New Approaches for the synthesis of heterocyclic compounds derived from cyclohexan-1,3-dione with anti-proliferative activities

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Abstract In the present work a series of heterocyclization reactions were adopted using cyclohexan-1,3-dione through its reaction with either furan-2-aldehyde or thiophene-2-aldehyde to give the corresponding ylidene derivatives **3a,b**. The latter compounds underwent heterocyclization reactions to give thiophene and pyran derivatives **5a-d** and **6a-d**, respectively. Moreover, compounds **3a,b** reacted with elemental sulfur and phenylisothiocyanate to give the fused thiazole derivatives **8a,b**. In addition, the reaction with either of hydrazine hydrate or phenylhydrazine to give the 4-hydrazono-4,5,6,7-tetrahydro-2*H*-indazole derivatives **10a-d**, respectively. Similarly, the reaction of either **3a** or **3b** with hydroxylamine hydrochloride gave the 6,7-dihydrobenzo[*c*]isoxazol-4(5*H*)-one oxime derivatives **12a** and **12b**, respectively. Other fused heterocyclic compounds were produced and their structures were elucidated. Evaluation of the synthesized compounds against selected cancer cell lines was performed. The most active compounds were further evaluated against tyrosine kinases and Pim-1 kinase inhibitions.

Keyword: cyclohexan-1,3-dione, thiophene, pyrazole, isoxazole, cytotoxicity

1. Introduction

With the last few years the synthesis of heterocyclic compounds attract the attention due to the wide spectrum of their high biological activities. In addition, many compounds were considered as good synthons for fused systems that characterized by different pharmaceutical

applications.[1-10] Therefore, organic chemists have been making extensive efforts to produce heterocyclic compounds by developing new and efficient synthetic transformations. Within the field of pharmaceutical chemistry, many pyrazoles, thiophenes and thiazoles were reported with a wide spectrum of biological activities that including potent analgesic, anti-convulsant, anti-inflammatory and anti-bacterial, anti-pyretics, anti-tumor, anti-parasitic, anti-microbial, anti-histaminic (H1), anti-anexiety test in mice, anti-arrhythmic and serotonin antagonist.[11-23] The present work dealing with the current application of pyrazole, thiophene, pyrimidine and oxazine cores in the designing of anticancer agents within tumor progression. In our search it was possible to verify that such compounds readily applicable to provide new insights and valuable inspiration in the research of new drugs and development and contribute to the management of cancer. Based on such importance of heterocyclic compounds, therefore, we studied the reaction of cyclohexane-1,3-dione with some heterocyclic aldehydes and cyanomethylene reagents followed by heterocyclization of the products together with the anti-tumor evaluations of the resulting compounds towards cancer cell lines were recorded.

2. Experimental

2.1. General

The obtained compounds showed their melting points using Electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were measured on a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer (PyeUnicam, UK, Cambridge). ^{1}H NMR spectra were obtained using Varian Gemini-300 (300 MHz, Varian UK) using DMSO-d_6 as a solvent and Tetraethylsilane (TMS) as internal standard chemical shifts are expressed as δ ppm. The mass spectra were measured with Hewlett Packard 5988 A GC/MS system (Hewlett Packard, Agilent, USA) instrument.

2.1.1. General procedure for the synthesis of the 2-methylenecyclohexane-1,3-dione derivatives 3a,b

Either of furan-2-aldehyde (0.96 g, 0.01 mol) of thiophene-2-aldehyde (1.12 g, 0.01 mol) was added to a solution of compound **1** (1.12 g, 0.01 mol) in absolute ethanol (40 mL) containing piperdine (0.50 mL). The reaction mixture, in each case,was heated under reflux for 3 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.1.1. 2-(Furan-2-ylmethylene)cyclohexane-1,3-dione (3a)

Yellow crystals from ethanol, yield (1.46 g, 77 %), m.p.185-188 °C. IR (KBr) υ max cm^{-1}: 3053 (CH, aromatic), 1704, 1687 (2 CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.45-1.69 (m, 2H, CH_2), 2.63-2.76 (m, 4H, $2CH_2$), 6.82 (s, 1H, CH), 6.80-7.83 (m, 3H, furan H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.4, 36.5, 39.2 ($3CH_2$), 112.4, 158.1 (C=CH), 135.8, 140.2, 142.6, 146.1 (furan C), 177.1, 179.4 (2CO). Anal. Calculated for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.31; H, 5.52. MS: m/e 190 (M^+, 28 %).

2.1.1.2. 2-(Thiophen-2-ylmethylene)cyclohexane-1,3-dione (3b)

Orange crystals from ethanol, yield (1.44 g, 70 %), m.p. 201-204 °C. IR (KBr) υ max cm^{-1}: 3055 (CH, aromatic), 1703, 1689 (2 CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 1.43-1.68 (m, 2H, CH_2), 2.61-2.74 (m, 4H, $2CH_2$), 6.80 (s, 1H, CH), 6.82-7.88 (m, 3H, thiophene H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8, 36.2, 39.1 ($3CH_2$), 112.6, 158.8 (C=CH), 135.6, 140.5, 142.3, 146.2 (thiophene C), 177.6, 179.2 (2CO). Anal. Calculated for $C_{11}H_{10}O_2S$: C, 64.05; H, 4.89; S, 15.55. Found: C, 63.80; H, 4.73; 15.38. MS: m/e 206 (M^+, 32 %).

2.1.2. General procedure for the synthesis of the 6,7-dihydrobenzo[*b*]-thiophen-5(4*H*)-one derivatives 5a-d

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added to a solution of either compound **3a** (1.90 g, 0.01 mol) or **3b** (2.06 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.2.1. 2-Amino-4-(furan-2-ylmethylene)-5-oxo-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-3-carbonitrile (5a)

Pale yellow crystals from ethanol, yield (1.94 g, 70 %), m.p. 130-132 °C. IR (KBr) υ max cm^{-1}: 3482-3353 (NH_2), 3056 (CH, aromatic), 2220 (CN), 1686 (CO), 1630 (C=C). ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 2.62-2.78 (2t, 4H, $2CH_2$), 4.73 (s, 2H, D_2O exchangeable, NH_2), 6.84 (s, 1H, CH), 6.82-7.86 (m, 3H, furan H).^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.6, 36.3, 39.5 ($3CH_2$), 112.6, 158.4 (C=CH), 116.8 (CN), 135.4, 140.6, 141.4, 142.2, 142.7, 144.8, 145.6, 146.5 (thiophene, furan C), 179.3 (CO). Anal. Calculated for $C_{14}H_{10}N_2O_2S$: C, 62.21; H, 3.73; N, 10.36; S, 11.86. Found: C, 62.36; H, 3.80; N, 10.41; S, 12.04. MS: m/e 270 (M^+, 32 %).

2.1.2.2. Ethyl 2-amino-4-(furan-2-ylmethylene)-5-oxo-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxylate (5b)

Yellow crystals from ethanol, yield (2.21 g, 70 %), m.p.125-127 °C. IR (KBr) υ max cm^{-1}: 3494-3368 (NH_2), 3058 (CH, aromatic), 2931, 2972 (CH_2, CH_3), 1689, 1688 (2 CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.12 (t, 3H, J =7.28 Hz, CH_3), 2.68-2.74 (2t, 4H, $2CH_2$), 4.21 (q, 2H, J = 7.28 Hz, CH_2), 4.76 (s, 2H, D_2O exchangeable, NH_2), 6.86 (s, 1H, CH), 6.84-7.92 (m, 3H, furan H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.3 ($OCH_2\underline{CH_3}$), 16.4, 36.8, 39.3 ($3CH_2$), 52.8 ($O\underline{CH_2}CH_3$), 112.3, 158.6 (C=CH), 135.6, 18.0, 140.8, 141.6, 142.3, 143.5, 144.2, 146.7 (thiophene, furan C), 164.8, 178.3 (2CO). Anal. Calculated for $C_{16}H_{15}NO_4S$: C, 60.55; H, 4.76; N, 4.41; S, 10.10. Found: C, 60.80; H, 4.83; N, 4.60; S, 10.26. MS: m/e 317 (M^+, 38 %).

2.1.2.3. 2-Amino-5-oxo-4-(thiophen-2-ylmethylene)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carbonitrile (5c)

Orange crystals from ethanol, yield (1.94 g, 68 %), m.p. 202-205 °C. IR (KBr) υ max cm^{-1}: 3459-3342 (NH_2), 3058 (CH, aromatic), 2221 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.64-2.75 (2t, 4H, $2CH_2$), 4.74 (s, 2H, D_2O exchangeable, NH_2), 6.88 (s, 1H, CH), 6.80-7.87 (m, 3H, thiophene H); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 39.8 ($2CH_2$), 112.3, 158.8 (C=CH), 116.9 (CN), 134.2, 138.6, 140.3, 142.4, 143.1, 143.6, 145.2, 146.7 (two thiophene C), 179.8 (CO). Anal. Calculated for $C_{14}H_{10}N_2OS_2$: C, 58.72; H, 3.52; N, 9.78; S, 22.39. Found: C, 58.36; H, 3.80; N, 9.68; S, 22.41. MS: m/e 288 (M^+, 46 %).

2.1.2.4. Ethyl 2-amino-5-oxo-4-(thiophen-2-ylmethylene)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxylate (5d)

Pale yellow crystals from ethanol, yield (2.13 g, 66 %), m.p. 189-192 °C. IR (KBr) υ max cm^{-1}: 3486-3342 (NH_2), 3054 (CH, aromatic), 2938, 2893 (CH_2, CH_3), 1689, 1686 (2 CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.13 (t, 3H, J =7.59 Hz, CH_3), 2.64-2.78 (2t, 4H, $2CH_2$), 4.23 (q, 2H, J = 7.59 Hz, CH_2), 4.79 (s, 2H, D_2O exchangeable, NH_2), 6.84 (s, 1H, CH), 7.29-7.85 (m, 3H, thiophene H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.1 ($OCH_2\underline{CH_3}$), 16.5, 36.4, 39.7 ($3CH_2$), 52.3 ($O\underline{CH_2}CH_3$), 112.1, 158.4 (C=CH), 135.3, 136.7, 138.3, 140.5, 141.5, 142.0, 143.8, 144.6, 146.8 (twothiophene C), 164.5, 178.9 (2CO). Anal. Calculated for $C_{16}H_{15}NO_3S_2$: C, 57.64; H, 4.53; N, 4.20; S, 19.23. Found: C, 57.80; H, 4.71; N, 4.38; S, 19.46. MS: m/e 333 (M^+, 26 %).

2.1.3. General procedure for the synthesis of the 2*H*-chromen-5-one derivatives 6a-d

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added to a solution of either compound **3a** (1.90 g, 0.01 mol) or **3b** (2.06 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (1.0 mL). The reaction mixture in each case, was heated under reflux for 3 h then was the excess solvent was removed under vacuum. The remaining product was triturated with diethylether and the formed solid product was collected by filtration.

2.1.3.1. 2-Amino-4-(furan-2-yl)-5-oxo-5,6,7,8-tetrahydro-2H-chromene-3-carbonitrile (6a)

Pale yellow crystals from ethanol, yield (1.76 g, 69 %), m.p. 194-196 °C. IR (KBr) υ max cm^{-1}: 3470-3328 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1632 (C=C); ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 1.41-1.66 (m, 2H, CH_2), 2.61-2.76 (m, 4H, $2CH_2$), 4.71 (s, 2H, D_2O exchangeable, NH_2), 6.04 (s, 1H, pyran H-4), 6.87-7.83 (m, 3H, furan H); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.3, 39.5 ($3CH_2$), 116.8 (CN), 134.3, 141.5, 141.8, 142.6, 1431.4, 143.8, 145.2, 146.4 (pyran, furan C), 178.4 (CO). Anal. Calculated for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.72; N, 10.93. Found: C, 65.38; H, 4.92; N, 11.29. MS: m/e 256 (M^+, 28 %).

