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Synthesis and Cytotoxic Activity of Some Novel Thieno[2,3-*d*:4,5-*d'*]Dipyrimidine Derivatives

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Abstract

Synthesis of some novel tricyclic compounds bearing thienodipyrimidine moiety by the reaction of ethyl 6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**1**) with different types of aromatic amines to give the corresponding carboxamide derivatives **2–11**, which cyclized with triethyl orthoformate in acetic anhydride to afford thieno[2,3-*d*:4,5-*d'*]dipyrimidine derivatives **12–15** is described. Similarly, thieno[2,3-*d*:4,5-*d'*]dipyrimidine derivative **17** was also prepared *via* the reaction of carboxamide derivative **2** with chloroacetyl chloride in chloroform to give thieno[3,2-*d*]pyrimidine-7-carboxamide derivative **16**, followed by a cyclization in boiling acetic anhydride. The structure of these compounds was confirmed on the basis of their spectral and analytical data such as IR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectral data. The synthesized compounds were screened for their cytotoxic activity.

Keywords: Thienopyrimidine; thienodipyrimidine; cytotoxic activity

1. Introduction

Thienopyrimidines represent structural analogues for biogenic purines and potential nucleic acid antimetabolites.[1] In particular, they are currently an important group of compounds that display anticancer activity, especially against solid tumors (*e.g.*, breast and ovarian).[2–4] On the other hand, the classes of tricyclic compounds bearing thienopyrimidine scaffolds have been the focus of great interest because of their pharmacological activities, such as antitumor,[5–9] anti-allergic, anti-inflammatory, analgesic, antifungal and antibacterial activities.[10–20] Consequently, thienopyrimidines have become an important class of compounds in drug discovery programs and the medicinal chemistry.[21–25]

In continuation of our previous work on biologically active nitrogen and sulfur heterocycles,[26–29] it was of interest to synthesize some novel thieno[2,3-*d*:4,5-*d*`]dipyrimidine derivatives and evaluate these compounds for their cytotoxic activity.

2. Experimental

All melting points are uncorrected and were determined on Gallenkamp electric melting point device. Pre-coated Merck silica gel 60F-254 plates were utilized for thin-layer chromatography (TLC) and the spots were visualized under UV light (254 nm). The infrared (IR) spectra were recorded (KBr disk) on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron Co. Egelsbach, Germany. The ^{1}H-NMR spectra were obtained on Varian Gemini 400 MHz (Varian Co., Cairo university, Egypt), and ^{13}C-NMR on the same instrument at 100 MHz. Deuterated DMSO-d_6 was used as the solvent, tetramethylsilane (TMS) was used as the internal standard and chemical shifts were measured in δ ppm. Mass spectra were determined on a GC-MS.QP-100 EX Shimadzu (Japan). Elemental analyses were recorded on Perkin-Elmer 2400 Elemental analyzer at the Micro-analytical Center at Cairo University, Cairo, Egypt.

General Procedure for the Reaction of Compound 1 with Different Aromatic Amines

An equimolar amount of compound **1** (2.83 g, 0.01 mol) and different aromatic amines namely, 2-aminopyridine (0.94 g, 0.01 mol), 4-aminobenzenesulfonamide (1.72 g, 0.01 mol), 2-aminothiazole (1.00 g, 0.01 mol), 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide (2.55 g, 0.01 mol), 4-aminoantipyrine (2.03 g, 0.01 mol), creatinine (1.13 g,

0.01 mol), 6-amino-2-thioxo-4-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (2.44 g, 0.01 mol), ethyl 5-acetyl-4-amino-2-(phenylamino)thiophene-3-carboxylate (3.04 g, 0.01 mol), 2-amino-4-hydroxyquinoline-3-carbonitrile (1.85 g, 0.01 mol), or 2,6-diaminopyridine (1.09 g, 0.01 mol), in the presence of freshly fused AcONa were heated on an oil bath for 1 h, then dil. HCl was added to the mixture, the formed solids were collected by filtration, washed with water, dried and crystallized from EtOH/DMF to give compounds **2**–**11**, respectively.

6-Amino-1,3-dimethyl-2,4-dioxo-N-(pyridin-2-yl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (2)

Deep brown crystals; yield (68%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3435–3415 (NH_2), 3385 (NH), 1671 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.20 (s, 6H, 2*N*-CH_3), 7.34 (s, 2H, NH_2), 7.22–8.36 (m, 4H, Ar-H), 10.67 (s, 1H, CONH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.5, 30.3, 114.5, 116.8, 118.5, 121.2, 136.4, 143.2, 145.7, 146.6, 151.4, 155.6, 166.8, 171.6; MS (EI, 70 eV) *m/z* = 331 (M^+). *Anal. Calcd for* $C_{14}H_{13}N_5O_3S$ (331.35): C, 50.75; H, 3.95; N, 21.14; S, 9.68. Found: C, 50.71; H, 3.89; N, 21.09; S, 9.64.

6-Amino-1,3-dimethyl-2,4-dioxo-N-(4-sulfamoylphenyl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (3)

Brown powder; yield (78%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3476–3453 (2NH_2), 3343 (NH), 1653 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.24 (s, 6H, 2*N*-CH_3), 6.84 (s, 2H, NH_2), 7.33 (s, 2H, NH_2), 7.61 (d, 2H, *J* = 7.50 Hz, Ar-H), 8.25 (d, 2H, *J* = 7.50 Hz, Ar-H), 10.67 (s, 1H, CONH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.6, 30.5, 114.3, 117.5, 121.4, 131.4, 136.3, 143.5, 145.2, 151.8, 155.2, 166.7, 171.5; MS (EI, 70 eV) *m/z* = 410 (M^++1). *Anal. Calcd for* $C_{15}H_{15}N_5O_5S_2$ (409.44): C, 44.00; H, 3.69; N, 17.11; S, 15.66. Found: C, 44.68; H, 3.64; N, 17.15; S, 15.61.

6-Amino-1,3-dimethyl-2,4-dioxo-N-(thiazol-2-yl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (4)

Brown powder; yield (67%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3446–3422 (NH_2), 3381 (NH), 1681 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.25 (s, 6H, 2*N*-CH_3), 7.35 (s, 2H, NH_2), 7.42 (d, 1H, *J* = 7.10 Hz, Ar-H), 7.58 (d, 1H, *J* = 7.10 Hz, Ar-H), 10.67 (s, 1H, CONH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.5, 30.4, 114.7, 118.9, 121.2, 138.4, 143.3, 151.7, 155.5, 160.8, 166.5, 171.3; MS (EI, 70 eV) *m/z* = 337 (M^+). *Anal. Calcd for* $C_{12}H_{11}N_5O_3S_2$ (337.37): C, 42.72; H, 3.29; N, 20.76; S, 19.01. Found: C, 42.67; H, 3.23; N, 20.72; S, 18.94.

6-Amino-1,3-dimethyl-2,4-dioxo-N-(4-(N-(thiazol-2-yl)sulfamoyl)phenyl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (5)

Brown powder; yield (62%); mp 283–284 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3456–3475 (NH_2), 3234 (2NH), 1627 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.21 (s, 6H, 2 *N*-CH_3), 7.36 (s, 2H, NH_2), 7.40 (d, 1H, *J* = 7.10 Hz, thiazole H_4), 7.55 (d, 1H, *J* = 7.10 Hz, thiazole H_5), 7.72 (d, 2H, *J* = 7.50 Hz, Ar-H), 8.28 (d, 2H, *J* = 7.50 Hz, Ar-H), 10.66, 11.84 (s, 2H, 2NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.7, 30.6, 114.7, 117.3, 118.6, 121.5, 131.5, 136.4, 138.9, 143.6, 146.2, 151.6, 155.7, 166.4, 168.6, 171.6; MS (EI, 70 eV) *m/z* = 492 (M^+). *Anal. Calcd for* $C_{18}H_{16}N_6O_5S_3$ (492.54): C, 43.89; H, 3.27; N, 17.06; S, 19.53. Found: C, 43.81; H, 3.23; N, 16.98; S, 19.44.

