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Scientific paper

Antiproliferative and Antiprostate Cancer Activities of Heterocyclic Compounds Derived from Cyclohexane-1,4-dione

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Abstract

2-Amino-6-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**3**) was prepared from the reaction of cyclohexane-1,4-dione with elemental sulfur and malononitrile in 1,4-dioxane and triethylamine as catalyst. The latter compound reacted with triethyl orthoformate and either malononitrile or ethyl cyanoacetate in 1,4-dioxane in the presence of triethylamine to produce 4*H*-thieno[2,3-*f*]chromene derivatives **10a,b**. In addition, fused pyran and pyridine derivatives were synthesized starting from compound **3**. The cytotoxicities of the synthesized compounds were studied using the six cancer cell lines together with c-Met kinase and PC-3 cell line. The most active compounds were tested against five tyrosine kinases and Pim-1 kinase, most of which showed strong inhibition, encouraging further work.

Keywords: Cyclohexan-1,4-dione; thiophene; thiazole; cytotoxicity; tyrosine inhihibitions

1. Introduction

Sulfur-containing heterocyclic compounds have attracted much attention in recent years because of their great medicinal and pharmaceutical importance.[1,2] Benzo[*b*]thiophene derivatives are one type of such sulfur-containing heterocyclic compounds and are good candidates for anticancer applications.[3–7] In addition, benzo[*b*]thiophene derivatives exhibit numerous other pharmacological effects, including antitumor agents,[8] anti-inflammatory agents,[9,10] antimicrobial agents,[11,12] anti-leishmanial agents,[13,14] antioxidants,[15] anti-anxiety agents, serotonin antagonists, and antiarrhythmic agents.[16] In addition, the combination of benzo[*b*]thiophene with other heterocyclic rings such as thiazole, thiophene, pyran, or pyridine rings increases the biological significance of such compound series.[17–20] Recently, our research group focused on benzo[*b*]thiophene derivatives by performing further heterocyclization reactions and then investigating their anticancer activities; in particular, some compounds showed inhibition of kinase and Pim-1.[21–25] In extension of this work, in this manuscript we show the synthesis of 2-amino-6-hydroxy-4,7-dihydrobenzo[*b*]thiophene-3-carbonitrile (**3**) starting from cyclohexane-1,4-dione, followed by further heterocyclization to prepare compounds whose antiproliferative activities and kinase inhibitions were investigated.

2. Experimental

2. 1. Generral

^{13}C NMR and ^{1}H NMR spectra were recorded using a Bruker DPX300 instrument in DMSO with TMS as the internal standard for protons and solvent signals as the internal standard for carbon spectra. Chemical shift values are given in δ (ppm). Mass spectra were checked using EIMS (Shimadzu) and ESI-esquire 3000 from Bruker Daltonics. Elemental analyzes were performed using the Microanalytical Data Unit at Cairo University. All reactions were monitored by TLC on 2 × 5 cm, 0.25 mm thick, precoated silica gel 60 F254 plates (Merck).

2. 1. 1. Synthesisof 2-amino-6-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (3)

To a solution of cyclohexane-1,4-dione (1) (1.2 g, 0.01 mol) in 1,4-dioxane (30 mL) with triethylamine (0.50 mL) was added malononitrile (0.66 g, 0.01 mol) and ele-

mental sulfur (0.32 g, 0.01 mol). The reaction mixture was heated at reflux for 1 h, and the product was filtered and dried.

Light brown crystals from 1,4-dioxane, yield: 75%; m.p.: 160–163 °C; IR (KBr) υ_{max} (cm^{-1}): 3422–3236 (OH, NH_2), 2966 (CH aliphatic), 2196 (CN), 1706 (CO), 1624 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.65 (d,2H, J = 6.7 Hz, $\underline{CH_2}$-CH=C), 3.39 (s, 2H, D_2O exchangeable, NH_2), 5.54 (s, 2H, CH_2), 6.82 (t, 1H, J = 6.7 Hz, CH_2-$\underline{CH}$=C), 9.97 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.6 ($\underline{CH_2}$-CH=C), 50.3 (CH_2), 66.3 (CH_2-$\underline{CH}$=C), 116.2 (CN), 118.4, 121.7, 128.9, 134.0 (thiophene C), 161.8 (CO); EIMS (m/z, %): 192 [M^+, 20]. Anal. Calcd. for $C_9H_8N_2OS$: C, 56.23; H, 4.19; N, 14.57; S, 16.68. Found: C, 55.94; H, 4.08; N, 14.39; S, 16.30.

2. 1. 2. Synthesis of 2-amino-7-benzylidene-6-hydroxy-4,7-dihydrobenzo[*b*]thiophene-3-carbonitrile (5)

Benzaldehyde (**4**) (1.06 g, 0.01 mol) was added to a solution of compound 3 (1.92 g, 0.01 mol) in 1,4-dioxane (30 mL) containing piperidine (0.50 mL) and heated for 1 h at reflux. The reaction mixture was cooled and poured into cold water containing a few drops of hydrochloric acid. The precipitated solid was filtered off, washed and dried.

Red crystals from 1,4-dioxane, yield: 76%; m.p.: 180–182 °C; IR (KBr) υ_{max} (cm^{-1}): 3428–3231 (OH,NH_2), 2923 (CH aliphatic), 2201 (CN), 1625 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.89 (d, 2H, J = 4.6 Hz, $\underline{CH_2}$-CH=C), 3.44 (s, 2H, D_2O exchangeable, NH_2), 7.21 (t, 1H, J = 4.6 Hz, CH_2-$\underline{CH}$=C), 7.49–7.93 (m, 6H, C_6H_5 and C=$\underline{CH}$–C_6H_5), 10.01 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.2 ($\underline{CH_2}$-CH=C), 66.3 (CH_2-$\underline{CH}$=C), 77.2, 114.5 (C=C), 116.2 (CN), 119.8, 120.4, 126.2, 128.4, 129.5, 131.2, 133.4, 134.5, 154.5 (C_6H_5, thiophene C); EIMS (m/z, %): 280 [M^+, 32]. Anal. Calcd. for $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99; S, 11.44. Found: C, 68.60; H, 4.29; N, 10.29; S, 11.09.

2. 1. 3. Synthesis of 2-amino-6-hydroxy-7-(2-hydroxybenzylidene)-4,7-dihydrobenzo-[*b*] thiophene-3-carbonitrile (7)

A solution of compound **3** (1.92 g, 0.01 mol) in 1,4-dioxane (30 mL) containing piperidine (0.50 mL) was refluxed with salycilalhyde (**6**) (1.22 g, 0.01 mol) for 1 h, the precipitated solid was filtered and dried after addition of cold water containing a few drops of hydrochloric acid.

Reddish brown crystals from 1,4-dioxane, yield: 77%; m.p.: 190–192 °C; IR (KBr) υ_{max} (cm^{-1}): 3423–3231 (OH-NH_2), 2925 (CH aliphatic), 2210 (CN), 1605 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.99 (d, 2H, $\underline{CH_2}$-CH=C), 3.32 (s, 2H, D_2O exchangeable, NH_2), 6.95 (m, 2H, 2 $\underline{CH}$=C), 7.40–7.78 (m, 4H, C_6H_4), 10.25, 11.26 (2s, 2H, D_2O exchangeable, 2OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8 ($\underline{CH_2}$-CH=C), 66.9 (CH_2-$\underline{CH}$=C), 77.9, 118.9 (C=C), 116.5 (CN), 120.2, 125.9, 127.9, 128.8, 129.6, 131.1, 133.6, 134.2, 146.7 (C_6H_4, thiophene C); EIMS (m/z, %): 296 [M^+, 51]. Anal. Calcd. for $C_{16}H_{12}N_2O_2S$: C, 64.85; H, 4.08; N, 9.45; S, 10.82. Found: C, 64.60; H, 4.29; N, 9.79; S, 10.58.

2. 1. 4. Synthesis of 4*H*-thieno[2,3-*f*]chromene derivatives 10a,b

Triethyl orthoformate (**8**) (1.48 mL, 0.01 mol) and either malononitrile (**2**) (0.66 g, 0.01 mol) or ethyl cyanoacetate (**9**) (1.13 mL, 0.01 mol) were added to a solution of compound 3 (1.92 g, 0.01 mol) in 1,4-dioxane (30 mL) with triethylamine (0.50 mL). The reaction mixture was heated at reflux for 2 h, cooled, and neutralized with cold water containing a few drops of hydrochloric acid; the precipitated product was filtered off and dried.

2,7-Diamino-4*H*-thieno[2,3-*f*]chromene-3,8-dicarbonitrile (10a)

Light brown crystals from 1,4-dioxane, yield: 47%; m.p.: >300 °C; IR (KBr) υ_{max} (cm^{-1}): 3424–3228 ($2NH_2$), 2924 (CH aliphatic), 2215, 2201 (2CN), 1626 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.39 (s, 2H, D_2O exchangeable, NH_2), 7.09–7.54 (m, 4H, pyran H-4 and Ar-H),7.91 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 77.1 (pyran C-4), 116.5, 117.3 (2CN), 114.7, 118.9, 120.2, 125.9, 127.9, 128.8, 131.1, 133.6, 134.2, 136.7 (Ar-C, pyran, thiophene); EIMS (m/z, %): 268 [M^+, 44]. Anal. Calcd. For $C_{13}H_8N_4OS$: C, 58.20; H, 3.01; N, 20.88; S, 11.95. Found: C, 58.50; H, 3.39; N, 20.62; S, 11.69.