2.1.3.2. 4-(Furan-2-yl)-2-hydroxy-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (6b)

Pale brown crystals from ethanol, yield (1.90 g, 74 %), m.p. 198-200 °C. IR (KBr) υ max cm^{-1}: 3568-3339 (OH), 3055 (CH, aromatic), 2222 (CN), 1687 (CO), 1630 (C=C); ^{1}H NMR (DMSO- d_6, 200 MHz): δ = 1.32-1.68 (m, 2H, CH_2), 2.63-2.74 (m, 4H, $2CH_2$), 6.02 (s, 1H, pyran H-4), 6.84-7.85 (m, 3H, furan H), 9.80 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 36.5, 39.3 ($3CH_2$), 116.7 (CN), 134.1, 138.0, 140.2, 141.7, 142.3, 142.9, 143.3, 146.6 (furan, pyran C), 178.8 (CO). Anal. Calculated for $C_{14}H_{11}NO_4$: C, 65.37; H, 4.31; N, 5.44. Found: C, 65.38; H, 4.92; N, 5.26. MS: m/e 257 (M^+, 36 %).

2.1.3.3. 2-Amino-4-(furan-2-yl)-5-oxo-5,6,7,8-tetrahydro-2H-chromene-3-carbonitrile (6c)

Brown crystals from ethanol, yield (1.95 g, 72 %), m.p. 211-214 °C. IR (KBr) υ max cm^{-1}: 3445-3372 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO- d_6, 200 MHz): δ = 1.41-1.64 (m, 2H, CH_2), 2.61-2.77 (m, 4H, $2CH_2$), 4.73 (s, 2H, D_2O exchangeable, NH_2), 6.07 (s, 1H, thiophene H-4), 6.91-7.73 (m, 3H, thiophene H); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 36.6, 39.8 ($3CH_2$), 116.4 (CN), 134.6, 138.7, 140.2, 141.6, 142.3, 142.8, 143.6, 146.2 (thiophene, pyran C), 178.6 (CO). Anal. Calculated for $C_{14}H_{12}N_2O_2S$: C,

61.75; H, 4.44; N, 10.29; S, 11.77. Found: C, 61.80; H, 4.46; N, 10.48; S, 11.58. MS: m/e 272 (M^+, 40 %).

2.1.3.4. 2-Hydroxy-5-oxo-4-(thiophen-2-yl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (6d)

Orange crystals from ethanol, yield (2.07 g, 76 %), m.p. 177-179 °C. IR (KBr) υ max cm^{-1}: 3542-3329 (OH), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 200 MHz): δ = 1.34-1.66 (m, 2H, CH_2), 2.61-2.76 (m, 4H, $2CH_2$), 6.04 (s, 1H, pyran H-4), 6.76-7.89 (m, 3H, furan H), 9.83 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.3, 39.6 ($3CH_2$), 116.9 (CN), 134.4, 139.2, 140.8, 141.5, 143.7, 144.3, 145.2, 146.8 (furan, pyran C), 178.6 (CO). Anal. Calculated for $C_{14}H_{11}NO_3S$: C, 61.52; H, 4.06; N, 5.12; S, 11.73. Found: C, 61.72; H, 5.04; N, 11.59. MS: m/e 273 (M^+, 42 %).

2.1.4. General procedure for thesynthesis 2-thioxohexahydrobenzo[*d*]thiazole derivatives 8a,b

Each of elemental sulfur (0.32 g, 0.01 mol) and phenylisothiocyanate (1.30 g, 0.01 mol) were added to a solution of either compound **3a** (1.90 g, 0.01 mol) or **3b** (2.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.0 mL). The reaction mixture was heated under reflux for 2 h then was left to cool and the formed solid product, in each case, was collected by filtration.

2.1.4.1. 4-(Furan-2-ylmethylene)-3-phenyl-2-thioxo-2,3,6,7-tetrahydrobenzo[d]-thiazol-5(4H)-one (8a)

Yellow crystals from ethanol, yield (2.50 g, 74 %), m.p. 168-169 °C. IR (KBr) υ max cm^{-1}: 3055 (CH, aromatic), 1688 (CO), 1630 (C=C), 1205 (C=S); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.63-2.78 (2t, 4H, $2CH_2$), 6.87 (s, 1H, CH), 6.82-7.85 (m, 8H, C_6H_5, furan H); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.6, 39.5 ($3CH_2$), 112.1, 158.4 (C=CH), 120.3, 122.6, 124.8, 127.2, 134.6, 140.8, 142.2, 143.2, 143.8, 146.9 (C_6H_5, furan, thiazole C), 179.6 (CO), 181.3 (C=S). Anal. Calculated for $C_{18}H_{13}NO_2S_2$: C, 63.69; H, 3.86; N, 4.13; S, 18.89. Found: C, 63.80; H, 3.69; N, 4.32; S, 19.18. MS: m/e 339 (M^+, 48 %).

2.1.4.2. 3-phenyl-4-(thiophen-2-ylmethylene)-2-thioxo-2,3,6,7-tetrahydrobenzo-[*d*]thiazol-5(4H)-one (8b)

Plae yellow crystals from 1,4-dioxane, yield (2.84 g, 80 %), m.p. 222-225 °C. IR (KBr) υ max cm^{-1}: 3055 (CH, aromatic), 1689 (CO), 1630 (C=C), 1205 (C=S); ^1H NMR (DMSO- d_6,

200 MHz): δ = 2.61-2.79 (2t, 4H, $2CH_2$), 6.88 (s, 1H, CH), 6.96-7.89 (m, 8H, C_6H_5, thiophene H); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.5, 39.7 ($2CH_2$), 112.4, 158.6 (C=CH), 120.7, 122.5, 124.3, 126.8, 136.2, 140.6, 141.3, 142.6, 143.4, 146.6 (C_6H_5, thiophene, thiazole C), 179.3 (CO), 181.3 (C=S). Anal. Calculated for $C_{18}H_{13}NOS_3$: C, 60.81; H, 3.69; N, 3.94; S, 27.06. Found: C, 60.69; H, 3.74; N, 4.12; S, 26.86. MS: m/e 355 (M^+, 59 %).

2.1.5. General procedure for thesynthesisofthe 4-hydrazono-4,5,6,7-tetrahydro-2H-indazole derivatives 10a-d

Either hydrazine hydrate (0.1 mL, 0.02 mol) or phenylhydrazine (2.16 g, 0.02 mol) was added to a solution of either of compound **3a** (1.90 g, 0.01 mol) or **3b** (2.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing. The reaction mixture was heated under reflux for 3h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.5.1. 3-(Furan-2-yl)-4-hydrazono-4,5,6,7-tetrahydro-2*H*-indazole (10a)

Yellow crystals from ethanol, yield (1.55 g, 72 %), m.p. 158-161 °C. IR (KBr) υ max cm^{-1}: 3472-3328 (NH, NH_2), 3055 (CH, aromatic), 1658 (exocyclic C=N), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.32-1.64 (m, 2H, CH_2), 2.60-2.78 (m, 4H, $2CH_2$), 4.86 (s, 2H, D_2O exchangeable, NH_2), 7.14-7.84 (m, 3H, furan H), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.5, 36.3, 39.7 ($3CH_2$), 112.4, 158.6 (C=CH), 136.4, 138.0, 139.2, 140.3, 143.8, 146.2 (furan, pyrazole C), 168.3, 172.8 (2C=N). Anal. Calculated for $C_{11}H_{12}N_4O$: C, 61.10; H, 5.59; N, 25.91. Found: C, 60.93; H, 5.29; N, 25.77. MS: m/e 216 (M^+, 36 %).

2.1.5.2. 3-(Furan-2-yl)-2-phenyl-4-(2-phenylhydrazono)-4,5,6,7-tetrahydro-2*H*-indazole (10b)

Pale yellow crystals from 1,4-dioxane, yield (2.50 g, 68 %), m.p. 177-180 °C. IR (KBr) υ max cm^{-1}: 3463-3341 (NH), 3055 (CH, aromatic), 1652 (exocyclic C=N), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.31-1.67 (m, 2H, CH_2), 2.63-2.77 (m, 4H, $2CH_2$), 7.26-7.89 (m, 13H, $2C_6H_5$, furan H), 8.30 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.2, 36.1, 39.4 ($3CH_2$), 120.2, 121.6, 123.2, 123.8, 124.0, 126.3, 128.3, 1290.1, 136.4, 140.3, 140.8, 141.2, 143.4, 146.8 ($2C_6H_5$, pyrazole, furan C), 176.2, 178.3 (2C=N). Anal. Calculated for $C_{23}H_{20}N_4O$: C, 74.98; H, 5.47; N, 15.21. Found: C, 75.19; H, 5.31; N, 15.02. MS: m/e 368 (M^+, 41 %).

2.1.5.3. 4-Hydrazono-3-(thiophen-2-yl)-4,5,6,7-tetrahydro-2*H*-indazole (10c)

Pale yellow crystals from 1,4-dioxane, yield (1.76 g, 76 %), m.p. 233-236 °C. IR (KBr) υ max cm^{-1}: 3458-3342 (NH, NH_2), 3055 (CH, aromatic), 1656 (exocyclic C=N), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.33-1.67 (m, 2H, CH_2), 2.62-2.75 (m, 4H, $2CH_2$), 4.80 (s, 2H, D_2O exchangeable, NH_2), 6.96-7.88 (m, 3H, thiophene H) 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.5, 36.3, 39.7 ($3CH_2$), 112.4, 158.6 (C=CH), 136.6, 1328.4, 139.2, 140.1, 143.5, 146.4 (thiphene, pyrazole C), 176.4, 178.6 (2C=N). Anal. Calculated for $C_{11}H_{12}N_4S$: C, 56.87; H, 5.21; N, 24.12; S, 13.80. Found: C, 56.93; H, 5.30; N, 24.31; S, 13.98. MS: m/e 232 (M^+, 28 %).

2.1.5.4. 2-Phenyl-4-(2-phenylhydrazono)-3-(thiophen-2-yl)-4,5,6,7-tetrahydro-2H-indazole (10d)

Orange crystals from ethanol, yield (2.61 g, 68 %), m.p. 180-184 °C. IR (KBr) υ max cm^{-1}: 3484-3326 (NH), 3055 (CH, aromatic), 1655 (exocyclic C=N), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.32-1.69 (m, 2H, CH_2), 2.60-2.78 (m, 4H, $2CH_2$), 7.23-7.85 (m, 13H, $2C_6H_5$, thiophene H), 8.29 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.4, 36.6, 39.4 ($3CH_2$), 120.8, 122.2, 123.6, 123.9, 125.8, 126.6, 128.1, 129.7, 135.8, 140.6, 142.6, 142.9, 143.1, 146.5 ($2C_6H_5$, pyrazole, thiophene C), 176.4, 178.6 (2C=N). Anal. Calculated for $C_{23}H_{20}N_4S$: C, 71.85; H, 5.24; N, 14.57; S, 8.34. Found: C, 72.13; H, 5.42; N, 14.38; S, 8.62. MS: m/e 384 (M^+, 26 %).

2.1.6. General procedure for the synthesis of the 6,7-dihydrobenzo[*c*]isoxazol-4(5*H*)-one oxime derivatives 12a,b

Hydroxylamine hydrochloride (1.40 g, 0.02 mol) was added to a solution of either compound **3a** (1.90 g, 0.01 mol) or **3b** (2.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium acetate (2.0 g). The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture and the formed solid product, in each case, was collected by filtration.

2.1.6.1. 3-(Furan-2-yl)-6,7-dihydrobenzo[c]isoxazol-4(5H)-one oxime (12a)

Yellow crystals from 1,4-dioxane, yield (1.52 g, 70 %), m.p. 177-180 °C. IR (KBr) υ max cm^{-1}: 3524-3376 (OH), 3055 (CH, aromatic), 1655 (exocyclic C=N), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.31-1.68 (m, 2H, CH_2), 2.63-2.77 (m, 4H, $2CH_2$), 6.93-7.81 (m, 3H, furan H), 10.29 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.2, 36.3, 39.2 ($3CH_2$), 132.3, 135.1, 140.6, 141.5, 142.8, 143.6 (isoxazole , furan C), 176.1, 178.4

(2C=N). Anal. Calculated for $C_{11}H_{10}N_2O_3$: C, 60.55; H, 4.62; N, 12.84. Found: C, 60.39; H, 4.80; N, 12.93. MS: m/e 218 (M^+, 32 %).