6-Amino-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (6)

Brown powder; yield (78%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3447–3423 (NH_2), 3384 (NH), 1680, 1650 (4C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.34 (s, 3H, CH_3), 3.21 (s, 6H, 2 *N*-CH_3), 3.26 (s, 3H, *N*-CH_3), 7.43 (s, 2H, NH_2), 7.27–7.70 (m, 5H, Ar-H), 10.64 (s, 1H, NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 14.6, 27.4, 30.3, 32.8, 108.4, 114.5, 121.6, 122.8, 124.9, 127.7, 130.2, 137.3, 143.4, 151.8, 155.7, 163.7, 166.8, 171.5; MS (EI, 70 eV) *m/z* = 441 (M^++1). *Anal. Calcd for* $C_{20}H_{20}N_6O_4S$ (440.48): C, 54.54; H, 4.58; N, 19.08; S, 7.28. Found: C, 54.49; H, 4.60; N, 18.99; S, 7.25.

6-Amino-1,3-dimethyl-N-(1-methyl-4-oxo-4,5-dihydro-1H-imidazol-2-yl)-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (7)

Brown powder; yield (60%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3447–3422 (NH_2), 3249 (NH), 1637 (4C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.16 (s, 3H, *N*-CH_3), 3.19 (s, 3H, *N*-CH_3), 3.22 (s, 3H, *N*-CH_3), 3.60 (s, 2H, imidazole H_5), 7.31 (s, 2H, NH_2), 9.51 (s, 1H, NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.6, 30.3, 38.3, 61.6, 114.6, 121.5, 143.4, 151.8, 153.2, 155.6, 163.8, 166.7, 171.4; MS (EI, 70 eV) *m/z* = 351 (M^++1). *Anal. Calcd for* $C_{13}H_{14}N_6O_4S$ (350.35): C, 44.57; H, 4.03; N, 23.99; S, 9.15. Found: C, 44.56; H, 4.03; N, 23.97; S, 9.13.

6-Amino-N-(5-cyano-2-thioxo-6-(p-tolyl)hexahydropyrimidin-4-yl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (8)

Brown powder; yield (69%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3447–3422 (NH_2), 3380, 3249 (3NH), 2218 (CN), 1641 (3C=O) 1383 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.18 (s, 3H, CH_3), 3.13 (m, 1H, pyrimidine H_5), 3.21 (s, 6H, 2*N*-CH_3), 3.67 (d,

1H, *J* = 7.50 Hz, pyrimidine H_6), 3.77 (d, 1H, *J* = 7.50 Hz, pyrimidine H_4), 7.68 (s, 2H, NH_2), 7.50 (d, 2H, *J* = 6.90 Hz, Ar-H), 7.89 (d, 2H, *J* = 6.90 Hz, Ar-H), 8.96, 10.15, 10.36 (s, 3H, 3NH, D_2O exchangeable); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.3, 27.4, 30.5, 45.6, 58.8, 78.4, 114.8, 117.6, 121.5, 126.4, 130.3, 134.2, 140.3, 143.4, 151.6, 155.3, 166.7, 171.5, 176.8; MS (EI, 70 eV) *m/z* = 483 (M^+). *Anal. Calcd for* $C_{21}H_{21}N_7O_3S_2$ (483.57): C, 52.16; H, 4.38; N, 20.28; S, 13.26. Found: C, 52.08; H, 4.32; N, 20.21; S, 13.18.

Ethyl 5-Acetyl-4-(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamido)-2-(phenylamino)thiophene-3-carboxylate (9)

Brown powder; yield (71%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3446–3418 (NH_2), 3221 (2NH), 1703, 1683, 1641 (5C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.33 (t, 3H, *J* = 7.10 Hz, CH_3), 3.14 (s, 6H, 2*N*-CH_3), 3.77 (s, 3H, $COCH_3$), 4.43 (q, 2H, *J* = 7.10 Hz, CH_2), 7.47 (s, 2H, NH_2), 7.27–7.58 (m, 5H, Ar-H), 9.31, 10.25 (s, 2H, 2NH, D_2O exchangeable); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 14.6, 26.2, 27.8, 30.6, 61.3, 114.3, 118.6, 121.4, 124.3, 126.6, 128.4, 139.5, 140.6, 143.5, 145.3, 151.6, 155.7, 160.2, 162.3, 166.3, 171.4, 186.3; MS (EI, 70 eV) *m/z* = 541 (M^+). *Anal. Calcd for* $C_{24}H_{23}N_5O_6S_2$ (541.60): C, 53.22; H, 4.28; N, 12.93; S, 11.84. Found: C, 53.18; H, 4.25; N, 12.88; S, 11.77.

6-Amino-N-(3-cyano-4-hydroxyquinolin-2-yl)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (10)

Brown powder; yield (64%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3417–3331 (NH_2, OH), 3222 (NH), 2219 (CN), 1676 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.21 (s, 6H, 2*N*-CH_3), 7.37 (s, 2H, NH_2), 7.68–8.46 (m, 4H, Ar-H), 10.21 (s, 1H, OH), 10.67 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.6, 30.4, 79.8, 110.6, 114.5, 117.3, 120.0, 121.6, 124.6, 126.4, 134.3, 143.8, 148.6, 150.2, 151.7, 155.9, 161.4, 166.2, 171.3; MS (EI, 70 eV) *m/z* = 422 (M^+). *Anal. Calcd for* $C_{19}H_{14}N_6O_4S$ (422.42): C, 54.02; H, 3.34; N, 19.90; S, 7.59. Found: C, 53.96; H, 3.38; N, 19.84; S, 7.51.

N,N'-(Pyridine-2,6-diyl)bis(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide) (11)

Reddish brown powder; yield (61%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3574–3475 (2NH_2), 3368 (2NH), 1687 (6C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.21 (s, 6H, 2*N*-CH_3), 3.25 (s, 6H, 2*N*-CH_3), 7.46 (s, 4H, 2NH_2), 7.21–7.64 (m, 3H, Ar-H), 10.74 (s, 2H, 2CONH, D_2O exchangeable); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.8, 30.5, 114.2, 116.8, 121.3, 136.6, 143.5, 145.4, 151.6, 155.7, 166.5, 171.3; MS (EI, 70 eV) *m/z* = 585 (M^++2). *Anal. Calcd for* $C_{23}H_{21}N_9O_6S_2$ (583.60): C, 47.34; H, 3.63; N, 21.60; S, 10.99. Found: C, 47.28; H, 3.59; N, 21.55; S, 10.92.

General Procedure for the Reaction of Compounds 2, 4, 5, 7 and 1 with Triethyl Orthoformate

To a solution of compound **2** (3.31 g, 0.01 mol), compound **4** (4.09 g, 0.01 mol), compound **5** (3.37 g, 0.01 mol), compound **7** (4.40 g, 0.01 mol) or compound **1** (2.83 g, 0.01 mol) in acetic anhydride (20 mL) triethyl orthoformate (1.48 g, 0.01 mol) was added. The reaction mixture was heated on water bath for 8–10 hours, then poured onto iced water and the formed solid product was collected by filtration, and crystallized from EtOH/DMF to give compounds **12**–**15** and **23**, respectively.

1,3-Dimethyl-8-(pyridin-2-yl)thieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1H,3H,8H)-trione (12)

Brownish blue powder; yield (67%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1667 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 7.26–8.46 (m, 4H, Ar-H), 8.56 (s, 1H, pyrimidine-H_7); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 28.2, 30.3, 114.4, 118.3, 122.6, 130.3, 136.5, 143.2, 145.3, 146.6, 148.5, 151.6, 155.5, 166.4, 168.6; MS (EI, 70 eV) *m/z* = 341 (M^+). *Anal. Calcd for* $C_{15}H_{11}N_5O_3S$ (341.35): C, 52.78; H, 3.25; N, 20.52; S, 9.39. Found: C, 52.74; H, 3.22; N, 20.46; S, 9.41.

