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Synthesis of novel 2,4-diamino-6-(arylaminomethyl) thieno[2,3-*d*]pyrimidines as potential antifolates

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Synthesis of 2,4-diamino-6-(arylaminomethyl)thieno[2,3-*d*]pyrimidines as potential lipophilic antifolates has been developed. The synthetic strategy is based on a sequential transformation of readily available ethyl 2-amino-4-chlorothieno[2,3-*d*]pyrimidine-6-caboxylate to 2,4-diaminothieno[2,3-*d*]pyrimidine-6-carbaldehyde, which in the reaction with the corresponding anilines in titanium isopropoxide in the presence of sodium borohydride furnished the title compounds.

Keywords: heterocycles, thienopyrimidines, synthesis, DHFR inhibitors

INTRODUCTION

Being an isoster of biogenic purines, thieno[2,3-d] pyrimidine has been recognised as an important scaffold for the development of biologically active compounds. Many thieno[2,3-d]pyrimidine derivatives have been established to possess a wide range of biological activities like the inhibitory activity of ATP competitive Hsp90 molecular chaperone [1], epidermal growth factor receptor (EGFR) kinase, Fas-activated serine/threonine kinase (FASTK) [2, 3], human farnesyl pyrophosphate synthase [4], and deregulated NRF2 transcriptional activity in cancer [5]. Some derivatives of thieno[2,3-d]pyrimidine show anticancer [6, 7] and antimicrobial activities [8], are promising as negative allosteric modulators of the dopamine D2 receptor [9], cannabinoid receptor type 2 (hCB2) agonists [10], anti-inflammatory and analgesic agents [11], and dihydrofolate reductase (DHFR) inhibitors [12–14]. Potent DHFR inhibitors usually contain a pyrimidine or fused pyrimidine heterocycle moiety, amino groups in positions 2 and 4 of the pyrimidine ring, a methylene or aminomethylene linker between the heterocyclic and aromatic parts of a molecule, and a lipophilic aromatic fragment. Trimetrexate (TMQ), piritrexim (PTX) and trimethoprim (TMP) are approved lipophilic DHFR inhibitors (antifolates) and have been clinically used for the prophylaxis and treatment of *Pneumocystis carinii* and *Toxoplasma gondii* infections in patients with the compromised immune system [15] (Figure).

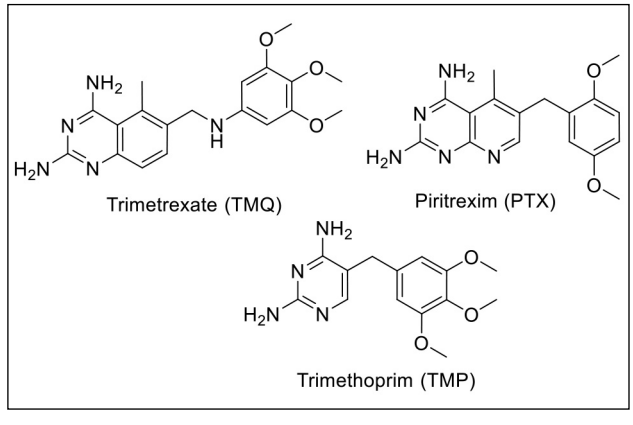

Figure. Structures of lipophilic antifolates

Taking into account these structural features of the known inhibitors and in connection with our program aimed at the development of efficient

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methods for the synthesis of functionalised nitrogen heterocycles [16–19], we present herein the synthesis of novel thieno[2,3-d]pyrimidine-based analogues of lipophilic antifolates.

EXPERIMENTAL

Melting points were determined in open capillaries with a digital melting point IA9100 series apparatus (ThermoFischer Scientific). All reactions and the purity of the synthesised compounds were monitored by TLC using Silica gel 60 F_{254} aluminum plates (Merck). Visualisation was accomplished by UV light. Column chromatography was performed using Silica gel 60 (0.040–0.063 mm) (Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA spectrometer (300 and 75 MHz, respectively). Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Elemental analyses were performed at the Elemental Analysis Laboratory of the Department of Organic Chemistry of Vilnius University.

