Synthesis of *N*-Aryl-2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides

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Synthetic routes for the preparation of methyl 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylate (**4**) - useful intermediate for lipophilic and classical antifolates from 2-amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) have been studied. It has been shown that more efficient synthesis of compound **4** includes the preparation of 4-methoxy derivative **7** and subsequent tandem substitution/annulation reaction with methyl mercaptoethanoate in dimethylformamide in the presence of potassium carbonate and molecular sieves 4 Å. Compound **4** was used for the synthesis of *N*-aryl 2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]-pyrimidine-6-carboxamides **10a-c**, including an analog of folic acid with amide bridge - *N*-(4-{[(2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)carbonyl]amino}-benzoyl)-L-glutamic acid (**10c**).

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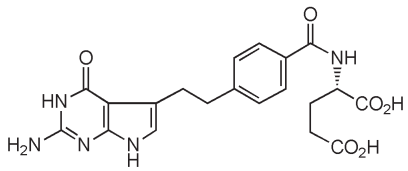
Thymidylate synthase, which mediates the reductive methylation of 2'-deoxyuridine-5'-monophosphate to give 2'-deoxythymidine-5'-monophosphate utilising the cofactor 5,10-methylenetetrahydrofolate and is thus essential for *de novo* DNA biosynthesis, has long been recognised as a prime objective for the development of antitumor agents. Numerous folate cofactor analogues have been synthesised and several of them have found use in the clinic as antitumor, antibacterial, and antiprotozoan agents [1]. Derivatives of 6/5-fused heterocycles, such as pyrrolo-, thieno- and furo[2,3-*d*]pyrimidines have a considerable interest since the discovery of multitargeted antifolate (Figure 1) [2].



MTA

Figure 1

In search of more potent and selective lipophilic and classical antifolates structural modifications concerning the bridge and aromatic part of molecules have been made in position 5 of the mentioned heterocycles [3-8]. However, to the best of our knowledge, there are only a few reports on the synthesis of potent dihydrofolate reductase inhibitors - 2,4-diaminothieno[2,3-*d*]pyrimidines, bearing arylalkyl substituents in the position 6 of the heterocycle [5,6,9,10] and no work has been done on the synthesis of the corresponding 6-substituted 2-amino-4-oxothieno[2,3-*d*]pyrimidines. In this connection and as a part of our program designed towards fused heterosystems incorporating the pyrimidine ring [11-15] we report herein results on a synthesis of *N*-aryl 2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides as novel potential antifolates. The title compounds contain a new type of bridge and, therefore, could have a considerable interest in structure-activity relationship studies. They were also expected to undergo reduction reaction into derivatives with the natural methyleneamino bridge between hetaryl and aryl moieties.

For the synthesis of derivatives of 2-amino-4-substituted-thieno[2,3-*d*]pyrimidine-6-carboxylic acids the corresponding esters are of considerable interest. According to the retrosynthetic analysis they could be produced from 2-amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) by synthetic routes **I** and **II**, differing in sequence of the thiophene ring formation and functionalisation of the position 4 of the pyrimidine ring (Figure 2).