Ethyl 2,7-diamino-3-cyano-4*H*-thieno[2,3-*f*]chromene-8-carboxylate (10b)

Pale brown crystals from 1,4-dioxane, yield: 62%; m.p.: >300 °C; IR (KBr) υ_{max} (cm^{-1}): 3423–3211 ($2NH_2$), 2923 (CH aliphatic), 2201 (CN), 1706 (CO), 1621 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.16 (t, 3H, J = 7.21 Hz, $OCH_2\underline{CH_3}$), 3.41 (s, 2H, D_2O exchangeable, NH_2), 4.20 (q, 2H, J = 7.21 Hz, $O\underline{CH_2}CH_3$), 7.26–7.61 (m, 4H, pyran H-4 and Ar-H), 7.83 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ8.5 ($OCH_2\underline{CH_3}$), 45.5 ($O\underline{CH_2}CH_3$), 77.4 (pyran C-4), 116.2 (CN), 115.6, 118.3,120.8, 121.9, 122.3, 128.1, 130.3, 132.0, 133.4, 147.4 (Ar-C, pyran, thiophene), 162.6 (CO); EIMS (m/z, %): 315 [M^+, 56]. Anal. Calcd. for $C_{15}H_{13}N_3O_3S$: C, 57.13; H, 4.16; N, 13.33; S, 10.17. Found: C, 57.40; H, 4.39; N, 13.62; S, 10.49.

2. 1. 5. Synthesis of *N*'-(2-amino-3-cyano-4,7-dihydrobenzo[*b*]thiophen-6-yl)-2-cyanoacetohydrazide (12)

To a solution of compound **3** (1.92 g, 0.01 mol) in 1,4-dioxane (30 mL) was added cyanoacetylhydrazine (**11**) (0.99 g, 0.01 mol) and the reaction mixture was heated un-

der refulx for 3 h and the resulting precipitate was collected by filtration after cooling.

Pale brown crystals from 1,4-dioxane, yield: 41%; m.p.: >300 °C; IR (KBr) υ_{max} (cm^{-1}): 3418–3205 (NH_2, 2NH), 2923 (CH aliphatic), 2210, 2197 (2CN), 1698 (CO), 1621 (C=C); ^{1}H NMR (300 MHz,DMSO-d_6) δ 2.71 (d, 2H, J = 6.8 Hz, $\underline{CH_2}$-CH=), 3.37 (s, 2H, D_2O exchangeable, NH_2), 3.76 (s, 2H, CO-$\underline{CH_2}$-CN), 5.54 (s, 2H, CH_2), 6.83 (t, 1H, J = 6.8 Hz, CH_2-$\underline{CH}$=), 8.13, 9.93 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 35.8 (CH_2), 66.3 (CH_2), 77.4, 118.5 (C=C), 98.9 (CO-$\underline{CH_2}$-CN), 115.7, 116.2 (2CN), 129.9, 133.3, 136.6, 154.7 (thiophene C), 162.6 (CO); δ EIMS (m/z, %): 273 [M^+, 24]. Anal. Calcd. for $C_{12}H_{11}N_5OS$: C, 52.73; H, 4.06; N, 25.62; S, 11.73. Found: C, 52.50; H, 4.39; N, 25.82; S, 11.69.

2. 1. 6. Synthesis of ethyl 2,7-diamino-3,8-dicyano-9-hydroxy-4,5-dihydronaphtho[1,2-*b*]thiophene-6-carboxylate (13)

A solution of compound **3** (1.92 g, 0.01 mol) (30 mL) and ethyl cyanoacetate (**9**) (1.13 mL, 0.01 mol) in 1,4-dioxane was heated at reflux with triethylamine (0.50 mL) for 3 hours. The solid formed was filtered off and dried after neutralizing the reaction mixture with cold water containing a few drops of hydrochloric acid.

Pale brown crystals from 1,4-dioxane, yield: 46%; m.p.: >300 °C; IR (KBr) υ_{max} (cm^{-1}): 3521–3209 (OH, $2NH_2$), 2928 (CH aliphatic), 2208, 2199 (2CN), 1704 (CO), 1624 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20 (t, 3H, J = 6.90 Hz, $OCH_2\underline{CH_3}$), 2.65 (m, 4H, CH_2-CH_2), 3.36 (s, 2H, D_2O exchangeable, NH_2), 4.19 (q, 2H, J = 6.90 Hz, $O\underline{CH_2}CH_3$), 7.84 (s, 2H, D_2O exchangeable, NH_2), 9.91 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.5 ($OCH_2\underline{CH_3}$), 46.9 ($O\underline{CH_2}CH_3$), 56.0, 67.3 (CH_2-CH_2), 115.5, 116.2 (2CN), 118.1, 119.7, 120.7, 122.5, 128.4, 129.3, 132.2, 133.3, 152.4 (Ph, thiophene C), 162.6 (CO); EIMS (m/z, %): 354 [M^+, 52]. Anal. Calcd. for $C_{17}H_{14}N_4O_3S$: C, 57.62; H, 3.98; N, 15.81; S, 9.05. Found: C, 57.50; H, 3.87; N, 15.53; S, 8.84.

2. 1. 7. Synthesis of 4,7-dihydrobenzo[*b*]thiophene derivatives 15a,b

A cold solution (0–5 °C) of compound **3** (1.92 g, 0.01 mol) in ethanol (30 mL) containing sodium acetate (2.5 g) was added to a cold solution of either benzenediazonium chloride (**14a**) (0.01 mol) or 4-methylbenzenediazonium chloride (**14b**) (0.01 mol) [prepared by adding sodium nitrite solution (0.7 g, 0.01 mol in 10 mL water) to a cold solution of either aniline oil (0.93 g, 0.01 mol) or 4-methylaniline (1.07 g, 0.01 mol) in concentrated hydrochloric acid (8 mL, 18%) with constant stirring]. The whole mixture was kept at room temperature for 1 hour and the resulting product was collected by filtration.

2-Amino-6-hydroxy-7-(2-phenylhydrazono)-4,7-dihydrobenzo[*b*]thiophene-3-carbonitrile (**15a**)

Black crystals from ethanol, yield: 81%; m.p.: >300 °C; IR (KBr) υ_{max} (cm^{-1}): 3518–3214 (OH, NH_2, NH), 2924 (CH aliphatic), 2199 (CN), 1625 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.79 (d, 2H, CH_2), 3.44 (s, 2H, D_2O exchangeable, NH_2), 7.17–7.63 (m, 6H, C_6H_5 and CH=C), 7.94 (s, 1H, D_2O exchangeable, NH), 9.01(s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.8(CH_2), 67.2, 115.2 (CH=C), 116.4 (CN), 119.4, 121.7, 126.1, 128.4, 128.9, 132.1, 133.4, 137.1 (C_6H_5 and thiophene), 182.8 (C=N); EIMS (m/z, %): 296 [M^+, 61]. Anal. Calcd. for $C_{15}H_{12}N_4OS$: C, 60.79; H, 4.08; N, 18.91; S, 10.82. Found: C, 60.49; H, 3.87; N, 18.53; S, 10.54.

2-Amino-6-hydroxy-7-(2-(p-tolyl)hydrazono)-4,7-dihydrobenzo[*b*]thiophene-3-carbonitrile (**15b**)

Dark brown crystals from ethanol, yield: 84%; m.p.: >300 °C; IR (KBr, υ_{max} cm^{-1}): 3524–3226 (OH, NH_2, NH), 2922 (CH aliphatic), 2200 (CN), 1626 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H, CH_3), δ3.05 (d, 2H, CH_2), 3.40 (s, 2H, D_2O exchangeable, NH_2), 7.17–7.59 (m, 5H, C_6H_4 and CH=C), 7.92 (s, 1H, D_2O exchangeable, NH), 9.21 (s, 1H, D_2O exchangeable, OH); ^{13}CNMR (75 MHz, DMSO-d_6) δ 16.5 (CH_3), 20.8 (CH_2), 66.5, 114.6 (CH=C), 117.4 (CN), 119.4, 121.7, 125.5, 128.2, 130.8, 132.7, 133.5, 137.0 (C_6H_5 and thiophene), 184.1 (C=N); EIMS (m/z, %): 310 [M^+, 57]. Anal. Calcd. for $C_{16}H_{14}N_4OS$: C, 61.92; H, 4.55; N, 18.05; S, 10.33. Found: C, 62.20; H, 4.24; N, 18.37; S, 10.41.

2. 1. 8. Synthesis of dihydrobenzo[*b*]thiophene derivatives 19 and 20

A solution of compound **3** (1.92 g, 0.01 mol) in dimethylformamide (30 mL) and phenyl isothiocyanate (**16**) (1.35 mL, 0.01 mol) was cooled overnight in the presence of potassium hydroxide (0.5 g). To the reaction mixture either α-chloroacetone (**18a**) (0.92 mL, 0.01 mol) or ethyl chloroacetate (**18b**) (1.22 mL, 0.01 mol) was added and allowed to stand overnight. The synthesized product was obtained by neutralizing the reaction mixture with a solution of cold water and a few drops of hydrochloric acid, filtered and dried.

2-Amino-6-hydroxy-7-(4-methyl-3-phenylthiazol-2(3*H*)-ylidene)-4,7-dihydrobenzo[*b*]thiophene-3-carbonitrile (19)

Dark brown crystals from ethanol, yield: 79%; m.p.: 182 °C; IR (KBr) υ_{max} (cm^{-1}): 3518–3220 (OH, NH_2), 2924 (CH aliphatic), 2188 (CN), 1629 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, CH_3), 2.72 (d, 2H, J = 4.5 Hz, CH_2), 3.30 (s, 2H, D_2O exchangeable, NH_2), 7.06 (t, 1H, J = 4.5 Hz, CH), 7.09–7.61 (m, 6H, C_6H_5 and thiazole H-5), 10.07 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 22.5 (CH_3), 34.3 (CH_2),

74.2, 118.1 (CH=C), 116.1 (CN), 121.7, 123.6, 124.4, 125.7, 127.8, 128.4, 128.7, 129.2, 129.4, 137.5, 139.4, 153.2 (C_6H_5, thiazole, thiophene); EIMS (m/z, %): 365 [M^+, 24]. Anal. Calcd. for $C_{19}H_{15}N_3OS_2$: C, 62.44; H, 4.14; N, 11.50; S, 17.55. Found: C, 62.59; H, 4.50; N, 11.22; S, 17.31.

