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Scientific paper

Cytotoxic Activities and Morphological Sudies of Thiophene, Thiazole and Pyridazine Derivatives Synthesized from Benzo[*d*]thiazole Derivatives

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Abstract

The benzo[*d*]thiazole derivatives **4a–c** were synthesized and used for the synthesis of thiophene derivatives **6a–f**. They form the arylhydrazone derivatives **8a–i** which were capable to form pyridazine derivatives **9a–i** and **10a–i**. The latter compounds reacted with thioglycolic acid to produce the thiazole derivatives **12a–i** and **13a–i**, respectively. The thiazole derivatives **15a–c** were also produced from **4a–c** through their reactions with elemental sulfur and phenylisothiocyanate. Further heterocyclization reactions of **15a** were carried out to produce thiophene derivatives. Evaluations of the synthesized products were carried out against some selected cancer cell lines and the most active compounds were further evaluated against the seventeen cancer cell lines classified according to the disease. Morphological changes of A549 cell line by the effect of compounds **13c** and **13h** were studied using microenvironment of the lung tissue where an excellent result was obtained.

Keywords: Benzo[*d*]thiazole, thiophene, thiazole, pyridazine, cytotoxicity, morphological studies

1. Introduction

In the field of synthetic and medicinal chemistry, heterocyclic compounds are important compounds that are considered not only as versatile set of scaffolds but also for being part of natural products. As a result, different substituents attached to the ring system can enhance the results of many studies of structure–activity relationship and as a result there is a possibility to tailor the physicochemical properties of the molecules. From a number of pharmacological areas benzo[*d*]thiazole is present in many bioactive compounds. Compounds containing the benzo[*d*]thiazole showed variety of biological activities these include antibacterial,[1] antifungal,[2] and anticancer agents,[3] and they have antidiabetic,[4] antidepressant,[5] anticonvulsant,[6] and radioprotective activities,[7] as well as neuroprotective properties useful for treating Alzheimer's disease[8] and Parkinson's disease.[9] Structure bottom right on the Figure 1 presents riluzole, a market drug that contains benzo[*d*]thiazole skeleton within its structure characterized by neuroprotective, anticonvulsant, and sedative properties[10,11] being very useful to treat amyotrophic lateral sclerosis.[12] Luciferin, another drug known in the market containing the benzo[*d*]thiazole moiety, extracted from firefly characterizes the bioluminescence of firefly species.[13] For that reason some of the compounds containing the benzo[*d*]thiazole nucleus can be used as fluorescent probes.[14]

Benzo[*d*]thiazole derivatives can be synthesized through cyclization or condensation reactions by the use of different catalysts like ammonium chloride, iodine, bromine, palladium acetate and copper catalysts. In addition, there are different reaction conditions that can be used for their synthesis like microwave, acidic and basic conditions and sometimes polymer-supported condensation reactions were adopted.[15–22] In recent years, our research group was concerned with the synthesis of varieties of new

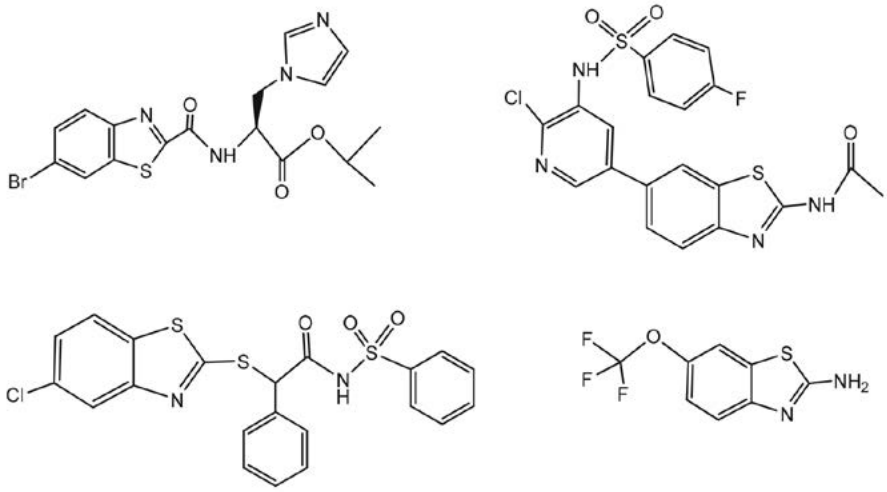


Figure 1. Representative examples of pharmacologically active benzo[*d*]thiazoles.

heterocyclic compounds followed by studying of their cytotoxicity against different cancer cell lines. Our previous work demonstrated that many of the synthesized compounds were useful in future drug designing.[23–26] In continuation of the previous work we are describing here a rapid and efficient preparation of a set of new benzo[*d*] thiazole derivatives starting from *ortho*-aminothiophenol that reacted with either ethyl cyanoacetate, diethylmalonate or ethyl acetoacetate. This new approach enables more rapid generation of a higher number of benzo[*d*]thiazole derivatives that were obtained using simple reactions using readily available reagents. The newly synthesized products were evaluated against six cancer cell lines together with studying c-Met kinase inhibitions.

2. Experimental

2. 1. General

Dry solvents were used through this work and all melting points of the synthesized compounds were recorded on Büchi melting point apparatus D-545. The IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. ^{13}C NMR and ^{1}H NMR spectra were measured on Bruker DPX300 instrument in DMSO-d_6 with TMS as the internal standard. Mass spectra were measured using EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were measured in the Micro-analytical Data center at Cairo University. All reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck) for getting complete reactions.

2. 1. 1. General Procedure for the Synthesis of the Benzo[d]thiazol-2-yl Derivatives 4a–c

Either ethyl cyanoacetate (1.13 g, 0.01 mol), diethylmalonate (1.60 g, 0.01 mol) or ethyl 3-oxobutanoate (1.30 g, 0.01 mol) was added to *ortho*-aminothiophenol (1.25 g, 0.01 mol). The whole reaction mixture was heated in an oil bath at 120 °C for 30 min, then was left to cool. The remaining product was triturated with ethanol and the formed solid product was collected by filtration.

2-(Benzo[*d*]thiazol-2-yl)acetonitrile (4a)

Yellow crystals from 1,4-dioxane, yield (1.39 g, 80%), m.p. 191–193 °C. IR (KBr) ν_{max} (cm^{-1}): 3050 (CH-aromatic), 2951 (CH-aliphatic), 2220 (CN), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.80 (s, 2H, CH_2), 7.25–7.39 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3 (CH_2), 116.8 (CN), 120.4, 121.8, 122.2, 122.7, 123.3, 123.6, 123.8, 124.2 (C_6H_4), 168.3 (C=N). Anal. Calcd for: $C_9H_6N_2S$ (174.22): C, 62.05; H, 3.47; N, 16.08; S, 18.40. Found: C, 61.87; H, 3.52; N, 15.80; S, 18.26%. EIMS: *m/z* 174 [M]$^+$ (62%).

Ethyl 2-(benzo[*d*]thiazol-2-yl)acetate (4b)

Yellow crystals from ethanol, yield 1.85 g (84%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3053 (CH-aromatic), 2951 (CH-aliphatic), 1688 (CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.16 (t, 3H, J = 7.25 Hz, $OCH_2\underline{CH_3}$), 3.83 (s, 2H, CH_2), 4.23 (q, 2H, J = 7.25 Hz, OCH_2CH_3), 7.26–7.43 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 36.3 (CH_2), 50.2 ($O\underline{CH_2}CH_3$), 120.1, 121.5, 121.7, 121.9, 122.8, 123.0, 123.4, 124.7 (C_6H_4), 165.2 (CO), 168.1 (C=N). Anal. Calcd for: $C_{11}H_{11}NO_2S$ (221.28): C, 59.71; H, 5.01; N, 6.33; S, 14.49. Found: C, 59.92; H, 4.83; N, 6.51; S, 14.62%. EIMS: *m/z* 221 [M]$^+$ (62%).

1-(Benzo[*d*]thiazol-2-yl)propan-2-one (4c)

Pale yellow crystals from ethanol, yield 1.43 g (75%), m.p. 210–212 °C. IR (KBr) ν_{max} (cm^{-1}): 3050 (CH-aromatic), 2954 (CH-aliphatic), 1689 (CO), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.28 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.27–7.45 (m, 4H, C_6H_4). ^{13}C

NMR (DMSO-d_6, 75 MHz): δ 24.6 (CH_3), 36.6 (CH_2), 119.8, 120.4, 120.7, 121.8, 122.3, 122.8, 123.4, 124.4 (C_6H_4), 165.8 (CO), 168.3 (C=N). Anal. Calcd for: $C_{10}H_9NOS$ (191.25): C, 62.80; H, 4.74; N, 7.32; S, 16.77. Found: C, 62.69; H, 4.81; N, 7.42; S, 16.58%. EIMS: *m/z* 191 $[M]^+$ (68%).

2. 1. 2. General Procedure for the Synthesis of the Thiophene Derivatives 6a–f

To a solution of either compound **4a** (1.74 g, 0.01 mol), **4b** (2.21 g, 0.01 mol) or **4c** (1.91 g, 0.01 mol) in absolute ethanol (60 mL) containing triethylamine (1.0 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The whole reaction mixture was heated under the reflux conditions for 1 h then was left to cool to room temperature and the produced solid product was collected by filtration.

2,4-Diamino-5-(benzo[*d*]thiazol-2-yl)thiophene-3-carbonitrile (6a)

Yellow crystals from 1,4-dioxane, yield 1.79 g (66%), m.p. 193–195 °C. IR (KBr) ν max (cm^{-1}): 3458-3323 (NH_2), 3054 (CH-aromatic), 2954 (CH-aliphatic), 2220 (CN), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 3.83, 4.28 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.24–7.52 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.7 (CN), 121.5, 122.2, 122.9, 123.5, 123.6, 123.8, 124.8 (C_6H_4), 138.3, 138.9, 140.2, 142.6 (thiophene C), 168.6 (C=N). Anal. Calcd for: $C_{12}H_8N_4S_2$ (272.35): C, 52.92; H, 2.96; N, 20.57; S, 23.55. Found: C, 52.87; H, 3.15; N, 20.72; S, 23.62%. EIMS: *m/z* 272 $[M]^+$ (68%).

2-Amino-5-(benzo[*d*]thiazol-2-yl)-4-hydroxythiophene-3-carbonitrile (6b)

Yellow crystals from 1,4-dioxane, yield 1.79 g (66%), m.p. 183–185 °C. IR (KBr) ν_{max} (cm^{-1}): 3549–3321 (OH, NH_2), 3054 (CH-aromatic), 2954 (CH-aliphatic), 2221 (CN), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.84 (s, 2H, D_2O exchangeable, NH_2), 7.27–7.42 (m, 4H, C_6H_4), 10.22 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.7 (CN), 121.5, 122.2, 122.9, 123.5, 123.6, 123.8, 124.8 (C_6H_4), 138.1, 138.4, 140.1, 142.8 (thiophene C), 168.8 (C=N). Anal. Calcd for: $C_{12}H_7N_3OS_2$ (273.33): C, 52.73; H, 2.58; N, 15.37; S, 23.46. Found: C, 52.88; H, 2.67; N, 15.48; S, 23.39%. EIMS: *m/z* 273 $[M]^+$ (80%).

2-Amino-5-(benzo[*d*]thiazol-2-yl)-4-methylthiophene-3-carbonitrile (6c)

Yellow crystals from ethanol, yield 1.76 g (65%), m.p. 153–155 °C. IR (KBr) ν_{max} (cm^{-1}): 3486–3323 (NH_2), 3056 (CH-aromatic), 2953 (CH-aliphatic), 2220 (CN), 1582 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.89 (s, 3H, CH_3), 3.81 (s, 2H, D_2O exchangeable, NH_2), 7.24–7.46 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.4 (CH_3), 116.4 (CN), 120.1, 122.5, 122.6, 123.2, 123.4, 123.7, 125.2 (C_6H_4), 138.0, 138.6, 140.1, 142.7 (thiophene C), 168.7 (C=N). Anal. Calcd for: $C_{13}H_9N_3S_2$ (271.36): C, 57.54; H, 3.34; N, 15.48; S, 23.63. Found: C, 57.72; H, 3.50; N, 15.16; S, 23.72%. EIMS: *m/z* 271 $[M]^+$ (72%).

Ethyl 2,4-Diamino-5-(benzo[*d*]thiazol-2-yl)thiophene-3-carboxylate (6d)

Orange crystals from ethanol, yield 1.91 g (60%), m.p. 203–205 °C. IR (KBr) ν_{max} (cm^{-1}): 3496–3341 (NH_2), 3055 (CH-aromatic), 2953 (CH-aliphatic), 2220 (CN), 1689 (CO), 1582 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.14 (t, 3H, J = 6.93 Hz, $OCH_2\underline{CH_3}$), 3.81, 4.25 (2×s, 4H, D_2O exchangeable, 2×NH_2), 4.23 (q, 2H, J = 6.93 Hz, $O\underline{CH_2}CH_3$), 7.24–7.46 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.2 ($OCH_2\underline{CH_3}$), 50.2 ($O\underline{CH_2}CH_3$), 121.5, 122.7, 122.6, 123.3, 123.5, 124.1, 125.6 (C_6H_4), 138.1, 139.2, 140.5, 142.8 (thiophene C), 165.4 (CO), 168.8 (C=N). Anal. Calcd for: $C_{14}H_{13}N_3O_2S_2$ (319.40): C, 52.65; H, 4.10; N, 13.16; S, 20.08. Found: C, 52.71; H, 3.96; N, 13.42; S, 19.83%. EIMS: *m/z* 319 $[M]^+$ (68%).

Ethyl 2-Amino-5-(benzo[*d*]thiazol-2-yl)-4-hydroxythiophene-3-carboxylate (6e)

Pale brown crystals from ethanol, yield 2.17 g (68%), m.p. 165–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3536–3358 (OH, NH_2), 3055 (CH-aromatic), 2952 (CH-aliphatic), 1688 (CO), 1588 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.13 (t, 3H, J = 6.11 Hz, $OCH_2\underline{CH_3}$), 4.28 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, J = 6.11 Hz, $O\underline{CH_2}CH_3$), 7.24–7.46 (m, 4H, C_6H_4), 10.30 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.4 ($OCH_2\underline{CH_3}$), 50.5 ($O\underline{CH_2}CH_3$), 121.6, 122.4, 122.9, 123.1, 123.7, 124.8, 125.6 (C_6H_4), 138.5, 138.2, 140.7, 142.7 (thiophene C), 164.9 (CO), 168.6 (C=N). Anal. Calcd for: $C_{14}H_{12}N_2O_3S_2$ (320.39): C, 52.48; H, 3.78; N, 8.74; S, 20.02. Found: C, 52.56; H, 3.87; N, 8.90; S, 19.91%. EIMS: *m/z* 320 $[M]^+$ (70%).

Ethyl 2-Amino-5-(benzo[*d*]thiazol-2-yl)-4-methylthiophene-3-carboxylate (6f)

Pale brown crystals from ethanol, yield 1.90 g (60%), m.p. 204–206 °C. IR (KBr) ν_{max} (cm^{-1}): 3451–3346 (NH_2), 3058 (CH-aromatic), 2952 (CH-aliphatic), 1689 (CO), 1588 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.12 (t, 3H, J = 7.11 Hz, $OCH_2\underline{CH_3}$), 2.69 (s, 3H, CH_3), 4.29 (s, 2H, D_2O exchangeable, NH_2), 4.24 (q, 2H, J = 6.11 Hz, $O\underline{CH_2}CH_3$), 7.26–7.49 (m, 4H, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.1 ($OCH_2\underline{CH_3}$), 36.8 (CH_3), 50.1 ($O\underline{CH_2}CH_3$), 121.4, 122.8, 123.4, 123.5, 124.1, 124.8, 125.5 (C_6H_4), 138.5, 138.7, 140.5, 142.9 (thiophene C), 165.3 (CO), 168.3 (C=N). Anal. Calcd for: $C_{15}H_{14}N_2O_2S_2$ (318.41): C, 56.58; H, 4.43; N, 8.80; S, 20.14. Found: C, 56.32; H, 4.62; N, 8.73; S, 19.88%. EIMS: *m/z* 318 $[M]^+$ (66%).

2. 1. 3. General Procedure for the Synthesis of the Arylhydrazone Derivatives 8a–i

To a cold solution (0–5 °C) of either compound **4a** (1.74 g, 0.01 mol), **4b** (2.21 g, 0.01 mol) or **4c** (1.91 g, 0.01 mol) in ethanol (60 mL) containing sodium acetate (3.0 g) either of benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride or 4-methoxybenzenediazonium chloride [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of either aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methoxyaniline (1.23 g, 0.01 mol) in concentrated hydrochloric acid (8.0 mL, 18%) with continuous stirring] was added with continuous stirring. The solid product formed upon stirring for 1 h was collected by filtration.

N′-Phenylbenzo[*d*]thiazole-2-carbohydrazonoyl Cyanide (8a)

Yellow crystals from 1,4-dioxane, yield 1.61 g (58%), m.p. 247–249 °C. IR (KBr) ν_{max} (cm^{-1}): 3348–3321 (NH), 3050 (CH-aromatic), 2951 (CH-aliphatic), 2220 (CN), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.23–7.42 (m, 9H, C_6H_5, C_6H_4), 8.52 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.8 (CN), 120.2, 121.5, 122.0, 122.5, 122.8, 123.1, 123.6, 124.7, 124.9, 125.3 (C_6H_5, C_6H_4), 168.5, 170.0 (2×C=N). Anal. Calcd for: $C_{15}H_{10}N_4S$ (278.33): C, 64.73; H, 3.62; N, 20.13; S, 11.52. Found: C, 64.90; H, 3.42; N, 20.07; S, 11.65%. EIMS: *m/z* 278 $[M]^+$ (68%).