2.1.6.2. 3-(Thiophen-2-yl)-6,7-dihydrobenzo[c]isoxazol-4(5H)-one oxime (12b)

Pale yellow crystals from 1,4-dioxane, yield (1.52 g, 65 %), m.p. 222-225 °C. IR (KBr) υ max cm^{-1}: 3551-3349 (OH), 3055 (CH, aromatic), 1653 (exocyclic C=N), 1630 (C=C),; ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 1.32-1.65 (m, 2H, CH_2), 2.61-2.74 (m, 4H, $2CH_2$), 7.22-7.80 (m, 3H, thiophene H), 10.29 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.4, 36.6, 39.8 ($3CH_2$), 132.1, 134.3, 138.8, 140.9, 141.6, 144.2 (isoxazole , thiophene C), 176.0, 178.6 (2C=N). Anal. Calculated for $C_{11}H_{10}N_2O_2S$: C, 56.39; H, 4.30; N, 11.96; S, 13.69. Found: C, 56.42; H, 4.49; N, 12.05; S, 13.83 MS: m/e 234 (M^+, 42 %).

2.1.7. 2-(Ethoxymethylene)cyclohexane-1,3-dione (14)

Ethyl orthoformate (1.68 g, 0.01 mol) was added to a solution of cyclohexan-1,3-dione (1.12 g, 0.01 mol) in acetic acid (40 mL). The reaction mixture was heated under reflux for 2 h then evaporated in vacuum and the remaining product was triturated with ethanol and the formed solid product was collected by filtration.

Yellow crystals from ethanol, yield (1.27 g, 76 %), m.p. 282-258 °C. IR (KBr) ν max cm^{-1}: 2980 (CH_2), 1689, 1686 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.28 (t, 3H, J = 6.83 Hz, CH_3), 1.49-1.67 (m, 2H, CH_2), 2.65-2.73 (m, 4H, $2CH_2$), 3.89 (q, 2H, J = 6.83 Hz, CH_2), 6.79 (s, 1H, CH), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.3 ($OCH_2\underline{CH_3}$), 16.2, 36.8, 39.0 ($3CH_2$), 62.8 ($O\underline{CH_2}CH_3$), 112.3, 158.2 (C=CH), 177.3, 179.2 (2CO). AnalysisCalcd for $C_9H_{12}O_3$ (168.19): C, 64.27; H, 7.19 %. Found: C, 64.08; H, 7.33 %. MS: m/e 168 (M^+, 22 %).

2.1.8. General procedure for the synthesis of the 2-(aminomethylene)cyclohexane-1,3-dione derivatives 16a-c

Equimolar amounts of aniline (0.93 g, 0.01 mol), 4-methylaniline (1.08 g, 0.01 mol) or 4-methoxyaniline (1.34 g, 0.01 mol) and compound **14** (1.68 g, 0.01 mol) in 1,4-dioxane (50 mL) was were under reflux for 4 h. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol and the product solid product, in each case, was collected by filtration.

2.1.8.1. 2-((Phenylamino)methylene)cyclohexane-1,3-dione (16a)

Yellow crystals from ethanol, yield (1.24 g, 58 %), m.p. 165-167 °C. IR (KBr) ν max cm^{-1}: 3480-3363 (NH), 3055 (CH aromatic), 2980 (CH_2), 1688, 1686 (2CO), 1632 (C=C); ^{1}H NMR

(DMSO-d_6, 300 MHz): δ = 1.42-1.69 (m, 2H, CH_2), 2.63-2.75 (m, 4H, $2CH_2$), 6.05 (s, 1H, CH), 7.28-7.39 (s, 5H, C_6H_5), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5, 36.8, 39.4 ($3CH_2$), 94.3 (CH), 120.3, 123.1, 124.8, 126.4 (C_6H_5), 177.1, 179.8 (2CO). AnalysisCalcd for $C_{13}H_{13}NO_2$ (215.25): C, 72.54; H, 6.09; N, 6.51 %. Found: C, 72.31; H, 6.29; N, 6.38 %.%. MS: m/e 215 (M^+, 35 %).

2.1.8.2. 2-((Phenylamino)methylene)cyclohexane-1,3-dione (16b)

Yellow crystals from ethanol, yield (1.67 g, 73 %), m.p. 214-217 °C. IR (KBr) ν max cm^{-1}: 3459-3341 (NH), 3054 (CH aromatic), 2982, 2886 (CH_3, CH_2), 1688, 1686 (2CO), 1631 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.43-1.67 (m, 2H, CH_2), 2.61-2.76 (m, 4H, $2CH_2$), 2.88 (s, 3H, CH_3), 6.08 (s, 1H, CH), 7.24-7.45 (s, 4H, C_6H_4), 8.26 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.3, 36.6, 39.5 ($3CH_2$), 23.8 (CH_3), 90.6 (CH), 120.8, 122.9, 123.4, 127.9 (C_6H_4), 177.3, 179.5 (2CO). AnalysisCalcd for $C_{14}H_{15}NO_2$ (229.27): C, 73.34; H, 6.59; N, 6.11 %. Found: C, 73.29; H, 6.41; N, 6.26 %.%. MS: m/e 229 (M^+, 40 %).

2.1.8.3. 2-(((4-Methoxyphenyl)amino)methylene)cyclohexane-1,3-dione (16c)

Yellow crystals from 1,4-dioxane, yield (1.47 g, 60 %), m.p. 193-196 °C. IR (KBr) ν max cm^{-1}: 3463-3329 (NH), 3055 (CH aromatic), 2982, 2886 (CH_3, CH_2), 1689, 1686 (2CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.41-1.69 (m, 2H, CH_2), 2.61-2.78 (m, 4H, $2CH_2$), 3.68 (s, 3H, OCH_3), 6.06 (s, 1H, CH), 7.27-7.48 (s, 4H, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.3, 36.6, 39.5 ($3CH_2$), 50.3 (OCH_3), 90.2 (CH), 120.3, 122.4, 125.6, 128.7 (C_6H_4), 177.1, 179.3 (2CO). AnalysisCalcd for $C_{14}H_{15}NO_3$ (245.27): C, 68.56; H, 6.16; N, 5.71 %. Found: C, 68.80; H, 6.24; N, 5.93 %.%. MS: m/e 245 (M^+, 38 %).

2.1.9. General procedure for the synthesis of the 6,7-dihydrobenzo[b]thiophen-5(4H)-one derivatives 17a-f

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added to a solution of either compound **16a** (2.15 g, 0.01 mol), **16b** (2.29 g, 0.01 mol) or **16c** (2.45 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.9.1. 2-Amino-5-oxo-4-((phenylamino)methylene)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (17a)

Pale yellow crystals from ethanol, yield (2.06 g, 70 %), m.p. 127-129 °C. IR (KBr) υ max cm^{-1}: 3472-3353 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1686 (CO), 1630 (C=C); ^{1}H

NMR (DMSO- d_6, 300 MHz): δ = 2.60-2.79 (2t, 4H, $2CH_2$), 4.78 (s, 2H, D_2O exchangeable, NH_2), 6.89 (s, 1H, CH), 7.26-7.42 (m, 5H, C_6H_5), 8.39 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 36.0, 39.8 ($2CH_2$), 112.2, 158.4 (C=CH), 116.8 (CN), 121.6, 122.4, 124.8, 127.2, 132.7, 134.2, 138.0, 142.6 (C_6H_5, thiophene C), 179.6 (CO). Anal. Calculated for $C_{16}H_{13}N_3OS$: C, 65.06; H, 4.44; N, 14.23; S, 10.86. Found: C, 65.18; H, 4.60; N, 14.19; S, 11.17. MS: m/e 295 (M^+, 38 %).

2.1.9.2. Ethyl 2-amino-5-oxo-4-((phenylamino)methylene)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxylate (17b)

Yellow crystals from ethanol, yield (2.18 g, 64 %), m.p. 96-98 °C. IR (KBr) υ max cm^{-1}: 3486-3351(NH_2), 3058 (CH, aromatic), 2930, 2972 (CH_2, CH_3), 1689, 1686 (2 CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.13 (t, 3H, J =7.08 Hz, CH_3), 2.68-2.76 (2t, 4H, $2CH_2$), 4.22 (q, 2H, J = 7.08 Hz, CH_2), 4.77 (s, 2H, D_2O exchangeable, NH_2), 6.86 (s, 1H, CH), 7.22-7.40 (m, 5H, C_6H_5), 8.33 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3 ($OCH_2\underline{CH_3}$), 36.8, 39.6 ($2CH_2$), 52.5 ($O\underline{CH_2}CH_3$), 112.4, 158.8 (C=CH), 121.3, 123.6, 125.2, 126.8, 132.9, 133.3, 138.0, 142.7 (C_6H_5, thiohene C), 164.2, 179.8 (2CO). Anal. Calculated for $C_{18}H_{18}N_2O_3S$: C, 63.14; H, 5.30; N, 8.18; S, 9.36. Found: C, 62.91; H, 5.49; N, 8.25; S, 9.60. MS: m/e 342 (M^+, 27 %).

2.1.9.3. 2-Amino-5-oxo-4-((p-tolylamino)methylene)-4,5,6,7-tetrahydrobenzo-[*c*]thiophene-3-carbonitrile (17c)

Orange crystals from 1,4-dioxane, yield (2.31 g, 75 %), m.p. 177-179 °C. IR (KBr) υ max cm^{-1}: 3493-3323 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.62-2.77 (2t, 4H, $2CH_2$), 2.79 (s, 3H, CH_3), 4.77 (s, 2H, D_2O exchangeable, NH_2), 6.82 (s, 1H, CH), 7.28-7.46 (m, 4H, C_6H_4), 8.37 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 36.0, 39.6 ($2CH_2$), 24.3 (CH_3), 112.0, 158.6 (C=CH), 116.9 (CN), 120.3, 123.7, 124.8, 128.9, 132.9, 134.7, 138.3, 142.2 (C_6H_4, thiophene C), 179.3 (CO). Anal. Calculated for $C_{17}H_{15}N_3OS$: C, 66.00; H, 4.89; N, 13.58; S, 10.36. Found: C, 65.85; H, 4.91; N, 13.70; S, 10.42. MS: m/e 309 (M^+, 24 %).

2.1.9.4. Ethyl 2-amino-5-oxo-4-((p-tolylamino)methylene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate (17d)

Brown crystals from 1,4-dioxane, yield (2.45 g, 69 %), m.p. 203-206 °C. IR (KBr) υ max cm^{-1}: 3459-3337 (NH_2), 3055 (CH, aromatic), 2930, 2970 (CH_2, CH_3), 1689, 1687 (2 CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.12 (t, 3H, J = 6.59 Hz, CH_3), 2.65-2.78 (2t, 4H, $2CH_2$), 2.80 (s, 3H, CH_3), 4.24 (q, 2H, J = 6.59 Hz, CH_2), 4.79 (s, 2H, D_2O exchangeable, NH_2),

6.83 (s, 1H, CH), 7.21-7.45 (m, 4H, C_6H_4), 8.36 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1 ($OCH_2\underline{CH_3}$), 36.3, 39.7 ($3CH_2$), 23.9 (CH_3), 52.1 ($O\underline{CH_2}CH_3$), 112.2, 158.7 (C=CH), 120.1, 123.8, 124.9, 128.3, 134.8, 137.2, 140.2, 142.6 (C_6H_5, thiophene C), 164.3, 179.5 (2CO). Anal. Calculated for $C_{19}H_{20}N_2O_3S$: C, 64.02; H, 5.66; N, 7.86; S, 9.00. Found: C, 63.91; H, 5.53; N, 8.01; S, 9.26. MS: m/e 356 (M^+, 32 %).

2.1.9.5. 2-Amino-4-(((4-methoxyphenyl)amino)methylene)-5-oxo-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (17e)

Yellow crystals from ethanol, yield (2.30 g, 71 %), m.p. 166-169 °C. IR (KBr) υ max cm^{-1}: 3474-3329 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.61-2.77 (2t, 4H, $2CH_2$), 3.68 (s, 3H, OCH_3), 4.75 (s, 2H, D_2O exchangeable, NH_2), 6.87 (s, 1H, CH), 7.24-7.46 (m, 4H, C_6H_4), 8.35 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.2, 39.4 ($3CH_2$), 50.21(OCH_3), 112.5, 158.4 (C=CH), 116.7 (CN), 120.1, 122.9, 125.3, 127.6, 132.2, 134.7, 137.6, 143.3 (C_6H_4, thiophene C), 179.6 (CO). Anal. Calculated for $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91; S, 9.85. Found: C, 62.53; H, 4.79; N, 12.83; S, 10.01. MS: m/e 325 (M^+, 32 %).