Ethyl 2-(((2-amino-3-cyano-6-oxo-5,6-dihydrobenzo[*b*]thiophen-7(4*H*)-ylidene)(phenyl-amino)methyl)thio)acetate (20)

Dark brown crystals from ethanol, yield: 78%; m.p.: 150 °C; IR (KBr) υ_{max} (cm^{-1}): 3518–3220 (OH, NH_2, NH), 2929 (CH aliphatic), 2127 (CN), 1722 (CO), 1635 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, 3H, J = 7.1 Hz, $OCH_2\underline{CH_3}$), 2.72 (d, 2H, CH_2), 3.06 (s, 2H, CH_2), 3.30 (s, 2H, D_2O exchangeable, NH_2), 4.15 (q, 2H, J = 7.1 Hz, $O\underline{CH_2}CH_3$), 7.06–7.72 (m, 6H, C_6H_5 and CH), 8.96 (s, 1H, D_2O exchangeable, NH), 10.07 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9 ($OCH_2\underline{CH_3}$), 28.7 (CH_2), 45.5 ($O\underline{CH_2}CH_3$), 50.1 (CH_2), 72.4, 118.7 (CH=C), 116.1 (CN), 121.2, 121.7, 123.6, 124.4, 125.7, 127.8, 128.7, 130.4, 137.5, 139.4 (C=C, C_6H_5, thiophene C), 163.2 (CO); EIMS (m/z, %): 413 [M^+, 24]. Anal. Calcd. for $C_{20}H_{19}N_3O_3S_2$: C, 58.09; H, 4.63; N, 10.16; S, 15.51. Found: C, 58.36; H, 4.50; N, 10.22; S, 15.31.

2. 1. 9. Synthesis of ethyl 2-amino-3-cyano-8-(phenylamino)-4,5-dihydrobenzo[1,2-*b*:5,6-*c'*]dithiophene-6-carboxylate (21)

Compound **20** (4.13 g, 0.01 mol) was heated in a solution of 1,4-dioxane containing triethylammine (0.50 mL) for 2 h under reflux. The resulting solution was neutralized with an ice water solution containing a few drops of hydrochloric acid to give the synthesized solid, which was filtered and dried.

Brown crystals from ethanol, yield: 78%; m.p.: 225 °C; IR (KBr) υ_{max} (cm^{-1}): 3418–3220 (NH_2, NH), 2924 (CH aliphatic), 2199 (CN), 1722 (CO), 1633 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, 3H, J = 7.2 Hz,$OCH_2\underline{CH_3}$), 3.06 (m, 4H, CH_2-CH_2), 3.30 (s, 2H, D_2O exchangeable, NH_2), 4.15 (q, 2H, J = 7.2 Hz, $O\underline{CH_2}CH_3$), 7.26–7.79 (m, 5H, C_6H_5), 8.96 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.3 ($OCH_2\underline{CH_3}$), 45.5 ($O\underline{CH_2}CH_3$), 61.5, 62.9 (CH_2-CH_2), 116.1 (CN), 118.0, 121.7, 128.8, 129.2, 129.9, 130.5, 131.6, 132.4, 133.0, 134.7, 136.4, 140.0 (C_6H_5, thiophene) 161.5 (CO); EIMS (m/z, %): 395 [M^+, 24]. Anal. Calcd. for $C_{20}H_{17}N_3O_2S_2$: C, 60.74; H, 4.33; N, 10.62; S, 16.22. Found: C, 60.49; H, 4.21; N, 10.82; S, 15.93.

2. 1. 10. Synthesis of 5,9-dihydro-4*H*-thieno[2,3-*f*]chromene derivatives 23a-f

A mixture of compound **3** (1.92 g, 0.01 mol), either malononitrile (**2**) (0.66 g, 0.01 mol) or ethyl cyanoacetate (**9**) (1.13, 0.01 mol) and either benzaldehyde (**4**) (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (**22a**) (1.4 g, 0.01 mol) or 4-methoxybenzaldehyde (**22b**) (1.36 g, 0.01 mol) in 1,4-dioxane (40 mL) and triethylamine (0.5 mL) was heated under reflux for 3 h and the precipitated product was kept under reflux. The precipitated product was recovered by adding cold water and a few drops of hydrochloric acid to the resulting mixture, filtered and dried.

2,7-Diamino-9-phenyl-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-3,8-dicarbonitrile (**23a**)

Reddish brown crystals from ethanol, yield: 40%; m.p.: 230 °C; IR (KBr) υ_{max} (cm^{-1}): 3422–3210 ($2NH_2$), 2924 (CH aliphatic), 2227, 2198 (2CN), 1625 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09 (m, 4H, CH_2-CH_2), 3.34 (s, 2H, D_2O exchangeable, NH_2), 7.24–7.96 (m, 6H, pyran H-4 and C_6H_5), 8.54 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 62.1, 65.3 (CH_2-CH_2), 76.5 (pyran C-4), 115.6, 116.6 (2CN), 118.4, 119.3, 122.6, 123.6, 128.9, 129.5, 129.9, 130.1, 131.9, 132.5, 133.7, 154.4 (C_6H_5, pyran, thiophene C); EIMS (m/z, %): 346 [M^+, 34]. Anal. Calcd. for $C_{19}H_{14}N_4OS$: C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 65.59; H, 3.88; N, 16.32; S, 9.09.

2,7-Diamino-9-(4-chlorophenyl)-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-3,8-dicarbonitrile (**23b**)

Red crystals from ethanol, yield: 77%; m.p.: 160 °C; IR (KBr) υ_{max} (cm^{-1}): 3421–3206 ($2NH_2$), 2959 (CH aliphatic), 2225, 2195 (2CN), 1621 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09 (m, 4H, CH_2-CH_2), 3.40 (s, 2H, D_2O exchangeable, NH_2), 7.26 (s, 1H, pyran H-4), 7.40–7.97 (m, 4H, C_6H_4), 8.53 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 63.2, 65.3 (CH_2-CH_2), 76.8 (pyranC-4), 115.9, 116.2 (2CN), 118.4, 119.1, 120.3, 121.9, 123.9, 129.4, 129.8, 130.7, 131.4, 132.5, 133.1, 148.1 (C_6H_4, pyran, thiophene C); EIMS (m/z, %): 380 [M^+, 45]. Anal. Calcd. for $C_{19}H_{13}ClN_4OS$: C, 59.92; H, 3.44; N, 14.71; S, 8.42. Found: C, 59.95; H, 3.24; N, 14.56; S, 8.73.

2,7-Diamino-9-(4-methoxyphenyl)-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-3,8-dicarbonitrile (**23c**).

Reddish brown crystals from ethanol, yield: 82%; m.p.: 120 °C; IR (KBr) υ_{max} (cm^{-1}): 3418–3220 ($2NH_2$), 2924 (CH aliphatic), 2214, 2199 (2CN), 1633 (C=C); ^{1}H NMR (300 MHz,DMSO-d_6) δ 3.06 (m, 4H, CH_2-CH_2), 3.36 (s, 2H, D_2O exchangeable, NH_2), 3.88 (s, 3H, OCH_3), 7.11–7.20 (m, 3H, pyran H-4 and Ar-H), 7.85–7.99 (m, 2H, Ar-H), 8.38 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.0 (OCH_3), 62.9, 66.3 (CH_2-CH_2), 77.3 (pyran C-4), 115.8, 116.8 (2CN), 114.0, 114.8, 119.4, 122.1, 124.0, 129.6, 129.7, 130.5, 131.7, 132.7, 133.3, 157.2 (C_6H_4, pyran, thiophene C); EIMS (m/z, %): 376 [M^+, 56]. Anal. Calcd. for $C_{20}H_{16}N_4O_2S$: C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 63.69; H, 3.90; N, 14.60; S, 8.82.

Ethyl 2,7-diamino-3-cyano-9-phenyl-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-8-carboxylate (23d)

Brown crystals from acetic acid, yield: 83%; m.p.: 161 °C; IR (KBr) υ_{max} (cm^{-1}): 3425–3211 ($2NH_2$), 2933 (CH aliphatic), 2198 (CN), 1733 (CO), 1612 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.07 (t, 3H, J = 7.2 Hz, $OCH_2\underline{CH_3}$), 3.17 (m, 4H, CH_2-CH_2), 3.39 (s, 2H, D_2O exchangeable, NH_2), 4.18 (q, 2H, J = 7.2 Hz, $O\underline{CH_2}CH_3$),7.08 (s, 1H, pyran H-4), 7.25–7.62 (m, 5H, C_6H_5), 8.23 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.5 ($OCH_2\underline{CH_3}$), 44.4 ($O\underline{CH_2}CH_3$), 61.9, 65.1 (CH_2-CH_2),97.9 (pyran C-4), 116.5 (CN), 121.7, 122.9, 123.7, 124.7, 127.9, 129.2, 130.9, 131.4, 133.8, 138.9, 147.5, 155.3 (C_6H_5, pyran, thiophene C), 163.1 (CO); EIMS (m/z, %): 393 [M^+, 32]. Anal. Calcd. for $C_{21}H_{19}N_3O_3S$: C, 64.10; H, 4.87; N, 10.68; S, 8.15. Found: C, 64.39; H, 4.60; N, 10.90; S, 8.31.

Ethyl 2,7-diamino-9-(4-chlorophenyl)-3-cyano-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-8-carboxylate (23e).

