New Approaches for the Synthesis of Heterocyclic Compounds Derived from Cyclohexan-1,3-dione with Anti-proliferative Activities

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Received: 06-11-2020

Abstract

In the present work a series of heterocyclization reactions were adopted using cyclohexan-1,3-dione through its reaction with either furan-2-carbaldehyde or thiophene-2-carbaldehyde 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 phenyl isothiocyanate to give the fused thiazole derivatives 8a,b. In addition, the reaction with either of hydrazine hydrate or phenylhydrazine has given the 4-hydrazono-4,5,6,7-tetrahydro-2H-indazole derivatives 10a–d, respectively. Similarly, the reaction of either 3a or 3b with hydroxylamine hydrochloride gave the 6,7-dihydrobenzo[c]isoxazol-4(5H)-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

Within the last few years the synthesis of heterocyclic compounds attracted 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 were 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 included potent analgesic, anti-convulsant, anti-inflammatory and anti-bacterial, anti-pyretic, anti-tumor, anti-parasitic, anti-microbial, anti-histaminic (H1), anti-anxiety activities in tests in mice, anti-arrhythmic and as serotonin antagonists.11–23 The present work is dealing with the current application of pyrazole, thio-
2. Experimental

2.1 General

The melting points of the obtained compounds were determined using Electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were measured on an FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer (PyeUnicam, UK, Cambridge).

1H NMR spectra were obtained using Varian Gemini-300 (300 MHz, Varian UK) using DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the 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).

2.1.1 General Procedure for the Synthesis of the 2-Methylenecyclohexane-1,3-dione Derivatives 3a,b

Either of furan-2-carbaldehyde (0.96 g, 0.01 mol) or thiophene-2-carbaldehyde (1.12 g, 0.01 mol) was added to a solution of cyclohexane-1,3-dione (1) (1.12 g, 0.01 mol) in absolute ethanol (40 mL) containing piperidine (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-(Furan-2-ylmethylene)cyclohexane-1,3-dione (3a)

Yellow crystals from ethanol, yield 1.46 g (77%), m.p. 206–209 °C. IR (KBr) ν max (cm–1) 3486–3342 (NH2), 2220 (CN), 1648, 1689 (CO), 1630 (C=C); 1H NMR (DMSO-d_6, 300 MHz) δ 1.45–1.69 (m, 2H, CH2), 2.63–2.76 (m, 4H, 2CH2), 6.82 (s, 1H, CH), 6.80–7.83 (m, 3H, furan H); 13C NMR (DMSO-d_6, 75 MHz) δ 16.8, 36.2, 39.1 (3CH2), 112.6, 158.1 (C=CH), 135.6, 140.2, 142.6, 146.1 (furan C), 177.1, 179.4 (2CO). Anal. Calcd for C11H10O3: C, 69.46; H, 5.52. Found: C, 69.31; H, 5.52. MS: m/z 270 (M+, 32%).

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

Orange crystals from ethanol, yield 1.44 g (70%), m.p. 185–188 °C. IR (KBr) ν max (cm–1) 3055 (CH, aromatic), 1704, 1687 (2 CO), 1632 (C=C); 1H NMR (DMSO-d_6, 300 MHz) δ 1.45–1.69 (m, 2H, CH2), 2.63–2.76 (m, 4H, 2CH2), 6.82 (s, 1H, CH), 6.80–7.83 (m, 3H, furan H); 13C NMR (DMSO-d_6, 75 MHz) δ 16.8, 36.2, 39.1 (3CH2), 112.6, 158.1 (C=CH), 135.6, 140.2, 142.6, 146.1 (furan C), 177.1, 179.4 (2CO). Anal. Calcd for C14H10N2O2S: C, 60.80; H, 4.83; N, 4.60; S, 10.26. MS: m/z 270 (M+, 28%).