2.1.9.6. Ethyl 2-amino-4-(((4-methoxyphenyl)amino)methylene)-5-oxo-4,5,6,7-tetra-hydrobenzo[*b*]-thiophene-3-carboxylate (17f)

Yellow crystals from ethanol, yield (2.40 g, 65 %), m.p. 203-206 °C. IR (KBr) υ max cm^{-1}: 3459-3337 (NH_2), 3055 (CH, aromatic), 2930, 2970 (CH_2, CH_3), 1689, 1687 (2 CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 1.13 (t, 3H, J = 7.59 Hz, CH_3), 2.62-2.75 (2t, 4H, $2CH_2$), 3.61 (s, 3H, OCH_3), 4.22 (q, 2H, J = 7.59 Hz, CH_2), 4.79 (s, 2H, D_2O exchangeable, NH_2), 6.83 (s, 1H, CH), 7.24-7.48 (m, 4H, C_6H_4), 8.34 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1 ($OCH_2\underline{CH_3}$), 36.3, 39.7 ($3CH_2$), 50.2 (OCH_3), 52.2 ($O\underline{CH_2}CH_3$), 112.2, 158.7 (C=CH), 120.2, 122.2, 124.3, 128.1, 134.6, 136.7, 138.1, 140.8 (C_6H_5, thiophene C), 164.1, 179.7 (2CO). Anal. Calculated for $C_{19}H_{20}N_2O_4S$: C, 61.27; H, 5.41; N, 7.52; S, 8.61. Found: C, 60.91; H, 5.23; N, 7.39; S, 8.59. MS: m/e 372 (M^+, 36 %).

2.1.10. General procedure for the synthesis of the 6,7-dihydrobenzo[b]thiophen-5(4*H*)-one derivatives 19a-c

Either compound **18a** (1.12 g, 0.01 mol), **18b** (1.74 g, 0.01 mol) or **18c** (1.94 g, 0.01 mol) was added to a solution of compound **1** (1.12 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.10.1. 2-Amino-5-oxo-N-phenyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxamide (19a)

Pale yellow crystals from ethanol, yield (2.14 g, 75 %), m.p. 120-123 °C. IR (KBr) υ max cm^{-1}: 3484-3337 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1689, 1686 (2CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.62-2.79 (2t, 4H, $2CH_2$), 3.02 (s, 2H, CH_2), 4.75 (s, 2H, D_2O exchangeable, NH_2), 7.28-7.40 (m, 5H, C_6H_5), 8.38 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 36.6, 40.3 ($3CH_2$), 116.6 (CN), 120.8, 122.2, 125.3, 127.3, 132.2, 134.5, 138.0, 142.9 (C_6H_5, thiophene C), 164.3, 179.6 (2CO). Anal. Calculated for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78; S, 11.20. Found: C, 63.22; H, 4.75; N, 9.59; S, 11.36. MS: m/e 286 (M^+, 42 %).

2.1.10.2. 2-Amino-5-oxo-N-(p-tolyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (19b)

Yellow crystals from ethanol, yield (1.98 g, 66 %), m.p. 194-196 °C. IR (KBr) υ max cm^{-1}: 3449-3327 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1689, 1687 (2CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.63-2.77 (2t, 4H, $2CH_2$), 2.80 (s, 3H, CH_3), 3.06 (s, 2H, CH_2), 4.78 (s, 2H, D_2O exchangeable, NH_2), 7.26-7.46 (m, 4H, C_6H_4), 8.38 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 36.6, 40.3 ($3CH_2$), 116.6 (CN), 120.8, 122.2, 125.3, 127.3, 132.7, 134.7, 138.4, 142.6 (C_6H_4, thiophene C), 164.3, 179.6 (2CO). Anal. Calculated for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33; S, 10.67. Found: C, 63.72; H, 5.75; N, 9.59; S, 10.36. MS: m/e 300 (M^+, 36 %).

2.1.10.3. 2-Amino-N-(4-chlorophenyl)-5-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (19c)

Brown crystals from 1,4-dioxane, yield (2.49 g, 78 %), m.p. 205-208 °C. IR (KBr) υ max cm^{-1}: 3473-3351 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1689, 1686 (2CO), 1630 (C=C); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.64-2.76 (2t, 4H, $2CH_2$), 3.06 (s, 2H, CH_2), 4.79 (s, 2H, D_2O exchangeable, NH_2), 7.23-7.48 (m, 4H, C_6H_4), 8.36 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO - d_6, 75 MHz): δ 16.1, 36.4, 40.6 ($3CH_2$), 116.9 (CN), 120.2, 123.5, 125.8, 128.6, 132.3, 134.7, 138.0, 142.7 (C_6H_4, thiophene C), 164.3, 179.8 (2CO). Anal. Calculated for $C_{15}H_{13}ClN_2O_2S$: C, 56.16; H, 4.08; N, 8.73; S, 10.00. Found: C, 56.29; H, 4.26; N, 8.93; S, 10.26. MS: m/e 320 (M^+, 28 %).

2.1.11. General procedure for the synthesis of the tetrahydrobenzo[*b*]thiophene-3-carboxamide derivatives 20a-c

A mixture of either **19a** (2.86 g, 0.01 mol), **19b** (3.00 g, 0.01 mol) or **19c** (3.20 g, 0.01 mol) in dimethylformamide (30 mL) and ethyl cyanoacetate (1.07 g, 0.01 mL) was heated under

reflux for 3 h. The solid product, formed in each case, produced upon pouring onto ice/water mixture was collected by filtration.

2.1.11.1. 2-(2-Cyanoacetamido)-5-oxo-N-phenyl-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxamide (20a)

Dark brown crystals from ethanol, yield (3.28 g, 78 %), m.p 118-120 °C. IR (KBr) υ max cm^{-1}: 3469-3329 (NH), 3054 (CH, aromatic), 2256 (CN), 1690-1686 (3CO), 1630 (C=C); ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 2.61-2.82 (2t, 4H, $2CH_2$), 3.08 (s, 2H, CH_2), 3.68 (s, 2H, CH_2), 7.29-7.42 (m, 5H, C_6H_5), 8.24, 8.34 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.6, 36.2, 40.5 ($3CH_2$), 60.3 (CH_2), 170.1 (CN), 120.3, 123.5, 125.9, 126.5, 132.8, 133.3, 138.6, 142.8 (C_6H_5, thiophene C), 164.3, 166.1, 179.6 (3CO). Anal. Calculated for $C_{18}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89; S, 9.07. Found: C, 61.31; H, 4.29; N, 11.68; S, 8.85. MS: m/e 353 (M^+, 20 %).

2.1.11.2. 2-(2-Cyanoacetamido)-5-oxo-N-phenyl-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxamide (20b)

Brown crystals from ethanol, yield (2.27 g, 62 %), m.p 211-214 °C. IR (KBr) υ max cm^{-1}: 3453-3340 (NH), 3055 (CH, aromatic), 2256 (CN), 1693-1685 (3CO), 1630 (C=C); ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 2.63-2.80 (2t, 4H, $2CH_2$), 2.70 (s, 3H, CH_3), 3.10 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 7.25-7.38 (m, 4H, C_6H_4), 8.23, 8.38 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.3, 36.4, 40.8 ($3CH_2$), 60.5 (CH_2), 168.3 (CN), 120.6, 122.1, 126.4, 127.1, 132.8, 134.2, 138.6, 142.9 (C_6H_4, thiophene C), 164.2, 166.1, 179.8 (3CO). Anal. Calculated for $C_{19}H_{17}N_3O_3S$: C, 62.11; H, 4.66; N, 11.44; S, 8.73. Found: C, 62.26; H, 4.48; N, 11.52; S, 8.90. MS: m/e 367 (M^+, 28 %).

2.1.11.3. N-(4-Chlorophenyl)-2-(2-cyanoacetamido)-5-oxo-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxamide (20c)

White crystals from ethanol, yield (2.12 g, 55 %), m.p 118-121 °C. IR (KBr) υ max cm^{-1}: 3470-3338 (NH), 3055 (CH, aromatic), 2256 (CN), 1691-1685 (3CO), 1630 (C=C); ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 2.62-2.82 (2t, 4H, $2CH_2$), 3.12 (s, 2H, CH_2), 3.62 (s, 2H, CH_2), 7.22-7.49 (m, 4H, C_6H_4), 8.26, 8.32 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.6, 40.4 ($3CH_2$), 60.2 (CH_2), 168.9 (CN), 120.4, 124.2, 125.1, 128.6, 132.5, 134.9, 138.4, 142.6 (C_6H_4, thiophene C), 164.4, 166.3, 179.6 (3CO). Anal. Calculated for $C_{18}H_{14}ClN_3O_3S$: C, 55.74; H, 3.64; N, 10.83; S, 8.27. Found: C, 55.80; H, 3.80; N, 11.01; S, 8.38. MS: m/e 387 (M^+, 30 %).

2.1.12. General procedure for the synthesis of the phenylthioureido derivatives 21a-c

Equimolar amounts of either **19a** (2.86 g, 0.01 mol), **19b** (3.00 g, 0.01 mol) or **19c** (3.20 g, 0.01 mol) and phenylisothiocyanate (1.30 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (1.0 mL) were heated under reflux for 3h then left to cool. The formed solid crystals, in each case, were collected by filtration.

2.1.12.1. 5-Oxo-N-phenyl-2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (21a)

Yellow crystals from ethanol, yield (2.52 g, 60 %), m.p. 115-117 °C. IR (KBr) υ max cm^{-1}: 3464-3331 (NH), 3054 (CH, aromatic), 1689, 1687 (2CO), 1632 (C=C), 1205 (C=S); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.63-2.84 (2t, 4H, $2CH_2$), 3.66 (s, 2H, CH_2), 7.28-7.40 (m, 10H, $2C_6H_5$), 8.21, 8.23, 8.38 (3s, 3H, D_2O exchangeable, 3NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.6, 40.6 ($3CH_2$), 120.8, 122.4, 125.8, 126.0, 126.3, 127.5, 127.9, 128.2, 131.9, 133.8, 139.3, 142.9 ($2C_6H_5$, thiophene C), 164.2, 179.2 (2CO),180.2 (C=S). Anal. Calculated for $C_{22}H_{19}N_3O_2S_2$: C, 62.68; H, 4.54; N, 9.97; S, 15.21. Found: C, 62.77; H, 4.49; N, 10.21; S, 15.49. MS: m/e 421 (M^+, 32 %).

2.1.12.2. 5-oxo-2-(3-phenylthioureido)-N-(p-tolyl)-4,5,6,7-tetrahydrobenzo-[b]thiophene-3-carboxamide (21b)

Pale yellow crystals from 1,4-dioxane, yield (2.70 g, 60 %), m.p. 205-208 °C. IR (KBr) υ max cm^{-1}: 3485-3329 (NH), 3055 (CH, aromatic), 1689, 1687 (2CO), 1638 (C=C), 1208 (C=S); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.61-2.89 (2t, 4H, $2CH_2$), 2.80 (s, 3H, CH_3), 3.67 (s, 2H, CH_2), 7.23-7.48 (m, 9H, C_6H_5, C_6H_4), 8.26, 8.21, 8.36 (3s, 3H, D_2O exchangeable, 3NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.5, 36.6, 40.6 ($3CH_2$), 30.6 (CH_3), 120.2, 121.6, 124.5, 126.7, 126.8, 128.1, 128.7, 129.3, 133.5, 135.2, 138.6, 142.6 (C_6H_5, C_6H_4, thiophene C), 164.6, 179.4 (2CO), 180.6 (C=S). Anal. Calculated for $C_{23}H_{21}N_3O_2S_2$: C, 63.42; H, 4.86; N, 9.65; S, 14.72. Found: C, 63.52; H, 4.68; N, 9.74; S, 14.80. MS: m/e 435 (M^+, 28 %).