Brown crystals from ethanol, yield: 79%; m.p.: 102 °C; IR (KBr) υ_{max} (cm^{-1}): 3423–3221 ($2NH_2$), 2921 (CH aliphatic), 2194 (CN), 1721 (CO), 1608 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.31 (t, 3H, J = 6.9 Hz, $OCH_2\underline{CH_3}$), 2.95 (m, 4H, CH_2-CH_2), 3.38 (s, 2H, D_2O exchangeable, NH_2), 4.31 (q, 2H, J = 6.9 Hz, $O\underline{CH_2}CH_3$), 7.15 (s, 1H, pyran H-4), 7.66–8.07 (m, 4H, C_6H_4), 8.40 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.1 ($OCH_2\underline{CH_3}$), 55.4 ($O\underline{CH_2}CH_3$), 62.4, 66.3 (CH_2-CH_2), 96.5 (pyran C-4), 116.0 (CN), 117.9, 121.8, 122.9, 124.1, 128.7, 129.6, 130.5, 131.9, 133.1, 137.9, 147.4, 154.9 (C_6H_4, pyran, thiophene C), 163.9 (CO); EIMS (m/z, %): 427 [M^+, 41]. Anal. Calcd. for $C_{21}H_{18}ClN_3O_3S$: C, 58.94; H, 4.24; N, 9.82; S, 7.49. Found: C, 59.09; H, 4.50; N, 10.02; S, 7.31.

Ethyl 2,7-diamino-3-cyano-9-(4-methoxyphenyl)-5,9-dihydro-4*H*-thieno[2,3-*f*]chromene-8-carboxylate (23f)

Reddish brown crystals from ethanol, yield: 68%; m.p.: 89 °C; IR (KBr) υ_{max} (cm^{-1}): 3413–3212 ($2NH_2$), 2915 (CH aliphatic), 2205 (CN), 1714 (CO), 1621 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (t, 3H, J = 7.2 Hz, $OCH_2\underline{CH_3}$), 2.91 (m, 4H, CH_2-CH_2), 3.33 (s, 2H, D_2O exchangeable, NH_2), 3.86 (s, 3H, OCH_3), 4.29 (q, 2H, J = 7.2 Hz, $O\underline{CH_2}CH_3$), 7.09–7.17 (m, 3H, pyran H-4 and Ar-H), 7.81–8.10 (m, 2H, Ar-H), 8.31 (s, 2H, D_2O exchangeable, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9 ($OCH_2\underline{CH_3}$), 55.1 (OCH_3), 55.9 ($O\underline{CH_2}CH_3$), 62.7, 66.3 (CH_2-CH_2), 98.5 (pyran C-4), 116.1 (CN), 114.8, 121.2, 122.6, 123.9, 128.1, 129.4, 130.7, 131.7, 133.4, 138.7, 147.8, 154.3 (C_6H_4, pyran, thiophene C), 163.5 (CO); EIMS (m/z, %): 423 [M^+, 54]. Anal. Calcd. for $C_{22}H_{21}N_3O_4S$: C, 62.40; H, 5.00; N, 9.92; S, 7.57. Found: C, 62.70; H, 4.72; N, 9.92; S, 7.81.

2. 1. 11. Synthesis of 4,5,6,9-tetrahydrothieno [2,3-*f*]quinoline derivatives 24a-f.

A mixture of compound **3** (1.92 g, 0.01 mol), either malononitrile (**2**) (0.66 g, 0.01 mol) or ethyl cyanoacetate (**9**) (1.13, 0.01 mol) and either benzaldehyde (**4**) (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (**22a**) (1.4 g, 0.01 mol), or 4-methoxybenzaldehyde (**22b**) (1.36 g, 0.01 mol) in 1,4-dioxane (40 mL) containing ammonuim acetate (0.5 g) was heated for 3-5 h under reflux. The obtained solution was neutralized by adding a few drops of hydrochloric acid and cold water. The product was precipitated, filtered off, washed with water and dried.

2,7-Diamino-9-phenyl-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-3,8-dicarbonitrile (24a).

Crimson red crystals from ethanol, yield: 72%; m.p.: 110 °C; IR (KBr) υ_{max} (cm^{-1}): 3424–3208 ($2NH_2$, NH), 2919 (CH aliphatic), 2214, 2194 (2CN), 1620 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.81 (m, 4H, CH_2-CH_2), 3.42 (s, 2H, D_2O exchangeable, NH_2), 7.10 (s, 1H, pyridine H-4), 7.26–8.07 (m, 5H, C_6H_5), 8.54 (s, 2H, D_2O exchangeable, NH_2), 10.01 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 60.4, 64.5 (CH_2-CH_2), 76.1 (pyridine C-4), 116.1, 116.6 (2CN), 114.7, 115.5, 121.4, 124.8, 129.3, 132.5, 133.8, 135.7, 138.2, 139.3, 148.3, 154.3 (C_6H_5, pyridine, thiophene C); EIMS (m/z, %): 345 [M^+, 34]. Anal. Calcd. for $C_{19}H_{15}N_5S$: C, 66.07; H, 4.38; N, 20.27; S, 9.28. Found: C, 66.18; H, 4.50; N, 20.23; S, 8.98.

2,7-Diamino-9-(4-chlorophenyl)-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-3,8-dicarbo-nitrile (24b)

Brick red crystals from ethanol, yield: 89%; m.p.: 140 °C; IR (KBr) υ_{max} (cm^{-1}): 3424–3209 ($2NH_2$, NH), 2920 (CH aliphatic), 2221, 2197 (2CN), 1622 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94 (m, 4H, CH_2-CH_2), 3.36 (s, 2H, D_2O exchangeable, NH_2), 7.22 (s, 1H, pyridine H-4), 7.63–8.05 (m, 4H, C_6H_4), 8.54 (s, 2H, D_2O exchangeable, NH_2), 10.03 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 60.3, 65.6 (CH_2-CH_2), 76.3 (pyridine C-4), 116.2, 117.1 (2CN), 114.3, 115.5, 123.9, 125.6, 129.7, 131.9, 133.7, 134.9, 137.9, 139.2, 147.3, 154.9 (C_6H_4, pyridine, thiophene); EIMS (m/z, %): 379 [M^+, 64]. Anal. Calcd. for $C_{19}H_{14}ClN_5S$: C, 60.07; H, 3.71; N, 18.44; S, 8.44. Found: C, 60.12; H, 3.49; N, 18.29; S, 8.54.

2,7-Diamino-9-(4-methoxyphenyl)-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-3,8-dicarbonitrile (24c)

Orange crystals from ethanol, yield: 77%; m.p.: 117 °C ; IR (KBr, υ_{max} cm^{-1}): 3421– 3207 ($2NH_2$, NH), 2925 (CH aliphatic), 2217, 2193 (2CN), 1614 (C=C); ^{1}H NMR (300 MHz,DMSO-d_6): δ 2.83 (m, 4H, CH_2-CH_2), 3.32 (s, 2H, D_2O exchangeable, NH_2), 3.88 (s, 3H, OCH_3), 7.12–7.20 (m, 3H, pyridine H-4 and Ar-H), 7.96-8.10 (m, 2H, Ar-H), 8.38 (s, 2H, D_2O exchangeable, NH_2), 9.98 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 55.8 (OCH_3), 62.6,66.1 (CH_2-CH_2), 76.8 (pyridine C-4),116.4, 116.9 (2CN), 114.7, 115.1, 124.0, 128.4, 129.7, 132.1, 133.3, 135.1, 138.6, 139.7, 147.8, 157.3 (C_6H_4, pyridine, thiophene C); EIMS (m/z, %): 375 [M^+, 49]. Anal.

Calcd. for $C_{20}H_{17}N_5OS$: C, 63.98; H, 4.56; N, 18.65; S, 8.54. Found: C, 64.29; H, 4.80; N, 18.42; S, 8.31.

Ethyl 2,7-diamino-3-cyano-9-phenyl-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-8-carboxylate (24d)

Pale brown crystals from acetic acid, yield: 69%; m.p.: 145 °C; IR (KBr) υ_{max} (cm^{-1}): 3419–3207 ($2NH_2$, NH), 2981 (CH aliphatic), 2196 (CN), 1719 (CO), 1606 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 (t, 3H, J = 6.3 Hz, $OCH_2\underline{CH_3}$), 2.78 (m, 4H, CH_2-CH_2), 3.42 (s, 2H, D_2O exchangeable, NH_2), 4.33(q, 2H, J = 6.3 Hz, $O\underline{CH_2}CH_3$), 7.14 (s, 1H, pyridine H-4), 7.56–8.06 (m, 5H, C_6H_5), 8.39 (s, 2H, D_2O exchangeable, NH_2), 9.83 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1 ($OCH_2\underline{CH_3}$), 55.3 ($O\underline{CH_2}CH_3$), 61.3, 63.5 (CH_2-CH_2), 98.1 (pyridine C-4), 116.6 (CN), 115.7, 121.0, 123.4, 124.3, 127.9, 130.9, 133.7, 135.8, 137.1, 139.8, 147.3, 154.7 (C_6H_5, pyridine, thiophene C), 163.1 (CO); EIMS (m/z, %): 392 [M^+, 34]. Anal. Calcd. for $C_{21}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.28; S, 8.17. Found: C, 64.50; H, 4.92; N, 14.56; S, 8.44.

Ethyl 2,7-diamino-9-(4-chlorophenyl)-3-cyano-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-8-carboxylate (24e)

Reddish brown crystals from ethanol, yield: 77%; m.p.: 98–100 °C; IR (KBr) υ_{max} (cm^{-1}): 3422–3209 ($2NH_2$, NH), 2978 (CH aliphatic), 2198 (CN), 1720 (CO), 1610 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (t, 3H, J = 6.93 Hz, $OCH_2\underline{CH_3}$), 2.84 (m, 4H, CH_2-CH_2), 3.36 (s, 2H, D_2O exchangeable, NH_2), 4.31 (q, 2H, J = 6.93 Hz, $O\underline{CH_2}CH_3$), 7.15 (s, 1H, pyridine H-4), 7.44–8.07 (m, 4H, C_6H_4), 8.40 (s, 2H, D_2O exchangeable, NH_2), 10.01 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 14.5 ($OCH_2\underline{CH_3}$), 55.1 ($O\underline{CH_2}CH_3$), 61.7, 63.4 (CH_2-CH_2), 97.6 (pyridine C-4), 116.4 (CN), 115.2, 121.6, 123.5, 124.5, 128.1, 131.6, 133.2, 135.6, 137.4, 139.8, 147.5, 154.7 (C_6H_4, pyridine, thiophene C), 163.8 (CO); EIMS (m/z, %): 426 [M^+, 66]. Anal. Calcd. for $C_{21}H_{19}ClN_4O_2S$: C, 59.08; H, 4.49; N, 13.12; S, 7.51. Found: C, 59.30; H, 4.41; N, 13.45; S, 7.81.