2.1.2 General Procedure for the Synthesis of the 6,7-Dihydrobenzo[b]thiophen-5(4H)-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-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) ν max (cm–1) 3494–3368 (NH2), 3058 (CH, aromatic), 2931, 2972 (CH2, CH3), 1689, 1688 (2 CO), 1630 (C=C); 1H NMR (DMSO-d_6, 300 MHz) δ 1.12 (t, 3H, J = 7.28 Hz, CH3), 2.68–2.74 (2t, 4H, 2CH2), 4.21 (q, 2H, J = 7.28 Hz, CH2), 4.76 (s, 2H, D2O exchangeable, NH2), 6.84 (s, 1H, CH), 6.84–7.92 (m, 3H, thiophene H); 13C NMR (DMSO-d_6, 75 MHz) δ 16.9, 36.9, 39.3 (3CH2), 112.6, 158.4 (C=CH), 135.4, 140.6, 141.4, 142.7, 144.8, 145.6, 146.5 (thiophene, furan C), 179.3 (CO). Anal. Calcd for C14H10N2O2S: C, 62.36; H, 3.80; N, 10.41; S, 12.04. Found: C, 62.36; H, 3.80; N, 10.41; S, 11.86. Formula: C14H10N2O2S; S: C, 62.21; H, 3.73; N, 10.36; S, 11.86. MS: m/z 270 (M+, 32%).
CO), 1630 (C=C); 1H NMR (DSMO-d$_6$, 300 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$_2$O exchangeable, NH$_2$), 6.84 (s, 1H, CH), 7.29–7.85 (m, 3H, thiophene H); 13C NMR (DSMO-d$_6$, 75 MHz) δ 16.1 (OCH$_2$CH$_3$), 65.5, 36.4, 39.7 (3CH$_2$), 112.1, 158.4 (C=CH), 135.3, 136.7, 138.3, 140.5, 141.5, 142.0, 143.8, 144.6, 146.8 (two thiophene C), 164.5, 178.9 (2CO). Anal. Calcd for C$_{16}$H$_{15}$NO$_3$S$_2$: 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/z 333 (M$^+$, 26%).

2. 1. 3. General Procedure for the Synthesis of the 2H-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 the excess solvent was removed under vacuum. The remaining product was triturated with diethyl ether and the formed solid product was collected by filtration.

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

Pale yellow crystals from ethanol, yield 1.66 g (69%), m.p. 194–196 ºC. IR (KBr) ν max (cm –1) 3470–3328 (NH$_2$), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); 1H NMR (DMSO-d$_6$, 6, 300 MHz) δ 2.76 (m, 4H, 2CH$_2$), 4.71 (s, 2H, D$_2$O exchangeable, NH$_2$), 6.07 (s, 1H, thiophene H-4), 6.91–7.73 (m, 3H, thiophene H); 13C NMR (DSMO-d$_6$, 75 MHz) δ 16.3, 36.6, 37.2, 39.8 (4CH$_2$), 116.4 (CN), 134.6, 138.7, 140.2, 141.6, 142.3, 143.6, 146.2 (thiophene, pyran C). Anal. Calcd for C$_{14}$H$_{13}$NO$_2$S: C, 64.72; H, 5.24; N, 12.59. MS: m/z 259 (M$^+$, 40%).

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

Orange crystals from ethanol, yield 1.96 g (76%), m.p. 177–179 ºC. IR (KBr) ν max (cm –1) 3542–3329 (OH), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); 1H NMR (DSMO-d$_6$, 300 MHz) δ 1.34–1.66 (m, 4H, 2CH$_2$), 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$_2$O exchangeable, OH); 13C NMR (DSMO-d$_6$, 75 MHz) δ 16.1, 36.3, 37.4, 39.6 (4CH$_2$), 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. Calcd for C$_{14}$H$_{13}$NO$_2$S: C, 64.80; H, 5.42; N, 12.36. Found: C, 64.72; H, 5.24; N, 12.59. MS: m/z 259 (M$^+$, 42%).

2. 1. 4. General Procedure for the Synthesis of 2-Thioxohexahydrobenzo[d]thiazole Derivatives 8a,b

Each of elemental sulfur (0.32 g, 0.01 mol) and phenyl isothiocyanate (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.

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 ºC. IR (KBr) ν max (cm –1) 1574–1504 (C=O), 1388–1346 (C=S), 1200–1158 (C–H). Anal. Calcd for C$_{13}$H$_{12}$NO$_2$S: C, 63.80; H, 3.69; N, 4.32; S, 19.18. MS: m/z 339 (M$^+$, 48%).