N′-(4-Chlorophenyl)benzo[*d*]thiazole-2-carbohydrazonoyl Cyanide (8b)

Orange crystals from ethanol, yield 1.69 g (63%), m.p. 211–213 °C. IR (KBr) ν_{max} (cm^{-1}): 3352–3341 (NH), 3050 (CH-aromatic), 2951 (CH-aliphatic), 2221 (CN), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.25–7.58 (m, 8H, 2×C_6H_4), 8.49 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.9 (CN), 120.0, 120.7, 121.3, 122.5, 122.6, 123.3, 123.8, 124.2, 124.5, 125.9 (2×C_6H_4), 168.7, 170.1 (2×C=N). Anal. Calcd for: $C_{15}H_9ClN_4S$ (312.78): C, 57.60; H, 2.90; N, 17.91; S, 10.25. Found: C, 57.83; H, 3.19; N, 18.21; S, 10.36%. EIMS: *m/z* 312 $[M]^+$ (70%).

N′-(4-Methoxyphenyl)benzo[*d*]thiazole-2-carbohydrazonoyl Cyanide (8c)

Orange crystals from ethanol, yield 1.90 g (62%), m.p. 197–199 °C. IR (KBr) ν_{max} (cm^{-1}): 3362–3336 (NH), 3050 (CH-aromatic), 2951 (CH-aliphatic), 2220 (CN), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.69 (s, 3H, OCH_3), 7.23–7.53 (m, 8H, 2×C_6H_4), 8.47 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.8 (OCH_3), 116.7 (CN), 120.3, 120.9, 121.1, 122.4, 122.8, 123.0, 123.6, 124.5, 124.8, 125.3 (2×C_6H_4), 168.9, 170.3 (2×C=N). Anal. Calcd for: $C_{16}H_{12}N_4OS$ (308.36): C, 62.32; H, 3.92; N, 18.17; S, 10.40. Found: C, 62.49; H, 3.75; N, 18.36; S, 10.42%. EIMS: *m/z* 308 $[M]^+$ (68%).

Ethyl 2-(Benzo[*d*]thiazol-2-yl)-2-(2-phenylhydrazono) acetate (8d)

Red crystals from ethanol, yield 1.78 g (55%), m.p. 215–217 °C. IR (KBr) ν_{max} (cm^{-1}): 3349–3327 (NH), 3050 (CH-aromatic), 2951 (CH-aliphatic), 2220 (CN), 1688 (CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.15 (t, 3H, *J* = 7.28 Hz, $OCH_2\underline{CH_3}$), 4.25 (q, 2H, *J* = 7.28 Hz, $O\underline{CH_2}CH_3$), 7.27–7.40 (m, 9H, C_6H_5, C_6H_4), 8.47 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.3 ($O\underline{CH_2}CH_3$), 50.2 ($OCH_2\underline{CH_3}$), 120.1, 120.8, 121.4, 122.6, 122.2, 123.5, 123.8, 124.1, 124.5, 125.1 (C_6H_5, C_6H_4), 165.4 (CO), 168.7, 170.0 (2×C=N). Anal. Calcd for: $C_{17}H_{15}N_3O_2S$ (325.38): C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 62.88; H, 4.80; N, 13.21; S, 10.16%. EIMS: *m/z* 325 $[M]^+$ (78%).

Ethyl 2-(Benzo[*d*]thiazol-2-yl)-2-(2-(4-chlorophenyl) hydrazono)acetate (8e)

Red crystals from 1,4-dioxane, yield 2.13 g (60%), m.p. 188–190 °C. IR (KBr) ν_{max} (cm^{-1}): 3354–3343 (NH), 3050 (CH-aromatic), 2954 (CH-aliphatic), 1669 (CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.13 (t, 3H, *J* = 6.68 Hz, $OCH_2\underline{CH_3}$), 4.22 (q, 2H, *J* = 6.68 Hz, $O\underline{CH_2}CH_3$), 7.23–7.56 (m, 8H, 2 C_6H_4), 8.51 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.6 ($O\underline{CH_2}CH_3$), 50.3 ($OCH_2\underline{CH_3}$), 120.3, 120.5, 121.6, 122.9, 122.1, 123.7, 123.8, 124.3, 124.8, 125.9 (2×C_6H_4), 165.4 (CO), 168.9, 170.1 (2×C=N). Anal. Calcd for: $C_{17}H_{14}ClN_3O_2S$ (359.83): C, 56.74; H, 3.92; N, 11.68; S, 8.91. Found: C, 56.83; H, 4.15; N, 11.80; S, 9.25%. EIMS: *m/z* 359 $[M]^+$ (86%).

Ethyl 2-(Benzo[*d*]thiazol-2-yl)-2-(2-(4-methoxyphenyl) hydrazono)acetate (8f)

Orange crystals from 1,4-dioxane, yield 2.48 g (70%), m.p. 148–150 °C. IR (KBr) ν_{max} (cm^{-1}): 3369–3353 (NH), 3053 (CH-aromatic), 2954 (CH-aliphatic), 1688 (CO), 1588 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.16 (t, 3H, *J* = 6.48 Hz, $OCH_2\underline{CH_3}$), 3.80 (s, 3H, OCH_3), 4.25 (q, 2H, *J* = 6.48 Hz, $O\underline{CH_2}CH_3$), 7.23–7.56 (m, 8H, 2×C_6H_4), 8.49 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.4 ($O\underline{CH_2}CH_3$), 50.1 ($OCH_2\underline{CH_3}$), 50.8 (OCH_3), 120.1, 120.8, 121.3, 122.6, 122.1, 123.9, 124.1, 124.5, 125.2, 125.7 (2×C_6H_4), 165.8 (CO), 168.9, 169.8 (2×C=N). Anal. Calcd for: $C_{18}H_{17}N_3O_3S$ (355.41): C, 60.83; H, 4.82; N, 11.82; S, 9.02. Found: C, 60.77; H, 4.79; N, 11.97; S, 9.28%. EIMS: *m/z* 355 $[M]^+$ (60%).

1-(Benzo[*d*]thiazol-2-yl)-1-(2-phenylhydrazono)propan-2-one (8g)

Orange crystals from 1,4-dioxane, yield 1.79 g (61%), m.p. 208–210 °C. IR (KBr) ν_{max} (cm^{-1}): 3353–3348 (NH), 3053 (CH-aromatic), 2950 (CH-aliphatic), 1688 (CO), 1584 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.87 (s, 3H, CH_3), 7.26–7.48 (m, 9H, C_6H_5, C_6H_4), 8.46 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DM-

SO-d_6, 75 MHz): δ 36.6 (CH_3), 120.0, 120.6, 121.5, 122.8, 123.4, 123.5, 123.6, 124.2, 124.8, 125.6 (C_6H_5, C_6H_4), 165.5 (CO), 168.8 (C=N). Anal. Calcd for: $C_{16}H_{13}N_3OS$ (295.36): C, 65.06; H, 4.44; N, 14.23; S, 10.86. Found: C, 65.15; H, 4.60; N, 14.37; S, 11.07%. EIMS: *m/z* 295 $[M]^+$ (58%).

1-(Benzo[*d*]thiazol-2-yl)-1-(2-(4-chlorophenyl)hydrazono)propan-2-one (8h)

Orange crystals from 1,4-dioxane, yield 2.13 g (64%), m.p. 166–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3372–3350 (NH), 3053 (CH-aromatic), 2950 (CH-aliphatic), 1688 (CO), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.93 (s, 3H, CH_3), 7.23–7.49 (m, 8H, 2×C_6H_4), 8.43 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3 (CH_3), 120.2, 120.5, 121.1, 122.6, 123.1, 123.4, 124.0, 124.2, 124.5, 125.8 (2×C_6H_4), 165.8 (CO), 168.5, 170.3 (2×C=N). Anal. Calcd for: $C_{16}H_{12}ClN_3OS$ (329.80): C, 58.27; H, 3.67; N, 12.74; S, 9.72. Found: C, 58.31; H, 3.80; N, 12.42; S, 9.59%. EIMS: *m/z* 329 $[M]^+$ (66%).

1-(Benzo[*d*]thiazol-2-yl)-1-(2-(4-methoxyphenyl)hydrazono)propan-2-one (8i)

Orange crystals from 1,4-dioxane, yield 2.17 g (67%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3389–3334 (NH), 3058 (CH-aromatic), 2950 (CH-aliphatic), 1689 (CO), 1581 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.96 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 7.22–7.58 (m, 8H, 2×C_6H_4), 8.45 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.2 (CH_3), 120.3, 120.8, 121.4, 122.8, 123.4, 123.9, 124.2, 124.5, 124.6, 125.2 (2×C_6H_4), 165.7 (CO), 168.9, 170.1 (2×C=N). Anal. Calcd for: $C_{17}H_{15}N_3O_2S$ (325.38): C, 62.75 H, 4.65; N, 12.91; S, 9.85. Found: C, 62.51; H, 4.75; N, 12.72; S, 9.69%. EIMS: *m/z* 325 $[M]^+$ (68%).

2. 1. 4. General Procedure for the Synthesis of the Pyridazine Derivatives 9a–i

To a solution of either **8a** (2.78 g, 0.01 mol), **8b** (3.12 g, 0.01 mol), **8c** (3.08 g, 0.01 mol), **8d** (3.25 g, 0.01 mol), **8e** (3.59 g, 0.01 mol), **8f** (3.55 g, 0.01 mol), **8g** (2.95 g, 0.01 mol), **8h** (3.29 g, 0.01 mol) or **8i** (3.25 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), malononitrile (0.66 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-imino-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (9a)

Pale yellow crystals from 1,4-dioxane, yield 1.72 g (50%), m.p. 166–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3354–3360 (NH), 3050 (CH-aromatic), 2220 (CN), 1658 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.96 (s, 2H, D_2O echangeable, NH_2), 7.25–7.43 (m, 9H, C_6H_5, C_6H_4), 8.58 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 117.1 (CN), 120.2, 120.4, 121.1, 122.5, 122.9, 123.2, 123.7, 124.7, 125.4, 125.8 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 168.6, 170.2, 170.3 (3×C=N). Anal. Calcd for: $C_{18}H_{12}N_6S$ (344.39): C, 62.77; H, 3.51; N, 24.40; S, 9.31. Found: C, 62.82; H, 3.69; N, 24.65; S, 19.52%. EIMS: *m/z* 344 $[M]^+$ (72%).

5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-imino-2,3-dihydropyridazine-4-carbonitrile (9b)

Pale yellow crystals from 1,4-dioxane, yield 2.49 g (66%), m.p. 240–243 °C. IR (KBr) ν_{max} (cm^{-1}): 3361–3343 (NH), 3050 (CH-aromatic), 2220 (CN), 1658 (C=N), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.85 (s, 2H, D_2O echangeable, NH_2), 7.28–7.52 (m, 8H, 2×C_6H_4), 8.42 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 117.3 (CN), 120.3, 120.7, 121.2, 122.6, 122.9, 122.8, 122.5, 124.4, 125.8, 125.9 (C_6H_5, C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 168.4, 170.1, 170.6 (3×C=N). Anal. Calcd for: $C_{18}H_{11}ClN_6S$ (378.84): C, 57.07; H, 2.93; N, 22.18; S, 8.46. Found: C, 57.26; H, 3.18; N, 22.31; S, 8.69%. EIMS: *m/z* 378 $[M]^+$ (68%).

5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-imino-2-(4-methoxyphenyl)-2,3-dihydropyridazine-4-carbonitrile (9c)

Pale yellow crystals from 1,4-dioxane, yield 2.20 g (59%), m.p. 196–198 °C. IR (KBr) ν_{max} (cm^{-1}): 3349–3362 (NH), 3050 (CH-aromatic), 2220 (CN), 1662 (C=N), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.66 (s, 3H, OCH_3), 4.88 (s, 2H, D_2O echangeable, NH_2), 7.25–7.58 (m, 8H, 2×C_6H_4), 8.46 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.6 (OCH_3), 116.9 (CN), 120.6, 120.6, 121.4, 122.6, 122.5, 123.8 123.9, 124.1, 125.5, 125.6 (2×C_6H_4), 140.5, 142.3 (pyridazine C-3, C-4), 168.4, 170.3, 170.5 (3×C=N). Anal. Calcd for: $C_{19}H_{14}N_6OS$ (374.42): C, 60.95; H, 3.77; N, 22.45; S, 8.56. Found: C, 61.28; H, 3.86; N, 22.29; S, 8.69%. EIMS: *m/z* 374 $[M]^+$ (58%).

6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-imino-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (9d)

Yelow crystals from 1,4-dioxane, yield 2.07 g (60%), m.p. 188–190 °C. IR (KBr) ν_{max} (cm^{-1}): 3349–3352 (OH, NH), 3050 (CH-aromatic), 2220 (CN), 1663 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.25–7.43 (m, 9H, C_6H_5, C_6H_4), 8.44 (s, 1H, D_2O exchangeable, NH), 10.30 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.7 (CN), 120.2, 120.4, 121.8, 122.1, 122.8, 123.2 123.9, 124.1, 125.3, 125.9 (C_6H_5, C_6H_4), 140.1, 142.6 (pyridazine C-3, C-4), 168.8, 170.1, 170.6 (3×C=N). Anal. Calcd for: $C_{18}H_{11}N_5OS$ (345.38): C, 62.60; H, 3.21; N, 20.28; S, 9.28. Found: C, 62.73; H, 3.36; N, 20.42; S, 9.31%. EIMS: *m/z* 345 $[M]^+$ (70%).

6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-hydroxy-3-imino-2,3-dihydropyridazine-4-carbonitrile (9e)

Yellow crystals from 1,4-dioxane, yield 2.41 g (70%), m.p. 155–157 °C. IR (KBr) ν_{max} (cm^{-1}): 3356–3332 (OH, NH), 3050 (CH-aromatic), 2220 (CN), 1661 (C=N), 1584 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.23–7.56 (m, 8H, 2×C_6H_4), 8.46 (s, 1H, D_2O exchangeable, NH), 10.28 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.9 (CN), 120.1, 120.5, 121.5, 122.7, 122.8, 123.2 123.9, 124.6, 125.1, 125.6 (2×C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 168.9, 170.0, 170.8 (3×C=N). Anal. Calcd for: $C_{18}H_{10}ClN_5OS$ (379.82): C, 56.92; H, 2.65; N, 18.44; S, 8.44. Found: C, 57.13; H, 2.80; N, 18.72; S, 8.24%. EIMS: *m/z* 379 $[M]^+$ (62%).

6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-imino-2-(4-methoxyphenyl)-2,3-dihydropyridazine-4-carbonitrile (9f)

Pale yellow crystals from 1,4-dioxane, yield 2.36 g (63%), m.p. 196–198 °C. IR (KBr) ν_{max} (cm^{-1}): 3358–3337 (OH, NH), 3050 (CH-aromatic), 2220 (CN), 1660 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.68 (s, 3H, OCH_3), 7.23–7.56 (m, 8H, 2×C_6H_4), 8.45 (s, 1H, D_2O exchangeable, NH), 10.22 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.3 (OCH_3), 116.7 (CN), 120.2, 120.8, 121.5, 122.1, 122.6, 123.3, 123.5, 124.7, 125.2, 125.5 (2×C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 168.6, 170.1, 170.3 (3×C=N). Anal. Calcd for: $C_{19}H_{13}N_5O_2S$ (375.40): C, 60.79; H, 3.49; N, 18.66; S, 8.54. Found: C, 60.59; H, 3.52; N, 18.73; S, 8.72%. EIMS: *m/z* 375 $[M]^+$ (60%).

6-(Benzo[*d*]thiazol-2-yl)-3-imino-5-methyl-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (9g)

Pale yellow crystals from 1,4-dioxane, yield 2.05 g (60%), m.p. 213–215 °C. IR (KBr) ν_{max} x (cm^{-1}): 3349–3353 (NH), 3050 (CH-aromatic), 2220 (CN), 1663 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.83 (s, 3H, CH_3), 7.27–7.48 (m, 9H, C_6H_5, C_6H_4), 8.43 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.2 (CH_3), 116.9 (CN), 120.4, 120.6, 121.3, 122.4, 122.8, 123.2, 123.7, 124.7, 125.0, 125.8 (C_6H_5, C_6H_4), 168.8, 170.2, 170.4 (3×C=N). Anal. Calcd for: $C_{19}H_{13}N_5S$ (343.41): C, 66.45; H, 3.82; N, 20.39; S, 9.34. Found: C, 66.53; H, 3.69; N, 20.41; S, 9.55%. EIMS: *m/z* 343 $[M]^+$ (50%).

6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-imino-5-methyl-2,3-dihydropyridazine-4-carbonitrile (9h)

Pale brown crystals from 1,4-dioxane, yield 2.48 g (66%), m.p. 149–151 °C. IR (KBr) ν_{max} (cm^{-1}): 3358–3328 (NH), 3050 (CH-aromatic), 2220 (CN), 1660 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.85 (s, 3H, CH_3), 7.25–7.52 (m, 8H, 2×C_6H_4), 8.46 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.8 (CH_3), 117.2 (CN), 120.2, 120.8, 121.1, 122.6, 122.9, 123.1, 123.8, 124.7, 125.4, 125.6 (2×C_6H_4), 140.3, 142.6 (pyridazine C-3, C-4), 168.4, 170.1, 170.2 (3×C=N). Anal. Calcd for: $C_{19}H_{12}ClN_5S$ (377.85): C, 60.40; H, 3.20; N, 18.53; S, 8.49. Found: C, 60.63; H, 3.36; N, 18.70; S, 8.52%. EIMS: *m/z* 377 $[M]^+$ (68%).