Initial ethyl 2-amino-4-chlorothieno[2,3-d]pyrimidine-6-carboxylate (**1**) was synthesised from 2-amino-4,6-dichloropyrimidine-5-carbaldehyde by using the procedures described previously [20].

(2-Amino-4-chlorothieno[2,3-*d*]pyrimidin-6-yl) methanol (**2**)**.** Compound **1** (1.83 g, 7.5 mmol) was added portionwise to a suspension of lithium aluminium hydride (0.57 g, 15 mmol) in tetrahydrofuran (100 mL) under argon flow at 0°C (ice bath) over 1.5 h. The reaction mixture was stirred for another 15 min. Sodium sulfate decahydrate was added portionwise until the evolution of hydrogen ceased. The mixture was filtered through a short pad of celite. Filter cake was washed with tetrahydrofuran until the product could not be detected by TLC. The filtrate was concentrated under reduced presure until dryness to give 1.18 g (73%) of **2** as a colourless solid pure enough to use further without purification. The analytical sample was purified by column chromatography (chloroform:acetone, 3:1); m.p. 189–191°C; R_f = 0.28; IR (KBr): 3418, 3315 (NH_2, OH); ^{1}H NMR: 4.66 (dd, J = 5.7 Hz, 1.2 Hz, 2H, CH_2), 5.71 (t, J = 5.7 Hz, 1H, OH), 7.04 (t, J = 1.2 Hz, 1H, C_5-H), 7.20 (br.s, 2H, NH_2); ^{13}C NMR: 59.5, 115.7, 120.6, 141.8, 153.9, 160.6, 171.8.

Anal. calcd. for $C_7H_6ClN_3OS$: C, 38.98; H, 2.80; N, 19.48. Found: C, 38.90; H, 3.23; N, 19.14.

(5-Aminotetrazolo[1,5-*c*]thieno[3,2-*e*]pyrimidin-8-yl)methanol (**4**)**.** A mixture of compound **2** (1.40 g, 6.3 mmol), sodium azide (0.437 g, 6.7 mmol) and ammonium chloride (0.358 g, 6.7 mmol) in dimethyl sulfoxide (26 mL) was stirred at 80°C for 10 h. After cooling to room temperature, the mixture was poured into water (*ca*. 500 mL) and left for several hours in a fridge. The precipitate was filtered off, washed with water and dried to give 1.24 g (86%) of **4** as a colourless solid pure enough to use further without purification. The analytical sample was purified by recrystallisation from a mixture of dimethylformamide and water; m.p. 208°C (dec.); IR (KBr): 3500, 3341 (NH_2, OH); IR (DMSO): 3433, 3300 (NH_2, OH); ^{1}H NMR: 4.75 (d, J = 6.0 Hz, 2H, CH_2), 5.73 (t, J = 6.0 Hz, 1H, OH), 7.51 (s, 1H, C_5-H), 8.51 (br.s, 2H, NH_2); ^{13}C: 59.4, 109.5, 116.3, 142.6, 143.7, 147.7, 159.9.

Anal. calcd. for $C_7H_6N_6OS$: C, 37.83; H, 2.72; N, 37.82. Found: C, 37.91; H, 2.64; N, 37.92.

(2,4-Diaminothieno[2,3-*d*]pyrimidin-6-yl) methanol (**5**). **Method A.** A mixture of **4** (0.100 g, 0.450 mmol) and triphenylphosphine (0.177 g, 0.675 mmol) in dimethylsulfoxide (2 mL) was stired at 80°C for 2.5 h. The reaction mixture was poured into water (*ca*. 20 mL) and left in a fridge for several hours. The resulted precipitate was collected by filtration, washed with water and transferred to a reaction vessel. Dichloromethane (4 mL), conc. hydrochloric acid (0.2 mL) were added and the reaction mixture was refluxed for 10 h. Volatiles were removed under reduced pressure, the remained solid was triturated with water, the resulted suspension neutralised with sat. sodium hydrocarbonate, filtered, washed with water and acetone (3 mL), dried and purified by column chromatography to give 65 mg (74%) of **5** as a yellowish solid, m.p. 225°C (dec.); R_f = 0.30 (chloroform:acetone, 5:3); IR (KBr): 3444, 3377, 3315 (NH_2, OH); ^{1}H NMR: 4.55 (d, J = 5.1 Hz, 2H, CH_2), 5.43 (t, J = 5.1 Hz, 1H, OH), 5.94 (br.s, 2H, NH_2), 6.87 (br.s, 2H, NH_2), 7.15 (s, 1H, C_5-H); ^{13}C NMR: 59.8, 108.9, 116.9, 134.9, 159.3, 161.5, 169.8.