2.1.12.3. N-(4-Chlorophenyl)-5-oxo-2-(3-phenylthioureido)-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxamide (21c)

Yellow crystals from ethanol, yield (2.95 g, 65 %), m.p. 200-203 °C. IR (KBr) υ max cm^{-1}: 3442-3338 (NH), 3054 (CH, aromatic), 1689, 1685 (2CO), 1630 (C=C), 1208 (C=S); ^1H NMR (DMSO- d_6, 300 MHz): δ = 2.62-2.86 (2t, 4H, $2CH_2$), 3.68 (s, 2H, CH_2), 7.22-7.48 (m, 10H, $2C_6H_5$), 8.22, 8.25, 8.41 (3s, 3H, D_2O exchangeable, 3NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.0, 36.6, 40.9 ($3CH_2$), 120.3, 124.6, 125.5, 126.6, 127.2, 128.3, 128.7, 128.9, 132.5, 134.7, 138.0, 142.6 ($2C_6H_5$, thiophen C), 164.5, 179.8 (2CO), 180.5 (C=S). Anal. Calculated for $C_{22}H_{18}ClN_3O_2S_2$: C, 57.95; H, 3.98; N,9.22; S, 14.06. Found: C, 57.63; H, 3.77; N, 9.39; S, 14.26. MS: m/e 455 (M^+, 28 %).

2.1.13. General procedure for thesynthesisofthehexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-onederivatives 22a-c

A suspension of either compound **21a** (4.21 g, 0.01 mol), **21b** (4.35 g, 0.01 mol), **21c** (4.55 g, 0.01 mol) in sodium ethoxide [prepared through dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (50 mL)] was heated in a boiling water bath for 6h. The reaction mixture was poured onto ice/water then triturated with hydrochloric acid (till pH 7) and the formed solid product was collected by filtration.

2.1.13.1. 3-Phenyl-4-(phenylimino)-2-thioxo-1,2,3,4,7,8-hexahydrobenzo[4,5]-thieno[2,3-*d*]pyrimidin-6(5*H*)-one (22a)

Yellow crystals from ethanol, yield (3.14 g, 78 %), m.p. 111-113 °C. IR (KBr) υ max cm^{-1}: 3458-3324 (NH), 3055 (CH, aromatic), 1688 (CO), 1632 (C=C), 1205 (C=S); ^{1}H NMR (DMSO- d_6, 300 MHz): δ = 2.61-2.86 (2t, 4H, $2CH_2$), 3.68 (s, 2H, CH_2), 7.28-7.40 (m, 10H, $2C_6H_5$), 8.21 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.1, 36.6, 40.6 ($3CH_2$), 120.8, 122.4, 125.8, 126.0, 126.3, 127.5, 127.9, 128.2, 132.2, 134.7, 137.2, 142.9 ($2C_6H_5$, thiophene C), 179.1 (CO), 180.3 (C=S). Anal. Calculated for $C_{22}H_{17}N_3OS_2$: C, 65.48; H, 4.25; N, 10.41; S, 15.89. Found: C, 65.63; H, 4.19; N, 10.32; S, 15.74. MS: m/e 403 (M^+, 38 %).

2.1.13.2. 3-Phenyl-2-thioxo-4-(p-tolylimino)-1,2,3,4,7,8-hexahydrobenzo[4,5]thieno-[2,3-*d*]pyrimidin-6(5*H*)-one (22b)

Brown crystals from ethanol, yield (2.46 g, 59 %), m.p. 273-275 °C. IR (KBr) υ max cm^{-1}: 3479-3336 (NH), 3055 (CH, aromatic), 1688 (CO), 1631 (C=C), 1209 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.60-2.88 (2t, 4H, $2CH_2$), 2.93 (s, 3H, CH_3), 3.65 (s, 2H, CH_2), 7.22-7.45 (m, 9H, C_6H_5, C_6H_4), 8.25 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.4, 36.8, 40.9 ($3CH_2$), 32.6 (CH_3), 120.2, 122.6, 125.5, 125.9, 126.9, 127.7, 127.3, 128.1, 132.2, 134.9, 137.6, 142.3 (C_6H_5, C_6H_4, thiophene C), 179.6 (CO), 180.8 (C=S). Anal. Calculated for $C_{23}H_{19}N_3OS_2$: C, 66.16; H, 4.59; N, 10.06; S, 15.36. Found: C, 66.39; H, 4.42; N, 10.15; S, 15.80. MS: m/e 417 (M^+, 42%).

2.1.13.3. 4-((4-Chlorophenyl)imino)-3-phenyl-2-thioxo-1,2,3,4,7,8-hexahydrobenzo-[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (22c)

Pale yellow crystals from ethanol, yield (3.49 g, 80 %), m.p. 119-122 °C. IR (KBr) υ max cm^{-1}: 3459-3328 (NH), 3054 (CH, aromatic), 1688, 1685 (2CO), 1630 (C=C), 1210 (C=S); ^{1}H

NMR (DMSO- d_6, 200 MHz): δ = 2.62-2.88 (2t, 4H, $2CH_2$), 3.68 (s, 2H, CH_2), 7.22-7.48 (m, 9H, C_6H_5, C_6H_4), 8.22 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 16.0, 36.6, 40.9 ($3CH_2$), 120.3, 124.6, 125.5, 126.6, 127.2, 128.3, 128.7, 128.9, 132.8, 134.3, 137.2, 142.9 (C_6H_5, C_6H_4, thiophene C), 164.8, 179.4 (2CO), 180.2 (C=S). Anal. Calculated for $C_{22}H_{16}ClN_3OS_2$: C, 60.33; H, 3.68; N, 9.59; S, 14.64. Found: C, 60.26; H, 3.49; N, 9.41; S, 14.52. MS: m/e 437 (M^+, 32 %).

2.2. Biology section

2.2.1. Cell proliferation assay

Foretinib was used as the positive control[24-26] during measuring the anti-proliferative activities of the newly synthesized compounds (Table 1). The newly synthesized compounds were evaluated against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460 using the standard MTT assay *in vitro*.

The IC_{50}'s were measured through three independent experiments and the data were expressed through Table 1. It is clear that many of the tested compounds showed potent anti-proliferative activity with IC_{50} values less than 6.00 μM. Generally, the variations of substituent's within the aryl moiety together with the hetero cycle ring being attached have a notable effect and a positive impact on the anti-proliferative activity.

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

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
3a	6.62±2.34	5.42±2.31	7.73±2.59	6.72±2.21	6.82±1.73	5.01±2.32
3b	0.40±0.25	0.39±0.17	0.29±0.16	0.24±0.16	0.19±0.17	0.42±0.23
5a	4.16±1.49	4.18±1.26	5.43±2.71	5.32±1.41	6.53±251	6.80±1.49
5b	1.07±0.68	1.17±0.63	2.04±0.95	2.27±1.33	1.95±0.49	2.17±0.52
5c	0.19±0.07	0.26±0.11	0.37±0.21	0.26±0.08	0.52±0.23	0.62±0.25
5d	0.39±0.15	0.36±0.21	0.27±0.18	0.29±0.17	0.36±0.22	0.35±0.18

6a	6.41±2.26	5.73±2.17	6.42±2.31	8.92±3.41	6.22±2.73	5.82±1.39
6b	1.08±0.69	0.82±0.26	0.63±0.37	0.38±0.26	1.82±0.79	0.63±0.31
6c	0.32±0.21	0.37±0.19	0.40±0.15	0.25±0.07	0.62±0.14	0.51±0.23
6d	0.32±0.15	0.42±0.26	0.16±0.09	0.26±0.14	0.37±0.17	0.28±0.08
8a	0.33±0.12	0.28±0.15	0.28±3.19	6.28±1.08	7.89±2.63	9.39±2.37
8b	0.65±0.18	0.29±0.12	0.43±0.25	0.36±0.19	0.26±0.18	0.56±0.18
10a	6.48±1.54	7.60±2.42	6.63±2.29	6.16±2.59	5.26±1.83	6.29±2.28
10b	2.48±1.01	3.80±1.18	2.47±1.14	4.52±2.16	0.93±0.42	2.83±1.02
10c	0.26±0.18	0.43±0.18	0.41±0.20	0.38±0.16	0.46±0.26	0.38±0.13
10d	0.65± 0.32	0.58±0.21	1.08±0.62	0.73±0.32	0.46±0.29	0.53±0.25
12a	1.82±0.78	1.06±0.62	0.84±0.38	0.61±0.19	0.72±0.36	0.59±0.16
12b	0.22±0.15	0.36±0.12	0.43±0.19	0.29±0.16	0.42±0.25	0.58±0.24
14	8.68±2.59	6.70±2.63	7.28±1.62	5.63±1.26	6.92±2.37	7.29±2.62
16a	4.91±1.56	5.41±1.28	3.52±1.15	3.30±1.86	5.02±2.80	4.69±1.38
16b	6.53±2.38	7.24±2.49	6.80 ±1.92	7.49±2.63	5.60±1.32	8.05±3.26
16c	0.76±0.39	0.68±0.27	0.43±0.26	0.51±0.23	0.60±0.29	0.39±0.24
17a	4.83±1.53	6.28±2.54	6.22±2.26	3.42±1.60	5.23±1.21	4.72±1.38
17b	3.41±1.25	4.61±1.28	4.82±1.52	2.16±0.73	2.37±1.29	3.42±1.53
17c	3.42±1.80	2.63±1.03	4.81±1.68	3.68±1.29	3.62±1.26	2.46±1.82
17d	1.02± 0.71	1.65 ± 0.83	0.93± 0.42	0.85±0.31	0.72± 0.29	0.69±0.23
17e	0.82±0.18	0.51±0.23	0.42±0.19	0.31±0.25	0.39±0.13	0.58±0.13
17f	0.21±0.09	0.33±0.17	0.48±0.31	0.35±0.12	0.59±0.23	0.23±0.17
19a	7.28±2.62	8.29±2.17	6.39±1.02	5.86±2.23	7.43±2.49	6.32±2.34

19b	2.34±1.14	2.28±0.82	3.51±1.50	3.46± 1.63	3.68±1.23	2.18±1.02
19c	0.24±0.13	0.32±0.17	0.48±0.23	0.29±0.36	0.26±0.15	0.43±0.29
20a	8.52±3.50	7.62±2.40	6.39±3.60	7.80±2.68	5.18±2.70	6.48±2.37
20b	4.26±1.05	5.72±2.83	6.93±2.40	4.70±1.52	5.94±1.29	1.04±0.89
20c	0.37± 0.16	0.35 ± 0.18	0.46 ±0.261	0.51 ±0.28	0.24 ±0.63	0.28±0.18
21a	8.24 ± 3.51	6.39 ± 2.73	4.46 ± 1.28	5.34 ± 1.60	6.70 ± 2.93	5.45±1.69
21b	1.42 ± 0.98	1.64 ± 0.52	0.68 ± 0.31	1.25 ± 0.79	2.35 ± 1.06	3.36±1.20
21c	6.43 ± 2.53	8.69 ±2.64	7.16 ± 1.69	8.69 ± 2.26	8.53 ± 2.38	8.76±2.49
22a	6.42 ± 2.26	8.25 ± 2.13	5.29 ± 2.30	3.27 ± 1.04	5.52 ± 1.31	4.50 ± 1.46
22b	2.43 ± 1.16	2.37 ± 1.28	3.45 ± 1.19	2.26 ± 1.16	4.58 ± 1.14	3.27 ± 3.16
22c	0.42 ± 0.23	0.29 ± 0.15	0.54 ± 0.29	0.19 ± 0.04	0.59 ± 0.31	0.42 ± 0.63
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

2.2.2. Structure activity relationship

It is clear from Table 1 that most of the compounds exhibited high cyctotoxicities against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460. Where in most cases, the presence of electronegative substituent's are responsible for the high inhibitions. Considering the 2-ylidenecyclohexane-1,3-dione derivatives **3a,b** it is clear that compound **3b** (X = S) showed higher cytotoxicities than compound **3a** (X = O). For the 4,5,6,7-tetrahydrobenzo[*b*]thiophene derivatives **5a-d**, compound **5a** (X = O, R =CN) exhibited relatively low inhibitions while compound **5b** (X = O, R = COOEt) showed moderate inhibitions. On the other side, compounds **5c** (X = S, R = CN) and **5d** (X = S, R = COOEt) showed extensively high inhibitions toward the six cancer cell lines. Similarly, for the 5,6,7,8-tetrahydro-4*H*-chromene derivatives **6a-d**, it is obvious that compound **6a** (X = O, Y = NH_2) showed low inhibitions, compound **6b** (X = O, Y = OH) with moderate inhibitions and compounds **6c** (X = S, Y = NH_2) and **6d** (X = S, Y = NH_2) with high inhibitions.