4-(1,3-Dimethyl-2,4,9-trioxo-1,2,3,4-tetrahydrothieno[2,3-d:4,5-d']dipyrimidin-8(9H)-yl)benzenesulfonamide (13)

Yellowish brown powder; yield (70%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3476–3445 (NH_2), 1668 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 6.82 (s, 2H, NH_2), 7.63 (d, 2H, *J* = 7.10 Hz, Ar-H), 8.16 (d, 2H, *J* = 7.10 Hz, Ar-H), 8.54 (s, 1H, pyrimidine-H_7); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.5, 30.6, 114.5, 117.3, 130.8, 131.9, 136.8, 143.7, 145.2, 148.3, 151.7, 155.4, 166.5, 168.8; MS (EI, 70 eV) *m/z* = 419 (M^+). *Anal. Calcd for* $C_{16}H_{13}N_5O_5S_2$ (419.43): C, 45.82; H, 3.12; N, 16.70; S, 15.29. Found: C, 45.73; H, 3.06; N, 16.63; S, 15.22.

4-(1,3-Dimethyl-2,4,9-trioxo-1,2,3,4-tetrahydrothieno[2,3-d:4,5-d']dipyrimidin-8(9H)-yl)-N-(thiazol-2-yl)benzenesulfonamide (14)

Gray crystals; yield (68%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3245 (NH), 1667 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 7.45 (d, 1H, *J* = 7.10 Hz, thiazole H_4), 7.62 (d, 1H, *J* = 7.10 Hz, thiazole H_5), 7.74 (d, 2H, *J* = 7.50 Hz, Ar-H), 8.24 (d, 2H, *J* = 7.50 Hz, Ar-H), 8.52 (s, 1H, pyrimidine-H_7), 11.68 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.8, 30.4, 114.8, 116.5, 118.8, 130.6, 131.7, 136.4, 138.3, 143.8, 145.2, 148.1, 151.5, 155.3, 166.7, 168.5, 170.2; MS (EI, 70 eV) *m/z* = 502 (M^+). *Anal. Calcd for* $C_{19}H_{14}N_6O_5S_3$ (502.54): C, 45.41; H, 2.81; N, 16.72; S, 19.14. Found: C, 45.32; H, 2.73; N, 16.66; S, 19.06.

8-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-1,3-dimethylthieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1H,3H,8H)-trione (15)

Red powder ; yield (72%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1667, 1655 (4C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.26 (s, 3H, CH_3), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.23 (s, 3H, *N*-CH_3), 7.38–7.78 (m, 5H, Ar-H), 8.51 (s, 1H, pyrimidine-H_7); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 14.6, 27.6, 30.5, 35.8, 105.6, 114.3, 118.1, 122.8, 128.6, 130.5, 131.7, 136.5, 143.3, 148.4, 151.6, 155.8, 163.7, 166.8, 168.6; MS (EI, 70 eV) *m/z* = 450 (M^+). *Anal. Calcd for* $C_{21}H_{18}N_6O_4S$ (450.47): C, 55.99; H, 4.03; N, 18.66; S, 7.12. Found: C, 55.97; H, 3.97; N, 18.59; S, 7.06.

Ethyl 6-((Ethoxymethylene)amino)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxylate (23).

Yellowish brown crystals; yield (63%); mp 123–125 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1708, 1659 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.22 (t, 3H, *J* = 7.50 Hz, $CH_2\underline{CH_3}$), 2.93 (t, 3H, *J* = 7.10 Hz, $CH_2\underline{CH_3}$), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.71 (q, 2H, *J* = 7.10 Hz, $\underline{CH_2}CH_3$), 4.15 (q, 2H, *J* = 7.50 Hz, $\underline{CH_2}CH_3$), 8.45 (s, 1H, N=CH); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.5, 18.9, 27.5, 30.4, 67.6, 68.7, 115.4, 123.4, 143.5, 151.8, 155.6, 158.5, 163.4, 169.5; MS (EI, 70 eV) *m/z* = 340 (M^++1). *Anal. Calcd for* $C_{14}H_{17}N_3O_5S$ (339.37): C, 49.55; H, 5.05; N, 12.38; S, 9.45. Found: C, 49.48; H, 4.97; N, 12.31; S, 9.37.

Synthesis of 6-(2-Chloroacetamido)-1,3-dimethyl-2,4-dioxo-N-(pyridin-2-yl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (16)

To a solution of compound **2** (3.31 g, 0.01 mol) in chloroform (25 mL) chloroacetyl chloride (1.12 g, 0.01 mol) was added at room temperature overnight. The reaction mixture was concentrated under reduced pressure and cooled. The obtained solid product was collected by filtration, dried and crystallized from EtOH/DMF to give compound **16.** Purple powder; yield (88%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3421 (2NH), 1698, 1671 (4C=O), 756 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.65 (s, 2H, CH_2), 7.24–8.44 (m, 4H, Ar-H), 10.67, 11.54 (s, 2H, 2NH); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.4, 30.5, 51.6, 114.3, 116.8, 118.6, 121.3, 136.5, 143.2, 145.7, 146.5, 151.6, 155.3, 166.9, 168.4, 178.8; MS (EI, 70 eV) *m/z* = 407 (M^+). *Anal. Calcd for* $C_{16}H_{14}ClN_5O_4S$ (407.83): C, 47.12; H, 3.46; N, 17.17; S, 7.86. Found: C, 47.11; H, 3.40; N, 17.09; S, 7.82.

Synthesis of 7-(Chloromethyl)-1,3-dimethyl-8-(pyridin-2-yl)thieno[2,3-d:4,5-d']dipyrimidine-2,4,9-(1H,3H,8H)-trione (17)

A solution of compound **16** (4.07 g, 0.01 mol) in acetic anhydride (20 mL) was refluxed for 6 h (TLC, mob. phase petroleum ether/ethyl acetate (8:2)). The reaction mixture was poured onto crushed ice water and the solid separated was collected by filtration, dried and crystallized from EtOH/DMF to give compound **17**. Pink powder; yield (78%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1657, 1645 (3C=O), 760 (C–Cl); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 4.45 (s, 2H, CH_2), 7.32–8.45 (m, 4H, Ar-H); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.8, 30.6, 48.5, 114.5, 118.3, 122.2, 130.4, 136.5, 143.2, 145.3, 146.7, 151.4, 155.8, 158.5, 164.8, 168.3; MS (EI, 70 eV) *m/z* = 389 (M^+). *Anal. Calcd for* $C_{16}H_{12}ClN_5O_3S$ (389.81): C, 49.30; H, 3.10; N, 17.97; S, 8.22. Found: C, 49.24; H, 3.06; N, 17.92; S, 8.17.

General Procedure for the Reaction of Compound 16 with Piperidine and Morpholine

To an equimolar amount of **16** (4.07 g, 0.01 mol) and piperidine (0.85 g, 0.01 mol) or morpholine (0.87 g, 0.01 mol) in dry xylene (20 mL) DMF (5 drops) was added. The reaction mixture was refluxed for 3 h, then poured onto iced water and the formed solid products, in each case, were collected by filtration and crystallized from EtOH/DMF to give compounds **18** and **19**, respectively.

1,3-Dimethyl-2,4-dioxo-6-(2-(piperidin-1-yl)acetamido)-N-(pyridin-2-yl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (18)

Greenish brown crystals; yield (64%); mp 270–272 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3435 (2NH), 1665 (4C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.13–1.45 (m, 6H, $3CH_2$), 2.27 (t, 4H, 2*N*CH_2), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.46 (s, 2H, $COCH_2N$), 7.29–8.56 (m, 4H, Ar-H), 10.86, 11.46 (s, 2H, 2NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 22.3, 25.5, 27.8, 30.7, 54.3, 67.6, 114.5, 116.2, 118.6, 121.4, 136.3, 143.3, 145.3, 146.5, 151.8, 155.6, 166.7, 168.5, 178.6; MS (EI, 70 eV) *m/z* = 456 (M^+). *Anal. Calcd for* $C_{21}H_{24}N_6O_4S$ (456.52): C, 55.25; H, 5.30; N, 18.41; S, 7.02. Found: C, 55.19; H, 5.27; N, 18.33; S, 6.96.