Anal. calcd. for $C_7H_8N_4OS$: C, 42.85; H, 4.11; N, 28.55. Found: C, 42.55; H, 4.08; N, 28.45.

Method B. A mixture of **4** (1.19 g 2.35 mmol) and tin dichloride (2.54 g, 13.4 mmol) in a mixture of 1,4-dioxane (237 mL) and water (71 mL) was refluxed for 1.5 h until a clear solution occurred. The mixture was concentrated to *ca*. 1/20

of the initial volume. 1 *M* solution of sodium hydroxide (*ca*. 2 mL) was added. The precipitate was collected by filtration, washed with water and acetone (3 × 3 mL) and dried to give 0.88 g (84%) of **5** whose properties were identical to those of the product obtained by method A and was pure enough to use in the next step without further purification.

2,4-Diaminothieno[2,3-*d*]pyrimidine-6-carbaldehyde (6). In a suspension of **5** (0.424 g, 2.16 mmol) and 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one (DMP) (1.1 g, 2.6 mmol) in dimethylformamide (5 mL), a drop of water was added and the reaction mixture was stirred at room temperature for 1.5 h. The reactiom mixture was quenched with a solution of sodium thiosulfate (0.41 g, 2.6 mmol) and sodium hydrogen carbonate (0.98 g, 11.7 mmol) in water (*ca*. 100 mL). The precipitate was collected by filtration, washed with water and methanol (3 × 5 mL) and dried to give 0.416 g (99%) of **6** as an off-white amorphous solid which was pure enough to use further without purification, m.p. 175°C (dec.); IR (KBr): 3431, 3326 (NH_2), 1688 (CO); ^{1}H NMR: 6.72 (br.s, 2H, NH_2), 7.50 (br.s, 2H, NH_2), 8.28 (s, 1H, C_5-H), 9.77 (s, 1H, CHO).

Anal. calcd. for $C_7H_6N_4OS$: C, 43.29; H, 3.11; N, 28.85. Found: C, 43.22; H, 3.23; N, 28.76.

2,4-Diamino-6-(arylaminomethyl)thieno[2,3-*d*]pyrimidines (7a–d). General procedure. 2,4-Diaminothieno[2,3-*d*]pyrimidine-6-carbaldehyde (**6**) (0.100 g, 0.515 mmol) and appropriate aniline (1.55 mmol) were added to a two neck round-bottom flask equipped with a magnetic stirrer and a condenser and capped with rubber septa. The flask was flushed with argon, titanium tetraisopropoxide was syringed (3 mL) and the reaction mixture was heated at 150°C (oil bath) for 8 h. The reaction mixture was cooled to room temperature, dry tetrahydrofuran (12 mL) was syringed and sodium borohydride (0.195 g, 5.15 mmol) was added under argon flow. Methanol was added dropwise (*ca*. a drop every 15 min) during 1–2 days by means of a syringe while stirring at room temperature. 0.1 *M* solution of sodium hydroxide (2 mL) was added, the suspension was filtered through a short pad of celite. A filter cake was washed with methanol until the product could not be detected by TLC. The filtrate was concentrated under reduced pressure, and residual water was removed by azeotropic distillation with 2-propanol under reduced pressure. The remained solid was purified by column chromatography, the products were dried in a Fisher drying pistol and stored under argon.

2,4-Diamino-6-[(4-methoxyphenylamino)methyl]thieno[2,3-*d*]pyrimidine (7a). Gray solid, yield 48 mg (31%); m.p. 152–153°C (dec.); R_f = 0.26 (chloroform:methanol, 9:1); IR (KBr): 3449, 3317 (NH_2, NH); ^{1}H NMR: 3.38 (s, 3H, OCH_3), 4.30 (d, *J* = 4.8 Hz, 2H, CH_2), 5.81 (t, *J* = 4.8 Hz, 1H, NH), 5.93 (br.s, 2H, NH_2), 6.60 (d, *J* = 9.3 Hz, 2H, $C_{2',6'}$-H), 6.72 (d, *J* = 9.3 Hz, 2H, $C_{3',5'}$-H), 6.86 (br.s, 2H, NH_2), 7.21 (s, 1H, C_5-H); ^{13}C NMR: 44.6, 56.0, 109.1, 114.3, 115.2, 117.1, 133.8, 143.3, 151.8, 159.1, 161.4, 169.4.