6-(Benzo[*d*]thiazol-2-yl)-3-imino-2-(4-methoxyphenyl)-5-methyl-2,3-dihydropyridazine-4-carbonitrile (9i)

Pale brown crystals from 1,4-dioxane, yield 2.16 g (58%), m.p. 201–203 °C. IR (KBr) ν_{max} (cm^{-1}): 3373–3346 (NH), 3050 (CH-aromatic), 2220 (CN), 1660 (C=N), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.87 (s, 3H, CH_3), 3.59 (s, 3H, OCH_3), 7.22–7.58 (m, 8H, 2×C_6H_4), 8.44 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.5 (CH_3), 50.8 (OCH_3), 117.1 (CN), 120.2, 120.6, 121.2, 122.4, 122.9, 123.5, 123.8, 124.4, 125.8, 125.8 (2×C_6H_4), 140.1, 142.2 (pyridazine C-3, C-4), 168.6, 170.3, 170.5 (3×C=N). Anal. Calcd for: $C_{20}H_{15}N_5OS$ (373.43): C, 64.33; H, 4.05; N, 18.75; S, 8.59. Found: C, 64.51; H, 3.87; N, 18.49; S, 8.68%. EIMS: *m/z* 373 $[M]^+$ (85%).

2. 1. 5. General Procedure for the Synthesis of the Pyridazin-6-one Derivatives 10a–i

To a solution of either **8a** (2.78 g, 0.01 mol), **8b** (3.12 g, 0.01 mol), **8c** (3.08 g, 0.01 mol), **8d** (3.25 g, 0.01 mol), **8e** (3.59 g, 0.01 mol), **8f** (3.55 g, 0.01 mol), **8g** (2.95 g, 0.01 mol), **8h** (3.29 g, 0.01 mol) or **8i** (3.25 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL), ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-oxo-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (10a)

Pale brown crystals from acetic acid, yield 2.00 g (58%), m.p. 179–181 °C. IR (KBr) ν_{max} (cm^{-1}): 3368–3343 (NH_2), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1658 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.98 (s, 2H, D_2O echangeable, NH_2), 7.24–7.41 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 117.3 (CN), 120.1, 120.5, 121.4, 122.8, 122.9, 123.5, 123.7, 124.2, 125.4, 125.9 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 165.8 (CO), 168.8, 170.2 (2×C=N). Anal. Calcd for: $C_{18}H_{11}N_5OS$ (345.38): C, 62.60; H, 3.21; N, 20.28; S, 9.28. Found: C, 62.87; H, 3.39; N, 20.53; S, 9.42%. EIMS: *m/z* 345 $[M]^+$ (60%).

5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10b)

Pale yellow crystals from 1,4-dioxane, yield 1.89 g (50%), m.p. 166–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3378–3323 (NH_2), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1658 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ

4.88 (s, 2H, D_2O echangeable, NH_2), 7.28–7.56 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.9 (CN), 120.1, 120.5, 121.2, 122.4, 122.7, 123.2, 123.7, 124.4, 125.4, 125.8 (C_6H_5, C_6H_4), 140.3, 142.2 (pyridazine C-3, C-4), 166.4 (CO), 170.1, 170.8 (2×C=N). Anal. Calcd for: $C_{18}H_{10}ClN_5OS$ (379.82): C, 56.92; H, 2.65; N, 18.44; S, 8.44. Found: C, 57.23; H, 2.80; N, 18.58; S, 8.64%. EIMS: *m/z* 379 $[M]^+$ (60%).

5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-methoxyphenyl)-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10c)

Pale yellow crystals from 1,4-dioxane, yield 2.25 g (60%), m.p. 230–233 °C. IR (KBr) ν_{max} (cm^{-1}): 3353–3322 (NH), 3050 (CH-aromatic), 2220 (CN), 1689 (CO), 1660 (C=N), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.68 (s, 3H, OCH_3), 4.83 (s, 2H, D_2O echangeable, NH_2), 7.27–7.56 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.6 (OCH_3), 116.7 (CN), 120.0, 120.1, 121.4, 122.2, 122.5, 123.4 123.9, 124.6, 125.2, 125.9 (2×C_6H_4), 140.1, 142.2 (pyridazine C-3, C-4), 165.3 (CO), 168.5, 170.2 (2×C=N). Anal. Calcd for: $C_{19}H_{13}N_5O_2S$ (375.40): C, 60.79; H, 3.49; N, 18.66; S, 8.54. Found: C, 60.73; H, 3.52; N, 18.73; S, 8.69%. EIMS: *m/z* 375 $[M]^+$ (68%).

6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-oxo-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (10d)

Yellow crystals from 1,4-dioxane, yield 2.00 g (58%), m.p. 168–170 °C. IR (KBr) ν_{max} (cm^{-1}): 3558–3336 (OH, NH), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1660 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.27–7.43 (m, 9H, C_6H_5, C_6H_4), 10.32 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 116.8 (CN), 120.1, 120.6, 121.2, 122.3, 122.8, 123.3 123.6, 124.3, 125.6, 125.7 (C_6H_5, C_6H_4), 140.4, 142.3 (pyridazine C-3, C-4), 166.3 (CO), 170.1, 170.4 (2×C=N). Anal. Calcd for: $C_{18}H_{10}N_4O_2S$ (346.36): C, 62.42; H, 2.91; N, 16.18; S, 9.26. Found: C, 62.53; H, 3.11; N, 16.28; S, 9.40%. EIMS: *m/z* 346 $[M]^+$ (80%).

6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10e)

Yellow crystals from 1,4-dioxane, yield 2.39 g (63%), m.p. 168–170 °C. IR (KBr) ν_{max} (cm^{-1}): 3359–3340 (OH, NH), 3053 (CH-aromatic), 2220 (CN), 1688 (CO), 1663 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.23–7.56 (m, 8H, 2×C_6H_4), 10.38 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 117.2 (CN), 120.1, 120.6, 121.3, 122.5, 122.8, 123.1 123.4, 124.3, 125.3, 126.53 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 166.5 (CO), 170.1, 170.5 (2×C=N). Anal. Calcd for: $C_{18}H_9ClN_4O_2S$ (380.81): C, 56.77; H, 2.38; N, 14.71; S, 8.42. Found: C, 56.53; H, 2.40; N, 14.59; S, 8.63%. EIMS: *m/z* 380 $[M]^+$ (77%).

6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-2-(4-methoxyphenyl)-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10f)

Pale yellow crystals from 1,4-dioxane, yield 2.36 g (63%), m.p. 196–198 °C. IR (KBr) ν_{max} (cm^{-1}): 3358–3337 (OH, NH), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1660 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.68 (s, 3H, OCH_3), 7.23–7.56 (m, 8H, 2×C_6H_4), 10.22 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.3 (OCH_3), 116.7 (CN), 120.2, 120.8, 121.5, 122.1, 122.6, 123.5, 123.3, 124.7, 125.2, 125.5 (2×C_6H_4), 168.6, 170.1 (2 X C=N). Anal. Calcd for: $C_{19}H_{12}N_4O_3S$ (376.39): C, 60.63; H, 3.21; N, 14.89; S, 8.52. Found: C, 60.59; H, 3.42; N, 14.73; S, 8.72%. EIMS: *m/z* 376 $[M]^+$ (64%).

6-(Benzo[*d*]thiazol-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydropyridazine-4-carbonitrile (10g)

Pale yellow crystals from 1,4-dioxane, yield 2.23 g (65%), m.p. 244–247 °C. IR (KBr) ν_{max} (cm^{-1}): 3361–3348 (NH), 3050 (CH-aromatic), 2220 (CN), 1689 (CO), 1663 (C=N), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.86 (s, 3H, CH_3), 7.23–7.45 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.8 (CH_3), 116.7 (CN), 120.1, 120.4, 121.5, 122.9, 123.0, 123.2, 123.6, 124.5, 125.20, 125.4 (C_6H_5, C_6H_4), 140.3, 142.3 (pyridazine C-3, C-4), 166.3 (CO), 168.8, 170.2 (2 X C=N). Anal. Calcd for: $C_{19}H_{12}N_4OS$ (344.39): C, 66.26; H, 3.51; N, 16.27; S, 9.31. Found: C, 66.35; H, 3.68; N, 16.32; S, 9.53%. EIMS: *m/z* 344 $[M]^+$ (67%).

6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-methyl-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10h)

Pale brown crystals from 1,4-dioxane, yield 2.07 g (55%), m.p. 180–183 °C. IR (KBr) ν_{max} (cm^{-1}): 3361–3328 (NH), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1660 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.88 (s, 3H, CH_3), 7.23–7.56 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.6 (CH_3), 117.0 (CN), 120.1, 120.6, 121.5, 121.8, 122.4, 123.1, 123.4, 124.2, 125.3, 125.6 (2×C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 165.3 (CO), 168.4, 170.3 (2 X C=N). Anal. Calcd for: $C_{19}H_{11}ClN_4OS$ (378.83): C, 60.24; H, 2.93; N, 14.79; S, 8.46. Found: C, 60.39; H, 3.16; N, 14.68; S, 8.58%. EIMS: *m/z* 378 $[M]^+$ (80%).

6-(Benzo[*d*]thiazol-2-yl)-2-(4-methoxyphenyl)-5-methyl-3-oxo-2,3-dihydropyridazine-4-carbonitrile (10i)

Pale brown crystals from 1,4-dioxane, yield 2.24 g (60%), m.p. 198–200 °C. IR (KBr) ν_{max} (cm^{-1}): 3380–3326 (NH), 3050 (CH-aromatic), 2220 (CN), 1688 (CO), 1660 (C=N), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.89 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 7.22–7.58 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.8 (CH_3), 50.6 (OCH_3), 117.1 (CN), 120.1, 120.4, 120.8, 122.4, 122.7, 123.3, 123.8, 124.4, 125.6, 125.9 (2× C_6H_4), 140.4, 142.3 (pyridazine C-3, C-4), 166.3 (CO), 168.6, 170.3 (2 X C=N). Anal. Calcd for: $C_{20}H_{14}N_4O_2S$ (374.42): C, 64.16; H, 3.77; N, 14.96; S, 8.56. Found: C, 64.28; H, 3.89; N, 15.42; S, 8.73%. EIMS: *m/z* 374 $[M]^+$ (75%).

2. 1. 6. General Method for the Synthesis of the Thiazole Derivatives 12a–i

To a solution of either **9a** (3.45g, 0.01 mol), **9b** (3.79g, 0.01 mol), **9c** (3.75 g, 0.01 mol), **9d** (3.46 g, 0.01 mol), **9e** (3.80 g, 0.01 mol), **9f** (3.76 g, 0.01 mol), **9g** (3.44 g, 0.01 mol), **9h** (3.78 g, 0.01 mol) or **9i** (3.74 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL) thioglycolic acid (0.92 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then was left to cool and the formed solid product was collected by filtration.

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-imino-2-phenyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12a)

Pale brown crystals from acetic acid, yield 2.50 g (58%), m.p. 244–246 °C. IR (KBr) ν_{max} (cm^{-1}): 3349–3343 (NH_2, NH), 3050 (CH-aromatic), 1689 (CO), 1658 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.96 (s, 2H, D_2O echangeable, NH_2), 5.31 (s, 2H, thiazole CH_2), 7.26–7.45 (m, 9H, C_6H_5, C_6H_4), 8.48 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.8 (thiazole CH_2), 120.2, 120.3, 121.1, 122.5, 122.8, 123.6, 123.9, 124.3, 124.4, 125.6 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 165.9 (CO), 168.8, 169.2, 170.0, 170.1 (4×C=N). Anal. Calcd for: $C_{20}H_{14}N_6OS_2$ (418.49): C, 57.40; H, 3.37; N, 20.08; S, 15.32. Found: C, 57.63; H, 3.51; N, 20.25; S, 15.52%. EIMS: *m/z* 418 $[M]^+$ (72%).

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-imino-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12b)

Yellow crystals from 1,4-dioxane, yield 2.71 g (60%), m.p. 177–180 °C. IR (KBr) ν_{max} (cm^{-1}): 3369–3343 (NH_2, NH), 3054 (CH-aromatic), 1689 (CO), 1656 (C=N), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.85 (s, 2H, D_2O echangeable, NH_2), 5.66 (s, 2H, thaizole CH_2), 7.23–7.56 (m, 8H, 2×C_6H_4), 8.39 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.6 (thiazole CH_2), 120.3, 120.6, 121.2, 122.6, 122.9, 123.2, 123.8, 124.1, 125.4, 125.6 (2×C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 166.6 (CO), 169.0, 169.5, 170.1, 170.9 (4×C=N). Anal. Calcd for: $C_{20}H_{13}ClN_6OS_2$ (452.94): C, 53.03; H, 2.89; N, 18.55; S, 14.16. Found: C, 52.96; H, 2.93; N, 18.68; S, 14.25%. EIMS: *m/z* 452 $[M]^+$ (66%).

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-imino-2-(4-methoxyphenyl)-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12c)

Pale yellow crystals from 1,4-dioxane, yield 2.77 g (62%), m.p. 166–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3368–3341 (NH_2, NH), 3050 (CH-aromatic), 1689 (CO), 1660 (C=N), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.68 (s, 3H, OCH_3), 4.83 (s, 2H, D_2O exchangeable, NH_2), 5.26 (s, 2H, thiazole CH_2), 7.25–7.56 (m, 8H, 2×C_6H_4), 8.36 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.6 (thiazole CH_2), 50.4 (OCH_3), 120.3, 120.2, 121.6, 122.2, 122.5, 123.6, 123.8, 124.2, 125.4, 125.8 (2×C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 165.9 (CO), 168.5, 169.3, 170.0, 170.3 (4×C=N). Anal. Calcd for: $C_{21}H_{16}N_6O_2S_2$ (448.52): C, 56.23; H, 3.60; N, 18.74; S, 14.30. Found: C, 56.48; H, 3.58; N, 18.82; S, 14.53%. EIMS: *m/z* 448 $[M]^+$ (60%).

2-(6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-imino-2-phenyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12d)

Yellow crystals from 1,4-dioxane, yield 2.30 g (55%), m.p. 183–185 °C. IR (KBr) ν_{max} (cm^{-1}): 3528–3338 (OH, NH), 3050 (CH-aromatic), 1689 (CO), 1660 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.60 (s, 2H, thiazole CH_2), 7.27–7.46 (m, 9H, C_6H_5, C_6H_4), 8.40 (s, 1H, D_2O exchangeable, NH), 10.32 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.6 (thiazole CH_2), 120.3, 120.4, 121.0, 121.7, 122.5, 123.7, 123.9, 124.1, 125.2, 125.6 (C_6H_5, C_6H_4), 140.4, 142.3 (pyridazine C-3, C-4), 166.1 (CO), 168.9, 169.3, 170.1, 170.3 (4×C=N). Anal. Calcd for: $C_{20}H_{13}N_5O_2S_2$ (419.48): C, 57.26; H, 3.12; N, 16.70; S, 15.29. Found: C, 57.36; H, 3.30; N, 16.59; S, 15.31%. EIMS: *m/z* 419 $[M]^+$ (73%).

2-(6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-hydroxy-3-imino-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12e)

Yellow crystals from 1,4-dioxane, yield 2.76 g (61%), m.p. 205–207 °C. IR (KBr) ν_{max} (cm^{-1}): 3541–3329 (OH, NH), 3050 (CH-aromatic), 1689 (CO), 1662 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.63 (s, 2H, thiazole CH_2), 7.25–7.56 (m, 8H, 2×C_6H_4), 8.43 (s, 1H, D_2O exchangeable, NH), 10.36 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.8 (thiazole CH_2), 120.1, 120.3, 121.0, 121.6, 122.8, 123.3, 123.9, 124.1, 125.2, 125.6 (2×C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 166.3 (CO), 168.9, 169.2, 170.0, 170.2 (4×C=N). Anal. Calcd for: $C_{20}H_{12}ClN_5O_2S_2$ (453.92): C, 52.92; H, 2.66; N, 15.43; S, 14.13. Found: C, 52.88; H, 2.80; N, 15.57; S, 14.08%. EIMS: *m/z* 453 $[M]^+$ (58%).

2-(6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-imino-2-(4-methoxyphenyl)-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12f)

Yellow crystals from 1,4-dioxane, yield 2.91 g (65%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3521–3349 (OH, NH), 3050 (CH-aromatic), 1689 (CO), 1660 (C=N), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.70 (s, 3H, OCH_3), 5.64 (s, 2H, thiazole CH_2), 7.26–7.53 (m, 8H, 2×C_6H_4), 8.45 (s, 1H, D_2O exchangeable, NH), 10.38 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.8 (thiazole CH_2), 50.8 (OCH_3), 120.1, 120.6, 121.3, 121.9, 122.4, 123.1, 123.5, 124.4, 124.8, 125.2 (2×C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 166.8 (CO), 168.5, 169.3, 169.6, 170.0 (4×C=N). Anal. Calcd for: $C_{21}H_{15}N_5O_3S_2$ (449.51): C, 56.11; H, 3.36; N, 15.58; S, 14.27. Found: C, 56.31; H, 3.42; N, 15.63; S, 14.48%. EIMS: *m/z* 449 $[M]^+$ (64%).