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

Pale yellow crystals from 1,4-dioxane, yield 2.84 g (80%), m.p. 222–225 ºC. IR (KBr) ν max (cm –1) 3055 (CH, aromatic), 1689 (CO), 1630 (C=C), 1205 (C=S); 1H NMR (DSMO-d$_6$, 300 MHz) δ 2.61–2.79 (2t, 4H, 2CH$_2$), 6.88 (s, 1H, CH), 6.96–7.89 (m, 8H, C$_{6}$H$_{5}$, thiophene H); 13C NMR (DSMO-d$_6$, 200 MHz) δ 1.41–1.64 (m, 4H, 2CH$_2$), 2.61–2.77 (m, 4H, 2CH$_2$), 4.73 (s, 2H, D$_2$O exchangeable, NH$_2$), 6.07 (s, 1H, thiophene H-4), 6.91–7.73 (m, 3H, thiophene H); 13C NMR (DSMO-d$_6$, 75 MHz) δ 16.3, 36.6, 37.2, 39.8 (4CH$_2$), 116.4 (CN), 134.6, 138.7, 140.2, 141.6, 142.3, 143.6, 146.2 (thiophene, pyran C). Anal. Calcd for C$_{15}$H$_{12}$N$_2$O$_2$: C, 65.09; H, 5.46; N, 10.84; S, 12.41. Found: C, 64.90; H, 5.62; N, 10.68; S, 12.58. MS: m/z 258 (M$^+$, 40%).
NMR (DMSO-$d_6$, 75 MHz) $\delta$ 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$_6$H$_5$, thiophene, thiazole C), 179.3 (CO), 181.3 (C=S). Anal. Calcd for C$_{11}$H$_{12}$N$_4$S: C, 71.85; H, 5.47; N, 15.21. Found: C, 72.13; H, 5.42; N, 15.02. MS: m/z 384 (M$^+$, 26%).

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) $\nu_{max}$ (cm$^{-1}$) 3458–3342 (OH), 3055 (CH, aromatic), 1655 (exocyclic C=N), 1650 (C=C); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 1.31–1.68 (m, 2H, CH$_2$), 2.63–2.77 (m, 4H, 2CH$_2$), 7.26–7.89 (m, 13H, 2C$_6$H$_5$, furan H), 8.30 (s, 1H, D$_2$O exchangeable, NH); $^1^3$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.2, 36.3, 39.4 (2CH$_2$), 132.3, 135.1, 140.6, 141.5, 142.8, 143.4, 146.8 (2C$_6$H$_5$, pyrazole, furan C), 176.2, 178.3 (2C=N). Anal. Calcd for C$_{23}$H$_{20}$N$_4$O: C, 71.85; H, 5.29; N, 15.21. Found: C, 71.73; H, 5.42; N, 15.40. MS: m/z 218 (M$^+$, 32%).

2. 1. 6. General Procedure for the Synthesis of the 6,7-Dihydrobenzo[4,5]isoxazol-4(5H)-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 was collected by filtration.

3-(Furan-2-yl)-3-(thiophen-2-yl)-4,5,6,7-tetrahydro-2H-indazole (10c)

Pale yellow crystals from 1,4-dioxane, yield 1.76 g (76%), m.p. 233–236 °C. IR (KBr) $\nu_{max}$ (cm$^{-1}$) 3458–3342 (NH, NH$_2$), 3055 (CH, aromatic), 1655 (exocyclic C=N), 1630 (C=C); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 1.33–1.67 (m, 2H, CH$_2$), 2.62–2.75 (m, 4H, 2CH$_2$), 4.80 (s, 2H, D$_2$O exchangeable, NH$_2$), 6.96–7.88 (m, 3H, thiophene H), 8.28 (s, 1H, D$_2$O exchangeable, NH); $^1^3$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 16.5, 36.3, 39.7 (3CH$_2$), 112.4, 158.6 (C=CH), 136.6, 138.4, 139.2, 140.1, 143.5, 146.4 (thiophene, pyrazole C), 176.4, 178.6 (2C=N). Anal. Calcd for C$_{11}$H$_{12}$N$_4$S: C, 56.87; H, 5.21; N, 12.42, S, 13.80. Found: C, 56.39; H, 5.30; N, 24.31; S, 13.98. MS: m/z 232 (M$^+$, 28%).