Anal. calcd. for $C_{14}H_{15}N_5OS$: C, 55.80; H, 5.02; N, 23.24. Found: C, 55.93; H, 4.97; N, 23.36.

2,4-Diamino-6-[(2,5-dimethoxyphenylamino)methyl]thieno[2,3-*d*]pyrimidine (7b). Gray solid, yield 61 mg (36%); m.p. 213°C (dec.); R_f = 0.30 (chloroform:methanol 10:1); IR (KBr): 3475, 3445, 3413, 3363, 3318 (NH_2, NH); ^{1}H NMR: 3.61 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.39 (d, *J* = 5.7 Hz, 2H, CH_2), 5.65 (t, *J* = 5.7 Hz, 1H, NH), 5.97 (br.s, 2H, NH_2), 6.09 (dd, *J* = 8.6 Hz, *J* = 3.0 Hz, 1H, $C_{4'}$-H), 6.15 (d, *J* = 3.0, 1H, $C_{6'}$-H), 6.71 (d, *J* = 8.6 Hz, 1H, $C_{3'}$-H), 6.89 (br.s, 2H, NH_2), 7.22 (s, 1H, C_5-H); ^{13}C NMR: 43.5, 55.6, 56.6, 98.7, 99.1, 109.1, 111.1, 117.2, 133.4, 139.2, 141.9, 154.8, 159.0, 159.3, 161.3.

Anal. calcd. for $C_{15}H_{17}N_5O_2S$: C, 54.36; H, 5.17; N, 21.13. Found: C, 54.51; H, 5.22; N, 21.03.

2,4-Diamino-6-[(4-chlorophenylamino)methyl]thieno[2,3-*d*]pyrimidine (7c). Gray solid, yield 54 mg (34%); m.p. 196–199°C (dec.); R_f = 0.27 (chloroform: methanol 9:1); IR (KBr): 3503, 3406, 3317 (NH_2, NH); ^{1}H NMR: 4.35 (d, *J* = 5.7 Hz, 2H, CH_2), 5.98 (br.s, 2H, NH_2), 6.48 (t, *J* = 5.7 Hz, 1H, NH), 6.64 (d, *J* = 8.7 Hz, 2H, $C_{2',6'}$-H), 6.89 (br.s, 2H, NH_2), 7.11 (d, *J* = 9.3 Hz, 2H, $C_{3',5'}$-H), 7.21 (s, 1H, C_5-H); ^{13}C NMR: 43.6, 109.1, 114.5, 117.5, 120.2, 129.3, 132.6, 147.9, 159.1, 161.4, 169.5.

Anal. calcd. for $C_{13}H_{12}ClN_5S$: C, 51.06; H, 3.96; Cl, 11.59; N, 22.90. Found: C. 51.24; H, 4.05; N, 23.02.

2,4-Diamino-6-[(2,5-dichlorophenylamino)methyl]thieno[2,3-*d*]pyrimidine (7d). Gray solid, yield 16 mg (9%); m.p. 193–195°C (dec.); R_f = 0.38 (chloroform:methanol 9:1); IR (KBr): 3412, 3395, 3364, 3321 (NH_2, NH); ^{1}H NMR: 4.52 (d, *J* = 6.0 Hz, 2H, CH_2), 5.98 (br.s, 2H, NH_2), 6.47 (t, *J* = 6.0 Hz, 1H, NH), 6.63 (dd, *J* = 8.4 Hz, *J* = 2.4 Hz, 1H, $C_{4'}$-H),