2-(6-(Benzo[*d*]thiazol-2-yl)-3-imino-5-methyl-2-phenyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12g)

Yellow crystals from 1,4-dioxane, yield 2.29 g (55%), m.p. 233–235 °C. IR (KBr) ν_{max} (cm^{-1}): 3436–3319 (NH), 3050 (CH-aromatic), 1689 (CO), 1660 (C=N), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.83 (s, 3H, CH_3), 5.65 (s, 2H, thiazole CH_2), 7.24–7.43 (m, 9H, C_6H_5, C_6H_4), 8.46 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.6 (CH_3), 45.6 (thiazole CH_2), 120.0, 120.4, 121.3, 121.6, 122.4, 123.3, 123.8, 124.4, 124.6, 125.5 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 166.5 (CO), 168.7, 169.3, 170.1, 170.3 (4×C=N). Anal. Calcd for: $C_{21}H_{15}N_5OS_2$ (417.51): C, 60.41; H, 3.62; N, 16.77; S, 15.36. Found: C, 60.59; H, 3.52; N, 16.80; S, 15.49%. EIMS: *m/z* 417 $[M]^+$ (70%).

2-(6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-imino-5-methyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12h)

Yellow crystals from 1,4-dioxane, yield 2.84 g (63%), m.p. 193–195 °C. IR (KBr) ν_{max} (cm^{-1}): 3448–3329 (NH), 3050 (CH-aromatic), 1689 (CO), 1660 (C=N), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.86 (s, 3H, CH_3), 5.67 (s, 2H, thiazole CH_2), 7.24–7.46 (m, 8H, 2×C_6H_4), 8.43 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.4 (CH_3), 45.9 (thiazole CH_2), 120.2, 120.5, 121.1, 121.7, 122.2, 122.8, 123.5, 124.4, 124.7, 125.8 (C_6H_5, C_6H_4), 140.2, 142.3 (pyridazine C-3, C-4), 166.8 (CO), 169.1, 169.5, 169.8, 170.2 (4×C=N). Anal. Calcd for: $C_{21}H_{14}ClN_5OS_2$ (451.95): C, 55.81; H, 3.12; N, 15.50; S, 14.19. Found: C, 55.93; H, 3.42; N, 15.68; S, 13.93%. EIMS: *m/z* 451 $[M]^+$ (80%).

2-(6-(Benzo[*d*]thiazol-2-yl)-3-imino-2-(4-methoxyphenyl)-5-methyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (12i)

Pale brown crystals from 1,4-dioxane, yield 2.68 g (62%), m.p. 155–157 °C. IR (KBr) ν_{max} (cm^{-1}): 3369–3346 (NH), 3050 (CH-aromatic), 1688 (CO), 1662 (C=N), 1584 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.87 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 5.66 (s, 2H, thiazole CH_2), 7.25–7.56 (m, 8H, 2×C_6H_4), 8.44 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.5 (CH_3), 50.3 (OCH_3), 120.5, 120.6, 120.8, 122.1, 122.7, 123.6, 123.8, 124.7, 124.9, 125.4 (2×C_6H_4), 140.1, 142.5 (pyridazine C-3, C-4), 166.6 (CO), 168.6, 169.2, 169.6, 170.1 (4×C=N). Anal. Calcd for: $C_{22}H_{17}N_5O_2S_2$ (447.53): C, 59.04; H, 3.83; N, 15.65; S, 14.33. Found: C, 58.79; H, 3.69; N, 15.52; S, 14.50%. EIMS: *m/z* 447.53 $[M]^+$ (82%).

2. 1. 7. General Method for the Synthesis of the Thiazole Derivatives 13a–i

To a solution of either **10a** (3.44g, 0.01 mol), **10b** (3.78g, 0.01 mol), **10c** (3.74 g, 0.01 mol), **10d** (3.45 g, 0.01 mol), **10e** (3.79 g, 0.01 mol), **10f** (3.75 g, 0.01 mol), **10g** (3.43 g, 0.01 mol), **10h** (3.77 g, 0.01 mol) or **10i** (3.73 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL) thioglycolic acid (0.92 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then was left to cool and the formed solid product was collected by filtration.

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13a)

Pale brown crystals from acetic acid, yield 2.51 g (60%), m.p. 210–212 °C. IR (KBr) ν_{max} (cm^{-1}): 3352–3341 (NH_2), 3050 (CH-aromatic), 1689, 1703 (2×CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.93 (s, 2H, D_2O echangeable, NH_2), 5.30 (s, 2H, thiazole CH_2), 7.26–7.48 (m, 9H, C_6H_5, C_6H_4). ^{3}C NMR (DMSO-d_6, 75 MHz): δ 48.5 (thiazole CH_2), 120.1, 120.4, 121.1, 122.7, 122.9, 123.3, 123.9, 124.1, 124.4, 125.8 (C_6H_5, C_6H_4), 140.1, 142.4 (pyridazine C-3, C-4), 165.8, 166.8 (2×CO), 168.5, 169.3, 170.3 (3×C=N). Anal. Calcd for: $C_{20}H_{13}N_5O_2S_2$ (419.48): C, 57.26; H, 3.12; N, 16.70; S, 15.29. Found: C, 57.31; H, 3.29; N, 16.58; S, 15.36%. EIMS: *m/z* 419 $[M]^+$ (60%).

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13b)

Yellow crystals from 1,4-dioxane, yield 2.26 g (50%), m.p. 199–201 °C. IR (KBr) ν_{max} (cm^{-1}): 3371–3346 (NH_2), 3054 (CH-aromatic), 1689, 1701 (2×CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.86 (s, 2H, D_2O echangeable, NH_2), 5.64 (s, 2H, thaizole CH_2), 7.23–7.59 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.8 (thiazole CH_2), 120.1, 120.4, 121.2, 122.3, 122.9, 123.6, 124.0, 124.3, 125.4, 125.6 (2×C_6H_4), 140.0, 142.3 (pyridazine C-3, C-4), 168.8, 169.3, 170.1 (3 X C=N). Anal. Calcd for: $C_{20}H_{12}ClN_5O_2S_2$ (453.92): C, 52.92; H, 2.66; N, 15.43; S, 14.13. Found: C, 52.86; H, 2.80; N, 15.57; S, 14.26%. EIMS: *m/z* 453 $[M]^+$ (68%).

2-(5-Amino-6-(benzo[*d*]thiazol-2-yl)-2-(4-methoxyphenyl)-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13c)

Pale yellow crystals from 1,4-dioxane, yield 3.14 g (70%), m.p. 210–212 °C. IR (KBr) ν_{max} (cm^{-1}): 3359–3331 (NH), 3050 (CH-aromatic), 1689, 1701 (2×CO), 1587 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.75 (s, 3H, OCH_3), 4.86 (s, 2H, D_2O echangeable, NH_2), 5.28 (s, 2H, thiazole CH_2), 7.25–7.58 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.3 (thiazole CH_2), 50.7 (OCH_3), 120.1, 120.5, 121.6, 122.4, 122.8, 123.6 123.8, 124.6, 125.3, 125.5 (2×C_6H_4), 140.5, 142.6 (pyridazine C-3, C-4), 166.1, 166.8 (2×CO), 168.6, 169.31, 170.3 (3×C=N). Anal. Calcd for: $C_{21}H_{15}N_5O_3S_2$ (449.51): C, 56.11; H, 3.36; N, 15.58; S, 14.27. Found: C, 56.26; H, 3.27; N, 15.63; S, 14.39%. EIMS: *m/z* 449 $[M]^+$ (66%).

2-(6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-3-imino-2-phe-

nyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13d)

Yellow crystals from 1,4-dioxane, yield 2.47 g (59%), m.p. 158–160 °C. IR (KBr) ν_{max} (cm^{-1}): 3546–3328 (OH, NH), 3054 (CH-aromatic), 1689, 1702 (2×CO), 1586 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.63 (s, 2H, thiazole CH_2), 7.25–7.46 (m, 9H, C_6H_5, C_6H_4), 10.38 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.8 (thiazole CH_2), 120.1, 120.4, 121.3, 121.7, 122.8, 123.7, 123.8, 124.6, 125.4, 125.7 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 166.1, 166.8 (2×CO), 168.9, 170.1, 170.5 (3×C=N). Anal. Calcd for: $C_{20}H_{12}N_4O_3S_2$ (420.46): C, 57.13; H, 2.88; N, 13.32; S, 15.25. Found: C, 57.38; H, 3.04; N, 13.55; S, 15.36%. EIMS: *m/z* 420 $[M]^+$ (68%).

2-(6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13e)

Yellow crystals from 1,4-dioxane, yield 2.72 g (60%), m.p. 196–198 °C. IR (KBr) ν_{max} (cm^{-1}): 3528–3339 (OH), NH), 3054 (CH-aromatic), 1689, 1701 (2×CO), 1583 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.66 (s, 2H, thiazole CH_2), 7.23–7.55 (m, 8H, 2×C_6H_4), 10.36 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.6 (thiazole CH_2), 120.2, 120.6, 121.2, 121.4, 122.3, 123.3, 123.9, 124.4, 125.1, 125.8 (2×C_6H_4), 140.1, 142.4 (pyridazine C-3, C-4), 166.6, 166.9 (2×CO), 168.9, 170.0, 170.2 (3×C=N). Anal. Calcd for: $C_{20}H_{11}ClN_4O_3S_2$ (454.91): C, 52.80; H, 2.44; N, 12.32; S, 14.10. Found: C, 52.98; H, 2.63; N, 12.57; S, 14.25%. EIMS: *m/z* 454 $[M]^+$ (65%).

2-(6-(Benzo[*d*]thiazol-2-yl)-5-hydroxy-2-(4-methoxyphenyl)-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13f)

Yellow crystals from 1,4-dioxane, yield 2.83 g (63%), m.p. 210–212 °C. IR (KBr) ν_{max} (cm^{-1}): 3363-3347 (OH), 3053 (CH-aromatic), 1688, 1702 (2×CO), 1585 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.72 (s, 3H, OCH_3), 5.65 (s, 2H, thiaazole CH_2), 7.23–7.58 (m, 8H, 2×C_6H_4), 10.22 (s, 1H, D_2Oexchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.8 (thaizole CH_2), 50.3 (OCH_3), 120.4, 120.6, 121.5, 122.5, 122.6, 123.2, 123.6, 124.7, 125.2, 125.8 (2×C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 165.8, 166.3 (2×CO), 168.8, 170.1, 170.5 (3×C=N). Anal. Calcd for: $C_{21}H_{14}N_4O_4S_2$ (450.49): C, 55.99; H, 3.13; N, 12.44; S, 14.24. Found: C, 56.15; H, 3.29; N, 12.60; S, 14.40%. EIMS: *m/z* 450 $[M]^+$ (68%).

2-(6-(Benzo[*d*]thiazol-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13g)

Yellow crystals from 1,4-dioxane, yield 2.59 g (62%), m.p. 244–246 °C. IR (KBr) ν_{max} (cm^{-1}): 3050 (CH-aromatic), 1689, 1700 (2×CO), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.86 (s, 3H, CH_3), 5.63 (s, 2H, thiazole CH_2), 7.28–7.45 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.8 (CH_3), 45.4 (thiazole CH_2), 120.2, 120.7, 121.1, 121.5, 122.2, 123.6, 123.8, 124.2, 124.3, 125.8 (C_6H_5, C_6H_4), 140.1, 142.3 (pyridazine C-3, C-4), 166.8, 167.3 (2×CO), 168.5, 169.3, 170.0 (3×C=N). Anal. Calcd for: $C_{21}H_{14}N_4O_2S_2$ (418.49): C, 60.27; H, 3.37; N, 13.39; S, 15.32. Found: C, 60.41; H, 3.47; N, 13.17; S, 15.50%. EIMS: *m/z* 418 $[M]^+$ (75%).

2-(6-(Benzo[*d*]thiazol-2-yl)-2-(4-chlorophenyl)-5-methyl-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13h)

Yellow crystals from 1,4-dioxane, yield 2.48 g (55%), m.p. 230–232 °C. IR (KBr) ν_{max} (cm^{-1}): 3055 (CH-aromatic), 1689, 1703 (2×CO), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.88 (s, 3H, CH_3), 5.62 (s, 2H, thiazole CH_2), 7.24–7.59 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.5 (CH_3), 45.6 (thiazole CH_2), 120.1, 120.4, 121.1, 121.8, 122.0, 123.4, 123.9, 124.2, 124.6, 125.5 (2×C_6H_4), 140.3, 142.3 (pyridazine C-3, C-4), 166.5, 167.6 (2×CO), 168.7, 169.3, 170.2 (3×C=N). Anal. Calcd for: $C_{21}H_{13}ClN_4O_2S_2$ (452.94): C, 55.69; H, 2.89; N, 12.37; S, 14.16. Found: C, 55.72; H, 3.07; N, 12.44; S, 13.96%. EIMS: *m/z* 452 $[M]^+$ (78%).

2-(6-(Benzo[*d*]thiazol-2-yl)-2-(4-methoxyphenyl)-5-methyl-3-oxo-2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one (13i)

Yellow crystals from 1,4-dioxane, yield 3.00 g (68%), m.p. 199–202 °C. IR (KBr) ν_{max} (cm^{-1}): 3453–3360 (NH), 3055 (CH-aromatic), 1688, 1703 (2×CO), 1580 (C=C). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.89 (s, 3H, CH_3), 3.77 (s, 3H, OCH_3), 5.65 (s, 2H, thiazole CH_2), 7.25–7.56 (m, 8H, 2×C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.5 (CH_3), 50.6 (OCH_3), 45.8 (thiazole CH_2), 120.3, 120.6, 121.4, 121.5, 122.0, 123.2, 123.4, 124.0, 124.6, 125.3 (2×C_6H_4), 140.2, 142.3 (pyridazine C-3, C-4), 166.7, 167.5 (2×CO), 168.7, 169.2, 170.2 (3×C=N). Anal. Calcd for: $C_{22}H_{16}N_4O_3S_2$ (448.52): C, 58.91; H, 3.60; N, 12.49; S, 14.30. Found: C, 59.15; H, 3.58; N, 12.37; S, 14.42%. EIMS: *m/z* 448 $[M]^+$ (80%).

2. 1. 8. General Procedure for the Synthesis of the Thiazole Derivatives 15a–c

To a solution of either **4a** (1.74 g, 0.01 mol), **4b** (2.21 g, 0.01 mol) or **4c** (1.91 g, 0.01 mol) in 1,4-dioxane (60 mL) containing triethylamine (1.0 mL) elemental sulfur (0.32 g, 0.01 mol) and phenylisothiocyanate (1.30 g, 0.01 mol) were added. The whole reaction mixture was heated under the reflux conditions for 1 h then was left to cool to room temperature and the obtained solid product was collected by filtration.

4-Amino-5-(benzo[*d*]thiazol-2-yl)-3-phenylthiazole-2(3*H*)-thione (15a)

Orange crystals from 1,4-dioxane, yield 1.97 g (58%), m.p. 202–204 °C. IR (KBr) ν_{max} (cm^{-1}): 3463, 3342 (NH_2), 3055 (CH-aromatic), 1580 (C=C), 1210 (C=S).

^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.93 (s, 2H, D_2O exchangeable, NH_2), 7.27–7.43 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.1, 120.4, 121.0, 121.8, 122.0, 123.6, 123.8, 124.0, 124.2, 125.0 (C_6H_5, C_6H_4), 143.2, 144.5 (thiazole C), 168.8 (C=N), 179.8 (C=S). Anal. Calcd for: $C_{16}H_{11}N_3S_3$ (341.46): C, 56.28; H, 3.25; N, 12.31; S, 28.17. Found: C, 56.06; H, 3.35; N, 12.42; S, 28.25%. EIMS: *m/z* 341 $[M]^+$ (70%).

5-(Benzo[*d*]thiazol-2-yl)-4-hydroxy-3-phenylthiazole-2(3*H*)-thione (15b)

Orange crystals from 1,4-dioxane, yield 2.12 g (62%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3571–3339 (OH), 3055 (CH-aromatic), 1583 (C=C), 1210 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.27–7.43 (m, 9H, C_6H_5, C_6H_4), (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.3, 120.6, 121.3, 121.5, 122.3, 122.7, 123.8, 124.0, 124.6, 125.3 (C_6H_5, C_6H_4), 143.4, 144.5 (thiazole C), 168.9 (C=N), 179.6 (C=S). Anal. Calcd for: $C_{16}H_{10}N_2OS_3$ (342.46): C, 56.12; H, 2.94; N, 8.18; S, 28.09. Found: C, 56.31,+; H, 3.16; N, 8.25; S, 28.18%. EIMS: *m/z* 342 $[M]^+$ (68%).

5-(Benzo[*d*]thiazol-2-yl)-4-methyl-3-phenylthiazole-2(3*H*)-thione (15c)

Orange crystals from 1,4-dioxane, yield 1.70 g (50%), m.p. 211–213 °C. IR (KBr) ν_{max} (cm^{-1}): 3055 (CH-aromatic), 1586 (C=C), 1213 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.78 (s, 3H, CH_3), 7.25–7.48 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.4 (CH_3), 120.4, 120.8, 121.2, 121.7, 122.5, 122.6, 123.8, 124.3, 124.8, 125.6 (C_6H_5, C_6H_4), 143.2, 144.1 (thiazole C), 168.6 (C=N), 179.9 (C=S). Anal. Calcd for: $C_{17}H_{12}N_2S_3$ (340.49): C, 59.97; H, 3.55; N, 8.23; S, 28.25. Found: C, 59.83; H, 3.46; N, 8.42; S, 28.30%. EIMS: *m/z* 340 $[M]^+$ (80%).