1,3-Dimethyl-6-(2-morpholinoacetamido)-2,4-dioxo-N-(pyridin-2-yl)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxamide (19)

Brown crystals; yield (61%); mp 258–260 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3433 (2NH), 1656 (4C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.29 (t, 4H, *J* = 6.88 Hz, 2*N*CH_2), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.47 (s, 2H, $COCH_2N$), 3.78 (t, 4H, *J* = 6.88 Hz, 2*O*CH_2), 7.31–8.44 (m, 4H, Ar-H), 10.87, 11.42 (s, 2H, 2NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.4, 30.6, 54.6, 65.3, 67.8, 114.3, 116.7, 118.8, 121.5, 136.5, 143.4, 145.5, 146.7, 151.3, 155.5, 166.5, 168.6, 177.2; MS (EI, 70 eV) *m/z* = 458 (M^+). *Anal. Calcd for* $C_{20}H_{22}N_6O_5S$ (458.49): C, 52.39; H, 4.84; N, 18.33; S, 6.99. Found: C, 52.36; H, 4.86; N, 18.25; S, 6.94.

Synthesis of 6-Amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxylic Acid (20)

A solution of compound **1** (2.83 g, 0.01 mol) in DMF (15 mL) and NaOH (25 mL, 0.5%) was refluxed for 3 h. The reaction mixture was allowed to cool. The obtained solid product was collected by filtration, washed by water, dried and crystallized from EtOH/DMF to give compound **20**. Brown powder; yield (81%); mp 251–253 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3435–3417 (NH_2), 3387 (OH), 1698, 1671 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 7.34 (s, 2H, NH_2), 11.85 (s, 1H, OH); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 27.3, 30.2, 114.6, 123.7, 143.8, 151.7, 155.4, 163.5, 169.6; MS (EI, 70 eV) *m/z* = 255 (M^+). *Anal. Calcd for* $C_9H_9N_3O_4S$ (255.25): C, 42.35; H, 3.55; N, 16.46; S, 12.56. Found: C, 42.30; H, 3.49; N, 16.38; S, 12.48.

Synthesis of 1,3,7-Trimethyl-9H-pyrimido[4',5':4,5]thieno[2,3-d][1,3]oxazine-2,4,9(1H,3H)-trione (21)

A solution of compound **20** (1.4 g, 0.005 mol) in Ac_2O (10 mL) was refluxed for 5 h. The reaction mixture was allowed to cool. The obtained solid product was collected by filtration, dried and crystallized from EtOH/DMF to give compound **21**. Gray crystals; yield (68%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1732, 1657 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.12 (s, 3H, CH_3), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 21.4, 28.5, 30.6, 114.2, 130.3, 143.6, 151.6, 155.5, 158.3, 164.5, 168.1; MS (EI, 70 eV) *m/z* = 279 (M^+). *Anal. Calcd for* $C_{11}H_9N_3O_4S$ (279.27): C, 47.31; H, 3.25; N, 15.05; S, 11.48. Found: C, 47.25; H, 3.31; N, 14.96; S, 11.38.

Synthesis of 1,3,7-Trimethyl-8-(pyridin-2-yl)thieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1H,3H,8H)-trione (22)

A solution of compound **21** (2.79 g, 0.01 mol) in acetic acid (15 mL) and 2-aminopyridine (0.94 g, 0.01 mol) was refluxed for 4 h. The reaction mixture was allowed to cool. The obtained solid product was collected by filtration, dried and crystallized from EtOH/DMF to give compound **22**. Brown powder; yield (66%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 1671 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.30 (s, 3H, CH_3), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 7.45–8.37 (m, 4H, Ar-H); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 21.8, 28.6, 30.7, 114.3, 118.4, 122.5, 130.5, 136.3, 143.8, 145.6, 146.8, 151.9, 155.6, 158.4, 166.8, 168.5; MS (EI, 70 eV) *m/z* = 355 (M^+). *Anal. Calcd for* $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08; H, 3.69; N, 19.71; S, 9.02. Found: C, 54.00; H, 3.61; N, 19.63; S, 8.98.

General Procedure for the Reaction of Compound 23 with Aniline, Phenylhydrazine and Hydrazine Hydrate

To a solution of compound **23** (3.39 g, 0.01 mol) in dry xylene (20 mL), aniline (0.93 g, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) or hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was refluxed for 3 h, then poured onto iced water and the formed solid products, in each case, were collected by filtration and crystallized from EtOH/DMF to give compounds **24**, **26** and **27**, respectively.

Ethyl 1,3-Dimethyl-2,4-dioxo-6-(((phenylamino)methylene)amino)-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxylate (24)

Gray fine powder; yield (61%); mp 180–182 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3285 (NH), 1701, 1653 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.00 (t, 3H, *J* = 7.10 Hz, CH_3), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.71 (q, 2H, *J* = 7.10 Hz, CH_2), 6.80–7.48 (m, 5H, Ar-H), 8.44 (s, 1H, N=CH), 10.47 (s, 1H, NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.3, 27.6, 30.2, 67.5, 115.3, 116.5, 121.5, 123.1, 130.1, 143.4, 145.8, 151.5, 155.4, 158.6, 163.2, 169.4; MS (EI, 70 eV) *m/z* = 386 (M^+). *Anal. Calcd for* $C_{18}H_{18}N_4O_4S$ (386.43): C, 55.95; H, 4.70; N, 14.50; S, 8.30. Found: C, 55.89; H, 4.62; N, 14.44; S, 8.25.

1,3-Dimethyl-8-(phenylamino)thieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1H,3H,8H)-trione (26)

Orange crystals; yield (77%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3283 (NH), 1652 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 6.82–7.74 (m, 5H, Ar-H), 8.45 (s, 1H, pyrimidine H_7) and 10.24 (s, 1H, NH, D_2O exchangeable); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 28.7, 30.1, 114.5, 116.3, 122.4, 128.5, 131.2, 143.2, 145.4, 148.6, 151.3, 155.8, 166.7, 168.5; MS (EI, 70 eV) *m/z* = 355 (M^+). *Anal. Calcd for* $C_{16}H_{13}N_5O_3S$ (355.37): C, 54.08; H, 3.69; N, 19.71; S, 9.02. Found: C, 54.00; H, 3.62; N, 19.68; S, 9.05.

Ethyl 6-((Hydrazinylmethylene)amino)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-d]pyrimidine-7-carboxylate (27)

Yellowish red crystals; yield (71%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3448–3419 (NH_2), 3285 (NH), 1701, 1649 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.00 (t, 3H, *J* = 7.10 Hz, CH_3), 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 3.71 (q, 2H, *J* = 7.10 Hz, CH_2), 4.45 (s, 2H, NH_2), 5.63 (s, 1H, NH), 8.49 (s, 1H, N=CH); ^{13} C-NMR (100 MHz, DMSO-d_6) δ (ppm): 18.5, 27.4, 30.5, 67.4, 115.2, 123.4, 143.2, 151.7, 155.6, 158.4, 163.5, 169.3; MS (EI, 70 eV) *m/z* = 325 (M^+). *Anal. Calcd for* $C_{12}H_{15}N_5O_4S$ (325.34): C, 44.30; H, 4.65; N, 21.53; S, 9.85. Found: C, 44.24; H, 4.59; N, 21.46; S, 9.76.

Synthesis of 8-Amino-1,3-dimethylthieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1H,3H,8H)-trione (28)

To a solution of compound **27** (2.79 g, 0.01 mol) in DMF (20 mL) TEA (5 drops) was added. The reaction mixture was heated for 5 hours then poured into iced wa-

ter and the formed solid product was collected by filtration, and crystallized from EtOH/DMF to give compound **28**. Greenish brown powder; yield (78%); mp > 300 °C (DMF-EtOH (1:2)); IR (KBr): ν/cm^{-1} = 3443–3385 (NH_2), 1649 (3C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.01 (s, 3H, *N*-CH_3), 3.09 (s, 3H, *N*-CH_3), 5.45 (s, 2H, NH_2), 8.43 (s, 1H, pyrimidine H_7); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 28.1, 30.8, 114.3, 131.4, 143.7, 148.8, 151.4, 155.6, 166.8, 168.3; MS (EI, 70 eV) *m/z* = 279 (M^+). *Anal. Calcd for* $C_{10}H_9N_5O_3S$ (279.27): C, 43.01; H, 3.25; N, 25.08; S, 11.48. Found: C, 43.05; H, 3.20; N, 25.00; S, 11.47.

