 4.08; N, 18.42.

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