***N*-(5-(Benzo[*d*]thiazol-2-yl)-3-phenyl-2-thioxo-2,3-dihydrothiazol-4-yl)-2-cyanoacetamide (16)**

To a solution of compound **15a** (3.41 g, 0.01 mol) in dimethylformamide, ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture was heated under the reflux conditions for 1 h then poured onto ice/water and the produced solid product was collected by filtration.

Orange crystals from 1,4-dioxane, yield 2.44 g (60%), m.p. 265–267 °C. IR (KBr) ν_{max} (cm^{-1}): 3055 (CH-aromatic), 2220 (CN), 1688 (CO), 1586 (C=C), 1211 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.32 (s, 2H, CH_2), 7.27–7.45 (m, 9H, C_6H_5, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 48.9 (CH_2), 117.3 (CN), 120.2, 120.6, 121.5, 121.9, 122.2, 122.8, 123.3, 123.6, 124.5, 125.3 (C_6H_5, C_6H_4), 143.3, 144.4 (thiazole C), 166.7 (CO), 168.8 (C=N), 180.2 (C=S). Anal. Calcd for: $C_{19}H_{12}N_4OS_3$ (408.52): C, 55.86; H, 2.96; N, 13.71; S, 23.55. Found: C, 55.74; H, 3.26; N, 13.89; S, 23.72%. EIMS: *m/z* 408 $[M]^+$ (75%).

2. 1. 9. General Procedure for the Synthesis of the Thiophene Derivatives 17a,b

To a solution of **16** (4.08 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL) elemental sulfur and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added. The reaction mixture was heated under the reflux conditions for 2 h then was poured onto ice/water containing a few drops of hydrochloric acid and the produced solid product was collected by filtration.

3,5-Diamino-*N*-(5-(benzo[*d*]thiazol-2-yl)-3-phenyl-2-thioxo-2,3-dihydro-thiazol-4-yl)-4-cyanothiophene-2-carboxamide (17a)

Yellow crystals from 1,4-dioxane, yield 3.03 g (60%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3480–3372 (NH_2), 3055 (CH-aromatic), 2220 (CN), 1689 (CO), 1586 (C=C), 1215 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.21, 5.60 (2×s, 4H, D_2O exchangeable, 2×NH_2), 7.27–7.50 (m, 9H, C_6H_5, C_6H_4), 8.20 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 117.0 (CN), 120.2, 120.8, 121.2, 121.4, 122.8, 122.9, 123.5, 124.7, 125.2, 125.4 (C_6H_5, C_6H_4), 139.5, 140.3, 141.5, 143.1, 144.3, 146.5 (thiazole, thiophene C), 166.2 (CO), 168.4 (C=N), 180.3 (C=S). Anal. Calcd for: $C_{22}H_{14}N_6OS_4$ (506.65): C, 52.15; H, 2.79; N, 16.59; S, 25.32. Found: C, 52.37; H, 2.83; N, 16.70; S, 25.49%. EIMS: *m/z* 506 $[M]^+$ (64%).

Ethyl 2,4-Diamino-5-((5-(benzo[*d*]thiazol-2-yl)-3-phenyl-2-thioxo-2,3-dihydrothiazol-4-yl)carbamoyl)thiophene-3-carboxylate (17b)

Yellow crystals from 1,4-dioxane, yield 3.04 g (55%), m.p. 148–150 °C. IR (KBr) ν_{max} (cm^{-1}): 3469–3328 (NH_2), 3055 (CH-aromatic), 2220 (CN), 1688, 1889 (2×CO), 1584 (C=C), 1215 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.16 (t, 3H, *J* = 7.21 Hz, $OCH_2\underline{CH_3}$), 4.24 (q, 2H, *J* = 7.21 Hz, $O\underline{CH_2}CH_3$), 5.25, 5.39 (2×s, 4H, D_2O exchangeable, 2×NH_2), 7.24–7.53 (m, 9H, C_6H_5, C_6H_4), 8.24 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.2 ($OCH_2\underline{CH_3}$), 50.6 ($O\underline{CH_2}CH_3$), 117.0 (CN), 120.3, 120.7, 121.0, 121.3, 122.5, 122.8, 123.5, 124.7, 125.1, 125.6 (C_6H_5, C_6H_4), 139.6, 140.1, 141.6, 143.1, 144.2, 146.8 (thiazole, thiophene C), 166.3, 166.7 (2×CO), 168.6 (C=N), 180.1 (C=S). Anal. Calcd for: $C_{24}H_{19}N_5O_3S_4$ (553.70): C, 52.06; H, 3.46; N, 12.65; S, 23.16. Found: C, 52.27; H, 3.59; N, 12.80; S, 23.27%. EIMS: *m/z* 553 $[M]^+$ (80%).

2. 1. 10. 1-(5-(Benzo[*d*]thiazol-2-yl)-3-phenyl-2-thioxo-2,3-dihydrothiazol-4-yl)-3-phenylthiourea (18)

To a solution of **15a** (3.41 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL) phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under the reflux conditions for 1 h then poured onto ice/water and the produced solid product was collected by filtration.

Yellow crystals from 1,4-dioxane, yield 2.38 g (50%), m.p. 188–190 °C. IR (KBr) ν_{max} (cm^{-1}): 3480-3341 (NH), 3055 (CH-aromatic), 1584 (C=C), 1211 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.26–7.57 (m, 14H, 2×C_6H_5, C_6H_4), 8.28, 8.32 (2×s, 2H, D_2O exchangeable, 2×NH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.1, 120.7, 120.9, 121.0, 121.1, 122.3, 122.8, 123.8, 124.6, 124.7, 125.0, 125.1, 125.4, 125.8 (2×C_6H_5, C_6H_4), 140.1, 143.1, 144.2, 146.8 (thiazole C), 168.6 (C=N), 180.1, 182.3 (2×C=S). Anal. Calcd for: $C_{23}H_{16}N_4S_4$ (476.66): C, 57.95; H, 3.38; N, 11.75; S, 26.91. Found: C, 58.13; H, 3.46; N, 11.83; S, 27.27%. EIMS: *m/z* 476 $[M]^+$ (72%).

2. 1. 11. General Procedure for the Synthesis of the 2*H*-[3,4'-Bithiazole]-2'(3'*H*)-thione Derivatives 20a–c

Either of ethyl chloroacetate (1.22 g, 0.01 mol), α-bromoacetone (1.36, 0.01 mol) or ω-bromoacetophenone (2.0 g, 0.01 mol) was added to a solution of compound **18** (4.76 g, 0.01 mol) in absolute ethanol (60 mL). The reaction mixture was heated under the reflux conditions for 2 h then was left to cool and the produced solid product was collected by filtration.

5'-(Benzo[*d*]thiazol-2-yl)-4-hydroxy-3'-phenyl-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (20a)

Yellow crystals from 1,4-dioxane, yield 3.14 g (61%), m.p. 222–225 °C. IR (KBr) ν_{max} (cm^{-1}): 3572–3352 (OH), 3055 (CH-aromatic), 1584 (C=C), 1209 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.25 (s, 1H, thiazole H-5), 7.26–7.52 (m, 14H, 2×C_6H_5, C_6H_4), 10.24 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.3, 120.6, 121.1, 121.3, 121.6, 122.3, 122.9, 123.5, 124.4, 124.9, 125.0, 125.3, 125.7, 125.8 (2×C_6H_5, C_6H_4), 142.3, 143.0, 144.1, 146.6 (two thiazole C), 168.8, 172.1 (2×C=N), 180.1 (C=S). Anal. Calcd for: $C_{25}H_{16}N_4OS_4$ (516.68): C, 58.11; H, 3.12; N, 10.84; S, 24.82. Found: C, 58.25; H, 3.31; N, 10.62; S, 24.75%. EIMS: *m/z* 516 $[M]^+$ (70%).

5'-(Benzo[*d*]thiazol-2-yl)-4-methyl-3'-phenyl-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (20b)

Yellow crystals from 1,4-dioxane, yield 3.28 g (64%), m.p. 158–160 °C. IR (KBr) ν_{max} (cm^{-1}): 3055 (CH-aromatic), 1582 (C=C), 1210 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.83 (s, 3H, CH_3), 6.28 (s, 1H, thiazole H-5), 7.24–7.50 (m, 14H, 2×C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.8 (CH_3), 120.1, 120.4, 121.1, 121.8, 121.9, 122.3, 122.9, 123.3, 124.4, 124.8, 125.0, 125.1, 125.3, 126.5 (2×C_6H_5, C_6H_4), 143.3, 143.6, 144.1, 146.6 (two thiazole C), 168.7, 169.1 (2 X C=N), 180.3 (C=S). Anal. Calcd for: $C_{26}H_{18}N_4S_4$ (514.71): C, 60.67; H, 3.52; N, 10.89; S, 24.92. Found: C, 60.80; H, 3.48; N, 10.66; S, 25.85%. EIMS: *m/z* 514 $[M]^+$ (60%).

5'-(Benzo[*d*]thiazol-2-yl)-3',4-diphenyl-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (20c)

Yellow crystals from 1,4-dioxane, yield 3.74 g (65%), m.p. 177–179 °C. IR (KBr) ν_{max} (cm^{-1}): 3055 (CH-aromatic), 1580 (C=C), 1213 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 6.27 (s, 1H, thiazole H-5), 7.22–7.54 (m, 19H, 3×C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 119.3, 119.6, 120.1, 120.4, 120.4, 120.7, 121.0, 121.6, 121.7, 122.3, 122.6, 123.3, 124.5, 124.9, 125.2, 125.4, 125.5, 126.2 (3C_6H_5, C_6H_4), 143.3, 143.8, 144.5, 146.9 (two thiazole C), 168.6 (C=N), 180.3 (C=S). Anal. Calcd for: $C_{31}H_{20}N_4S_4$ (576.78): C, 64.55; H, 3.50; N, 9.71; S, 22.24. Found: C, 64.49; H, 3.36; N, 9.83; S, 22.40%. EIMS: *m/z* 576 $[M]^+$ (65%).

2. 1. 12. General Procedure for the Synthesis of the Azo Derivatives 21a–c

To a cold solution (0–5 °C) of **20a** (5.16 g, 0.01 mol) in ethanol (60 mL) containing sodium acetate (3.0 g) either of benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride or 4-methoxybenzenediazonium chloride [prepared by the addition of sodium nitrite solution (0.70 g, 0.01 mol) to a cold solution of either aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methoxyaniline (1.23 g, 0.01 mol) in concentrated hydrochloric acid (8.0 mL, 18%) with continuous stirring] was added with continuous stirring. The solid product formed upon stirring for 1 h was collected by filtration.

5'-(Benzo[*d*]thiazol-2-yl)-4-hydroxy-3'-phenyl-5-((*Z*)-phenyldiazenyl)-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (21a)

Yellow crystals from 1,4-dioxane, yield 3.72 g (60%), m.p. 166–168 °C. IR (KBr) ν_{max} (cm^{-1}): 3561–3339 (OH), 3055 (CH-aromatic), 1584 (C=C), 1212 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.23–7.54 (m, 19H, 3×C_6H_5, C_6H_4), 10.29 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.3, 120.7, 121.0, 121.2, 121.3, 121.6, 122.3, 122.5, 122.9, 123.5, 123.7, 123.9, 124.4, 124.6, 125.2, 125.3, 125.7, 125.8 (3×C_6H_5, C_6H_4), 142.3, 143.0, 144.4, 146.9 (thiazole C), 168.6, 170.3 (2×C=N), 180.1 (C=S). Anal. Calcd for: $C_{31}H_{20}N_6OS_4$ (620.79): C, 59.98; H, 3.25; N, 13.54; S, 20.66. Found: C, 59.72; H, 3.41; N, 13.39; S, 20.59%. EIMS: *m/z* 620 $[M]^+$ (65%).

5'-(Benzo[*d*]thiazol-2-yl)-5-((*Z*)-(4-chlorophenyl)diazenyl)-4-hydroxy-3'-phenyl-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (21b)

Yellow crystals from 1,4-dioxane, yield 3.14 g (48%), m.p. 155–157 °C. IR (KBr) ν_{max} (cm^{-1}): 3532–3341 (OH), 3055 (CH-aromatic), 1582 (C=C), 1218 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.25–7.57 (m, 18H, 2×C_6H_5, 2×C_6H_4), 10.27 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 120.1, 120.5, 120.8, 121.2, 121.6, 121.8, 122.1, 122.7, 123.0, 123.5, 123.5, 123.9, 124.4, 124.8, 125.1, 125.2, 125.6, 125.9 (2×C_6H_5, 2×C_6H_4), 142.1, 143.5, 144.4, 146.7 (thiazole C), 168.7, 170.2 (2×C=N), 180.3

(C=S). Anal. Calcd for: $C_{31}H_{19}ClN_6OS_4$ (655.24): C, 56.82; H, 2.92; N, 12.83; S, 19.57. Found: C, 56.72; H, 2.83; N, 12.79; S, 19.42%. EIMS: *m/z* 655 $[M]^+$ (68%).

5'-(Benzo[*d*]thiazol-2-yl)-4-hydroxy-5-((*Z*)-(4-methoxyphenyl)diazenyl)-3'-phenyl-2-(phenylimino)-2*H*-[3,4'-bithiazole]-2'(3'*H*)-thione (21c)

Yellow crystals from 1,4-dioxane, yield 3.90 g (60%), m.p. 132–136 °C. IR (KBr) ν_{max} (cm^{-1}): 3548–3327 (OH), 3055 (CH-aromatic), 1582 (C=C), 1212 (C=S). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.77 (s, 3H, OCH_3), 7.28–7.59 (m, 18H, 2×C_6H_5, 2×C_6H_4), 10.29 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.4 (OCH_3), 120.2, 120.3, 120.5, 121.0, 121.4, 121.7, 122.1, 122.4, 123.2, 123.5, 123.6, 123.8, 124.1, 124.4, 125.0, 125.1, 125.6, 125.7 (2×C_6H_5, 2×C_6H_4), 142.3, 143.7, 144.2, 146.5 (thiazole C), 168.3, 170.3 (2×C=N), 180.1 (C=S). Anal. Calcd for: $C_{32}H_{22}N_6O_2S_4$ (650.82): C, 59.06; H, 3.41; N, 12.91; S, 19.71. Found: C, 58.92; H, 3.29; N, 12.82; S, 19.50%. EIMS: *m/z* 650 $[M]^+$ (58%).

3. Results and Discussion

3. 1. Chemistry

The reaction sequences for the synthesized compounds are depicted in Schemes 1–4 starting with the benzo[*d*]thiazole derivatives **4a**–**c**. The latter compounds were obtained through the reaction of *ortho*-aminothiophenol (**1**) with ethyl cyanoacetate (**2a**), diethylmalonate (**2b**) or ethyl 3-oxobutanoate (**2c**) in an oil bath at 120 °C. The structures of compounds **4a**–**c** were based on the obtained analytical and spectral data (see Experimental section). Compounds **4a**–**c** were used to produce thiophene derivatives through their reactions with elemental sulfur and either malononitrile (**5**) or ethyl cyanoacetate (**2a**) in ethanolic solution containing triethylamine to afford the thiophene derivatives **6a**–**f**, respectively (Scheme 1). Their structures were confirmed on the basis of their spectral data. Thus, the ^{1}H NMR spectrum showed two singlets at δ 3.84 and 4.26 ppm (D_2O exchangeable) indicating the two NH_2 groups and a multiplet at δ 7.27–7.42 ppm corresponding to the phenyl group. Moreover, the ^{13}C NMR spectrum which revealed the presence of a signal at δ 116.7 indicating the presence of the CN group, six signals at δ 121.5, 122.9, 123.5, 123.6, 123.8, and 124.8 corresponding to the C_6H_4 group, four signals at δ 138.3, 138.9, 140.2, 142.6 due to the thiophene moiety, and a signal at δ 168.6 for the C=N moiety.

Compounds **4a**–**c** were appropriate for arylhydrazone production *via* their reactions with aryldiazonium salts. Thus, the reaction of **4a**–**c** with either of the diazonium salts namely benzenediazonium chloride (**7a**), 4-chlorobenzenediazonium chloride (**7b**) or 4-methoxybenzenediazonium chloride (**7c**) gave the arylhydrazono derivatives **8a**–**i**, respectively. The latter compounds reacted with malononitrile in 1,4-dioxane solution containing triethylamine to give the pyridazin-2-imino derivatives **9a**–**i**, respectively (Scheme 2); the reaction took place according to the previously reported work.[27–30]

Similarly, the reaction of **8a**–**i** with ethyl cyanoacetate under the same reaction conditions gave the pyridazin-2-oxo derivatives **10a**–**i**, respectively. Interestingly, all of the pyridazin-2-imino derivatives **9a**–**i** were also converted to the pyridazin-2-oxo derivatives upon heating under the reflux conditions in ethanol containing sodium hydroxide (2.0 g, dissolved in 10 mL water) (confirmed by m.p. and mixed m.p.). All of compounds **9a**–**i** and **10a**–**i** with the α-oxocyano moiety reacted with thioglycolic acid affording the thiazole derivatives **12a**–**i** and **13a**–**i**, respectively (Schemes 3 and 4).