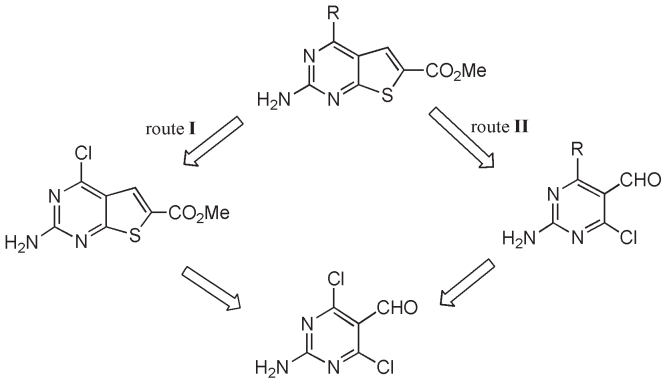


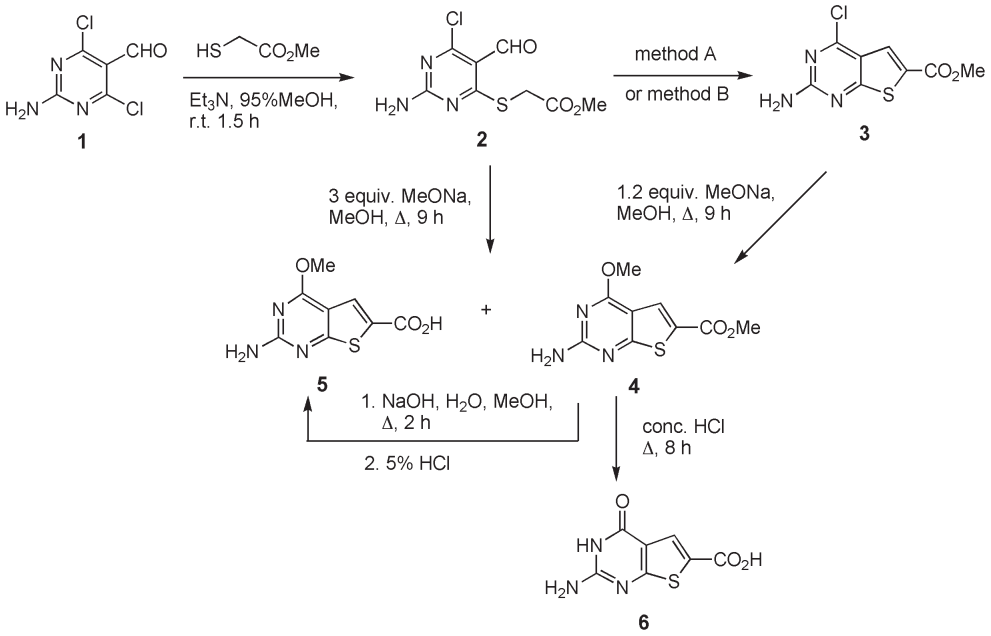
Figure 2

Firstly, synthesis of 2-amino-4-substituted-thieno[2,3-*d*]pyrimidine-6-carboxylates according to route **I** has been investigated. Reaction of carbaldehyde **1** with methyl mercaptoethanoate in anhydrous methanol in the presence of

triethylamine at room temperature gave a mixture of **2** and **3** in a ratio 1:0.7, as judged by ^{1}H-NMR. Performing the reaction of **1** with methyl mercaptoethanoate in a mixture of methanol and water resulted in the formation of **2** as the only reaction product (Scheme 1).

synthesis by route **II**. 2-Amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) was allowed to react with sodium methoxide in methanol. It was noticed that already at room temperature a mixture of monomethoxy **7** and dimethoxy derivatives **8** in a ratio 6:1 was formed (Scheme 2).

Scheme 1



Reagents and conditions: Method A: Et_3N, MeOH, Δ, 18 h; Method B: K_2CO_3, DMF, MS 4A, 60°, 1 h;

Reflux of **2** with triethylamine in methanol for 18 hours (Method A) or heating at 60 °C with potassium carbonate in dimethylformamide in the presence of molecular sieves 4 Å for 1 hour (Method B) afforded 4-chlorothienopyrimidine **3** (Scheme 1). Compound **3** reacted with sodium methoxide to give methyl 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylate (**4**) as the sole reaction product. Attempt to shorten the reaction pathway, *i.e*. to synthesise **4** by tandem substitution/annulation reaction of **2** with an excess of sodium methoxide in methanol led to a mixture of ester **4** and acid **5** in 34% and 33% yields, respectively. Ester **4** when heated with sodium hydroxide in aqueous methanol afforded 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylic acid (**5**), whereas acidic hydrolysis of **4** using concentrated hydrochloric acid gave 2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**6**). Thus, synthesis of ester **4** – a key intermediate for the preparation of 2-amino-4-oxothieno[2,3-*d*]-pyrimidines bearing various functional groups in the position 6 by route **I** could be performed by three-step procedure in overall 26% yield. Taking into account that overall yield of **4** is not high it was of interest to investigate its

Nevertheless we have found that the desired compound **7** can be synthesised in a reasonable yield by reflux of **1** in methanol in the presence of triethylamine.

2-Amino-6-chloro-4-methoxypyrimidine-5-carbaldehyde (**7**) reacted with methyl mercaptoethanoate in the presence of bases to give the thienopyrimidine **4**. Data presented in Table 1 indicate that the duration of the reaction and the yields of ester **4** depend on a solvent and base.