Ethyl 2,7-diamino-3-cyano-9-(4-methoxyphenyl)-4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline-8-carboxylate (24f)

Brown crystals from ethanol, yield: 89%; m.p.: 87 °C; IR (KBr) υ_{max} (cm^{-1}): 3417–3212 ($2NH_2$, NH), 2984 (CH aliphatic), 2203 (CN), 1716 (CO), 1625 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (t, 3H, J = 6.82 Hz, $OCH_2\underline{CH_3}$), 2.68 (m, 4H, CH_2-CH_2), 3.34 (s, 2H, D_2O exchangeable, NH_2), 3.87 (s, 3H, OCH_3), 4.31 (q, 2H, J = 6.82 Hz, $O\underline{CH_2}CH_3$), 7.08–7.22 (m, 3H, pyridine H-4 and Ar-H), 8.01-8.04 (m, 2H, Ar-H), 8.30 (s, 2H, D_2O exchangeable, NH_2), 9.91 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.9 ($OCH_2\underline{CH_3}$), 50.1 (OCH_3), 55.6 ($O\underline{CH_2}CH_3$), 62.0, 66.3 (CH_2-CH_2), 98.4 (pyridine C-4), 116.1 (CN), 115.9, 121.3, 123.8, 124.7, 127.9, 131.2,

Table 1. *In vitro* growth inhibitory effects IC_{50} ± SEM (μM) of the newly synthesized compounds against cancer cell lines.

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
3	6.29 ± 1.63	5.59 ± 2.35	4.29 ± 2.61	6.77 ± 2.37	7.18 ± 2.57	5.82 ± 1.31
5	6.27 ± 1.80	8.61 ± 2.29	4.36 ± 1.59	3.38 ± 1.62	5.80 ± 1.08	2.49 ± 0.68
7	3.18 ± 1.63	0.42 ± 0.30	1.52 ± 0.23	4.61 ± 2.51	2.63 ± 1.38	1.79 ± 0.83
10a	8.53 ± 2.36	8.29 ± 2.13	8.34 ± 3.70	8.39 ± 2.42	9.68 ± 3.37	8.27 ± 2.91
10b	1.22 ± 0.87	0.52 ± 0.32	0.73 ± 0.48	1.49 ± 0.41	2.46 ± 0.83	1.32 ± 0.42
12	0.24 ± 0.15	0.32 ± 0.22	0.34 ± 0.09	0.42 ± 0.33	0.24 ± 0.19	0.26 ± 0.14
13	4.26 ± 2.12	3.14 ± 1.39	8.14 ± 3.52	6.91 ± 2.42	3.62 ± 1.47	4.73 ± 2.68
15a	3.25 ± 1.08	2.18 ± 0.07	2.68 ± 1.17	2.69 ± 0.98	2.80 ± 1.32	5.54 ± 2.38
15b	4.65 ± 1.36	5.43 ± 2.25	1.39 ± 0.89	1.82 ± 0.96	2.34 ± 0.29	1.80 ± 0.28
19	1.23 ± 0.39	1.44 ± 0.83	2.31 ± 0.67	1.35 ± 0.68	0.89 ± 0.46	1.25 ± 0.59
20	3.12 ± 1.68	4.29 ± 2.39	5.27 ± 3.54	3.18 ± 1.26	4.31 ± 2.82	3.27 ± 1.57
21	1.02 ± 0.95	1.28 ± 0.79	1.08 ± 2.80	2.28 ± 1.23	1.67 ± 0.85	1.62 ± 0.63
23a	1.32 ± 0.88	1.43 ± 0.87	1.74 ± 0.69	1.52 ± 0.83	0.89 ± 0.35	1.63 ± 0.69
23b	0.27 ± 0.18	0.39 ± 0.19	0.62 ± 0.35	0.82 ± 0.63	0.72 ± 0.53	1.29 ± 0.83
23c	7.26 ± 2.58	3.18 ± 2.31	6.68 ± 2.40	5.62 ± 3.42	4.71 ± 1.26	6.80 ± 2.26
23d	8.53 ± 3.57	5.72 ± 3.86	6.48 ± 2.68	7.38 ± 1.87	4.69 ± 2.41	6.50 ± 2.81
23e	0.28 ± 0.15	0.32 ± 0.14	0.36 ± 0.15	0.19 ± 0.06	0.38 ± 0.15	0.17 ± 0.08
23f	4.53 ± 2.51	6.48 ± 2.63	6.59 ± 1.42	6.29 ± 1.38	6.75 ± 2.69	6.58 ± 2.80
24a	4.59 ± 2.26	5.53 ± 2.70	6.31 ± 2.29	6.50 ± 2.63	8.53 ± 2.72	6.32 ± 2.42
24b	0.40 ± 0.33	0.23 ± 0.18	0.52 ± 0.23	0.41 ± 0.25	0.26 ± 0.19	0.25 ± 0.08
24c	3.34 ± 1.24	4.67 ± 1.50	2.80 ± 0.77	2.53 ± 1.19	3.35 ± 1.64	4.49 ± 2.06
24d	6.40 ± 2.58	6.94 ± 2.39	6.29 ± 2.43	6.58 ± 2.30	5.68 ± 2.39	6.55 ± 1.90
24e	1.27 ± 0.53	0.82 ± 0.57	0.83 ± 0.82	1.72 ± 0.94	0.79 ± 0.26	0.59 ± 0.24
24f	0.48 ± 0.26	0.56 ± 0.32	0.42 ± 0.35	0.67 ± 0.40	0.29 ± 1.85	0.69 ± 0.42
Foretinib	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03±0.0055	0.90 ± 0.13	0.44 ± 0.062

133.4, 135.1, 137.6, 139.4, 147.8, 154.3 (C_6H_4, pyridine, thiophene C), 163.4 (CO); EIMS (m/z, %): 422 [M^+, 33]. Anal. Calcd. for $C_{22}H_{22}N_4O_3S$: C, 62.54; H, 5.25; N, 13.26; S, 7.59. Found: C, 62.77; H, 5.52; N, 13.09; S, 7.31.

2. 2. Biology Section

Materials

ATP (adenosine triphosphate) is used in this biology section. DMSO (dimethyl sulfoxide), MgCl2 (magnesium chloride) were purchased from Sigma. Receptor tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR were purchased from Carna Biosciences (Kobe, Japan).

2. 2. 1. Cell proliferation test

The antiproliferative activities of the newly synthesized compounds (Table 1) were evaluated against the five c-Met-dependent cancer cell lines (A549, HT -29, MKN-45, U87MG, and SMMC-7721) and one c-Met-independent cancer cell line (H460) with foretinib as a positive control using the standard MTT assay in vitro.[26] The experimental procedure was applied according to the previously reported work.[27–29]

***In vitro* cell experiments**

All compounds were tested for their cytotoxicity in the six cancer cell lines using the MTT method. The results, expressed as IC50 (average of at least three independent experiments), were summarized in Table 1. The data presented in Table 1 show that the tested compounds exhibited moderate to strong cytotoxicity against the six cancer cell lines in the single-digit lM range. Compounds **12**, **19**, **23a**, **23b**, **23e**, **24b**, **24e**, and **24f** exhibited higher cytotoxicity against U87MG than fortinib (the positive control).

2. 2. 2. Structure Activity Relationship

Table 1 shows the inhibitory effect of the new compounds on cancer cell lines A549, H460, HT -29, MKN-45, U87MG, and SMMC-7721. There are many compounds that showed high inhibitory values, such as **10b**, **12**, **23b**, **23e**, **24b**, **24e**, and **24f**. In addition, some compounds showed moderate inhibition, such as **7**, **20**, **21**, **23a**, and **24c**. The analysis of Table 1 shows that the substituted groups and the type of heterocyclic ring have a great influence on the inhibitions. Thiophene derivatives **3** and **5** had little inhibitory effect on the cancer cell lines tested. In contrast, fused derivative **7** showed moderate inhibition. Surprisingly, 4*H*-thieno[2,3-*f*]chromene derivatives **10a** and **10b** showed low inhibitory values, while compound **10a** (Y = COOEt) showed high inhibitory values, which was attributed to the presence of the COOEt group. Hydrazide-hydrazone derivative **12** showed strong inhibition against the tested cancer cell lines, while compounds **13** and **15a,b** showed moderate inhibition. In addition, compounds **19** and **21** showed moderate inhibition, with compound **19** exhibiting high inhibition against the U87MG cell line with an IC50 of 0.89M. For thieno[2,3-*f*]chromene derivatives **23a-f**, the different substituents played the major role in the inhibitions of the compounds. Compound **23a** (X = CN, Y = H) showed moderate inhibitions, while compounds **23b** (X = CN, Y = Cl) and **23e** (X = COOEt, Y = Cl) showed the strongest inhibitions against the tested cancer cell lines. In contrast, compounds **23c**, **23d**, and **23f** showed lower inhibitory activity. Interestingly, compounds **24a-f**, **24b**, **24e**, and **24f** showed the highest inhibitory values among the six compounds, as the high inhibitory values of compounds **24b** and **24e** were due to the electronegative Cl group. Compound **24f** showed high inhibition values despite the electron-donating OCH_3 group, while the inhibition values of compounds **24a**, **24c**, and **24d** decreased.