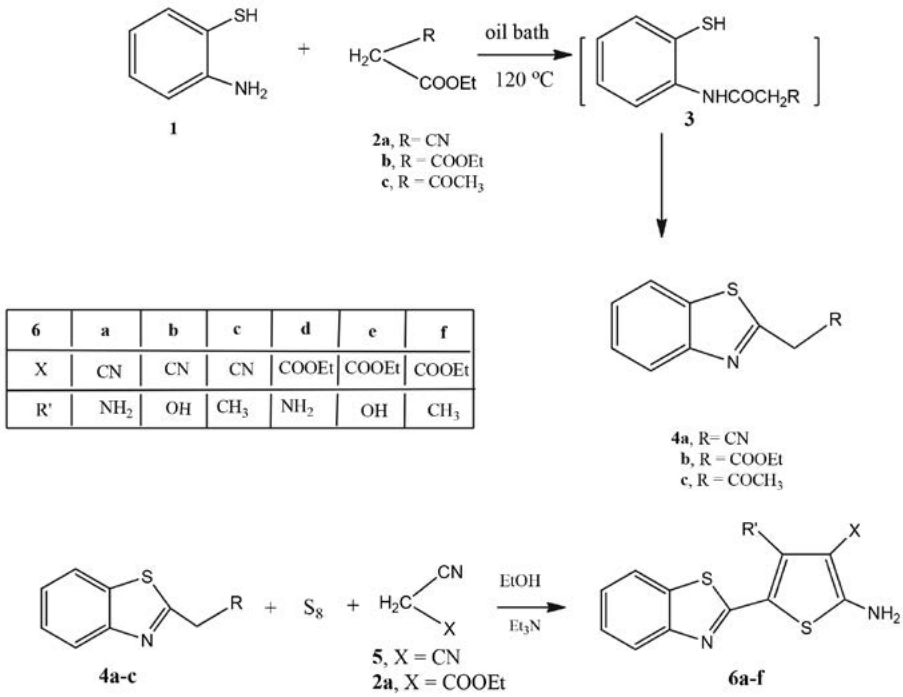
Compounds **4a**–**c** reacted with elemental sulfur and phenylisothiocynate (**14**) in 1,4-dioxane solution containing triethylamine to give the thiazole derivatives **15a**–**c**, respectively. Structures of the latter products were established on the basis of their respective analytical and spectral data. Thus, the ^{1}H NMR spectrum of **15a** showed the presence of a singlet at δ 4.93 ppm (D_2O exchangeable) corresponding to the NH_2 group and a multiplet at δ 7.27–7.43 indicating the two phenyl groups. In addition the ^{13}C NMR which revealed the presence of ten signals at δ 120.1, 120.4, 121.0, 121.8, 122.0, 123.6, 123.8, 124.0, 124.2, 125.0 for the C_6H_5 and C_6H_4 groups, two signals at δ 143.2, 144.5 for the thiazole C-4, C-5, a signal at δ 168.8 due to the C=N moiety and a signal at δ 179.8 due to the C=S group. Compound **15a** underwent acylation reaction through its reaction with ethyl cyanoacetate in dimethylformamide solution to give the *N*-cyanoacetamido derivative **16** (Scheme 4).

Compound **16** reacted with either malononitrile (**5**) or ethyl cyanoacetate (**2a**) and elemental sulfur in ethanol containing triethylamine to produce the thiophene derivatives **17a** and **17b**, respectively. Moreover, the reaction of **15a** with phenylisothiocyanate (**14**) gave the thiourea derivative **18**. Compound **18** reacted with the α-halocarbonyl derivatives namely ethyl chloroacetate (**19a**), α-bromoacetone (**19b**) or ω-bromoacetophenone (**19c**) in absolute ethanol solution under the reflux conditions affording the thiazole derivatives **20a**–**c**, respectively. Compound **20a** was found to be an active compound toward azo formation. Thus, its reaction with benzenediazonium chloride (**7a**), 4-chlorobenzenediazonium chloride (**7b**) or 4-methoxybenzenediazonium chloride (**7c**) produced the arylazo derivatives **21a**–**c**, respectively (Scheme 5).

3. 2. Biology

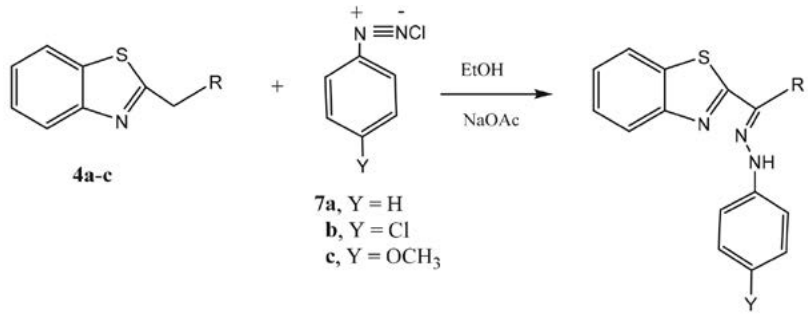
3. 2. 1. Cell Proliferation Assay

The anti-proliferative activities of the newly synthesized compounds (Table 1) were evaluated against the six cancer cell lines A549, HT-29, MKN-45, U87MG, SMMC-7721, and H460 using the standard MTT assay *in vitro*,

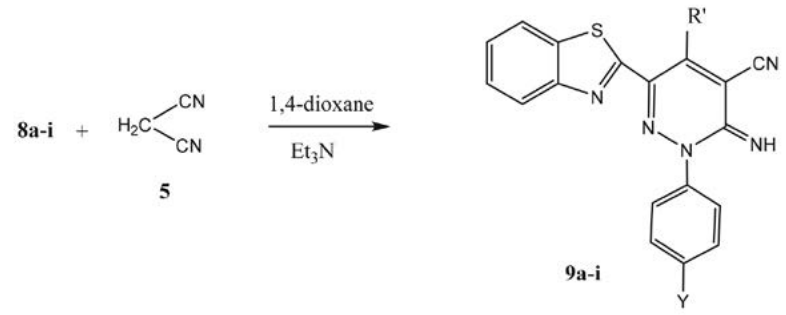


6	a	b	c	d	e	f
X	CN	CN	CN	COOEt	COOEt	COOEt
R'	NH_2	OH	CH_3	NH_2	OH	CH_3

Scheme 1. Synthesis of compounds **4a–c** and **6a–f**.

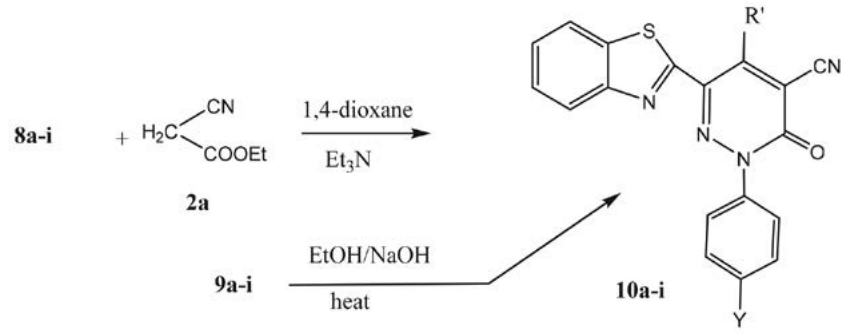


8	a	b	c	d	e	f	g	h	i
R	CN	CN	CN	COOEt	COOEt	COOEt	$COCH_3$	$COCH_3$	$COCH_3$
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

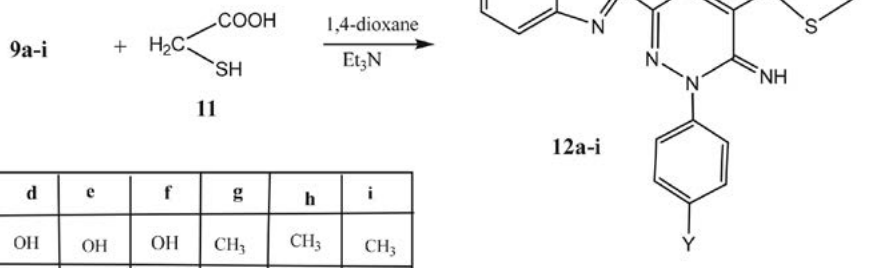


9	a	b	c	d	e	f	g	h	i
R'	NH_2	NH_2	NH_2	OH	OH	OH	CH_3	CH_3	CH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

Scheme 2. Synthesis of compounds **8a–i** and **9a–i**.

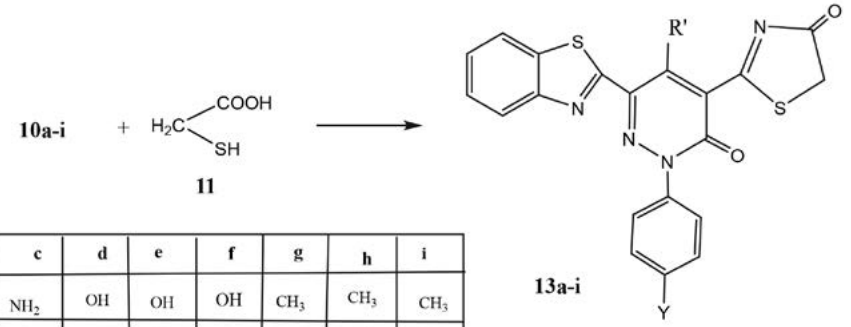


10	a	b	c	d	e	f	g	h	i
R	NH_2	NH_2	NH_2	OH	OH	OH	CH_3	CH_3	CH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

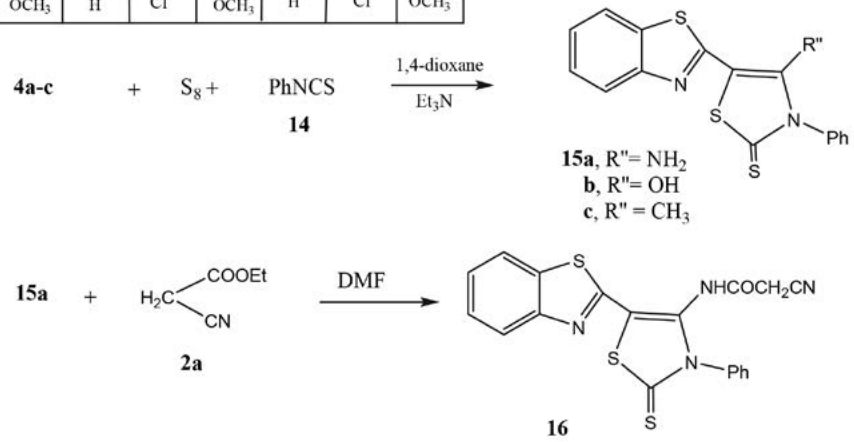


12	a	b	c	d	e	f	g	h	i
R	NH_2	NH_2	NH_2	OH	OH	OH	CH_3	CH_3	CH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

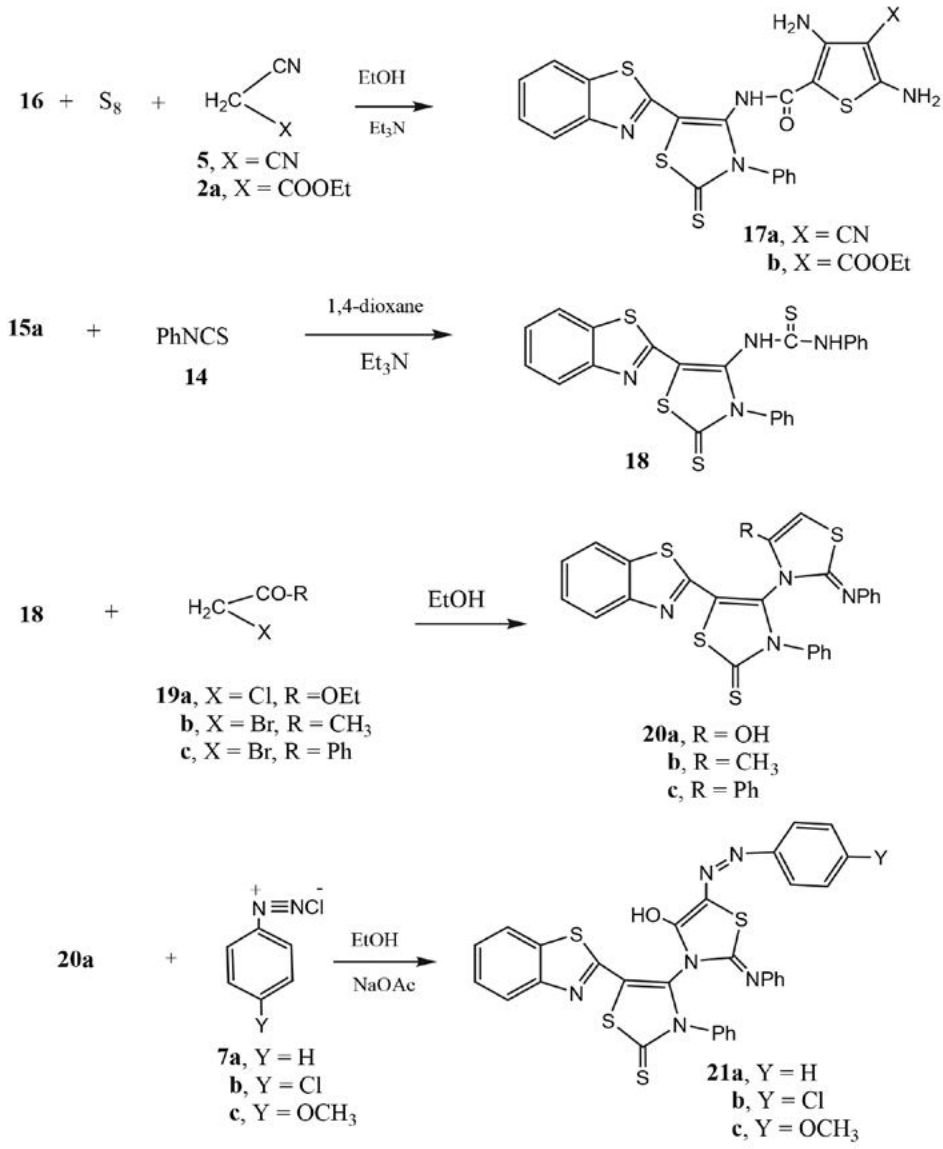
Scheme 3. Synthesis of compounds **10a–i** and **12a–i**.



13	a	b	c	d	e	f	g	h	i
R'	NH_2	NH_2	NH_2	OH	OH	OH	CH_3	CH_3	CH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3



Scheme 4. Synthesis of compounds **13a–i**, **15a–c** and **16**.



Scheme 5. Synthesis of compounds **17a**,**b**, **18**, **20a**–**c** and **21a**–**c**.

with foretinib as the positive control.[31–33] The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximately 4×10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures and were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 μL of DMSO in each well, and the absorbance at 492 nM (for absorbance of MTT formazan) and 630 nM (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each cell line. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

The mean values of three independent experiments, expressed as IC_{50} values, are presented in Table 1. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 30 μM. Generally, the variations of substituents within the aryl moiety together with the heterocycle ring being attached have a notable influence on the anti-proliferative activity.