2. 1. Cytotoxic Activity

The potential cytotoxicity of the tested compounds was evaluated using the MTT assay. The cell lines were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the prepared compounds to allow the attachment of cells to the wall of the plate. The tested compounds were dissolved in dimethylsulfoxide (DMSO) and diluted 1000-fold in the assay. Different concentrations of the tested compounds ranging from 1 to 50 μg/mL, were added to the cell monolayer. The monolayer cells were incubated with the compounds for 48 h at 37 °C, in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) sulforhodamine B (SRB). The excess stain was washed with 1% acetic acid and attached stain was recovered with base tris-(hydroxymethyl)aminomethane (tris-EDTA) buffer, color intensity was measured in a plate reader at 570 nm, the relation between surviving fraction and drug concentration was plotted to get the survival curve of tumor cell line and the IC_{50} was calculated. The obtained IC_{50} values are illustrated in Table 1.

3. Results and Discussion

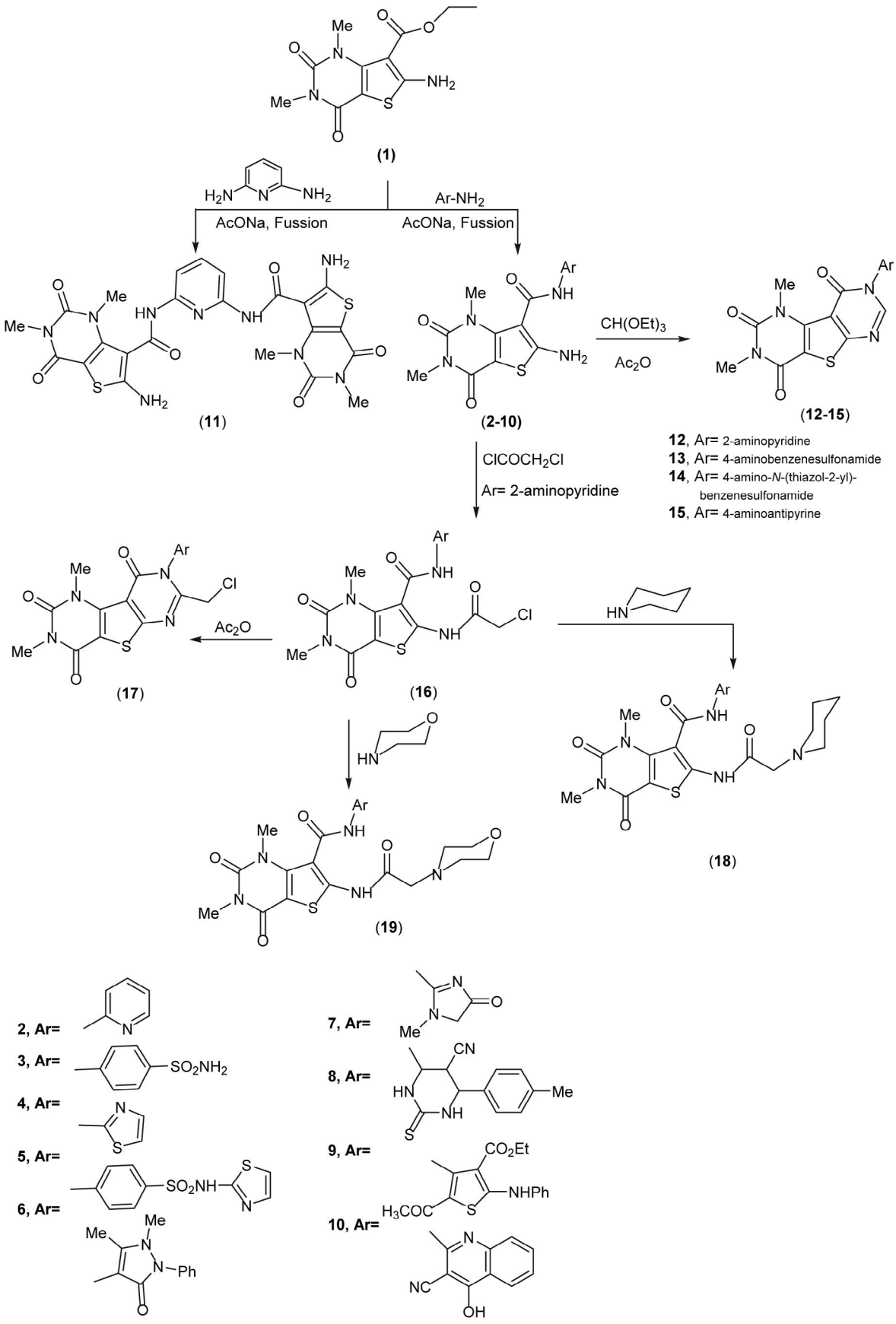
The synthetic procedures adopted to obtain the target compounds are depicted in Schemes 1–3. The reaction of ethyl 6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**1**),[29] with equimolar ratio of different types of aromatic amines, namely 2-aminopyridine, 4-aminobenzenesulfonamide, 2-aminothiazole, 4-amino-*N*-(thiazol-2-yl)benzenesulfonamide, 4-aminoantipyrine, creatinine, 6-amino-2-thioxo-4-(*p*-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carbonitrile, ethyl 5-acetyl-4-amino-2-(phenylamino)thiophene-3-carboxylate and 2-amino-4-hydroxyquinoline-3-carbonitrile in the presence of a catalytic amount of freshly fused sodium acetate furnished the corresponding carboxamide derivatives **2**–**10**. Analytical and spectral data for compounds **2**–**10** were in agreement with the proposed structures. In general, the IR spectra of these derivatives showed the absorption bands at 1687–1641 cm^{-1} for C=O amide groups instead of C=O ester groups.

Also, compounds **8** and **10** showed the absorption bands at 2218–2219 cm^{-1} due to CN groups. Whereas ^{1}H-NMR spectra revealed singlet signals at 3.21, 7.68 and 10.67 ppm assignable to two *N*-CH_3, NH_2 and NH protons. In addition, the ^{1}H-NMR spectrum of **9** exhibited a triplet signal at δ 1.33 ppm due to CH_3 and a quartet signal at 4.43 ppm due to CH_2 of ester group. Also, the mass spectra of these compounds **2**–**10** showed the molecular ion peaks at *m/z* = 331 (M^+), 410 (M^++1), 337 (M^+), 492 (M^+), 441 (M^++1), 351 (M^++1), 483 (M^+), 541 (M^+) and 422 (M^+), respectively, which are in agreement with their molecular formula.

Compound **1** reacted with 2,6-diaminopyridine to give *N,N'*-(pyridine-2,6-diyl)bis(6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxamide) (**11**). The assignment of structure **11** was supported by elemental analysis and spectral data. The IR spectrum showed absorption bands at 3574–3475, 3368, 1687 cm^{-1} due to two NH_2, two NH and six CO groups. Its ^{1}H-NMR spectrum revealed singlet signals at 3.21, 3.25, 7.46 and 10.74 ppm assignable to two *N*-CH_3, two NH_2 and two NH protons. The mass spectrum showed the molecular ion peak at *m/z* = 585 (M^++2), corresponding to the molecular formula $C_{23}H_{21}N_9O_6S_2$.

Next, we aimed to prepare the tricyclic compounds bearing thienopyrimidine scaffolds, being the focus of great interest because of their pharmacological activities. Thus, treatment of carboxamide derivatives **2**, **3**, **5** and **6** with triethyl orthoformate in acetic anhydride afforded thieno[2,3-*d*:4,5-*d'*]dipyrimidine derivatives **12**–**15**. Structures **12**–**15** were established on the basis of elemental and spectral analyses. In general, the IR spectra showed absorption bands at 1667–1655 cm^{-1} due to carbonyl groups. Its ^{1}H-NMR spectra revealed singlet signals at 3.01 and 3.09 assignable to two *N*-CH_3 protons, in addition to a singlet signal at 8.51–8.56 ppm assignable to pyrimidine-H_7. The mass spectra of these compounds showed the molecular ion peaks at *m/z* = 341 (M^+), 419 (M^+), 502 (M^+) and 450 (M^+) respectively, which are in agreement with their molecular formulas.