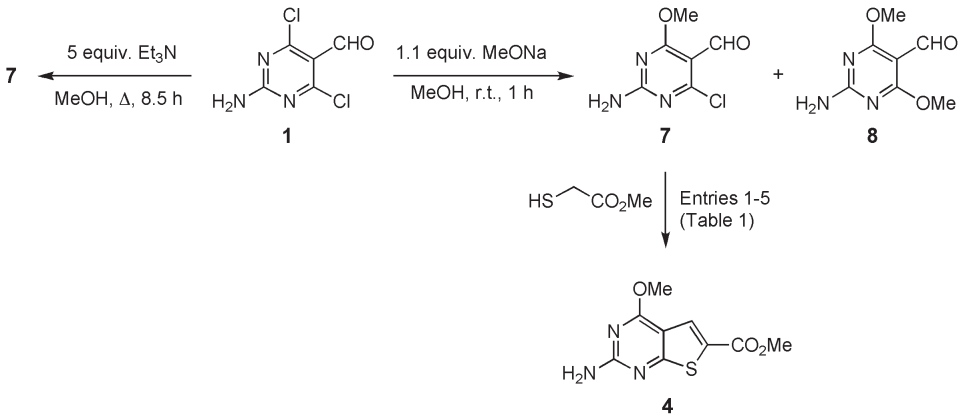
Table 1

Data of synthesis of methyl 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylate (**4**) from compound **7** and methyl mercaptoethanoate.[a]

Entry	Reaction conditions	Reaction time, h	Isolated yield, %
1	2.2 equiv. Et_3N, pyridine, reflux	23	30
2	2.2 equiv. Et_3N, MeOH, reflux	50	63
3	2.2 equiv. Et_3N, DMF, 80 °C	27	47
4	5 equiv. K_2CO_3, DMF, 60 °C	3	59
5	5 equiv. K_2CO_3, DMF, MS 4 Å, 60 °C	3	93

[a] in all experiments 1.1 equivalent of $HSCH_2CO_2Me$ was used.

Scheme 2

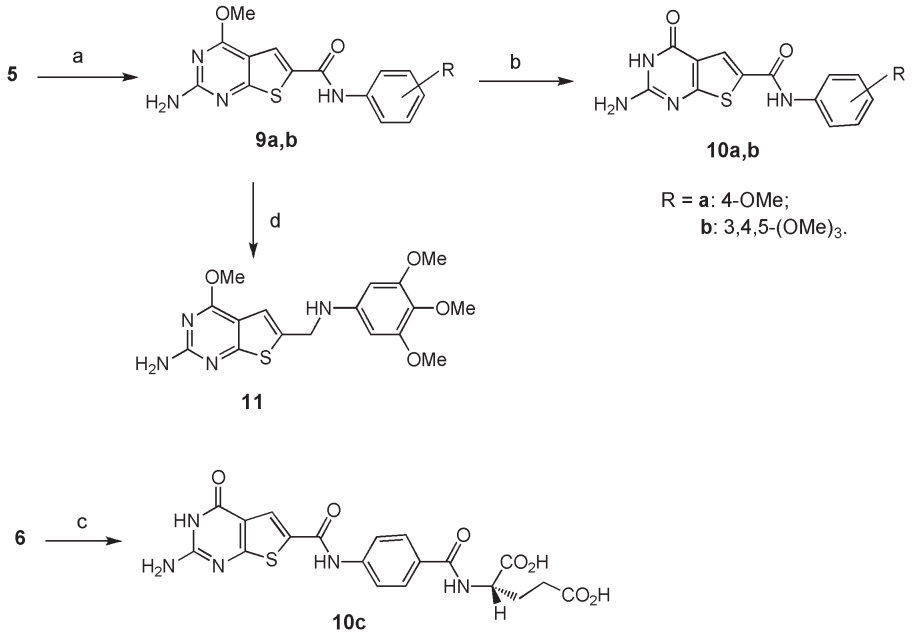


Almost quantitative conversion of **7** into **4** was achieved when the reaction was carried out in dimethylformamide at 60 °C in the presence of potassium carbonate and molecular sieves 4 Å (entry 5). Comparing results obtained in the synthesis of ester **4** from 2-amino-4,6-dichloropyrimidine-5-carbonitrile (**1**) by routes **I** and **II** one can envisage that route **II** is more efficient: compound **4** is produced by two-step procedure with overall 66% yield.