2. 2. 3. HTRF Kinase Assay

The c-Met kinase activity of the newly synthesized compounds was assayed using a homogeneous time-resolved fluorescence (HTRF) assay (Table 2), as reported previously.[30] In addition, the maximally active compounds **7**, **10a**, **10b**, **13**, **15a**, **21**, **24a**, **24b**, **24c**, **24d**, and **24e** were extra assayed using the same screening method for the five tyrosine kinases (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) (Table 3). The experimental technique and chemicals used were based on reported work.[31]

Enzymatic *in vitro* tests

All freshly prepared benzo[*b*]thiophene derivatives were evaluated for their inhibitory activity against c-Met enzyme[32] in a homogeneous time-resolved fluorescence (HTRF) assay, with foretinib serving as a positive control. The antiproliferative activity of all newly synthesized compounds against the human prostate cancer cell line PC-3 was calculated by MTT assay[33,34] using SGI-1776 as the reference drug. The results, reported as IC_{50} (average of at least three independent experiments) for both HTRF and antiproliferative activity, are shown in Table 2. Most of the compounds tested showed potent antiproliferative activity with IC_{50} values of less than 30 mM. In most cases, the heterocycles were associated with the benzothiophene moiety, and variations in substituents had a marked effect on antiproliferative activity. The most potent compounds against c-Met kinase were compounds **7**, **10b**, **13**, **15a**, **21**, **24b**, **24c**, **24d**, and **24e**. It is very surprising that compounds **10b**, **13**, **15a**, **24b**, **24c**, **24d** and **24e** showed stronger inhibition than the reference drug foretinib (IC_{50} 1.16 mM). On the other hand, screening with the prostate cancer cell line PC-3 showed that compounds **10b**, **23c**, **23e**, **24a**, **24b** and **24d** had the highest inhibition values. All tested compounds showed higher inhibition than the reference drug SGI-1776, except compounds **3**, **10a**, **15b** and **23f**.

Table 2. c-Met enzymatic activity of the newly synthesized compounds.

Compound No	IC_{50} (nM) c-Met	IC_{50} (nM) PC-3
3	4.65 ± 1.42	6.56 ± 1.38
5	10.23 ± 3.58	2.16 ± 1.13
7	1.18 ± 0.69	2.51 ± 0.34
10a	1.64 ± 0.89	6.42 ±2.51
10b	0.33 ± 0.16	0.28 ± 0.16
12	4.38 ±1.64	3.58 ±1.24
13	0.48 ± 0.15	2.48 ± 1.20
15a	0.32 ± 0.20	4.26 ± 1.42
15b	13.62 ± 4.53	8.37 ± 2.63
19	4.116 ± 5.41	8.57 ± 2.46
20	6.34 ± 2.62	2.17 ± 1.15
21	1.27 ± 0.71	2.08 ± 0.85
23a	8.32 ± 2.74	2.36 ± 1.27
23b	18.27 ± 4.58	2.39 ± 0.83
23c	5.82 ± 1.29	0.92 ± 0.32
23d	18.29 ± 4.70	1.06 ± 0.73
23e	2.41 ± 1.04	0.8 3 ± 0.41
23f	6.24 ± 2.38	8.41 ± 2.49
24a	2.08 ± 0.87	0.96 ± 0.42
24b	0.08 ± 0.03	0.16 ± 0.04
24c	0.32 ± 0.26	1.03 ± 0.69
24d	0.22 ± 0.08	0.59 ± 0.08
24e	0.06 ± 0.004	1.15 ± 0.72
24f	5.31 ± 2.62	4.33 ± 1.36
	Foretinib	**SGI-1776**
	1.16 ± 0.17	4.86 ± 0.16

2. 2. 4. Inhibition of Tyrosine Kinases (Enzyme IC_{50} (nM)

The five tyrosine kinesis c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR were used using sorafenib as the reference drug to test the inhibitions of the selected compounds. The selection of the compounds was based on their high inhibitory activity against the six cancer cell lines. Table 2 shows that compounds **7**, **10a**, **10b**, **13**, **15a**, **21**, **24a**, **24b**, **24c**, **24d**, and **24e** had the highest inhibitory values. The data in Table 3 show that compounds **10a**, **13**, **24b**, and **24a** had the highest inhibitory activity among the compounds tested.

Table 3 showed that compounds **10a**, **10b**, **24b**, **24c**, and **24e** inhibit the investigated tyrosine kinases most strongly, whereas compounds **7**, **21**, **24a**, and **24d** show only slight inhibition.

2. 2. 5. Inhibition of Selected Anti-Pim-1 Kinase Compounds

In addition, compounds **10b**, **12**, **19**, **21**, **23a**, **23b**, **23e**, **24b**, **24e**, and **24f** were selected to investigate their inhibitory effects on Pim-1 kinase (Table 4). Based on their IC_{50} values in a range of 10 concentrations, these compounds showed strong inhibition against both c-Met kinase and the cancer cell lines tested. The most active compounds were **10b**, **23a**, **23e**, **24b**, and **24f**, with IC_{50} values of 0.29, 0.036, 0.26, 0.43, and 0.31 mM, respectively.

Table 3. Inhibition of tyrosine kinases (Enzyme IC_{50} (nM) by compounds **7, 10a, 10b, 13, 15a, 21, 24a, 24b, 24c, 24d** and **24e**.

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
7	4.16	2.68	3.19	2.57	0.83
10a	0.43	0.29	0.61	0.39	0.71
10b	0.24	1.29	2.42	1.29	2.06
13	1.03	0.48	1.18	0.49	0.25
15a	1.69	1.22	0.63	0.52	0.69
21	2.72	4.53	5.62	3.41	1.58
24a	3.62	2.95	2.80	2.45	3.68
24b	0.36	0.42	0.53	0.29	0.31
24c	0.48	0.61	0.58	1.22	0.72
24d	1.08	2.40	2.35	3.06	2.69
24e	0.22	0.36	0.18	0.49	0.31
Foretinib	0.19	0.17	0.20	0.13	0.26

Table 4. The inhibitions of compounds **10b**, **12**, **19**, **21**, **23a**, **23b**, **23e**, **24b**, **24e** and **24f** toward Pim-1 kinase.

Compound	Inhibition ratio at 10 μ M	IC_{50} (μM)
10b	94	0.29
12	30	> 10
19	24	> 10
21	30	>10
23a	96	0.036
23b	26	>10
23e	95	0.26
24b	88	0.43
24e	28	>10
24f	89	0.31
SGI-1776	–	0.048

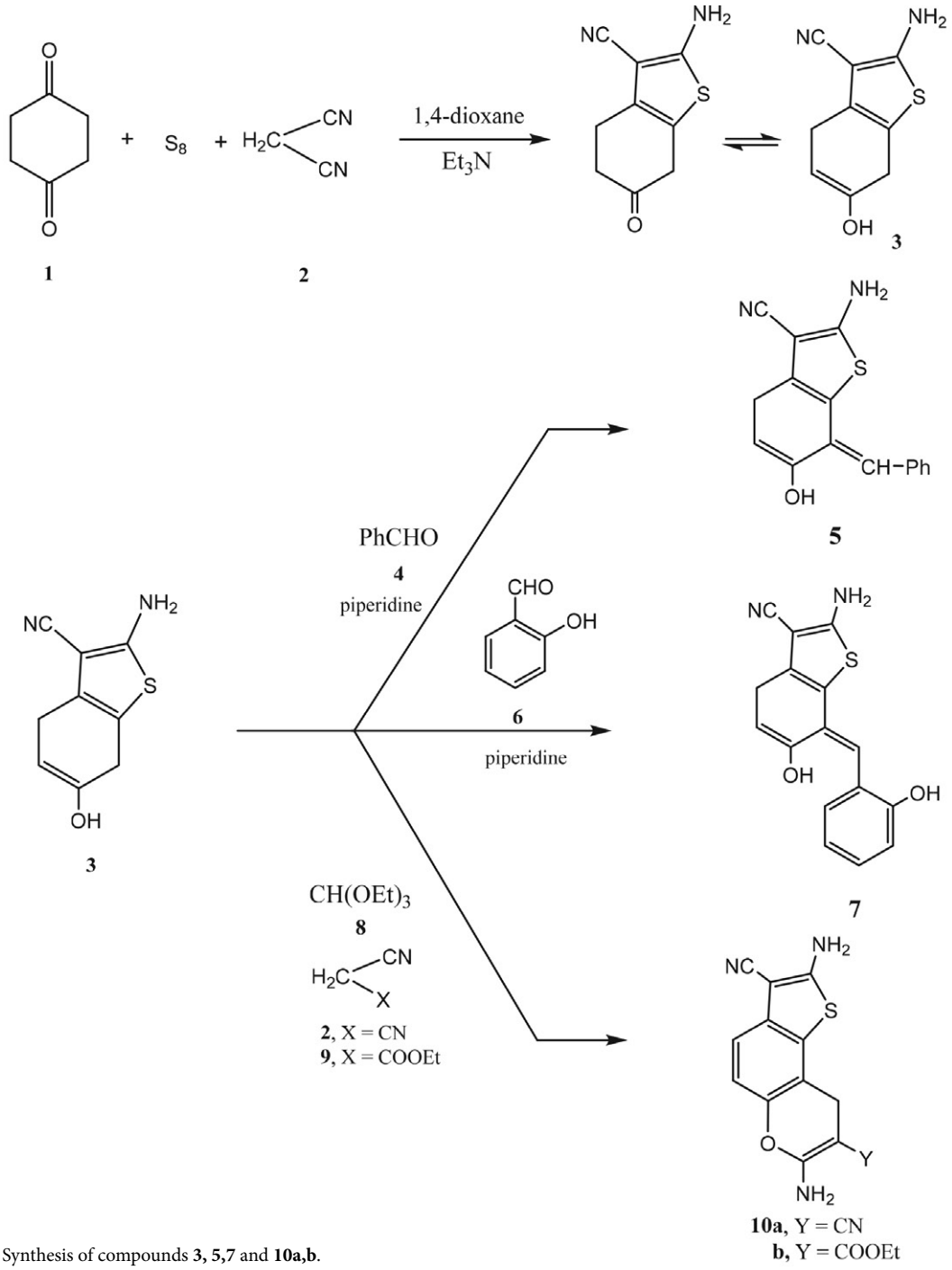
3. Results and Discussion

3. 1. Chemistry

In recent years, our research group has carried out numerous heterocyclic reactions with cyclohexanedione derivatives.[35–37] The aim of these reactions was the synthesis of thiophene derivatives by Gewald's thiophene method,[38–41] and the synthesis of hydrazide-hydrazone derivatives.[42,43] The prepared compounds showed interesting results as anticancer agents. As a continuation of our work here, we demonstrated the heterocyclization of cyclohexane-1,4-dione and then studied its biological evaluation. The reaction sequences for the synthesis of the final compounds **3** to **24a-f** are shown in Schemes 1-4. The chemical structures of the new compounds were secured by spectral data (IR, ^{1}H and ^{13}C NMR, MS). Cyclohexane-1,4-dione