Also, the reaction of carboxamide derivative **2** with chloroacetyl chloride in chloroform at room temperature gave 6-(2-chloroacetamido)-1,3-dimethyl-2,4-dioxo-*N*-(pyridin-2-yl)-1,2,3,4-tetrahydro-thieno[3,2-*d*]pyrimidine-7-carboxamide (**16**). The assignment of structure **16** was supported by elemental analysis and spectral data. The IR spectrum showed absorption band at 3421 cm^{-1} due to two NH groups, in addition to the stretching vibration of four carbonyl groups at 1698 and 1671 cm^{-1} and showed a band at 756 cm^{-1} due to C–Cl group. Its ^{1}H-NMR spectrum revealed singlet signals at 3.01, 3.09, 3.65, 10.67 and 11.54 ppm assignable to two *N*-CH_3, CH_2 and two NH protons. Also, ^{13}C-NMR spectra showed a signal at 51.6 ppm due to $\underline{CH_2}Cl$. The mass spectrum showed the molecular ion peak at *m/z* = 407 (M^+), corresponding to the molecular formula $C_{16}H_{14}ClN_5O_4S$.


(1)
AcONa, Fussion
Ar-NH2
AcONa, Fussion
(11)
(2-10)
CH(OEt)3
Ac2O
(12-15)
12, Ar= 2-aminopyridine
13, Ar= 4-aminobenzenesulfonamide
14, Ar= 4-amino-N-(thiazol-2-yl)-benzenesulfonamide
15, Ar= 4-aminoantipyrine
ClCOCH2Cl
Ar= 2-aminopyridine
(17)
Ac2O
(16)
(18)
(19)
2, Ar=
3, Ar=
4, Ar=
5, Ar=
6, Ar=
7, Ar=
8, Ar=
9, Ar=
10, Ar=

Scheme 1

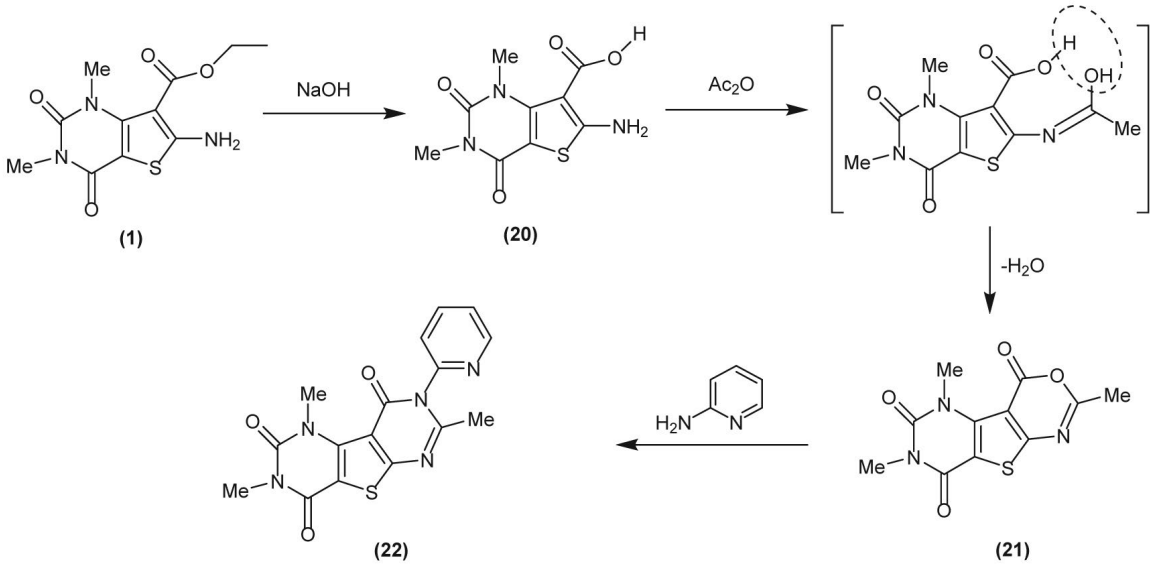
Cyclization of compound **16** in acetic anhydride afforded the corresponding 7-(chloromethyl)-1,3-dimethyl-8-(pyridin-2-yl)thieno[2,3-*d*:4,5-*d*']dipyrimidine-2,4,9-(1*H*,3*H*,8*H*)-trione (**17**). The assignment of structure **17** was supported by elemental analysis and spectral data. The IR spectrum showed bands at 1657 and 1645 cm^{-1} due to three carbonyl groups. In addition a band at 760 cm^{-1} due to C–Cl group was observed. ^{1}H-NMR spectrum of **17** revealed singlet signals at 3.01, 3.09 and 4.45 ppm assignable to two *N*-CH_3 and $\underline{CH_2}$-Cl protons. The mass spectrum showed the molecular ion peak at *m/z* = 389 (M^+), corresponding to the molecular formula $C_{16}H_{12}ClN_5O_3S$.

In contrast to the behaviour of compound **16** toward heterocyclic secondary amines, piperidine or morpholine in DMF reacted with **16** to furnish ethyl 1,3-dimethyl-2,4-dioxo-6-(2-(piperidin-1-yl)acetamido)-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**18**) and ethyl 1,3-dimethyl-6-(2-morpholino- acetamido)-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**19**), respectively. The assignment of structures **18** and **19** was supported by elemental analysis and spectral data. The IR spectra showed absorption bands at 3433–3435 and 1656–1665 cm^{-1} due to two NH and four CO groups. Their ^{1}H-NMR spectra revealed singlet signals at 3.46, 10.86 and 11.46 ppm assignable to CO$\underline{CH_2}$N and two NH protons. Also, ^{13}C-NMR spectra showed signals in the region 22.3–65.3 ppm due to the cyclic CH_2 groups and in addition a signal at 67.6 due to NHCO$\underline{CH_2}$. The mass spectra of compounds **18** and **19** showed their molecular ion peaks at *m/z* = 456 (M^+) and 458 (M^+), respectively, which are in agreement with their molecular formula.