Although ester group in **4** was able to undergo alkaline or acidic hydrolysis (Scheme 1) it appeared to be rather inert towards anilines. We did not succeed to obtain the corresponding amide by performing reaction of **4** with 4-methoxyaniline in different conditions including prolonged heating of reagents without solvent. Similar inertness of the ester group was also observed in a series of ethyl 4-dialkylamino-2-methylthiothieno[2,3-*d*]pyrimidine-6-carboxylates [15]. Therefore, arylamides of 2-amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylic acid (**9a,b**) were obtained by the reaction of acid **5** with the corresponding anilines in the presence of auxiliary reagents - dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) (Scheme 3). 4-Methoxy derivatives **9a,b** were converted into the corresponding 4-oxo derivatives **10a,b** by reflux in concentrated hydrochloric acid.

The thienopyrimidine analog of folic acid bearing amide bridge - *N*-(4-{[(2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]-

Scheme 3



Reagents and conditions: (a) substituted aniline, DCC, HOBt, DMF, r.t., Ar, 48 h, (b) conc. HCl, Δ, 4 h; (c) 1. diethyl N-(4-aminobenzoyl)-L-glutamate, DCC, HOBt, DMF, r.t., Ar, 7 days, 2. 1M NaOH, r.t., 15 min, 3. HCl; (d) **9b**, $LiAlH_4$, r.t., Ar, 96 h.

pyrimidin-6-yl)carbonyl]amino}benzoyl)-L-glutamic acid (**10c**) was synthesised by one-pot procedure according to the method applied for the synthesis of amides **9a,b** by the reaction of **6** with diethyl *N*-(aminobenzoyl)-L-glutamate and following alkaline hydrolysis of the ester groups (Scheme 3).

In order to obtain lipophilic antifolates with CH_2NH bridge between the thienopyrimidine and aryl fragments attempts to reduce amides **9,10** using B_2H_6 or $LiAlH_4$ were made. However, only compound **9b** in the reaction with $LiAlH_4$ furnished 2-amino-4-methoxy-6-{[(3,4,5-trimethoxyphenyl)amino]methyl}thieno[2,3-*d*]pyrimidine (**11**) in 26% yield.

In conclusion, we synthesised and characterised some *N*-aryl 2-amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides (**10a-c**) which could represent novel potential antifolates with amide bridge. It was also shown that reduction of amides **9a,b** and **10a,b** with $LiAlH_4$ is not applicable method for the synthesis of the corresponding methyleneamino derivatives. Investigation of biological properties of compounds **10**, **11** as well as study on the synthesis of the corresponding compounds with CH_2NH bridge 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. NMR spectra were recorded on a Varian INOVA spectrometer (300 MHz). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as internal standard. Optical rotation of **10c** was measured using a polarimeter Polamat-A. Enantiomeric excess was determined with a P/ACE 2100 apparatus (Beckman Instruments Inc.) using fused silica capillary (length - 50 cm till detector, internal diameter - 75 μm), electrolyte - 50 mM $Na_2B_4O_7$ solution in water, selector - β-cyclodextrine (5 mM), voltage +25 kV, and UV detector (λ = 254 nm). 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 60 F_{254} (Merck).

2-Amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) was prepared following the literature procedure [16].

Methyl [(2-Amino-4-chloro-5-formyl-6-pyrimidinyl)thio]-ethanoate (**2**).

To a stirred suspension of 2-amino-4,6-dichloropyrimidine-5-carbaldehyde (**1**) (0.2 g, 1.0 mmol) in methanol (6.3 ml) and water (0.3 ml) was added triethylamine (0.11 g, 1.1 mmol) and methyl mercaptoethanoate (0.12 g, 1.1 mmol). The reaction mixture was stirred at room temperature for 1.5 hours. The precipitate was collected by filtration, washed with water, dried in air and recrystallised from 2-propanol to give 0.18 g (66%) of **2** as a yellow needles, mp 174-179°; ir (Nujol): 3416, 3299 (NH_2), 1720, 1646 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.68 (s, 3H, CH_3), 4.01 (s, 2H, CH_2), 7.99 (br.s, 1H, NH), 8.25 (br.s, 1H, NH), 10.09 (s, 1H, CHO).