was subjected to Gewald's thiophene synthesis by reacting it with elemental sulfur and malononitrile (**2**) in 1,4-dioxane with triethylamine under reflux to give 2-amino-6-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-cabonitrile (**3**). The spectral data showed that compound **3** was present in both the keto and enol tautomeric structures. The presence of a broad signal at n 3422 cm^{-1} confirmed the presence of the OH group along with the appearance of a signal at n 1706 cm^{-1} due to the presence of the CO group in the IR spectrum. In addition, the ^1H NMR spectrum showed the appearance of a doublet and a triplet at δ 2.65 and 6.82 ppm for the CH_2–CH=C protons besides a singlet at δ 5.54 ppm for the CH_2 group between OH and the sp^2 carbon and two singlet at δ 3.39 and 9.97 ppm (D_2O interchangeable) corresponding to the NH_2 and OH groups, respectively. In addition, the ^{13}C NMR spectrum showed signals at d 22.6 (CH_2–CH=C), 50.3 (CH_2), 66.3 (CH_2–CH=C), 116.2 (CN), 118.4, 121.7, 128.9, 134.0 (thiophene C), and 161.8 (CO).

Compound **3** was the major starting compound for various heterocyclization reactions because it contains an active methylene moiety between the OH group and the

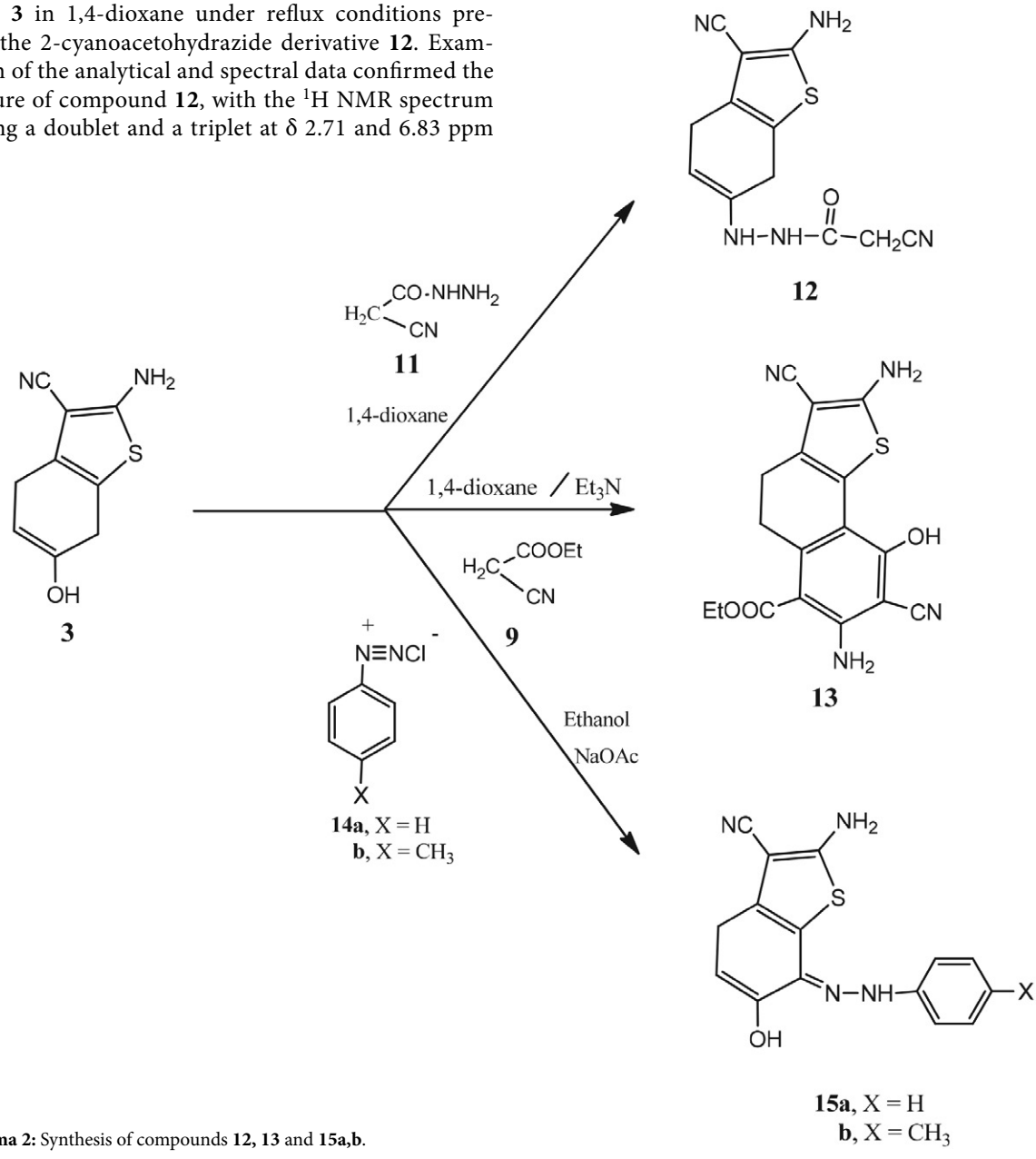


Schema 1: Synthesis of compounds **3, 5,7** and **10a,b**.

sp^2 carbon. For example, compound **3** reacted with benzaldehyde in 1,4-dioxane containing a catalytic amount of piperidine to give the arylidene derivative **5**. Similarly, the reaction of compound **3** with salicylaldehyde (**6**) formed the 2-hydroxybenzylidene derivative **7**.

The synthesis, reactions, and biological activities of 4*H*-pyran-containing molecules have been extensively studied. In addition, 4*H*-pyran derivatives are also an essential component of some pharmaceutical agents and natural products.[44–46] This inspired us to synthesize 4*H*-pyran derivatives via the multicomponent reaction of compound **3**. Thus, compound **3** was subjected to a multicomponent reaction with ethyl orthoformate and either malononitrile (**2**) or ethyl cyanoacetate (**9**) to give 4*H*-pyran derivatives **10a** and **10b**, respectively (Scheme 1).

Addition of cyanoacetylhydrazine (**11**) to compound **3** in 1,4-dioxane under reflux conditions prepared the 2-cyanoacetohydrazide derivative **12**. Examination of the analytical and spectral data confirmed the structure of compound **12**, with the ^{1}H NMR spectrum showing a doublet and a triplet at δ 2.71 and 6.83 ppm confirming the presence of CH2–CH=C protons, in addition to two singlet at δ 3.76 and 5.54 ppm for the protons CO–CH_2–CN and CH_2, respectively. In addition to the presence of three singlet (D_2O exchangeable) at δ 3.37, 8.13 and 9.93 ppm for NH_2 and two NH groups, respectively. The ^{13}C NMR spectrum showed signals at δ 35.8 (CH_2–CH=C), 66.3 (CH_2), 77.4, 118.5 (CH_2–CH=C), 98.9 (CO–CH_2–CN), 115.7, 116.2 (2CN), 129.9, 133.3, 136.6, 154.7 (thiophene C), 162.6 (CO). In addition, ethyl cyanoacetate (**9**) reacted with compound **3** in 1,4-dioxane containing a catalytic amount of triethylamine to produce the dihydronaphtho[1,2-*b*]thiophene derivative **13**. The study of analytical and spectral data confirmed the proposed structure of compound **13** as mentioned in the experimental section. On the other