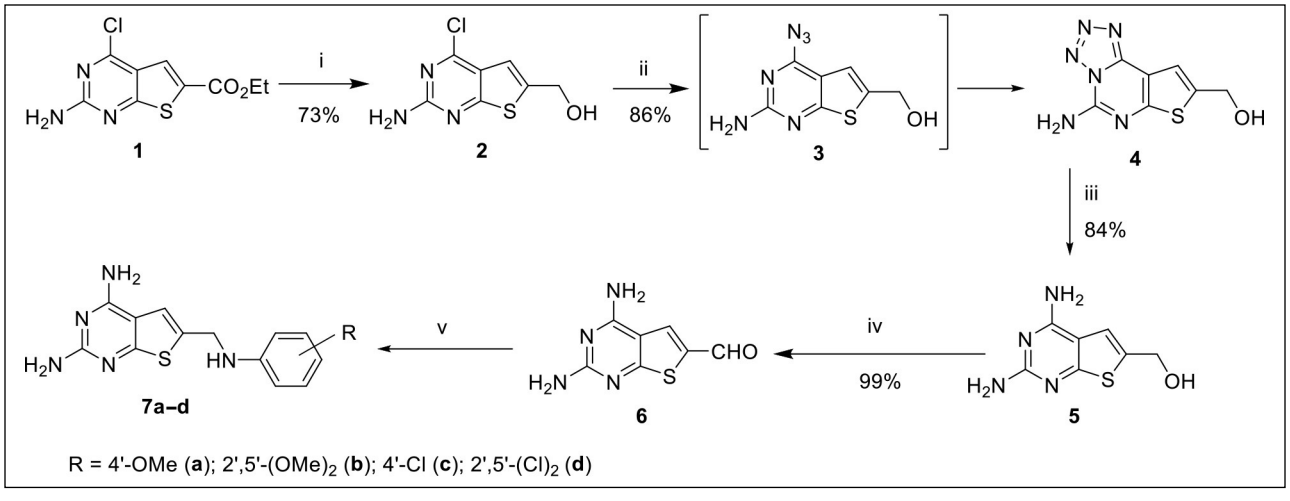
6.69 (d, J = 2.4 Hz, 1H, $C_{3'}$-H), 6.91 (br.s, 2H, NH_2), 7.30 (d, J = 8.4 Hz, 1H, $C_{6'}$-H); ^{13}C NMR: 43.1, 109.1, 111.6, 116.9, 117.4, 117.5, 130.9, 131.8, 133.1, 145.5, 159.1, 161.5, 169.5.

Anal. calcd. for $C_{13}H_{11}Cl_2N_5S$: C, 45.89; H, 3.26; N, 20.58. Found: C, 45.99; H, 3.21; N, 20.45.

RESULTS AND DISCUSSION

For the synthesis, an easily accessible ethyl 2-amino-4-chlorothieno[2,3-*d*]pyrimidine-5-carboxylate (**1**) [20] was used as a starting material. Compound **1** smoothly reacted with lithium aluminium hydride in tetrahydrofuran at 0°C to give alcohol **2** in 73% yield (Scheme). The latter under the treatment with sodium azide in dimethylsulfoxide in the presence of ammonium chloride furnished the corresponding tetrazolothienopyrimidine **4**. It is known that azides, in which the azido group is adjacent to a nitrogen atom, can undergo spontaneous cyclisation to form a tetrazole ring. The azide-tetrazole equilibrium is often observed in π-deficient heterocycles such as azidopyrimidines [21, 22], azidopurines [23] and azido-7-deazapurines [24]. The obtained tetrazolothienopyrimidine **4** was easily distinguished from its azido tautomer **3** by spectral data. In the ^{1}H NMR spectra of **4** only one set of signals was observed in deuterio dimethylsulfoxide and its IR spectra recorded in dimethylsulfoxide solution and in a solid state (KBr) did not contain a specific azido group absorption band.