Anal. Calcd. for $C_8H_8ClN_3O_3S$: C, 36.72; H, 3.08; N, 16.06. Found: C, 36.35; H, 2.84; N, 16.29.

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

Method A.

To a stirred suspension of compound **2** (1.00 g, 3.8 mmol) in methanol (30 ml) was added triethylamine (0.43 g, 4.2 mmol). The reaction mixture was refluxed while stirring for 18 hours and cooled to room temperature. The precipitate was collected by filtration, washed with water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.66 g (71%) of **3** as a yellow solid, mp 246-249°; ir (Nujol): 3405, 3328 (NH_2), 1714 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.89 (s, 3H, CH_3), 7.52 (br.s, 2H, NH_2), 7.81 (s, 1H, C5-H).

Anal. Calcd. for $C_8H_6ClN_3O_2S$: C, 39.43; H, 2.48. Found: C, 39.54; H, 2.56.

Method B.

To a stirred solution of compound **2** (0.70 g, 2.7 mmol) in dimethylformamide (17 ml) were added molecular sieves 4 Å (0.70 g) and potassium carbonate (1.87 g, 13.5 mmol). The reaction mixture was stirred at 60° for 1 hour, cooled to room temperature and poured into water (*ca*. 200 ml). The precipitate was collected by filtration, washed with water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.46 g (71%) of **3** as a yellow solid, whose properties are identical to those of the product obtained in method A.

Methyl 2-Amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylate (**4**) and 2-Amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylic Acid (**5**).

To a solution of sodium methoxide in methanol prepared from sodium (4.8 g, 20.9 mmoles) and methanol (45 ml) compound **2** (1.80 g, 6.9 mmoles) was added portionwise. The reaction mixture was refluxed for 9 hours. After cooling to room temperature the precipitate was collected by filtration, washed with water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.56 g (34%) of **4** as a yellowish solid, mp 235-238°. The filtrate was acidified with 5% hydrochloric acid until pH 5. The precipitate was collected by filtration, rinsed with cold water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.51 g (33%) of **5** as a colorless solid, mp > 300°.

Compound **4**: ir (Nujol): 3424, 3312 (NH_2), 1702 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.83 (s, 3H, CO_2CH_3); 3.99 (s, 3H, C4-OCH_3), 7.25 (br.s, 2H, NH_2), 7.73 (s, 1H, C5-H).

Anal. Calcd. for $C_9H_9N_3O_3S$: C, 45.18; H, 3.79; N, 17.56. Found: C, 45.38; H, 3.79; N, 17.59.

Compound **5**: ir (Nujol): 3508, 3475, 3297, 3178 (NH_2, NH, OH), 1629 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.96 (s, 3H, CH_3), 6.64 (br.s, 2H, NH_2), 7.23 (s, 1H, C5-H).

Anal. Calcd. for $C_8H_7N_3O_3S$: C, 42.66; H, 3.13; N, 18.66. Found: C, 42.32; H, 3.25; N, 18.30.

Methyl 2-Amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylate (**4**).

Method A.

To a solution of sodium methoxide in methanol prepared from sodium (0.02 g, 0.9 mmol) and methanol (10 ml) compound **3** (0.2 g, 0.8 mmol) was added portionwise. The reaction mixture was refluxed for 9 hours. The precipitate was collected by filtra-

tion, washed with water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.11 g (55%) of **4** as a yellowish solid, whose properties are identical to those of the product obtained from compound **2**.

Method B.

To a stirred solution of compound **7** (0.2 g, 1.1 mmol) in dimethylformamide (5 ml) were added molecular sieves 4 Å (0.3 g), methyl mercaptoethanoate (0.13 g, 1.2 mmoles) and potassium carbonate (0.76 g, 5.5 mmol). Mixture was heated while stirring at 60° for 3 hours, cooled to room temperature and poured into water (*ca*. 70 ml). The precipitate was collected by filtration, washed with water and dried to give 0.24 g (93%) of **4** as a yellowish solid, whose properties are identical to those of the product obtained from compound **2**. The substance was pure enough to use further without purification.