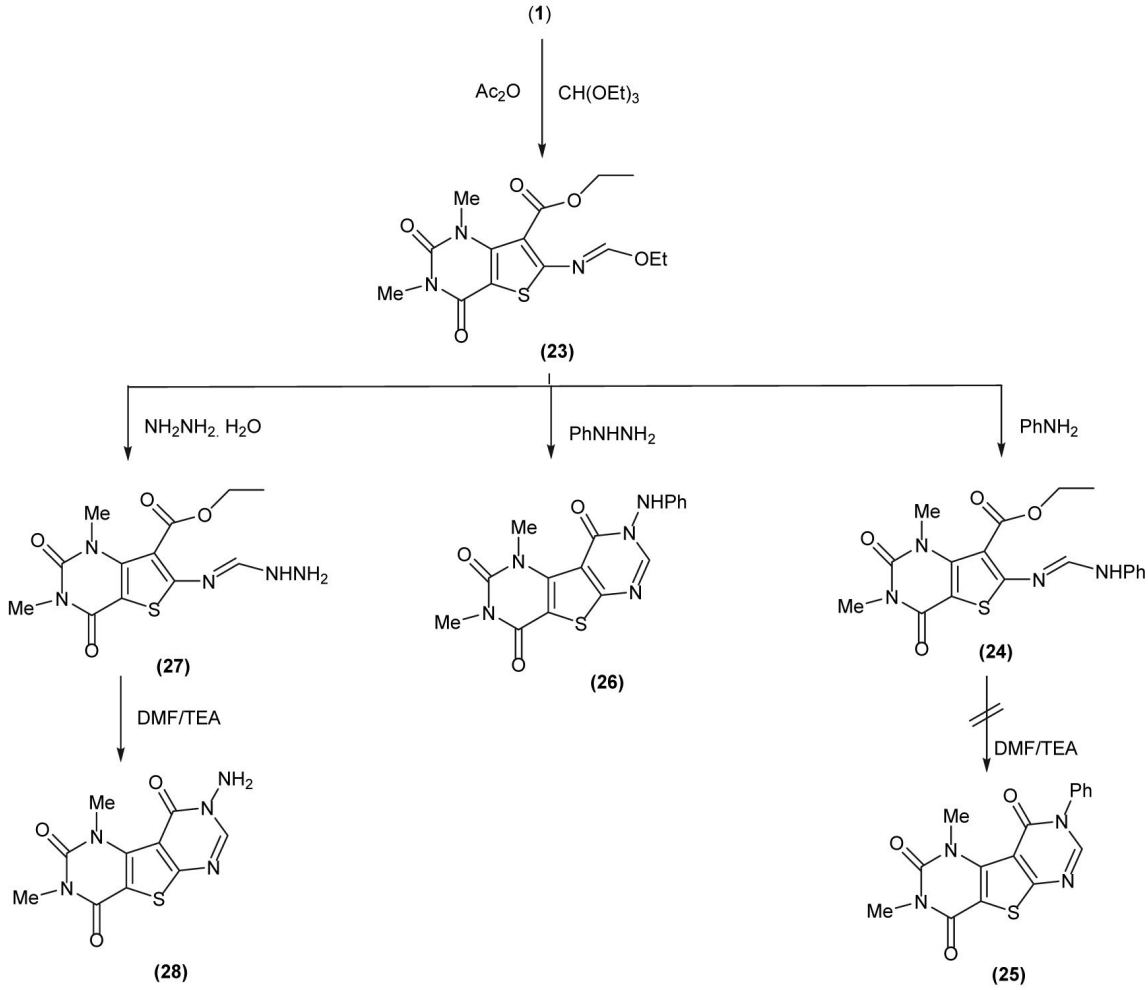
Thieno[2,3-*d*][1,3]oxazine heterocyclic frameworks have been found to be associated with diverse biological activities. Therefore, hydrolysis of *o*-aminoester derivative **1** in aqueous sodium hydroxide afforded the corresponding 6-amino-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylic acid (**20**). The assignment of structure **20** was supported by elemental analysis and spectral data. The IR spectrum showed bands at 3435, 3417 and 3387 cm^{-1} due to NH_2 and OH groups, in addition to the stretching vibration of three carbonyl groups at 1698 and 1671 cm^{-1}. Its ^{1}H-NMR spectrum revealed singlet signals at 3.01, 3.09, 7.34 and 11.85 ppm assignable to two *N*-CH_3, NH_2 and OH protons. The mass spectrum showed the molecular ion peak at *m/z* = 255 (M^+), corresponding to the molecular formula $C_9H_9N_3O_4S$.

Refluxing of the amino acid derivative **20** with acetic anhydride seemed to be a logical method for the preparation of the corresponding 1,3,7-trimethyl-9*H*-pyrimido[4',5':4,5]thieno[2,3-*d*][1,3]oxazine-2,4,9(1*H*,3*H*)trione (**21**). The assignment of structure **21** was supported by elemental analysis and spectral data. The IR spectrum showed the disappearance of NH_2 group and presence of the three carbonyl groups at 1732 and 1657 cm^{-1}. Its ^{1}H-NMR spectrum revealed singlet signals at 2.12, 3.01 and 3.09 ppm assignable to three CH_3 protons. Also, ^{13}C-NMR spectra showed signals at 21.4, 28.5 and 30.6 ppm due to three CH_3. The mass spectrum showed the molecular ion peak at *m/z* = 279 (M^+), corresponding to the molecular formula $C_{11}H_9N_3O_4S$.

1,3,7-Trimethyl-8-(pyridin-2-yl)thieno[2,3-*d*:4,5-*d*']dipyrimidine-2,4,9(1*H*,3*H*,8*H*)-trione (**22**) was obtained through the reaction of **21** with 2-aminopyridine in acetic acid. The assignment of structure **22** was supported by elemental analysis and spectral data. The IR spectrum showed the three carbonyl groups at 1671 cm^{-1}. Its ^{1}H-NMR spectrum revealed singlet signals at 2.30, 3.01 and 3.09 ppm assignable to three CH_3 protons. In addition, the mass spectrum showed the molecular ion peak at *m/z* = 355 (M^+), corresponding to the molecular formula $C_{16}H_{13}N_5O_3S$.



Scheme 2



Scheme 3

Refluxing of compound **1** in acetic anhydride and triethyl orthoformate formed ethyl 6-((ethoxymethylene) amino)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**23**). The assignment of structure **23** was supported by elemental analysis and spectral data. The IR spectrum showed absorption bands at 1708 and 1659 cm^{-1} due to the three carbonyl groups. Its ^{1}H-NMR spectrum revealed triplet signals at 1.22, 2.93 ppm assignable to two CH_3 protons, quartet signals at 3.71, 4.15 ppm assignable to two CH_2, two singlet signals at 3.01 and 3.09 ppm assignable to two *N*-CH_3 and a singlet signal at 8.45 ppm assignable to N=CH proton. The mass spectrum showed the molecular ion peak at *m/z* = 340 (M^++1), corresponding to the molecular formula $C_{14}H_{17}N_3O_5S$.

We studied the reactivity of *N*-ethoxymethino group with different types of amines. Thus, treatment of compound **23** with aniline gave ethyl 1,3-dimethyl-2,4-dioxo-6-(((phenylamino)methylene)amino)-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**24**). The assignment of structure **24** was supported by elemental analysis and spectral data. The IR spectrum showed an absorption band at 3285 cm^{-1} due to NH group, in addition to the stretching vibration of three carbonyl groups at 1701 and 1653 cm^{-1}. Its ^{1}H-NMR spectrum revealed a triplet signal at 3.00 ppm assignable to CH_3 protons, a quartet signal at 3.71 ppm assignable to CH_2, two singlet signals at 3.01 and 3.09 ppm assignable to two *N*-CH_3, a singlet signal at 8.44 ppm assignable to N=CH and a singlet signal at 10.47 ppm assignable to NH proton. Also, ^{13}C-NMR spectra showed signals at 18.3, 67.5 and 158 ppm due to OCH_2CH_3 and N=CH. The mass spectrum showed the molecular ion peak at *m/z* = 386 (M^+), corresponding to the molecular formula $C_{18}H_{18}N_4O_4S$. We tried to obtain the cyclic compound **25** by refluxing the compound **24** in DMF and TEA but failed.

On the other hand, the compound **23** was treated with phenylhydrazine to give 1,3-dimethyl-8-(phenylamino)thieno[2,3-d:4,5-d']dipyrimidine-2,4,9(1*H*, 3*H*,8*H*)-trione (**26**). The assignment of structure **26** was supported by elemental analysis and spectral data. The IR spectrum showed absorption bands at 3283 and 1652 cm^{-1} due to NH group and carbonyl group. Its ^{1}H-NMR spectrum revealed two singlet signals at 3.01 and 3.09 ppm assignable to two *N*-CH_3, a singlet signal at 8.45 ppm assign-

able to pyrimidine-H_7 and a singlet signal at 10.24 ppm assignable to NH proton. Also, ^{13}C-NMR spectra showed a signal at 148.6 ppm due to C7 of pyrimidine ring. The mass spectrum showed the molecular ion peak at *m/z* = 355 (M^+), corresponding to the molecular formula $C_{16}H_{13}N_5O_3S$.

Moreover, compound **23** reacted with hydrazine hydrate to afford ethyl 6-((hydrazinylmethylene)amino)-1,3-dimethyl-2,4-dioxo-1,2,3,4-tetrahydrothieno[3,2-*d*]pyrimidine-7-carboxylate (**27**). The assignment of structure **27** was supported by elemental analysis and spectral data. The IR spectrum showed absorption bands at 3448, 3419 and 3285 cm^{-1} due to NH_2 and NH groups, in addition to the stretching vibration of three carbonyl groups at 1701 and 1649 cm^{-1}. Its ^{1}H-NMR spectrum revealed a triplet signal at 3.00 ppm assignable to CH_3 protons, a quartet signal at 3.71 ppm assignable to CH_2, two singlet signals at 3.01 and 3.09 ppm assignable to two *N*-CH_3, a singlet signal at 4.45 ppm assignable to NH_2, a singlet signal at 5.63 ppm assignable to NH and a singlet signal at 8.49 ppm assignable to N=CH proton. The mass spectrum showed the molecular ion peak at *m/z* = 325 (M^+), corresponding to the molecular formula $C_{12}H_{15}N_5O_4S$.