Considering the 2,3,6,7-tetrahydrobenzo[*d*]thiazole derivatives **8a,b** where both of the two compounds exhibited high cytotoxicities and this is attributed to the presence of the thiazole moiety in both compounds. For the 4,5,6,7-tetrahydro-2*H*-indazole derivatives **10a-d** it is clear that compound **10a** (X = O, R = H) showed low inhibitions, compound **10b** with high inhibition only toward U87MG cell line with IC_{50} = 0.93 μM and moderate inhibitions toward the other five cell lines A549, H460, HT29, MKN-45 and SMMC-7721. Compounds **10c** (X = S, R = H) and **10d** (X = S, R = Ph) revealed high inhibitions toward the six cancer cell lines. On the other hand, the 6,7-dihydrobenzo[*c*]isoxazole derivatives **12a** and **12b** showed high inhibitions. Compound **14** the 2-(ethoxymethylene)cyclohexane-1,3-dione showed low inhibitions toward the six cancer cell lines. Considering the 2-(arylamino)methylene)cyclohexane-1,3-dione derivatives **16a-c** where compound **16c** (X = OCH_3) showed the highest inhibitions among the three compounds although compound **16a** (X = H) showed relatively higher inhibitions than compound **16b** (X = CH_3). For the 6,7-dihydrobenzo[*b*]thiophene derivatives **17a-f**, it is clear from Table 1 that compounds **17a**, **17b** and **17c** showed low inhibitions while compounds **17d** (X = CH_3, R = COOEt), **17f** (X = OCH_3, R = CN) and **17d** (X = OCH_3, R = COOEt) showed high inhibitions. Within the 6,7-dihydrobenzo[*b*]thiophene derivatives **19a-c** and **20a-c**, compounds **19c** (Y = Cl) and **20c** (X= Cl) showed the highest inhibitions among the six compounds. Surprisingly, for compounds **21a-c** where compound **21b** (Y = CH_3) showed the highest inhibitions than **21a** (Y = H) and **21c** (Y = Cl). Finally for the 1,2,3,4,7,8-hexahydrobenzo[4,5]thien[2,3-*d*]pyrimidine derivatives **22a-c**, where compound **22a** (Y = H) showed low inhibitions, compound **22b** (Y = CH_3) with moderate inhibitions and compound **22c** (Y = Cl) showed high inhibitions. It is of great value to mention that compounds **3b**, **5c**, **5d**, **6b**, **6c**, **6d**, **8a**, **8b**, **10c**, **10d**, **12a**, **12b**, **16c**, **17d**, **17e**, **17f**, **19c**, **20c** and **22c** were the most cytotoxic compounds among the tested compounds.

2.2.3. Inhibition of tyrosine kinases (Enzyme IC_{50} (nM)

Compounds **3b**, **5c**, **5d**, **6b**, **6c**, **6d**, **8a**, **8b**, **10c**, **10d**, **12a**, **12b**, **16c**, **17d**, **17e**, **17f**, **19c**, **20c** and **22c** were selected for inhibition of the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR. It is clear from Table 2 that compounds **3b**, **5d**, **6b**, **6d**, **8a**, **8b**, **10c**, **12a**, **12b**, **16c**, **17e**, **17f** and **19c** were the most potent of the tested compounds towards the five tyrosine kinases. Compound **5c** showed high potency

towards the two kinases c-EGFR and PDGFR with IC_{50}'s 0.96 and 0.68 nM, respectively. In addition the 2-(((4-Methoxyphenyl)amino)methylene)cyclohexane-1,3-dione (**16c)** showed activity towards the five kinases with IC_{50}'s 0.14, 0.32, 0.21, 0.36 and 0.40 nM, respectively. Compounds **6c**, **10d**, **20c** and **22c** showed the lowest potency among the tested compounds.

Table 2. Inhibition of tyrosine kinases (Enzyme IC_{50} (nM) by compounds **3b**, **5c**, **5d**, **6b**, **6c**, **6d**, **8a**, **8b**, **10c**, **10d**, **12a**, **12b**, **16c**, **17d**, **17e**, **17f**, **19c**, **20c** and **22c**

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
3b	0.80	0.37	0.42	0.58	0.38
5c	1.03	2.63	1.82	0.96	0.68
5d	0.23	0.26	0.42	0.69	0.72
6b	0.48	0.27	0.62	0.49	0.52
6c	1.42	2.58	1.61	1.80	2.31
6d	0.16	0.24	0.57	0.34	0.28
8a	0.58	0.42	0.38	0.27	0.19
8b	0.29	0.48	0.68	0.52	0.40
10c	0.16	0.13	0.28	0.31	0.28
10d	2.07	1.24	1.30	1.28	1.72
12a	0.36	0.24	0.62	0.18	0.24
12b	0.18	0.53	0.61	0.53	0.42
16c	0.14	0.32	0.21	0.36	0.40
17d	1.85	1.64	1.52	2.83	1.18
17e	0.26	0.23	0.37	0.28	0.46
17f	0.55	0.80	0.92	0.16	0.27
19c	0.26	0.42	0.31	0.50	0.62
20c	1.27	1.43	2.60	2.88	1.69
22c	2.49	2.61	1.96	2.37	3.39

2.2.4. Inhibition of selected compounds towards Pim-1 kinase

Compounds **3b**, **5d**, **6b**, **6d**, **8a**, **8b**, **10c**, **12a**, **12b**, **16c**, **17e**, **17f** and **19c** were selected to examine their Pim-1 kinase inhibition activity (Table 3) as these compounds showed high inhibition towards the tested cancer cell lines at a range of 10 concentrations and the IC_{50} values were calculated. Compounds **5d**, **6b**, **6d**, **10c**, **12a**, **17e** and **17f** were the most potent to inhibit Pim-1 activity with IC_{50} value of 0.24, 0.41, 0.30, 0.28, 0.45, 0.23 and 0.25μM, respectively. On the other hand, compounds **3b**, **8a, 8b**, **12b, 16c,** and **19c** were less effective (IC_{50}> 10 μM). SGI-1776 was used as positive control with IC_{50} 0.048 μM in the assay. These profiles in combination with cell growth inhibition data of compounds **3b**, **5d**, **6b**, **6d**, **8a**, **8b**,

10c, **12a**, **12b**, **16c**, **17e**, **17f** and **19c** were listed in Table 3 indicated that Pim-1 was a potential target of these compounds.

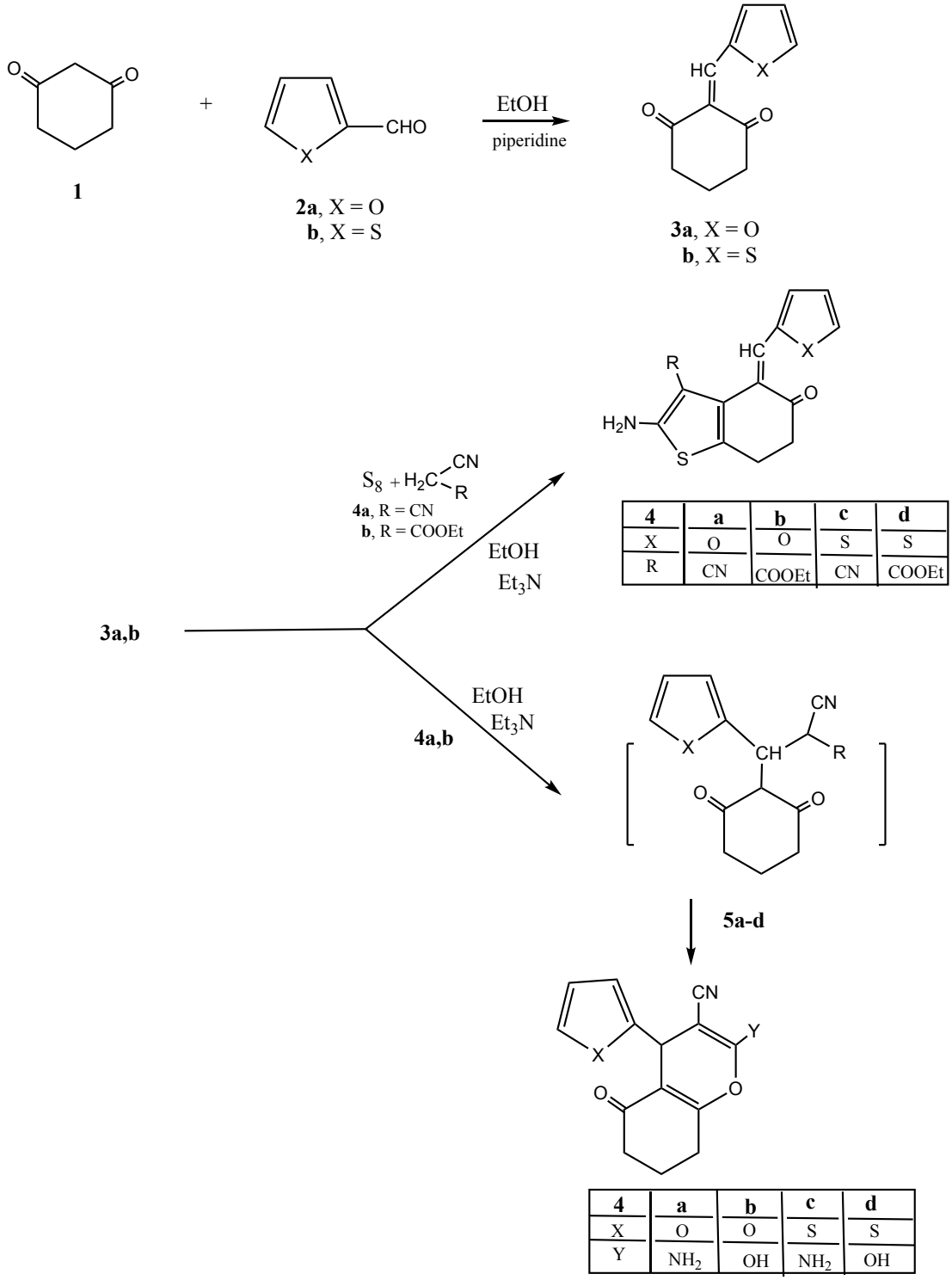
Table 3. The inhibitor activity of compounds **3b**, **5d**, **6b**, **6d**, **8a**, **8b**, **10c**, **12a**, **12b**, **16c**, **17e**, **17f** and **19c**toward Pim-1 Kinase.

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
3b	16	> 10
5d	96	0.24
6b	90	0.41
6d	89	0.30
8a	26	> 10
8b	24	> 10
10c	92	0.28
12a	0.88	0.45
12b	28	> 10
16c	26	> 10
17e	92	0.23
17f	90	0.25
19c	18	> 10
SGI-1776	-	0.048

3. Results and discussion

The synthesis of the 2-(hetero-2-ylmethylene)cyclohexane-1,3-dione derivatives **3a,b** has been accomplished as outlined in Scheme 1 starting from cyclohexan-1,3-dione. Compounds **3a** and **3b** were obtained through the reaction of cyclohexane-1,3-dione with either of furan-2-aldehyde or thiophene-2-aldehyde. The reaction of either of compound **3a** or **3b** with elemental sulfur and either of malononitrile (**4a**) or ethyl cyanoacetate (**4b**) gave the 6,7-dihydrobenzo[*b*]thiophen-5(4*H*)-one derivatives **5a-d**, respectively. The structures of the latter products were based on the analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **5a** (as an example) showed the presence of two triplets at δ 2.62-2.78 ppm for the two CH_2 groups, a singlet at δ 4.73 ppm (D_2O exchangeable) indicating the presence of the NH_2 group, a singlet at δ 6.84 for the pyran *H*-4 and a multiplet at δ 6.82-7.86 ppm for the furan protons. In addition, the ^{13}C NMR spectrum revealed three signals at δ 16.6, 36.3 and 39.5 equivalent to the three CH_2 groups, two signals at δ 112.6 and 158.4 for the C=CH group, a signal at δ 116.8 for the CN group, eight signals at δ 135.4, 140.6, 141.4, 142.2, 142.7, 144.8, 145.6, 146.5 for the thiophene and furan carbons, a signal at δ 179.3 indicating the CO group.