2-Amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylic Acid (**5**).

To a stirred suspension of compound **4** (0.56 g, 2.3 mmol) in methanol (15 ml) was added a solution of sodium hydroxide (0.23 g, 5.7 mmol) in water (3 ml). The reaction mixture was refluxed while stirring for 2 hours. After removal of the solvent under reduced pressure, the residue was dissolved in a minimal amount of water and acidified with 5% hydrochloric acid to pH 5. The precipitate was collected by filtration, rinsed with cold water, dried and recrystallised from a mixture of dimethylformamide and water to give 0.40 g (76%) of **5** as a colorless solid, whose properties are identical to those of the product obtained from compound **2**.

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

A mixture of compound **4** (0.20 g, 0.84 mmoles) and concentrated hydrochloric acid (10 ml) was refluxed for 8 hours. After cooling to room temperature the precipitate was collected by filtration, washed with water (3 x 10 ml), dried and recrystallised from a mixture of dimethylformamide and water to give 0.13 g (74%) of **6** as a colorless solid, mp > 300°; ir (potassium bromide): 3310, 3131 (NH_2, NH, OH), 1722, 1717 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 6.92 (br.s, 2H, NH_2), 7.68 (s, 1H, C5-H), 11.17 (br.s, 1H, CONH), 12.97 (br.s, 1H, CO_2H); ^{13}C nmr (dimethylsulfoxide-d_6): 116.8, 123.4, 128.6, 155.3, 158.9, 163.8, 172.2.

Anal. Calcd. for $C_7H_5N_3O_3S$: C, 39.81; H, 2.39; N, 19.90. Found: C, 40.17; H, 2.53; N, 19.75.

2-Amino-6-chloro-4-methoxypyrimidine-5-carbaldehyde (**7**).

To a stirred suspension of compound **1** (0.5 g, 2.6 mmol) in methanol (20 ml) was added triethylamine (1.32 g, 13.0 mmol). The reaction mixture was refluxed while stirring for 8.5 hours. The solvent was removed under reduced pressure. The solid was washed several times with water, dried and recrystallised from 2-propanol to give 0.347 g (71%) of **7** as a yellowish solid, mp 192-195°; ir (Nujol): 3288, 3112 (NH_2), 1692 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.94 (s, 3H, OCH_3), 8.08 (br.s, 2H, NH_2), 10.00 (s, 1H, CHO).

Anal. Calcd. for $C_6H_6ClN_3O_2$: C, 38.42; H, 3.22; N, 22.40. Found: C, 38.60; H, 3.29; N, 22.00.

N-(4-Methoxyphenyl)amide of 2-Amino-4-methoxythieno[2,3-*d*]-pyrimidine-6-carboxylic Acid (**9a**).

The reaction vessel was charged with argon and subsequently loaded with compound **5** (0.23 g, 1 mmol), dimethylformamide (10 ml), 4-methoxyaniline (0.18 g, 1.5 mmol), *N,N'*-dicyclohexylcarbodiimide (0.31 g, 1.5 mmol) and 1*H*-1,2,3-benzotriazol-1-ol (0.2 g, 1.5 mmol). The reaction mixture was stirred under argon at room temperature for 24 hours. Water (0.5 ml) was added and the precipitate was collected filtration after 1 hour. The filtrate was poured into water (*ca*. 100 ml). The precipitate was collected by filtration, dried and recrystallised from a mixture of dimethylformamide and water to give 0.19 g (56%) of **9a** as a colorless solid, mp 236-239° (dec.); ir (Nujol): 3442, 3308, 3164 (NH_2, NH), 1636 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.76 (s, 3H, C4'-OCH_3), 4.03 (s, 3H, C4-OCH_3), 6.89 (br.s, 2H, NH_2), 7.05 (d, *J* = 8.0 Hz, 2H, C3',5'-H), 7.66 (d, *J* = 8.0 Hz, 2H, C2',6'-H), 8.18 (s, 1H, C5-H), 10.18 (br.s, 1H, CONH).

Anal. Calcd. for $C_{15}H_{14}N_4O_3S$: C, 54.54; H, 4.27; N, 16.96. Found: C, 54.25; H, 4.64; N, 17.18.