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

Pale yellow crystals from 1,4-dioxane, yield 2.50 g (68%), m.p. 158–161 °C. IR (KBr) $\nu_{max}$ (cm$^{-1}$) 3472–3328 (NH, NH$_2$), 3055 (CH, aromatic), 1655 (exocyclic C=N), 1630 (C=C); $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 1.32–1.64 (m, 2H, CH$_2$), 2.60–2.78 (m, 4H, 2CH$_2$), 4.86 (s, 2H, D$_2$O exchangeable, NH$_2$), 7.14–7.84 (m, 3H, furan H), 8.28 (s, 1H, D$_2$O exchangeable, NH); $^1^3$C NMR (DMSO-$d_6$, 75 MHz) $\delta$ 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. Calcd for C$_{23}$H$_{20}$N$_4$O: C, 71.85; H, 5.29; N, 15.21. Found: C, 71.73; H, 5.42; N, 15.40. MS: m/z 384 (M$^+$, 26%).
thiophene H), 10.29 (s, 1H, D$_2$O exchangeable, OH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ 16.4, 36.6, 39.8 (3CH$_3$), 132.1, 134.3, 138.8, 140.9, 141.6, 144.2 (isoxazolyl, thiophene C), 176.0, 178.6 (2C=–N). Anal. Calcld for C$_{11}$H$_{10}$N$_2$O$_2$S: C, 56.39; H, 4.30; N, 13.83. MS: m/z 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/z 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 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$_3$), 6.79 (s, 1H, CH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ 16.3, 23.8 (CH$_3$), 90.6 (CH), 120.8, 122.9, 123.4, 127.9 (C$_{6}$H$_{4}$), 177.3, 179.5 (2CO). Analysis Calcld for C$_9$H$_{12}$O$_3$ (168.19): C, 64.27; H, 7.19%. Found: C, 64.08; H, 7.33%. MS: m/z 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 (2.29 g, 0.01 mol) and compound 14 (1.68 g, 0.01 mol) in 1,4-dioxane (50 mL) were refluxed for 4 h. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol and the solid product formed, in each case, was collected by filtration.

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$^{-1}$) 3470–3363 (NH), 3055 (CH aromatic), 2980 (CH$_3$), 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$_{6}$H$_{4}$), 8.28 (s, 1H, D$_2$O exchangeable, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ 16.3, 36.6, 39.4 (3CH$_2$), 112.3, 138.0, 142.6 (C$_{6}$H$_{4}$), 177.1, 179.3 (2CO). Analysis Calcld for C$_{14}$H$_{15}$NO$_2$ (229.27): C, 73.29; H, 6.41; N, 5.71%. Found: C, 68.80; H, 6.24; N, 5.93%. MS: m/z 229 (M$^+$, 40%).

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$_2$), 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$_{6}$H$_{4}$), 8.28 (s, 1H, D$_2$O exchangeable, NH); $^{13}$C NMR (DMSO-$d_6$, 75 MHz) δ 16.3, 36.6, 39.5 (3CH$_3$), 50.3 (OCH$_3$), 90.2 (CH), 120.3, 122.4, 125.6, 128.7 (C$_{6}$H$_{4}$), 177.1, 179.3 (2CO). Analysis Calcld for C$_{16}$H$_{13}$O$_3$N$_2$ (245.27): C, 68.56; H, 6.16; N, 5.71%. Found: C, 68.80; H, 6.24; N, 5.93%. MS: m/z 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-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) ν max (cm$^{-1}$) 3472–3353 (NH$_2$), 3055 (CH aromatic), 2982, 2886 (CH$_{3}$, CH$_2$), 1689, 1686 (2CO), 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$_2$O exchangeable, NH$_2$), 6.89 (s, 1H, CH), 7.26–7.42 (m, 5H, C$_{6}$H$_{4}$), 8.39 (s, 1H, D$_2$O 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$_{6}$H$_{4}$, thiophene C), 179.6 (CO). Anal. Calcld for C$_{16}$H$_{13}$N$_2$O$_2$: C, 75.06; H, 4.44; N, 14.23; S, 10.86. Found: C, 75.18; H, 4.60; N, 14.19; S, 11.17. MS: m/z 295 (M$^+$, 38%).