Finally, cyclization of compound **27** in DMF and TEA to afford the corresponding 8-amino-1,3-dimethylthieno[2,3-*d*:4,5-*d'*]dipyrimidine-2,4,9(1*H*,3*H*, 8*H*)-trione (**28**) succeeded. The assignment of structure **28** was supported by elemental analysis and spectral data. The IR spectrum showed absorption bands at 3443, 3385 cm^{-1} due to NH_2 groups, in addition to the stretching vibration of three carbonyl groups at 1649 cm^{-1}. Its ^{1}H-NMR spectrum revealed two singlet signals at 3.01 and 3.09 ppm assignable to two *N*-CH_3, a singlet signal at 5.45 ppm assignable to NH_2 and a singlet signal at 8.43 ppm assignable to pyrimidine-H_7. Also, ^{13}C-NMR spectra showed a signal at 148.8 ppm due to C7 of pyrimidine ring. The mass spectrum showed the molecular ion peak at *m/z* = 279 (M^+), corresponding to the molecular formula $C_{10}H_9N_5O_3S$.

3. 1. Cytotoxic Activity

The newly synthesized target compounds were evaluated for their *in vitro* anticancer effects *via* the standard MTT method,[30–32] against a panel of four human tumor cell lines namely; Hepatocellular carcinoma (HePG-2), Colorectal carcinoma (HCT-116), Mammary gland (MCF-7) and Epitheliod Carcinoma (Hela). The cell lines were obtained from ATCC *via* the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil (5-FU) was used as the standard anticancer drug for comparison. The data of cytotoxic activity are reported in Table 1.

The results are expressed as IC_{50} (inhibitory concentration 50%), the concentration of compounds which inhibits the tumor cell growth by 50%. The obtained results revealed that eight of the tested compounds namely; **2**, **3**, **5**, **7**, **9**, **10**, **26** and **27** exhibited variable degrees of inhibitory activity towards the four tested human tumor cell lines. The compounds **2**, **3** and **26** showed the highest cytotoxic activity against all four cell lines. In addition, compounds **5**, **9**, **10** and **27** exhibited a strong cytotoxic activity against the four cell lines, whereas, compound **7** had a moderate activity against HePG-2 cell line and a strong cytotoxic activity against remaining three cell lines. Finally, the rest of compounds showed the lowest activity against all four cell lines. On the other hand, compound **2** showed activity better than that of the standard drug against HePG-2 (IC_{50} = 6.9 ± 0.69), and the most potent activity

Table 1. *In vitro* cytotoxic activity of some new compounds against four cell lines

Compounds	*In vitro* Cytotoxicity IC_{50} (μg/ml)*			
	HePG2	HCT-116	MCF-7	Hela
5-FU	7.9 ± 0.41	5.5 ± 0.28	5.3 ± 0.38	4.8 ± 0.31
1	70.0 ± 4.78	32.7 ± 2.54	30.1 ± 2.79	48.9 ± 3.75
2	6.9 ± 0.69	9.5 ± 0.99	8.2 ± 0.87	8.7 ± 0.73
3	7.8 ± 0.80	5.6 ± 0.47	5.6 ± 0.59	9.7 ± 1.10
4	46.2 ± 3.64	34.9 ± 3.05	37.5 ± 3.12	27.6 ± 2.54
5	20.7 ± 1.86	20.1 ± 1.85	17.7 ± 1.58	18.8 ± 1.66
6	49.9 ± 3.21	40.3 ± 3.67	47.8 ± 4.15	30.3 ± 3.80
7	31.1 ± 2.57	13.2 ± 1.18	12.3 ± 1.03	19.8 ± 1.58
8	59.3 ± 4.31	49.2 ± 3.60	51.2 ± 4.35	39.9 ± 3.14
9	20.0 ± 1.52	22.8 ± 2.06	15.8 ± 1.24	16.1 ± 1.28
10	16.0 ± 1.35	15.8 ± 1.23	10.7 ± 1.37	14.9 ± 1.34
11	89.4 ± 5.10	62.9 ± 4.70	62.2 ± 5.30	87.1 ± 5.47
19	>100	94.1 ± 6.34	94.3 ± 5.89	>100
24	93.0 ± 5.12	79.9 ± 5.16	80.1 ± 5.45	94.3 ± 6.34
26	9.8 ± 0.84	7.7 ± 0.56	8.9 ± 0.57	10.8 ± 0.97
27	13.1 ± 1.04	11.4 ± 1.10	9.1 ± 0.98	12.2 ± 1.53

*IC_{50} (μg/ml): 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic), 5-FU = 5-Fluorouracil

against Hela (IC_{50} = 8.7 ± 0.73), whereas compound **3** had the most potent activity against HCT-116 and MCF-7 (IC_{50} = 5.6 ± 0.47) and (IC_{50} = 5.6 ± 0.59), respectively.

Regarding the structure–activity relationship we can reveal that compounds, such as **2**, containing a pyridine ring besides a thienopyrimidine ring and as the number of heterocyclic rings increases, especially when a six membered ring which contains one heteroatom is present, possess a high potency and their activity increases in comparison with the other compounds. In addition, compound **3** showed a more potent activity due to the presence of a sulfonamide moiety that was shown to be able to selectively concentrate in tumor tissues in addition to playing a unique role in carbonic anhydrase inhibition.[33,34]

Also, compound **26** has a strong activity due to the presence of phenylhydrazinyl moiety and compound **27** has a very good activity due to the hydrazenyl group. Moreover, compounds **5**, **9** and **10** showed very good activities due to the presence of sulfathiazole, thiophene and hydroxyquinoline rings, respectively; as a heterocyclic ring increases the activity otherwise, not very strong like compound **2** due to steric hindrance. Finally, compound **7** has a moderate activity due to the introduction of dihydroimidazol-4-one ring beside the thienopyrimidine reducing the activity towards the four cell lines.

4. Conclusion

The present study describes the synthesis of a series of novel tricyclic compounds bearing thienopyrimidine scaffolds having been the focus of great interest because of their promising pharmacological activities. This work has advantages of cheap starting materials, excellent yields, mild reaction conditions and simple experimental procedures. The compounds **2**, **3** and **26** showed the most potent antitumor activity against HepG2, HCT-116, MCF-7 and Hela cell lines.

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Povzetek

Opisujemo sintezo nekaterih novih tricikličnih spojin, ki vsebujejo tienodipirimidinski fragment. Sinteza temelji na reakciji etil 6-amino-1,3-dimetil-2,4-diokso-1,2,3,4-tetrahidrotieno[3,2-*d*]pirimidin-7-karboksilata (**1**) z različnimi vrstami aromatskih aminov **2**–**11**, ki daje ustrezne karboksamide, ki ob prisotnosti trietil ortoformata in acetanhidrida ciklizirajo v tieno[2,3-*d*:4,5-*d*']dipirimidinske derivate **12**–**15**. Podobno smo pripravili tudi tieno[2,3-*d*:4,5-*d*']dipirimidinski derivat **17**, ki je nastal s ciklizacijo v vrelem acetanhidridu iz tieno[3,2-*d*]pirimidin-7-karboksamidnega derivata **16**, ki smo ga pripravili iz karboksamida **2** z obdelavo s kloroacetil kloridom v kloroformu. Strukture pripravljenih spojin smo potrdili na osnovi njihovih spektroskopskih in analitskih podatkov, kot so IR, ^{1}H-NMR, ^{13}C-NMR spektroskopija in masna spektrometrija. Pripravljenim spojinam smo določili tudi njihove citotoksične aktivnosti.