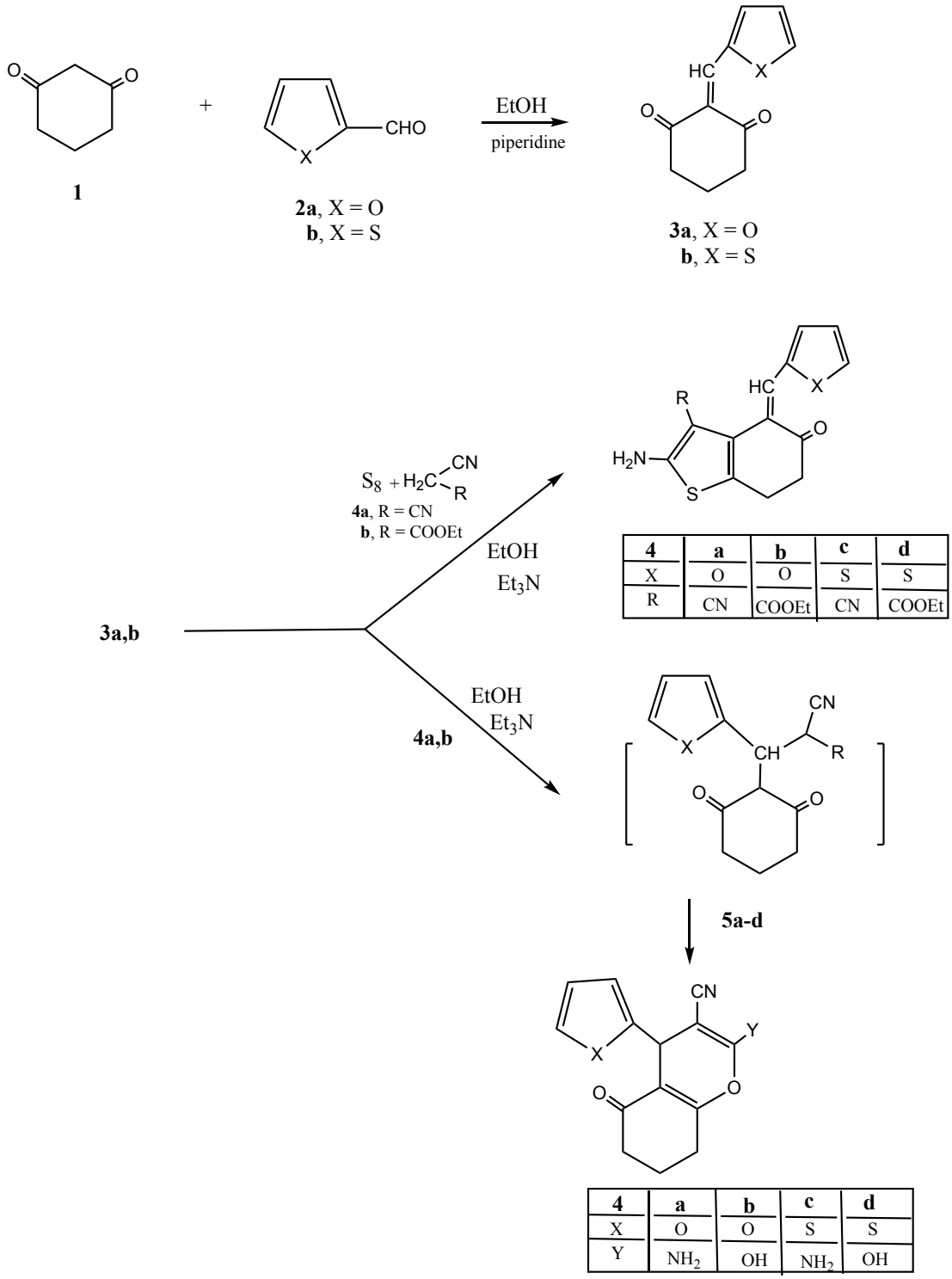
The reaction of either compound **3a** or **3b** with either of malononitrle (**4a**) or ethyl cyanoacetate (**4b**) in ethanol containing a catalytic amount of triethylamine gave the 2*H*-chromen-5-one derivatives **6a-d**, respectively (Scheme 1).



Scheme 1. Synthesis of compounds **3a,b**; **4,b**; **6a-d**.

The reactivity of compounds **3a** and **3b** toward thiazole synthesis was studied. Thus, the reaction of either compound **3a** or **3b** with elemental sulfur and phenylisothiocyanate gave the 2-thioxohexahydrobenzo[*d*]thiazole derivatives **8a** and **8b**, respectively.

The reaction of either of compound **3a** or **3b** with either hydrazine hydrate (**9a**) or phenylhydrazine (**9b**) gave the 4-hydrazono-4,5,6,7-tetrahydro-2*H*-indazole derivatives **10a-d**, respectively. Similarly, the reaction of either **3a** or **3b** with hydroxylamine hydrochloride (**11**) gave the 6,7-dihydrobenzo[*c*]isoxazol-4(5*H*)-one oxime derivatives **12a** and **12b**, respectively (Scheme 2).

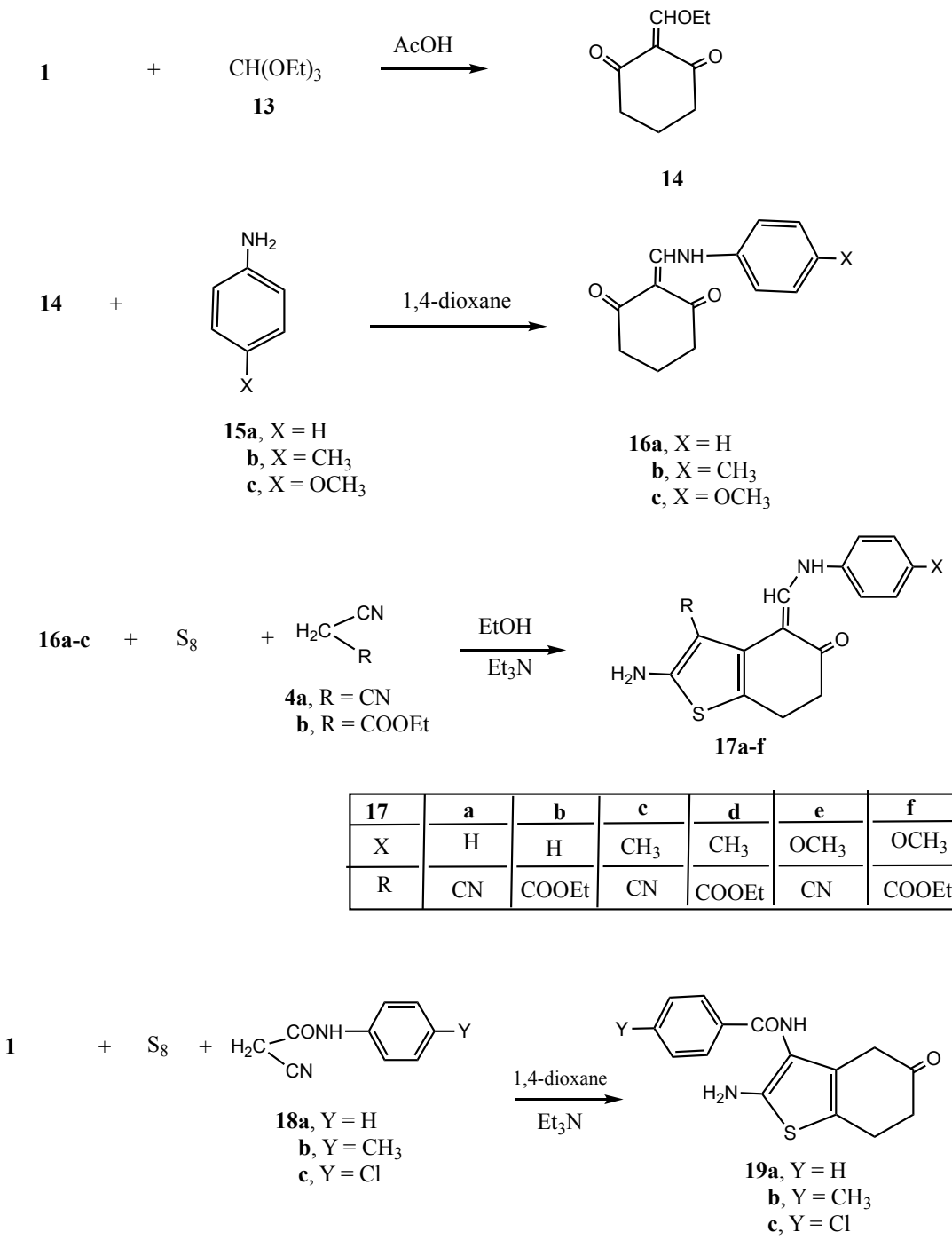


Scheme 1. Synthesis of compounds **3a,b**; **4,b**; **6a-d**.

Next, we moved toward studying of the use of 2-(ethoxymethylene)cyclohexane-1,3-dione (**14**), obtained according to the reported work[27] via the reaction of cyclohexane-1,3-dione with ethyl orthoformate in acetic acid solution, through different heterocyclization reactions. Thus, the reaction of compound **3** with any of the aromatic amines namely aniline (**15a**), 4-methylaniline (**15b**) or 4-methoxyaniline (**15c**) gave the 2-(aminomethylene)cyclohexane-1,3-

dione derivatives **16a-c**, respectively. Structures of compounds **16a-c** were confirmed on the basis of their respective analytical and spectral data (see experimental section). Compounds **16a-c** were used to synthesize thiophene derivatives using the Gewald's thiophene synthesis.[28-30] Thus, the reaction of any of compounds **16a**, **16b** or **16c** with elemental sulfur and either of malononitrile (**4a**) or ethyl cyanoacetate (**4b**) gave the 6,7-dihydrobenzo[*b*]thiophene derivatives **17a-f**, respectively.

The reaction of cycohexane-1,3-dione with elemental sulfur and any of cyanoacetanilide (**19a**), cyano-4-methylacetanilide (**19b**) or cyano-4-methoxyacetanilide (**19c**) gave the 6,7-dihydrobenzo[*b*]thiophene derivatives **19a-c**, respectively (Scheme 3). The analytical and spectral data of the latter compounds were consistent with their respective structures. Thus, the ^{1}H NMR of compound **19a** showed the presence of two triplets at δ 2.62-2.79 ppm equivalent to the two CH_2 groups, a singlet for the third CH_2 group, a singlet at δ 4.75 ppm (D_2O exchangeable for the NH_2 group, a multiplet at δ 7.28-7.40 ppm for the phenyl protons and a singlet at δ8.38 ppm, D_2O exchangeable, indicating the NH group. Moreover, the ^{13}C NMR spectrum showed the presence of three signals at δ 16.3, 36.6, 40.3 corresponding to the three CH_2 groups, a signal at δ 116.6 indicating the presence of the CN group, signals at δ 120.8, 122.2, 125.3, 127.3, 132.2, 134.5, 138.0, 142.9 for the C_6H_5 and thiophene carbons and two signal at δ 164.3, 179.6 confirming the presence of two CO groups.