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) ν max (cm$^{-1}$) 3486–3351 (NH$_2$), 3058...
(CH, aromatic), 2930, 2972 (CH₂, CH₃), 1689, 1686 (2 CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 1.13 (t, 3H, J = 7.08 Hz, CH₃), 2.68–2.76 (2t, 4H, 2CH₂), 4.22 (q, 2H, J = 7.08 Hz, CH₂), 4.77 (s, 2H, D₂O exchangeable, NH₂), 6.86 (s, 1H, CH), 7.22–7.40 (m, 5H, C₆H₅), 8.33 (s, 1H, D₂O exchangeable, NH), 1³C NMR (DMSO-d₆, 75 MHz) δ 16.3 (OCH₂CH₃), 36.8, 39.6 (2CH₂), 52.5 (OCH₂CH₃), 112.4, 158.8 (C=CH), 121.3, 136.2, 125.2, 125.4, 126.8, 133.0, 130.0, 142.7 (C₆H₅, thiophene C), 164.2, 179.8 (2CO). Anal. Calcd for C₁₈H₁₈N₂O₄S: C, 63.14; H, 14.39; N, 8.18; S, 10.36. Found: C, 62.92; H, 4.75; N, 9.78; S, 11.20. Found: C, 62.53; H, 4.79; N, 12.83; S, 10.01. MS: m/z 325 (M⁺, 32%).

Ethyl 2-Amino-4-(((4-methoxyphenyl)amino)methylene)-5-oxo-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate (17f)

Yellow crystals from ethanol, yield 2.40 g (65%), m.p. 203–206 °C. IR (KBr) v_max (cm⁻¹) 3459–3337 (NH₂), 3055 (CH, aromatic), 2930, 2970 (CH₂, CH₃), 1689, 1687 (2 CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 1.13 (t, 3H, J = 7.59 Hz, CH₃), 2.62–2.75 (2t, 4H, 2CH₂), 3.61 (s, 3H, OCH₃), 4.22 (q, 2H, J = 7.59 Hz, CH₂), 4.79 (s, 2H, D₂O exchangeable, NH₂), 6.83 (s, 1H, CH), 7.24–7.48 (m, 4H, C₆H₄), 8.34 (s, 1H, D₂O exchangeable, NH), 3.13C NMR (DMSO-d₆, 75 MHz) δ 16.1 (OCH₂CH₃), 36.3, 39.7 (2CH₃), 50.2 (OCH₂CH₃), 52.2 (OCH₂CH₃), 112.3, 158.7 (C=C), 120.2, 122.4, 124.3, 128.1, 134.6, 136.7, 138.1, 140.8 (C₆H₅, thiophene C), 164.1, 179.7 (2CO). Anal. Calcd for C₁₉H₁₆N₃O₅S: 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/z 372 (M⁺, 36%).

2. 1. 10. General Procedure for the Synthesis of the 6,7-Dihydrobenzo[b]thiophene-3-carboxamide Derivatives 19a–c

Either of compounds 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 cyclohexane-1,3-dione (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.

Ethyl 2-Amino-5-oxo-4-(((p-tolylamino)methylene)-4,5,6,7-tetrahydrobenzo[b]-thiophene-3-carboxylate (17d)

Brown crystals from 1,4-dioxiane, yield 2.45 g (69%), m.p. 203–206 °C. IR (KBr) v_max (cm⁻¹) 3459–3337 (NH₂), 3055 (CH, aromatic), 2930, 2970 (CH₂, CH₃), 1689, 1687 (2 CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 1.12 (t, 3H, J = 6.59 Hz, CH₃), 2.65–2.78 (2t, 4H, 2CH₂), 2.80 (s, 3H, CH₃), 4.24 (q, 2H, J = 6.59 Hz, CH₂), 4.79 (s, 2H, D₂O exchangeable, NH₂), 6.83 (s, 1H, CH), 7.21–7.45 (m, 4H, C₆H₄), 8.36 (s, 1H, D₂O exchangeable, NH); ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.1 (OCH₂CH₃), 36.3, 39.7 (2CH₃), 23.9 (CH₃), 52.1 (OCH₂CH₃), 112.2, 158.7 (C=C), 120.1, 123.8, 124.9, 128.3, 138.4, 137.2, 140.2, 142.6 (C₆H₄, thiophene C), 164.3, 179.5 (2CO). Anal. Calcd for C₁₇H₁₇N₃O₃S: 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/z 356 (M⁺, 32%).