N-(3,4,5-Trimethoxyphenyl)amide of 2-Amino-4-methoxythieno[2,3-*d*]pyrimidine-6-carboxylic Acid (**9b**).

This compound was prepared analogously to **9a** from compound **5** (0.45 g, 2.0 mmol). Yield 0.38 g (49%), colorless solid (2-propanol), mp 189-191° (dec.); ir (Nujol): 3450, 3334, 3216 (NH_2, NH), 1626 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.65 (s, 3H, C4'-OCH_3), 3.79 (s, 6H, C3',5'-OCH_3), 4.03 (s, 3H, C4-OCH_3), 7.11 (br.s, 2H, NH_2), 7.19 (s, 2H, C2',6'-H), 8.20 (s, 1H, C5-H), 10.16 (br.s, 1H, CONH); ^{13}C nmr (dimethylsulfoxide-d_6): 54.3, 56.4, 60.8, 98.3, 111.5, 121.7, 130.3, 134.3, 135.6, 153.4, 160.6, 162.4, 165.8, 172.4.

Anal. Calcd. for $C_{17}H_{18}N_4O_5S$: C, 52.30; H, 4.65; N, 14.35. Found: C, 52.51; H, 4.85; N, 14.17.

N-(4-methoxyphenyl)amide of 2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic Acid (**10a**).

A mixture of compound **9a** (0.10 g, 0.30 mmol) and concentrated hydrochloric acid (5 ml) was refluxed while stirring for 4 hours. After cooling to room temperature, the precipitate was collected by filtration, washed with water (3 x 5 ml), dried and recrystallised from a mixture of dimethylformamide and water to give 0.07 g (73%) of **10a** as a colorless solid, mp > 300° (dec); ir (Nujol): 3541, 3342, 3187 (NH_2, NH), 1671, 1636 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 3.75 (s, 3H, C4'-OCH_3), 6.92 (d, *J* = 9,3 Hz, 2H, C3',5'-H), 7.29 (br.s, 2H, NH_2), 7.64 (d, *J* = 9,3 Hz, 2H, C2',6'-H), 8.22 (s, 1H, C5-H), 10.17 (br.s, 1H, CONH), 11.48 (br.s, 1H, CONH); ^{13}C nmr (dimethylsulfoxide-d_6): 55.9, 114.5, 117.3, 122.3, 124.5, 130.0, 132.6, 154.7, 156.2, 158.6, 160.3, 168.6.

Anal. Calcd. for $C_{14}H_{12}N_4O_3S$: C, 53.16; H, 3.82; N, 17.71. Found: C, 53.24; H, 3.92; N, 17.49.

N-(3,4,5-trimethoxyphenyl)amide of 2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic Acid (**10b**).

This compound was prepared analogously to **10a** from compound **9b** (0.10 g, 0.26 mmole). Yield 0.068 g (71%), colorless solid (dimethylformamide-water), mp>300° (dec.); ir (potassium bromide): 3272, 3200 (NH_2, NH), 1704, 1669 (CO) cm^{-1}. ^1H nmr (dimethylsulfoxide-d_6): 3.65 (s, 3H, C4'-OCH_3), 3.78 (s, 6H, C3',5'-OCH_3), 6.92 (br.s, 2H, NH_2), 7.18 (s, 2H, C2',6'-H), 8.21 (s, 1H, C5-H), 10.11 (br.s, 1H, CONH), 11.12 (br.s, 1H, CONH); ^{13}C nmr (dimethylsulfoxide-d_6): 56.4, 60.8, 98.2, 117.1, 124.7, 129.1, 134.3, 135.7, 153.3, 155.0, 159.1, 160.6, 171.4.

Anal. Calcd. for $C_{16}H_{16}N_4O_5S$: C, 51.06; H, 4.28; N, 14.89. Found: C, 51.08; H, 4.57; N, 15.11.

N-(4-{[(2-Amino-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)carbonyl]amino}benzoyl)-L-glutamic Acid (**10c**).