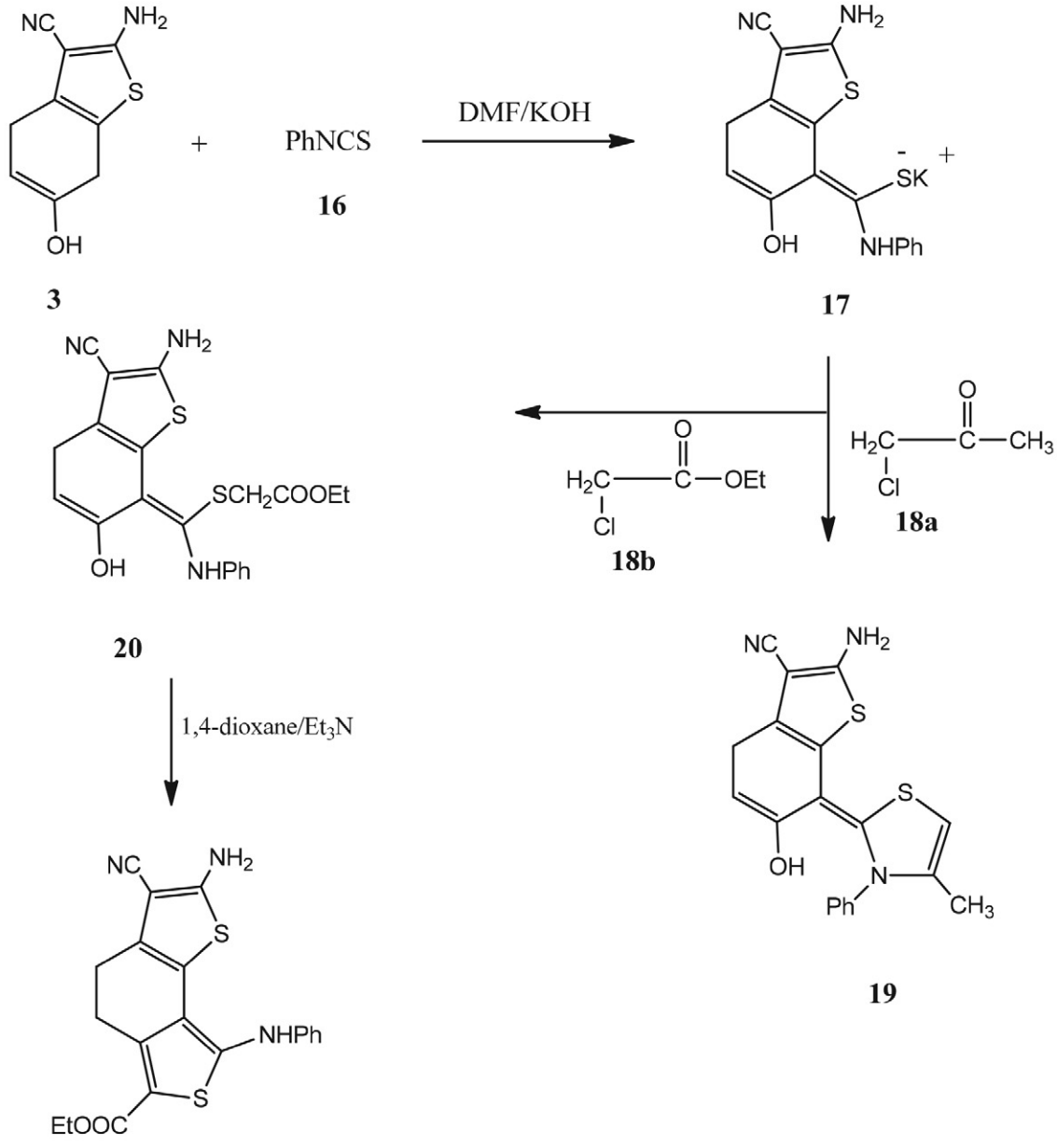


Schema 2: Synthesis of compounds **12, 13** and **15a,b**.

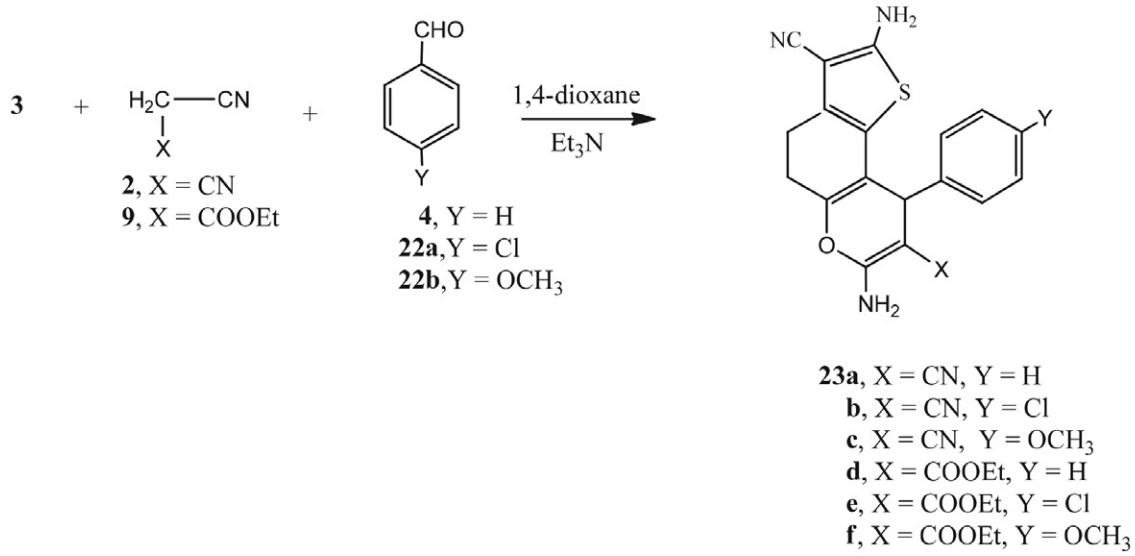
hand, compound **3** reacted with either benzenediazonium chloride or p-tolylbenzenediazonium chloride in ethanol solution containing sodium acetate at 0–5 °C to give the corresponding arylhydrazone derivatives **15a** and **15b**, respectively (Scheme 2).

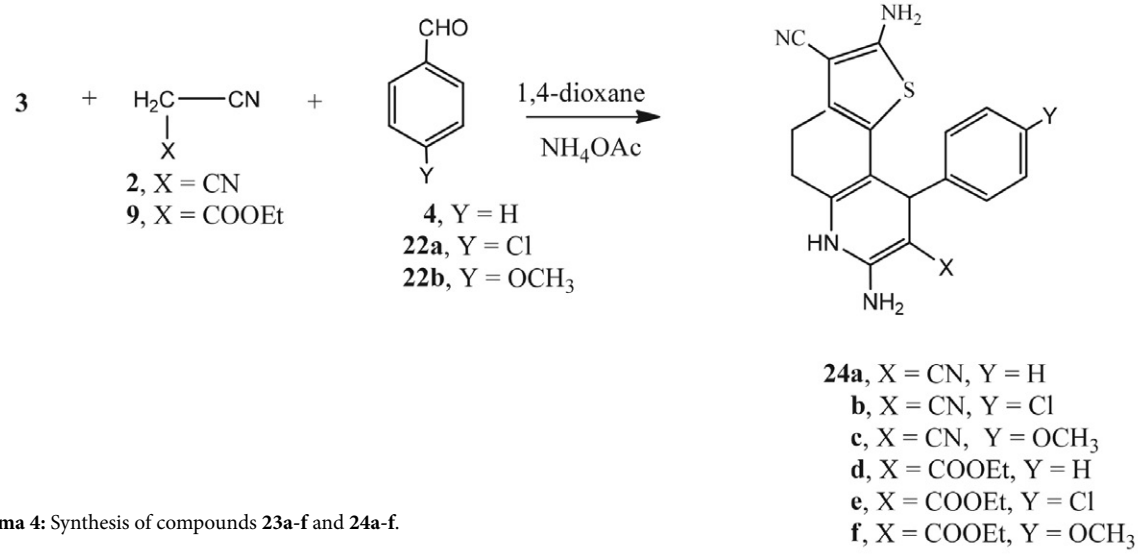
Continuing our previous work on the synthesis of thiophene or thiazole derivatives using phenyl isothiocyanate in basic dimethylformamide and subsequent heterocyclization of the intermediate potassium salt by a-haloketones,[47,48] in this work we have demonstrated such reactions with the aim of preparing heterocyclic compounds with predicted biological activity. For example, the reaction of compound **3** with phenyl isothiocyanate and potassium hydroxide in dimethylformamide gave the potassium salt intermediate **17**, followed by the addition of a–chloroacetone (**18a**) to the intermediate **17**, giving the thiazole derivative **19**. However, reaction with ethyl a-chloroacetate (**18b**) surprisingly gave the thioether derivative **20**. Heating of compound **20** in 1,4-dioxane with a catalytic amount of triethylamine gave the condensed dithiophene derivative **21** (Scheme 3). The structures of compounds **19**, **20**, and **21** were confirmed by the data reported in the experimental section.

Moreover, the multicomponent reaction of compound **3** with either malononitrile or ethyl cyanoacetate and either benzaldehyde, 4-chlorobenzaldehyde, or 4-methoxybenzaldehyde in 1,4-dioxane in the presence of trimethylamine as catalyst gave the 4*H*-thieno[2,3-*f*]chromene derivatives **23a-f**. Similarly, reaction of compound **3** with malononitrile or ethyl cyanoacetate and either benzald-



Schema 3: Synthesis of compounds **19, 20** and **21**.





Schema 4: Synthesis of compounds **23a-f** and **24a-f**.

hyde, 4-chlorobenzaldhyde or 4-methoxybenzaldhyde in 1,4-dioxane with ammonium acetate gave the 4,5,6,9-tetrahydrothieno[2,3-*f*]quinoline derivatives **24a-f**. The structure of compounds **23a-f** and **24a-f** (Scheme 4) was determined based on the study of their spectral data and elemental analyzes (see Experimental section).

4. Conclusion

The benzo[*b*]thiophene derivative was the major starting compound for several heterocyclization reactions. All new compounds were tested on the six cancer cell lines. In addition, the c-Met kinase activity of all compounds was calculated, and the most active compounds were tested against five other tyrosine kinases. In addition, compounds **10b**, **12**, **20**, **21**, **23a**, **23b**, **23e**, **24b**, **24e**, and **24f** were selected to investigate their inhibitory effect on Pim-1 kinase, as these compounds showed a large inhibitory effect on c-Met kinase and the cancer cell lines studied. The results obtained in this work will stimulate further work in the future.

Human and Animal Rights

No Animals/Humans were used for studies that are basis of this research.

Consent for Publication

This work is consent for publication through the Journal formats.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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Povzetek

V prispevku je opisana priprava 2-amino-6-okso-4,5,6,7-tetrahidrobenzo[*b*]tiofen-3-karbonitril (**3**) z reakcijo cikloheksan-1,4-diona z elementarnim žveplom in malononitrilom v 1,4-dioksanu in s trietilaminom kot katalizatorjem. Iz pripravljene spojine so z reakcijo s trietil ortoformatom in malononitrilom ali etil cianoacetatom v 1,4-dioksanu kot topilu, v prisotnosti trietilamina, nastali 4*H*-tieno[2,3-*f*]kromenski derivati **10a,b**. Poleg teh so iz spojine **3** pripravili tudi kondenzirane piranske in piridinske derivate. Citotoksičnost sintetiziranih spojin so preučevali na šestih rakavih celičnih linijah skupaj s c-Met kinazo in PC-3 celično linijo. Najbolj aktivne spojine so bile dodatno testirane na petih tirozin kinazah in Pim-1 kinazi. Večina testiranih spojin je pokazala močno inhibicijo, kar je dobra spodbuda za nadaljnje delo.