2-Amino-4-(((4-methoxyphenyl)amino)methylene)-5-oxo-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile (17e)

Yellow crystals from ethanol, yield 2.30 g (71%), m.p. 166–169 °C. IR (KBr) v_max (cm⁻¹) 3474–3329 (NH₂, NH), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 2.61–2.77 (2t, 4H, 2CH₂), 3.68 (s, 3H, OCH₃), 4.75 (s, 2H, D₂O exchangeable, NH₂), 6.87 (s, 1H, CH), 7.24–7.46 (m, 4H, C₆H₄), 8.35 (s, 1H, D₂O exchangeable, NH); ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.1, 36.2, 39.4 (3CH₂), 50.2 (OCH₂), 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 (C₆H₅, thiophene C), 179.6 (CO). Anal. Calcd for C₁₇H₁₅N₃O₂S: 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/z 325 (M⁺, 32%).

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

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Yellow crystals from ethanol, yield 1.98 g (66%), m.p. 194–196 °C. IR (KBr) ν_{max} (cm^{-1}) 3494–3327 (NH₂, NH), 3055 (CH, aromatic), 2220 (CN), 1689, 1687 (2 CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 2.63–2.77 (2t, 4H, 2CH₂), 2.80 (s, 3H, CH₃), 3.06 (s, 2H, CH₂), 4.78 (s, 2H, D₂O exchangeable, NH₂), 7.26–7.46 (m, 4H, C₆H₄), 8.38 (s, 1H, D₂O exchangeable, NH); ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.3, 36.6, 40.3 (3CH₂), 116.6 (CN), 120.8, 122.2, 125.3, 127.3, 132.7, 134.7, 138.4, 142.6 (C₆H₄, thiophene C), 164.3, 179.6 (2CO). Anal. Calcld for C₁₆H₁₆N₂O₂S: 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/z 300 (M⁺, 36%).

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) ν_{max} (cm^{-1}) 3473–3351 (NH₂, NH), 3055 (CH, aromatic), 2220 (CN), 1689, 1686 (2 CO), 1630 (C=C); ¹H NMR (DMSO-d₆, 300 MHz) δ 2.64–2.76 (2t, 4H, 2CH₂), 3.06 (s, 2H, CH₂), 4.79 (s, 2H, D₂O exchangeable, NH₂), 7.23–7.48 (m, 4H, C₆H₄), 8.36 (s, 1H, D₂O exchangeable, NH); ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.1, 36.4, 40.6 (3CH₂), 116.9 (CN), 120.2, 123.5, 125.8, 128.6, 132.3, 134.7, 138.0, 142.7 (C₆H₄, thiophene C), 163.4, 179.8 (2CO). Anal. Calcld for C₁₅H₁₄ClN₃O₃S: C, 56.29; H, 4.26; N, 8.93; S, 10.26. MS: m/z 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 N,N-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.

5-Oxo-N-phenyl-2-(3-phenylthiourea)-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) ν_{max} (cm^{-1}) 3464–3331 (NH), 3054 (CH, aromatic), 1689, 1687 (2 CO), 1632 (C=C), 1205 (C=S); ¹H NMR (DMSO-d₆, 300 MHz) δ 2.63–2.84 (2t, 4H, 2CH₂), 3.66 (s, 2H, CH₃), 7.28–7.40 (m, 10H, 2C₆H₄), 8.21, 8.23, 8.38 (3s, 3H, D₂O exchangeable, 3NH); ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.1, 36.6, 40.6 (3CH₃), 120.8, 122.4, 125.8, 126.0, 126.3, 127.5, 127.9, 128.2, 131.9, 133.8, 139.3, 142.9 (2C₆H₅, thiophene C), 164.2, 179.2 (2CO), 180.2 (C=S). Anal. Calcld for C₁₉H₁₉N₃O₃S: C, 66.8; H, 4.54; N, 9.97; S, 15.21. Found: C, 66.7; H, 4.49; N, 10.21; S, 15.49. MS: m/z 421 (M⁺, 32%).