3. 2. 2. Structure Activity Relationship

Table 1 demonstrates that many of the synthesized compounds revealed high inhibitions toward the used cancer cell lines. It is clear that most of the tested compounds

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

Compd No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
4a	3.31 ± 1.42	2.38 ± 1.16	2.48 ± 1.69	3.30 ± 1.38	2.18 ± 0.98	2.60 ± 1.05
4b	6.12 ± 1.26	4.35 ± 1.42	5.23 ± 1.34	4.62 ± 1.53	5.49 ± 1.29	3.28 ± 1.42
4c	2.58 ± 1.11	2.72 ± 1.15	3.26 ± 1.08	2.52 ± 1.16	2.41 ± 1.16	3.21 ± 1.22
6a	5.31± 1.46	3.16 ± 1.46	4.1 6 ± 1.41	5.19 ± 2.30	4.46 ± 1.23	5.64 ± 1.37
6b	0.46 ± 0.21	0.32 ± 0.19	0.28 ± 0.04	0.32 ± 0.18	0.42 ± 0.26	0.39 ± 0.20
6c	4.31 ± 1.24	3.28 ± 1.13	3.42 ± 1.27	5.36 ± 2.16	3.49 ± 1.16	4.38 ± 1.15
6d	1.36 ± 0.98	2.29 ± 0.87	1.38 ± 0.91	1.36 ± 0.77	3.67 ± 1.27	1.27 ± 0.88
6e	0.31 ± 0.51	0.62 ± 1.63	0.17 ± 0.16	0.43 ± 0.16	0.23 ± 0.06	0.31 ± 0.18
6f	0.41 ± 0.25	0.71 ± 0.57	0.42 ± 0.39	0.42 ± 0.22	0.28 ± 0.23	0.31 ± 0.21
8a	6.42 ± 2.28	5.37 ± 1.45	7.50 ± 1.87	6.65 ± 2.27	5.51 ± 2.39	6.34 ± 2.18
8b	0.22 ± 0.05	0.33 ± 0.21	0.39 ± 0.17	0.22 ± 0.16	0.30 ± 0.21	0.28 ± 0.17
8c	4.29 ± 1.52	3.38 ± 1.48	4.46 ± 2.19	3.85 ± 1.36	4.70 ± 1.39	5.54 ± 1.19
8d	5.31 ± 1.13	4.32 ± 1.26	3.29 ± 1.37	5.26 ± 2.26	4.36 ± 1.85	5.38 ± 1.39
8e	0.23 ± 0.18	0.39 ± 0.15	0.27 ± 0.09	0.23 ± 0.11	0.32 ± 0.13	0.22 ± 0.19
8f	5.86 ± 2.38	4.62 ± 1.64	3.76 ±1.32	4.42 ± 2.15	3.59 ± 1.26	4.68 ± 1.47
8g	1.84 ± 0.84	0.95 ± 0.36	1.58 ± 0.86	2.62 ± 1.07	3.59 ± 1.16	2.68 ± 1.03
8h	0.26 ± 0.15	0.35 ± 0.16	0.27 ± 0.15	0.29 ± 0.09	0.43 ± 0.22	0.39 ± 0.16
8i	4.63 ± 1.17	3.41 ± 1.34	5.33 ± 2.62	5.27 ± 1.29	4.31 ± 1.02	3.84 ± 1.32
9a	4.41 ± 2.08	6.31 ± 1.48	5.50 ± 1.83	4.68 ± 2.14	6.52 ± 2.41	5.38 ± 2.38
9b	0.24 ± 0.08	0.23 ± 0.11	0.29 ± 0.16	0.42 ± 0.19	0.35 ± 0.26	0.42 ± 0.16
9c	3.11 ± 1.37	3.68 ± 1.58	4.52 ± 2.30	4.80 ± 1.49	4.52 ± 1.63	5.26 ± 1.79
9d	0.35 ± 0.259	0.35 ± 0.17	0.26 ± 0.23	0.29 ± 0.17	0.46 ± 0.30	0.40 ± 0.24
9e	0.21 ± 0.15	0.29 ± 0.13	0.36 ± 0.15	0.28 ± 0.15	0.41 ± 0.17	0.24 ± 0.15
9f	3.22 ± 1.13	4.52 ± 1.14	5.60 ± 1.45	4.82 ± 2.31	4.52 ± 1.26	5.13 ± 1.16
9g	0.23 ± 0.14	0.25 ± 0.16	0.53 ± 0.26	0.31± 0.19	0.53 ± 0.16	0.42 ± 0.23
9h	0.23 ± 0.12	0.28 ± 0.15	0.34 ± 0.17	0.22 ± 0.08	0.31 ± 0.20	0.48 ± 0.17
9i	2.83 ± 1.13	2.80 ± 1.64	3.16 ± 1.45	3.87 ± 1.35	3.62 ± 1.12	3.78 ± 1.60
10a	2.81 ± 1.16	2.53 ± 1.25	3.62 ± 1.41	5.23 ± 2.31	2.70 ± 0.91	5.38 ± 2.38
10b	0.24 ± 0.08	0.23 ± 0.11	0.29 ± 0.16	0.42 ± 0.19	0.35 ± 0.26	0.51 ± 0.18
10c	5.34 ± 1.49	3.68 ± 1.32	5.42 ± 2.54	6.71 ± 1.29	6.31 ± 1.52	4.87 ± 1.23
10d	0.35 ± 0.23	0.42 ± 0.18	0.25 ± 0.14	0.29 ± 0.16	0.43 ± 0.21	0.35 ± 0.24
10e	0.21 ± 0.14	0.35 ± 0.16	0.26 ± 0.15	0.28 ± 0.15	0.41 ± 0.17	0.24 ± 0.15
10f	1.26 ± 0.93	2.40 ± 1.12	2.39 ± 0.86	1.53 ± 0.79	1.82 ± 1.43	1.62 ± 0.74
10g	0.25 ± 0.16	0.35 ± 0.14	0.41 ± 0.23	0.62 ± 0.25	0.41 ± 0.13	0.53 ± 0.19
10h	0.24 ± 0.15	0.38 ± 0.16	0.42 ± 0.15	0.35 ± 0.16	0.52 ± 0.23	0.35 ± 0.18
10i	1.62 ± 0.69	2.63 ± 1.14	2.17 ± 1.12	1.32 ± 1.15	2.42 ± 1.12	1.73 ± 0.93
12a	0.80 ± 0.25	0.21 ± 0.11	0.37 ± 0.20	0.42 ± 0.16	0.81 ± 0.20	0.62 ± 0.14
12b	0.38 ± 0.15	0.42 ± 0.27	0.23 ± 0.12	0.57 ± 0.25	0.41 ± 0.23	0.37 ± 0.13
12c	0.15 ± 0.26	0.31 ± 0.25	0.41 ± 0.42	0.72 ± 0.25	0.80 ± 0.36	0.53 ± 0.25
12d	0.35 ± 0.19	0.35 ± 0.17	0.26 ± 0.23	0.29 ± 0.17	0.46 ± 0.30	0.40 ± 0.24
12e	0.21 ± 0.15	0.29 ± 0.13	0.36 ± 0.15	0.28 ± 0.15	0.41 ± 0.17	0.24 ± 0.15
12f	3.22 ± 1.13	4.52 ± 1.14	5.60 ± 1.45	4.82 ± 2.31	4.52 ± 1.26	5.13 ± 1.16
12g	0.23 ± 0.14	0.25 ± 0.16	0.53 ± 0.26	0.31 ± 0.19	0.53 ± 0.16	0.42 ± 0.23
12h	0.23 ± 0.12	0.28 ± 0.15	0.34 ± 0.17	0.22 ± 0.08	0.31 ± 0.20	0.48 ± 0.17
12i	0.83 ± 0.13	0.80 ± 0.64	0.16 ± 0.05	0.87 ± 0.35	0.62 ± 0.12	0.78 ± 0.60
13a	0.80 ± 0.60	0.63 ± 0.26	0.57 ± 0.13	0.53 ± 0.39	0.61 ± 0.44	0.31 ± 0.16
13b	0.28 ± 0.13	0.31 ± 0.17	0.26 ± 0.15	0.32 ± 0.22	0.50 ± 0.24	0.15 ± 0.06
13c	0.16 ± 0.06	0.32 ± 0.15	0.42 ± 0.18	0.52 ± 0.23	0.53 ± 0.21	0.39 ± 0.24
13d	0.25± 0.17	0.32 ± 0.13	0.24 ± 0.15	0.25 ± 0.14	0.32 ± 0.23	0.29 ± 0.15
13e	0.39 ± 0.21	0.27 ± 0.12	0.27 ± 0.13	0.24 ± 0.12	0.30 ± 0.14	0.29 ± 0.11
13f	0.26 ± 0.13	0.32 ± 0.16	0.39 ± 0.25	0.32 ± 0.21	0.39 ± 0.23	0.35 ± 0.15
13g	0.33 ± 0.15	0.25 ± 0.18	0.40 ± 0.28	0.34 ± 0.17	0.29 ± 0.14	0.58 ± 0.24
13h	0.21 ± 0.14	0.27 ± 0.12	0.30 ± 0.19	0.28 ± 0.12	0.35 ± 0.16	0.28 ± 0.15
13i	0.23 ± 0.13	0.73 ± 0.34	0.36 ± 0.25	0.57 ± 0.15	0.36 ± 0.13	0.32 ± 0.21
15a	1.33 ± 1.03	1.62 ± 0.87	1.06 ± 0.85	1.62 ± 0.96	1.02 ± 0.82	1.38 ± 0.49
15b	0.24 ± 0.12	0.37 ± 0.13	0.29 ± 0.13	0.22 ± 0.13	0.32 ± 0.19	0.27 ± 0.14
15c	1.62 ± 0.59	1.13 ± 0.86	1.24 ± 0.83	0.51 ± 0.24	0.32 ± 0.16	0.78 ± 0.26

Compd No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
16	0.26 ± 0.16	0.27 ± 0.15	0.33 ± 0.27	0.28 ± 0.14	0.35 ± 0.15	0.35 ± 0.17
17a	0.24 ± 0.16	0.23 ± 0.18	0.24 ± 0.16	0.39 ± 0.12	0.38 ± 0.15	0.30 ± 0.17
17b	0.63 ± 0.32	0.51 ± 0.24	0.66 ± 0.29	0.45 ± 0.16	0.56 ± 0.16	0.68 ± 0.31
18	0.23 ± 0.15	0.29 ± 0.16	0.27 ± 0.14	0.26 ± 0.17	0.22 ± 0.17	0.38 ± 0.16
20a	0.22 ± 0.13	0.32 ± 0.27	0.14 ± 0.03	0.54 ± 0.23	0.25 ± 0.12	0.17 ± 0.08
20b	0.23 ± 0.14	0.35 ± 0.16	0.25 ± 0.16	0.27 ± 0.14	0.41 ± 0.16	0.26 ± 0.13
20c	0.68 ± 0.29	0.53 ± 0.26	0.24 ± 0.14	0.42 ± 0.25	0.53 ± 0.18	0.42 ± 0.23
21a	0.12 ± 0.03	0.22 ± 0.16	0.12 ± 0.08	0.32 ± 0.11	0.45 ± 0.22	0.27 ± 0.11
21b	0.25 ± 0.14	0.33 ± 0.14	0.35 ± 0.24	0.35 ± 0.16	0.21 ± 0.11	0.35 ± 0.17
21c	0.66 ± 0.23	0.83 ± 0.22	0.62 ± 0.16	0.50 ± 0.25	0.38 ± 0.15	0.38 ± 0.27
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

exhibited high inhibitions toward the six cancer cell lines. Considering the benzo[*d*]thiazole derivatives **4a–c**, it is clear that compound **4c** exhibited the highest cytotoxicity out of these three compounds; this might be attributed to the presence of the acetyl moiety. For the thiophene derivatives **6a–f**, all compounds showed high inhibitions except compounds **6a** (X = CN, R^- = NH_2) and **6c** (X = CN, R^- = CH_3). On the other hand compound **6d** (X = COOEt, R' = NH_2) showed moderate inhibitions toward the six cancer cell lines. Considering the aryl hydrazone derivatives **8a–i** it can be seen that compounds **8a**, **8c**, **8d**, **8f** and **8i** exhibited low inhibitions. However, compounds **8b** (R= CN, Y = Cl), **8e** (R= COOEt, Y = Cl), **8g** (R= $COCH_3$, Y = H) and **8h** ((R= $COCH_3$, Y = Cl), showed moderate to high inhibitions toward the six cancer cell lines. On the other hand, among the pyridazine derivatives **9–i**, all compounds exhibited from high to moderate inhibitions: compounds **9b**, **9d**, **9e**, **9g** and **9h** showed high inhibitions and compounds **9a**, **9c**, **9f** and **9i** exhibited moderate inhibition. In most cases the high inhibitions are attributed to the presence of either OH group at the pyridazine ring or of the Cl group attached to the aryl moiety. Similarly for the pyridazin-2-one derivatives **10a–i**: compounds **10b**, **10d**, **10e**, **10g** and **10h** showed high inhibitions and compounds **10a**, **10f** and **10i** exhibited moderate inhibition while compound **10c** exhibited low inhibitions. Interestingly, among the thiazole derivatives **12a–i** and **13a–i** all compounds exhibited high inhibitions toward the six cancer cell lines except compound **12f** which showed moderate inhibitions. Although some compounds contain electron donating groups like the OCH_3 or electron repealing groups like the CH_3 group it seemed that in these compounds the presence of the second thiazole moiety enhances the inhibitions. For the thiazol-2-thione derivatives **15a–c**, it was obvious that compound **15b** (R" = OH) exhibited the highest inhibitions among the three compounds, although compounds **15a** (R" = NH_2) and **15c** (R" = CH_3) showed moderate inhibitions. Moreover, the *N*-alkylated thiazol-thione derivative **16** exhibited high inhibitions toward the selected cancer cell lines. Compounds **17a** (X = CN), **17b** (X = COOEt) and **18** with the two thiazole rings and the thiophene ring showed high inhibitions toward the six cancer cell lines. Finally, for the trithiazolyl derivatives **20a–c** and **21a–c** it was seen that all compounds exhibited high inhibitions toward the six cancer cell lines. It was obvious that the three thiazole moieties had a strong effect on the activities of such compounds.

3. 2. 3. *In vitro* Evaluation of the Anticancer Activity of compounds 13b–g and 13i

The 2,3-dihydropyridazin-4-yl)thiazol-4(5*H*)-one derivatives **13b**, **13c**, **13d**, **13e**, **13f**, **13g** and **13i** were selected for an advanced assay against a panel of approximately seventeen tumor cell lines at 10-fold dilutions of five concentrations (100 μM, 10 μM, 1.0 μM, 0.1 μM and 0.01 μM).[34,35] The tested compounds exhibited inhibition activity (GI_{50} < 5 μM) against the cancer cell lines that are classified into groups according to the type of disease, the data are shown in Table 2. Throughout our work for compounds **13b**, **13c**, **13d, 13e**, **13f, 13g** and **13i** there are two factors, the substituent at C-4 of the pyridazine ring and the substituent at the position 4 of the aryl group. It is clear from Table 2 that all tested compounds exhibited high inhibitions toward the cell lines categorized according to the type of the disease. From the tested compounds compound **13e** (R" = OH, Y = Cl) exhibited the highest inhibitions among the tested compounds, it was obvious that the presence of the electronegative OH and Cl groups has a strong impact on the activity of this compound. On the other hand, compound **13i** (R" = CH3, Y = OCH_3) exhibited the lowest inhibitions toward the cancer cell lines. Such finding was attributed to the presence of both of the OCH_3 and CH_3 groups within the structure of the compound. Interestingly, compound **13c** (R = NH_2, Y = OCH_3) exhibited highest inhibition toward K-526 cell line with GI_{50} = 0.14 μM although it contains the OCH_3 moiety. On the other hand, compound **13f** (R" = OH, Y = OCH_3) exhibited the highest inhibition toward RPMI-8262 cell line.

Table 2. The influence of compounds **13b**–**g** and **13i** on the growth of individual tumor cell lines (GI_{50} < 5 μM).

Disease	Cell line	13b	13c	13d	13f	13e	13g	13i
Leukemia	CCRF-CEM	0.32	0.29	0.29	0.46	0.22	0.36	0.86
Leukemia	RPMI-8262	0.36	0.27	0.30	0.11	0.37	0.28	0.79
Leukemia	SR 0.44	0.29	0.57	0.41	0.31	0.62	0.97	
Leukemia	HI-60 (TB)	0.36	0.42	0.27	0.32	0.27	0.16	0.63
Leukemia	K-526	0.26	0.14	0.46	0.41	0.12	0.36	0.81
NCS-Lung cancer	HOP-62	0.60	0.51	0.47	0.312	0.16	0.52	1.97
NCS-Lung cancer	NCI-H460	0.37	0.42	0.37	0.26	0.66	0.52	2.18
Colon cancer	HCT-116	0.45	0.36	0.47	0.26	0.26	0.38	3.18
Colon cancer	HCT-15	0.25	0.38	0.29	0.39	0.42	0.31	1.42
Colon cancer	KM12	0.74	0.37	0.28	0.42	0.48	0.37	0.93
CNS cancer	SF-295	0.58	0.26	0.45	0.63	0.39	0.70	0.73
CNS cancer	U251	0.27	0.16	0.34	0.81	0.89	0.37	0.62
Melanoma	LOX IMVI	0.41	0.48	0.58	0.32	0.29	0.44	1.17
Melanoma	MDA-MB-435	0.40	0.27	0.59	0.53	0.40	0.77	1.23
Melanoma	UACC-62	0.38	0.47	0.44	0.46	0.20	0.56	0.79
Ovarian cancer	OVCAR-3	0.52	0.42	0.29	0.28	0.31	0.55	0.80
Renal cancer	786-0	0.53	0.43	0.19	0.28	0.27	0.43	0.99

3. 2. 4. HTRF Kinase Assay

Mesenchymal epithelial transition factor (c-Met) is a multifunctional transmembrane tyrosine kinase and acts as a receptor for hepatocyte growth factor/Scatter factor (HGF/SF).[36] There are multiple oncogenetic characteristics of c-Met that were outlined shortly after its discovery, including cell division and transportation through an extracellular matrix invasion.[37] Moreover, c-Met kinase activity has a high relationship to prostate cancer where c-Met played a key role in the transformation of prostate cancer from the primary androgen-sensitive to androgen-insensitive state along with the increase in radioresistance.[38–41] Based on these reported observations, the c-Met kinase activity of all compounds was evaluated using homogeneous time-resolved fluorescence (HTRF) assay as previously reported.[42] Taking foretinib as the positive control, the results are expressed as IC_{50} and summarized in Table 3. The anti-proliferative activities of all target compounds against the human prostatic cancer PC-3 cell line were measured by MTT assay[43] using anibamine as the reference drug. The mean values of three independent experiments were expressed as IC_{50} values and are presented in Table 3. Generally, the variations of substituents within the aryl moiety together with the heterocyclic ring being attached have a notable influence on the anti-proliferative activity.

As indicated in Table 3, all the tested compounds displayed potent c-Met enzymatic activity with IC_{50} values ranging from 0.12 to 8.93 nM and potent prostate PC-3 cell line inhibitions with IC_{50} values ranging from 0.11 to 12.91 μM. Compared with foretinib (IC_{50} = 1.16 nM), the forty compounds **4a**, **6c**, **6e**, **8b**, **8c**, **8e**, **8g**, **8h, 8i**, **9b**, **9c**, **9e**, **9h**, **9i**, **10b**, **10c**, **10e**, **10h**, **10i**, **12b**, **12c**, **12e**, **12g**, **12h**, **12i**, **13b**, **13c**, **13g**, **13h**, **13i**, **15b**, **15c**, **16**, **17a**, **18**, **20a**, **20c**, **21a**, **21b** and **21c** exhibited highest potency with IC_{50} values less than 1.16 nM. Remarkably, among the synthesized compounds **4a**, **4b**, **4c**, **6b**, **6c**, **6d**, **6e**, **8b**, **8c**, **8e**, **8g**, **8h**, **8i**, **9b**, **9c**, **9h**, **9i**, **10b**, **10c**, **10e**, **10h**, **10i**, **12b**, **12e**, **12h**, **12i**, **13b**, **13c**, **13g**, **13h**, **13i**, **15b**, **16**, **17a**, **17b**, **18**, **20a**, **20c** and **22b** displayed much higher anti-proliferation activities than the standard anibamine (IC_{50} = 3.26 μM). Analyzing the data in Table 3 showed that compound **9e** exhibited the lowest GI_{50} (GI_{50} = 0.17 μM) among the tested compounds. On the other hand, compound **9c** showed the highest inhibition toward PC-3 cell line with IC_{50} = 0.16 μM.