The reaction vessel was charged with argon and subsequently loaded with compound **6** (0.5 g, 2.4 mmol), dimethylformamide (25 ml), *N*-(4-aminobenzoyl)-L-glutamic acid diethyl ester (0.92 g, 2.85 mmol), *N,N'*-dicyclohexylcarbodiimide (0.98 g, 4.75 mmol), and 1*H*-1,2,3-benzotriazol-1-ol (0.49 g, 3.6 mmol). The reaction mixture was stirred under argon at room temperature for 7 days. Water (1.5 ml) was added and the reaction mixture was stirred for an additional 1 hour. The precipitate was collected by filtration, the filtrate was poured into water (*ca*. 250 ml). The precipitate was collected by filtration and suspended in 1 *M* sodium hydroxide solution (*ca*. 7 ml). The mixture was stirred at room temperature for 15 min and filtered. The filtrate was roughly acidified with concentrated hydrochloric acid to pH 7 and then adjusted to pH 4 with 5% hydrochloric acid. The precipitate that formed was collected by filtration, washed with water (3 x 5 ml), dried and recrystallised from a mixture of dimethylformamide and water to give 0.47 g (43%) of **10c** as a colorless solid, mp 255° (dec); $[\alpha]_{546}$ = +8 (c = 2, DMF); ee 95%; ir (Nujol): 3409, 3316, 3201, 3102 (NH_2, NH, OH), 1645 (CO) cm^{-1}; ^1H nmr (dimethylsulfoxide-d_6): 1.90-2.16 (m, 2H, CH_2), 2.37 (t, *J* = 7.4 Hz, 2H, CH_2), 4.36-4.44 (m, 1H, CH), 7.01 (br.s, 2H, NH_2), 7.84 (d, *J* = 9.0 Hz, 2H, Ar-H), 7.89 (d, *J* = 9.0 Hz, 2H, Ar-H), 8.28 (s, 1H, C5-H), 8.51 (d, *J* = 7.5 Hz, 1H, CONH), 10.42 (br.s, 1H, CONH), 11.25 (br.s, 1H, CONH), 12.46 (br.s, 2H, CO_2H); ^{13}C nmr (dimethylsulfoxide-d_6): 26.7, 31.2, 52.7, 117.1, 119.7, 125.5, 128.6, 129.0, 129.3, 142.4, 155.2, 159.1, 161.0, 166.7, 171.7, 174.2, 174.6.

Anal. Calcd. for $C_{19}H_{17}N_5O_7S$: C, 49.67; H, 3.73; N, 15.24. Found: C, 49.77; H, 3.71; N, 15.16.

2-Amino-4-methoxy-6-{[(3,4,5-trimethoxyphenyl)amino]-methyl}thieno[2,3-*d*]pyrimidine (**11**).

The reaction vessel was charged with argon and loaded with anhydrous tetrahydrofurane (15 ml) and lithium aluminium hydride (0.36 g, 9.2 mmoles). Compound **9b** (0.36 g, 0.92 mmoles) was added in portions and the reaction mixture was stirred under argon at ambient temperature for 96 hours. Water was added dropwise while stirring until evolution of hydrogen ceased. The solid was collected by filtration and washed with methanol (3 x 15 ml). The solvent from the filtrate was removed under reduced pressure and the crude product was recrystallised from a mixture of acetonitrile and water to give 0.09 g (26%) of **11** as a colorless solid, mp 136-137°; ir (potassium bromide): 3471, 3357, 3331 cm^{-1} (NH_2, NH). ^1H nmr (deuterated chloroform): 3.79 (s, 3H, C4'-OCH_3), 3.83 (s, 6H, C3',5'-OCH_3), 4.03 (s, 3H, C4-OCH_3), 4.07 (t, 1H, J = 4.8 Hz, NH), 4.48 (d, 2H, J = 4.8 Hz, CH_2), 4.99 (br.s, 2H, NH_2), 5.96 (s, 2H, C2',6'-H), 7.08 (s, 1H, C5-H); ^{13}C nmr (chloroform-d): 44.9, 53.9, 56.2, 61.3, 91.2, 112.3, 116.0, 131.0, 135.9, 144.4, 154.2, 160.1, 164.6, 170.7.

Anal. Calcd. for $C_{17}H_{20}N_4O_4S$: C, 54.24; H, 5.36; N, 14.08. Found: C, 54.45; H, 5.60; N, 14.16.

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