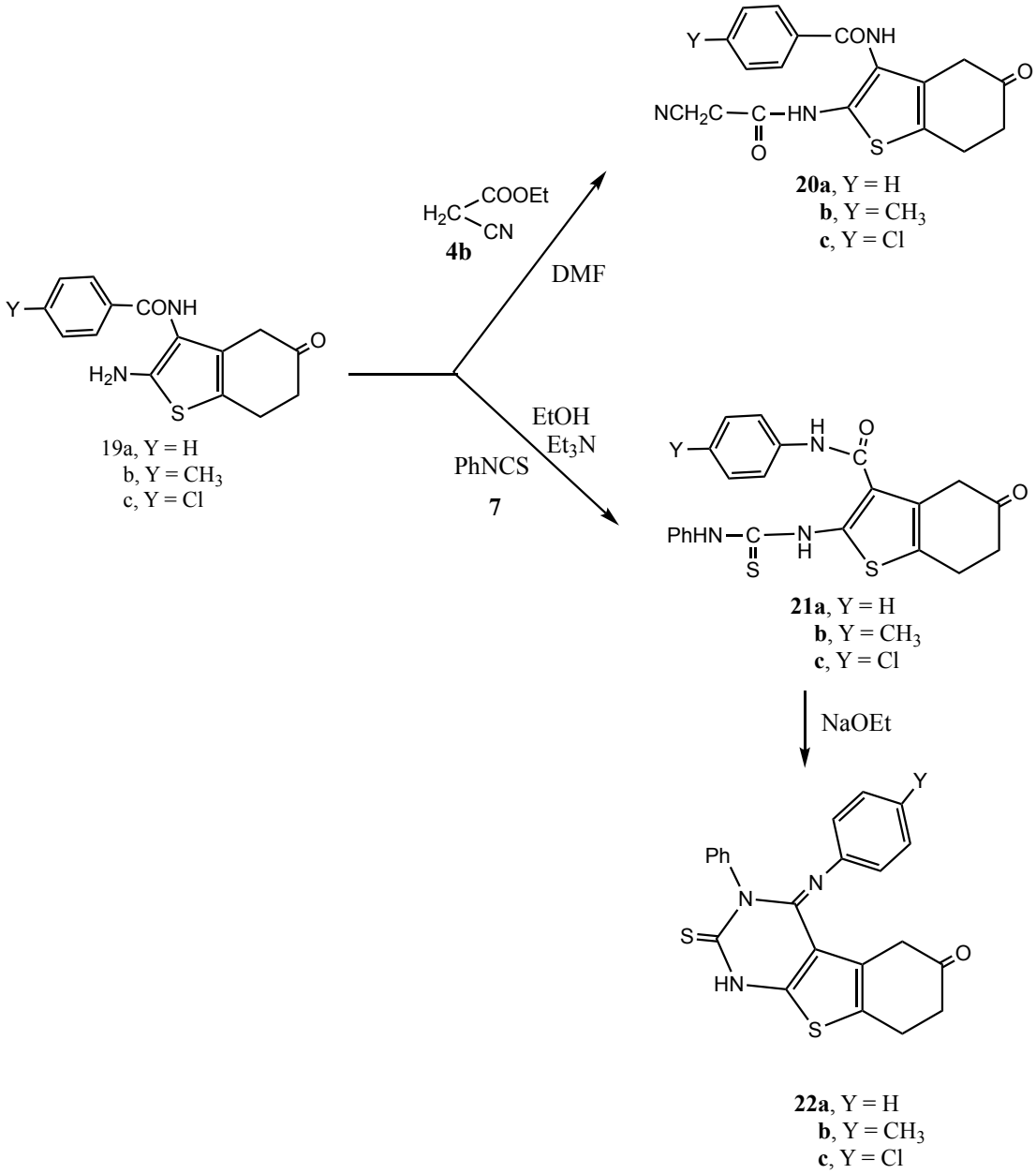


17	a	b	c	d	e	f
X	H	H	CH_3	CH_3	OCH_3	OCH_3
R	CN	COOEt	CN	COOEt	CN	COOEt

Scheme 3. Synthesis of compounds **14**, **16a-c**; **17a-f** and **19a-c**.

Compounds **19a-c** were ready to form amide derivatives through their reactions with cyanomethyleno esters. Thus, the reaction of either compounds **19a-c** with ethyl cyanoacetate in dimethylformamide solution to form the 4,5,6,7-tetrahydrobenzo[*b*]thiophen-2-yl)acetamide derivatives **20a-c**, respectively. On the other hand, the reaction of either compounds **19a**, **19b** or **9c** with phenylisothiocyanate gave the corresponding N-phenylthiourea derivatives **21a-c**, respectively. The analytical and spectral data of compounds **21a-c** were consistent with their respective structures. Compounds **20a-c** were ready for further cyclization to form biologically

active annulated compounds. Thus, heating of either compound**21a**, **21b** or **21c** in sodium ethoxide solution in a boiling water bath afforded the hexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidine derivatives **22a-c**, respectively (Scheme 4). Their structures were based on the analytical and spectral data (see experimental section).



Scheme 4. Synthesis of compounds **20a-c**; **21a-c** and **22a-c**.

4. Conclusion

Forty novel heterocyclic compounds bearing cyclohexanone were designed and synthesized. Their structures were confirmed by multiple techniques. The synthesized compounds were

screened for cytotoxic activity against a panel of six human cancer cell lines using MTT assay. Some intriguing structure–activity relationships were found and discussed and the most active compounds were selected for further screening against tyrosine kinases, Pim-1 kinase and the results indicated that these compounds were good condidates as anti-cancer agents this will encourange further work in the future.

HUMAN AND ANIMAL RIGHTS

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

CONSENT FOR PUBLICATION

This work is consent for publication through the Journal formats.

CONFLICT OF INTEREST

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

5. References

1. W. Pfau, H. Marquardt, *Toxicology* **2001**, *166*, 25-30.

DOI: 10.1016/S0300-483X(01)00435-8

2. V. P. Boyarskiy, K. V. Luzyanin, V. Y. Kukushkin, *Coord. Chem. Rev.* **2012**, *256*, 2029-2056.

DOI: 10.1016/j.ccr.2012.04.022

3. R. K. Singh, N. Sinha, S. Jain, M. Salman, F. Naqvi, N. Anand, *Tetrahedron* **2005**, *61,* 8868-8874.

DOI: 10.1016/j.tet.2005.07.018

4. V. Frenna, G. Macaluso, G. Consiglio,B. Cosimelli, D. Spinelli, *Tetrahedron* **1999**, *55*, 12885-12896.

DOI: 10.1016/S0040-4020(99)00762-0

5. B. S. Jursic, F. Douelle, K. Bowdy, E. D. Stevens, *Tetrahedron Lett.* **2002**, *43*, 5361-5365.

DOI: 10.1016/S0040-4039(02)00799-2

6. K. V. Padoley, S. N. Mudliar, R. A. Pandey, *Bioresource Technology* **2008**, *99*, 4029-4043.

DOI: 10.1016/j.biortech.2007.01.047

7. A. W. Erian, S. M. Sherif, A. A. Alassar, Y. M. Elkholy, *Tetrahedron* **1994**, *50*, 1877-1884.

DOI: 10.1016/j.biortech.2007.01.047

8. J. Bergman, S. Bergman, T. Brimert, *Tetrahedron* **1999**, *55*, 10447-10466.

DOI: 10.1016/S0040-4020(99)00570-0

9. J. Fuentes,W. Moreda, C. Ortiz, I. Robina, C. Welsh, *Tetrahedron* **1992**, *48*, 6413-6424.

DOI: 10.1016/S0040-4020(99)00570-0

10. S. Buscemi, A. Pace, I. Pibiri, N. Vivona, T. Caronna, *J. of Fluor. Chem.* **2004**, *125*, 165-173.

DOI: 10.1016/j.jfluchem.2003.07.006

11. M. R. Shaaban, T. S. Saleh, A. S. Mayhoub, A. Mansour, A. M. Farag, *Bioorg. & Med. Chem.* **2008**, *16,* 6344-6352.

DOI: 10.1016/j.bmc.2008.05.011

12. M. G. Rimoli, L. Avallone, P. de Caprariis, E. Luraschi, E. Abignente, W. Filippelli, L. Berrino, F. Rossi, *Eur. J. Med. Chem.* **1997**, *32,* 195-203.

DOI: 10.1016/S0223-5234(97)83971-2

13. L. Feng, K. W. Yang, L. S. Zhou, J. M. Xiao, X. Yang, L. Zhai, Y. L. Zhang, M. W. Crowder, *Bioorg. & Med. Chem. Lett.* **2012**, *22*, 5185-5189.

DOI: 10.1016/j.bmcl.2012.06.074

14. S. Günal, N. Kaloğlu, S.l. Özdemir, I. D. Özdemir, *Inorg. Chem. Comm.* **2012**, *21*, 142-146.

DOI: 10.1016/j.inoche.2012.04.033

15. S. K. Srivastava, W. Haq, P.M.S. Chauhan, *Bioorg. & Med. Chem. Lett.* **1999**, *9*, 965-966.

DOI: 10.1016/S0960-894X(99)00128-6

16. D. Z. Li,Y. Li, X.G. Chen, C. G. Zhu, J. Yang, H. Y. Liu, X. D. Pan, *Chin. Chem. Lett.* **2007**, *18*, 1335-1338.

DOI: 10.1016/j.cclet.2007.09.014

17. Z. Li, Q. Yang, X. Qian, *Bioorg. & Med. Chem.* **2005***, 13,* 3149-3155.

DOI: 10.1016/j.bmc.2005.02.045

18. R. J. Pagliero, S. L. usvarghi, A. B. Pierini, R. Brun, M. R. Mazzieri, *Bioorg. & Med. Chem.* **2010***, 18*, 142-150.

DOI: 10.1016/j.bmc.2009.11.010

19. S. Barbuceanu, G. Saramet, G. L. Almajan, C. Draghici, F. Barbuceanu, G. Bancescu*, Eur. J. Med. Chem.* **2012**, *49*, 417-423.

DOI: 10.1016/j.ejmech.2012.01.031

20. A. D. Settimo, G. Primofiore, F. D. Settimo, L. Calzolari, P. Cazzulani, A. Passoni, O. Tofanm, *Eur. J. Med Chem.* **1992**, *27*, 395-400.

DOI: 10.1016/0223-5234(92)90153-R

21. A. E. Amr, M. H. Sherif, M. G. Assy, M. A. Al-Omar, I. Ragab, *Eur. J. Med. Chem.* **2010**, *45*, 5935-5942.

DOI: 10.1016/j.ejmech.2010.09.059

22. T. Bányász, J. Magyar, A. Varró, A. Kovács, I. Gyönös, G. Szénási, P. Nánási, *Gen. Pharmac.: The Vasc. Sys.*, **1999**,*32,* 329-333.

DOI: 10.1016/S0306-3623(98)00197-9

23. D. M. Swanson, C.R. Shah, B. Lord, K. Morton, L. K. Dvorak, C. Mazur, R. Apodaca, W. Xiao, J. D. Boggs, M. Feinstein, S. Wilson, A. J. Barbier, P. Bonaventure, T. W. Lovenberg, N. I. Carruthers, *Eur. J. Med. Chem.* **2009**, *44*, 4413-4425.

DOI: 10.1016/j.ejmech.2009.06.007

24. L. Liu, A. Siegmund, N. Xi, P. Kaplan-Lefko, K. Rex, A. Chen. J. Lin, J. Moriguchi, L. Berry, L. Y. Huang, Y. Teffera, Y. J. Yang, Y. H. Zhang, S. F. Bellon, M. Lee, R. Shimanovich, A. Bak, C. Dominguez, M. H. Norman, J. C. Harmange, I. Dussault, T. S. Kim, *J. Med. Chem.* **2008**, *51*, 3688-3691.

DOI: 10.1021/jm800401t

25. M. L. Peach, N. Tan, N. Tan, S. J. Choyke, A. Giubellino, G. Athauda, T. R. Burke, M. C. Nicklaus, D. P. Bottaro, *J. Med. Chem.* **2009**, *52*, 943-951.

DOI: 10.1021/jm800791f

26. F. D. Bacco, P. Luraghi, E. Medico, G. Reato, F. Girolami, T. Perera, P. Gabriele, P. M. Comoglio, C. Boccaccio, *J. Natl. Cancer Inst.* **2011**, *103*, 645-661. **DOI**: 10.1093/jnci/djr093

27. R. M. Mohareb, K. M. H. Hilmy, Y. A. Elshehawy, *Bull. Chem. Soc. Ethiop.* **2018**, *32*, 285-308. **DOI**: 10.4314/bcse.v32i2.9

28. B. P. McKibben, C. H. Cartwright, A. L. Castelhano, *Tetrahedron Lett.***1999**, *40*, 5471-5474. **DOI**: 10.1016/S0040-4039(99)01108-9

29. K. Wang, D. Kim, A. Dömling, *J. Comb. Chem.* **2010**, *12*, 111-118. **DOI**: 10.1021/cc9001586

[30. R. Mishra, K. K. Jha, S. Kumar, I. Tomer, *Der Pharma Chemica* **2011**, *3*, 38-54.