For the conversion of a tetrazole ring to an amino group several methods were attempted. Compound **3** appeared to be inert towards sodium borohydride or lithium aluminium hydride. The reduction with zinc in acetic acid or Raney nickel in ethanol resulted in the formation of complex mixtures of unidentified products. Nevertheless, the Staudinger reaction of **4** with triphenylphosphine and the subsequent hydrolysis of the phosphine imine intermediate with aqueous hydrochloric acid were successful and afforded 2,4-diaminothieno[2,3-*d*]pyrimidine **5** in 74% yield. However, compound **5** showed a very poor solubility in various solvents and for its purification column chromatography was required. Thus, the method is convenient enough for the preparation of compound **5** in a milligram scale. For the synthesis of compound **5** in a larger scale, another more efficient method was developed. The reduction of tetrazolothienopyrimidine **4** with stannous chloride in a mixture of 1,4-dioxane and water gave the desired (2,4-diaminothieno[2,3-*d*]pyrimidin-6-yl)methanol (**5**) in the high 84% yield. Then, compound **5** reacted with Dess–Martin periodinane (DMP) to give carbaldehyde **6** in a quantitative yield. To carry out the reductive amination of **6** several techniques were attempted. Namely, the treatment of a mixture of carbaldehyde and aniline with sodium cianoborohydride in methanol or *p*-toluenesulfonic acid in toluene under reflux or titanium tetrachloride in toluene or dichloromethane as well as L-proline in dimethylsulfoxide did not afford the desirable compounds. The formation of imine intermediate seems



Scheme. Reagents and conditions: i – $LiAlH_4$, THF, 0°C, 1.5 h; ii – NaN_3, NH_4Cl, DMSO, 80°C, 10 h; iii – method A: Ph_3P, DMSO, 80°C, 2.5 h; 2. concd. HCl, dichloromethane, reflux, 10 h; method B: $SnCl_2$, 1,4-dioxane, H_2O, reflux, 1.5 h; iv – DMP, DMF, H_2O cat., r.t., 1.5 h; v – 1. aniline, Ti(OPr-iso)$_4$, 150°C, 8 h; 2. $NaBH_4$, THF, CH_3OH, r.t., 1–2 days

to be a limiting factor in the synthesis of **7**. The reductive amination of carbaldehyde **6** to give **7a–d** was successfully accomplished by heating **6** with the corresponding arylamine in titanium isopropoxide at 150°C, and the subsequent reduction of intermediates with sodium borohydride in the presence of methanol at room temperature.

The investigation of the synthesised compounds **7a–d** for the possible inhibition of *P. carinii*, *T. gondii* and *Mycobacterium avium* DHFR is under progress and will be reported elsewhere.

CONCLUSIONS

In summary, the synthesis of 2,4-diamino-6-(arylaminomethyl)thieno[2,3-*d*]pyrimidines as potential DHFR inhibitors has been developed. The proposed reaction pathway includes efficient transformations of functional groups to form 2,4-diaminothieno[2,3-*d*]pyrimidine-6-carbaldehyde and its reductive amination reaction with the substituted anilines in titanium tetraisopropoxide and sodium borohydride in the presence of methanol to furnish the target compounds. The obtained results can be useful for the development of the libraries of novel lipophilic antifolates among pyrimidine nucleus containing heterocycles.

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Mindaugas Dailidė, Sigitas Tumkevičius

NAUJŲ 2,4-DIAMINO-6-(ARILAMINOMETIL) TIENO[2,3-*D*]PIRIMIDINŲ, POTENCIALIŲ ANTIFOLATŲ, SINTEZĖ

S a n t r a u k a

Dihidrofolato reduktazės (DHFR) slopinančio aktyvumo tyrimams susintetinti 2,4-diamino-6-(arilaminometil)tieno[2,3-*d*]pirimidino dariniai. Pasiūlytas sintezės kelias susideda iš etil-2-amino-4-chlortieno[2,3-*d*]pirimidin-6-kaboksilato redukcijos reakcijos, gauto chlordarinio reakcijos su natrio azidu, susidarant 5-aminotetrazolo[1,5-*c*]tieno[3,2-*e*]pirimidin-8-il) metanoliui, pastarojo redukcijos alavo dichloridu reakcijos, ir gauto (2,4-diaminotieno[2,3-*d*]pirimidin-6-il)metanolio oksidacijos Dess-Martin periodinanu iki atitinkamo tieno[2,3-*d*]pirimidin-6-karbaldehido bei pastarojo redukcinio amininimo reakcija su pakeistais anilinais, naudojant natrio borhidridą ir titano triizopropoksidą. Gauti rezultatai gali būti naudingi, kuriant naujus lipofilinius antifolatus pirimidino fragmentą turinčių heterociklų pagrindu.