All synthesized compounds were tested against the VERO, Monkey Kidney normal cell line, where they showed low activities against the normal cell line. Interestingly, from Table 3 compounds **4b**, **4c**, **6b** and **6d** showed SI > 15, while the thirty three compounds **4a**, **6c**, **6e**, **8b**, **8c**, **8e**, **8i**, **8h**, **9b**, **9c**, **9h**, **9i**, **10b**, **10c**, **10e**, **10h**, **10i**, **12b**, **12e**, **12h**, **12i**, **13b**, **13c**, **13g**, **13h**, **13i**, **15b**, **16**, **17a**, **17b**, **18**, **20a** and **21b** exhibited SI > 100, while the rest of compounds showed SI < 15.

Table 3. c-Met enzymatic activity and PC-3 inhibition of the newly synthesized compounds

Compound No	IC_{50} (nM) c-Met	IC_{50}(μM) PC-3	VERO[a] (μM)	SI PC-3[b]
4a	0.43 ± 0.21	0.52 ± 0.25	66.23 ± 4.90	> 100
4b	1.13 ± 0.79	1.23 ± 0.93	33.52 ± 3.71	27.25
4c	1.86 ± 0.86	1.24 ± 0.93	34.32 ± 8.37	27.68
6a	2.63 ± 1.02	3.48 ± 1.14	28.92 ± 4.53	8.31
6b	2.42 ± 1.23	2.29 ± 1.31	36.52 ± 3.69	15.94
6c	0.21 ± 0.14	0.39 ± 0.24	69.43 ± 6.52	> 100
6d	1.62 ± 0.87	1.04 ± 0.74	40.58 ± 4.52	39.01
6e	0.26 ± 0.14	0.29 ± 0.13	68.49± 4.26	> 100
6f	8.52 ± 3.53	10.43 ± 3.65	26.31 ± 3.49	2.52
8a	4.83 ± 1.53	5.63 ± 2.73	23.82 ± 5.23	4.23
8b	0.29 ± 0.08	0.33 ± 0.15	60.43 ± 5.34	>100
8c	0.24 ± 0.16	0.38 ± 0.24	68.48 ± 4.51	> 100
8d	1.68 ± 1.12	3.29 ± 1.24	30.58 ± 3.84	1.08

Compound No	IC_{50} (nM) c-Met	IC_{50}(μM) PC-3	VERO[a] (μM)	SI PC-3[b]
8e	0.42 ± 0.25	0.42 ± 0.24	68.52 ± 5.38	> 100
8f	6.51 ± 1.32	7.25 ± 1.37	23.27 ± 2.39	3.21
8g	0.28 ± 0.11	0.33 ± 0.18	22.23 ± 3.45	67.36
8h	0.35 ± 0.15	0.27 ± 0.14	58.90 ± 6.84	> 100
8i	0.24 ± 0.16	0.31 ± 0.19	62.19 ± 4.32	> 100
9a	2.81 ± 1.13	4.68 ± 0.16	20.50 ± 2.33	4.38
9b	0.25 ± 0.14	0.24 ± 0.12	66.58 ± 5.31	> 100
9c	0.31 ± 0.16	0.17 ± 0.09	70.26 ± 4.24	> 100
9d	2.13 ± 1.01	8.62 ± 1.62	44.81 ± 2.16	5.42
9e	0.15 ± 0.02	8.46 ± 2.42	40.61 ± 2.69	4.80
9f	5.26 ± 2.15	10.32 ± 2.31	30.76 ± 4.31	2.98
9g	3.19 ± 2.25	5.68 ± 2.32	28.27 ± 2.63	4.97
9h	0.38 ± 0.13	0.24 ± 0.12	59.12 ± 3.32	> 100
9i	0.37 ± 0.17	0.36 ± 0.27	68.12 ± 4.35	> 100
10a	6.83 ± 1.28	10.63 ± 3.58	25.73 ± 4.61	2.42
10b	0.25 ± 0.13	0.32 ± 0.22	59.68 ± 5.21	> 100
10c	0.23 ± 0.13	0.30 ± 0.15	62.38 ± 4.23	> 100
10d	4.26 ± 1.85	6.65 ± 1.78	25.28 ± 4.80	3.80
10e	0.18 ± 0.06	0.21 ± 0.13	58.76 ± 4.13	> 100
10f	8.11 ± 2.52	12.62 ± 3.16	22.18 ± 4.60	1.75
10g	7.48 ± 2.43	6.82 ± 2.51	30.52 ± 3.62	4.47
10h	0.48 ± 0.16	0.29 ± 0.13	65.16 ± 4.63	>100
10i	0.12 ± 0.08	0.16 ± 0.07	70.58 ± 5.19	> 100
12a	7.38 ± 1.67	8.26 ± 2.17	31.26 ± 3.73	3.78
12b	0.29 ± 0.13	0.43 ± 0.26	59.32 ± 4.62	>100
12c	0.42 ± 0.21	5.29 ± 2.23	48.69 ± 1.62	9.20
12d	8.93 ± 2.53	7.85 ± 3.52	64.90 ± 5.43	8.26
12e	0.25 ± 0.13	0.39 ± 0.17	68.24 ± 6.43	> 100
12f	5.62 ± 1.34	6.36 ± 1.81	35.25 ± 2.79	5.54
12g	0.32 ± 0.18	4.64 ± 0.14	30.17 ± 4.52	6.50
12h	0.17 ± 0.09	0.27 ± 0.15	44.31 ± 4.62	>100
12i	0.32 ± 0.14	0.19 ± 0.08	77.48 ± 4.82	> 100
13a	6.31 ± 2.52	9.39 ± 1.42	26.54 ± 3.42	2.82
13b	0.28 ± 0.15	0.36 ± 0.19	58.37 ± 5.82	>100
13c	0.63 ± 0.26	0.58 ± 0.169	68.32 ± 4.51	> 100
13d	8.96 ± 2.81	9.41 ± 2.82	22.73 ± 3.51	2.41
13e	2.83 ± 1.64	4.58 ± 1.53	23.59 ± 2.71	5.15
13f	4.32 ± 1.54	12.91 ± 3.81	27.24 ± 2.94	2.10
13g	0.34 ± 0.28	0.29 ± 0.18	44.13 ± 4.53	>100
13h	0.15 ± 0.08	0.36 ± 0.14	65.54 ± 5.31	>100
13i	0.41 ± 0.24	0.38 ± 0.15	66.25 ± 3.52	> 100
15a	8.36 ± 2.51	10.61 ± 4.28	27.24 ± 2.94	2.56
15b	0.22 ± 0.14	0.11 ± 0.07	69.34 ± 5.20	> 100
15c	0.31 ± 0.22	8.71 ± 3.53	25.24 ± 3.82	2.89
16	0.29 ± 0.16	0.29 ± 0.08	66.31 ± 4.62	> 100
17a	0.48 ± 0.23	0.62 ± 0.18	69.28 ± 4.63	> 100
17b	1.46 ± 0.91	0.21 ± 0.08	57.28 ± 4.62	> 100
18	0.25 ± 0.13	0.23 ± 0.16	72.38 ± 6.80	> 100
20a	0.12 ± 0.07	0.41 ± 0.23	65.28 ± 5.73	> 100
20b	6.39 ± 2.18	8.25 ± 2.36	36.30 ± 3.52	4.39
20c	1.45 ± 0.88	2.82 ± 0.13	49.20 ± 5.71	5.57
21a	9.98 ± 0.26	8.82 ± 2.37	64.22 ± 4.82	7.28
21b	0.23 ± 0.16	0.25 ± 0.09	67.24 ± 4.81	> 100
21c	0.28 ± 0.16	12.25 ± 4.19	26.24 ± 5.62	2.14
	Foretinib	**Anibamine**		
	1.16 ± 0.17	3.26 ± 0.35	–	–

[a] VERO, Monkey Kidney cell line (Cat No-11095–080).
[b] Selectivity index (SI) were calculated by IC_{50} values in normal cell line divided by IC_{50} values in PC-3 cancer cell line.

3. 2. 5. Determination of Morphological Changes of A549 Cell Line by the Effect of Compounds 13c and 13h

The lung is a unique organ that should be protected against inhaled pathogens and toxins, without imbalanced immune responses or compromising its vital function. For that matter microenvironment of the lung tissue is regulated through complex and refined cell interactions.[44,45] For that reason we studied the effect of compound **13c** (Fig. 2) and **13h** (Fig. 3) toward A549 cell line which was selected for studying the morphological changes. There are many reports concerned with morphological changes of other cell lines.[46,47] To understand the ability of compound **13c** and **13h** in apoptosis induction, various qualitative (morphological) and quantitative assays were performed on the A549 lung cancer cell line. The changes in the morphological features of A549 cells were observed after the treatment with compound **13c** and **13h** at different concentrations along with the untreated control cells. Further, images shown in Fig. 2A and 3A were captured using phase-contrast microscopy after 72 h; revealing the characteristic apoptotic features like changes in morphology (shape, shrinkage) of the cell, reduction in the number of live cells. In the present study, A549 cells after treatment with compound **13c** and **13h** for 72 h exhibited the formation of apoptotic features such as the appearance of membrane blebs and inverse proportion in the number of cells with the concentration of compound tested as indicated in Fig. 2B and 3B, respectively. Compound **13c** and **13h** treated A549 cells after 72 h, on staining with DAPI visualized the chromatin condensation as well as pyknotic (inset of 1.25 μM) and condensed (bright colored: inset of 2.5 μM) nuclei formation as depicted in Fig. 2C and 3C, respectively.

4. Conclusion

Throughout this work we aimed to synthesize a set of novel compounds that were produced from benzo[*d*]thiazole derivatives. The structures of the synthesized compounds were confirmed by multiple techniques and they were screened for cytotoxic activity against six cancer cell lines. In addition, most of the tested compounds exhibited high inhibitions toward c-Met kinase and PC-3 cell lines. The results obtained in this assay indicate that these compounds are good candidates as anti-cancer agents thus encouraging 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.

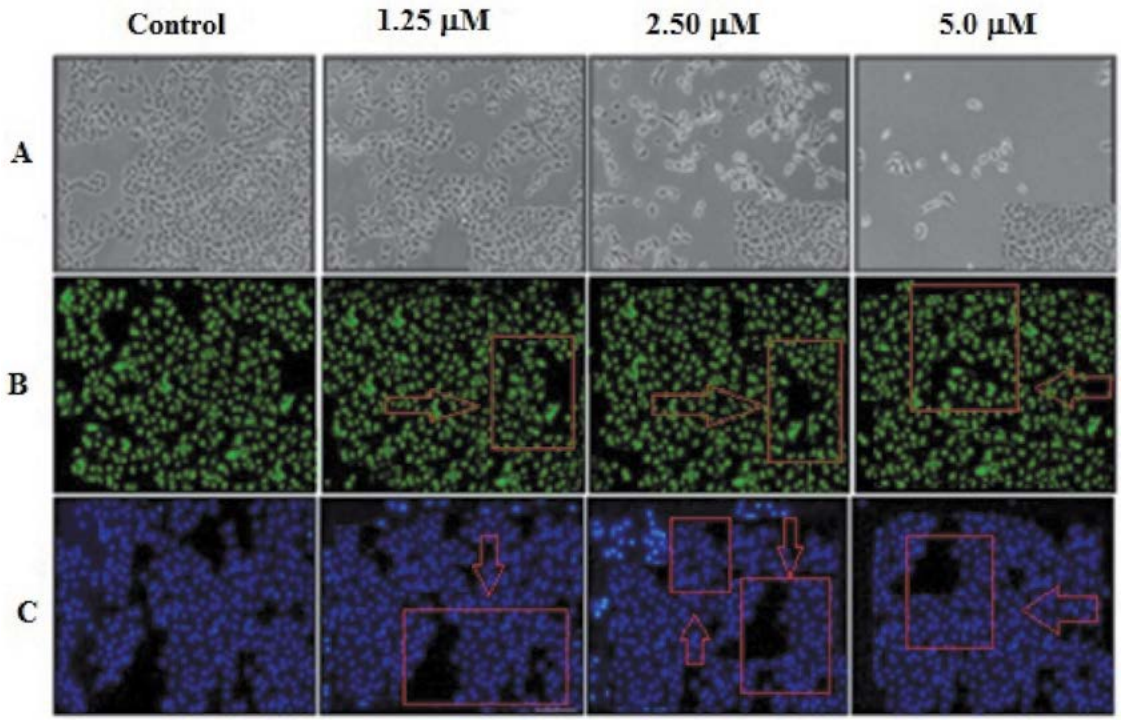


Figure 2. Microscopic observation of the effect of different concentrations (1.25, 2.5, and 5 µM) of compound **13c** in comparison to control (untreated) on A549 cells after 72 h of treatment. (A) Morphological changes were observed through phase-contrast microscopy. (B) Morphological changes such as membrane blebs were visualized through a fluorescence microscopy by performing acridine orange staining. (C) Nuclear changes were observed by DAPI staining using a fluorescence microscopy. Photos at different concentrations of treatment indicate the changes induced by treatment and red-colored arrows specify the area of effect.

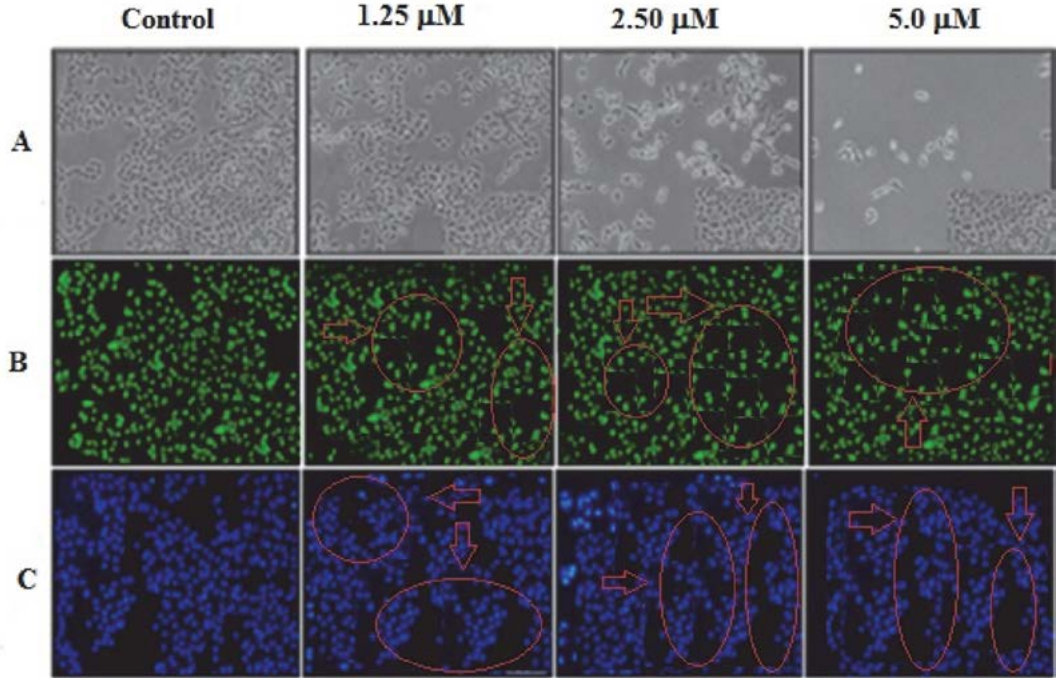


Figure 3. Microscopic observation of the effect of different concentrations (1.25, 2.5, and 5 µM) of compound **13h** in comparison to control (untreated) on A549 cells after 72 h of treatment.

Conflict of Interest

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

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Povzetek

Pripravili smo benzo[*d*]tiazolne derivate **4a**–**c** ter jih uporabili za nadaljnjo sintezo tiofenskih derivatov **6a**–**f**. Iz njih smo pripravili arilhidrazonske derivate **8a**–**i**, ki smo jih lahko pretvorili v piridazinske derivate **9a**–**i** in **10a**–**i**. Te spojine smo reagirali s tioglikolno kislino in tako pripravili tiazolne derivate **12a**–**i** in **13a**–**i**. Iz spojin **4a**–**c** smo z reakcijo z elementarnim žveplom in fenilizotiocianatom sintetizirali še tiazolne derivate **15a**–**c**. Z nadaljnjimi reakcijami heterociklizacij smo spojino **15a** pretvorili v tiofenske derivate. Sintetizirane spojine smo preizkusili na izbranih rakastih celičnih linijah in najbolj aktivne spojine testirali proti razširjenemu setu sedemnajstih rakastih celičnih linij, ki smo jih razvrstili glede na vrsto bolezni, ki jo povzročajo. Morfološke spremembe celične linije A549 kot posledico učinkovanja spojin **13c** in **13h** smo študirali v mikrookolju pljučnega tkiva ter prišli do odličnih rezultatov.