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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) v_{max} (cm⁻¹) 3485–3329 (NH), 3055 (CH, aromatic), 1689, 1687 (2 CO), 1638 (C=C), 1208 (C=S). ¹H NMR (DMSO-d₆, 300 MHz) δ 2.61–2.89 (2t, 4H, 2CH₂), 2.80 (s, 3H, CH₃), 3.67 (s, 2H, CH₂), 7.23–7.48 (m, 9H, C₆H₅, C₆H₄, C₆H₅, thiophene H), 8.26, 8.21, 8.36 (3s, 3H, D₂O exchangeable, NH); ¹³C NMR (DMSO-d₆) 75 MHz δ 16.5, 36.6, 40.6 (3CH₂), 30.6 (CH₃), 72.7–7.48 (m, 9H, C₆H₅, C₆H₄, C₆H₅, thiophene C), 120.2, 121.6, 124.5, 126.7, 126.8, 128.1, 128.7, 129.3, 133.5, 135.2, 138.6, 142.6 (C₆H₅, C₆H₄, thiophene C), 164.5, 179.4 (2CO), 180.6 (C=S). Anal. Calcld for C₂₂H₁₈ClN₃O₂S₂: 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/z 403 (M⁺, 38%).

2. 2. 1. Cell Proliferation Assay

Foretinib was used as the positive control 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.

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

Brown crystals from ethanol, yield 2.46 g (59%), m.p. 273–275 °C. IR (KBr) v_{max} (cm⁻¹) 3479–3336 (NH), 3055 (CH, aromatic), 1688, 1681 (2 CO), 1631 (C=C), 1209 (C=S). ¹H NMR (DMSO-d₆, 300 MHz) δ 2.60–2.88 (2t, 4H, 2CH₂), 2.93 (s, 3H, CH₃), 3.65 (s, 2H, CH₂), 7.22–7.45 (m, 9H, C₆H₅, C₆H₄, thiophene C), 8.25 (s, 1H, D₂O exchangeable, NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ 16.4, 36.8, 40.9 (3CH₂), 120.2, 122.6, 125.5, 125.9, 126.9, 127.7, 127.3, 128.1, 132.2, 134.9, 139.6, 142.8 (C₆H₅, C₆H₄, thiophene C), 179.6 (CO). Anal. Calcld for C₂₃H₂₁N₃O₂S₂: 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/z 417 (M⁺, 42%).

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compound 5b (X = O, R = COOEt) showed moderate inhibitions. On the other side, compounds 5c (X = S, R = COOEt) and 5d (X = S, R = COOEt) showed extensively high inhibitions toward the six cancer cell lines. Similarly, for the 5,6,7,8-tetrahydro-4H-chromene derivatives 6a–d, it is obvious that compound 6a (X = O, Y = NH₂) showed low inhibitions, compound 6b (X = O, Y = OH) displayed moderate inhibitions and compounds 6c (X = S, Y = NH₂) and 6d (X = S, Y = NH₂) showed high inhibitions. Considering the 2,3,6,7-tetrahydrobenzo[d]thiazole derivatives 8a,b, where both of the two compounds exhibited high cytotoxicities, this is at-
ttributed to the presence of the thiazole moiety in both compounds. For the 4,5,6,7-tetrahydro-2H-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 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 higher 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\(_3\)) displayed 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

| 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\(_{50}\) values of 0.24, 0.41, 0.30, 0.28, 0.45, 0.23 and 0.25 µM, 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 the 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 are listed in Table 3 indicating that Pim-1 is a potential target of these compounds.

### 3. Results and Discussion

The synthesis of the 2-(hetero-2-yl)methylene)cyclohexane-1,3-dione derivatives 3a,b has been accomplished as outlined in Scheme 1 starting from cyclohexan-1,3-dione (1). Compounds 3a and 3b were obtained through the reaction of 1 with either of furan-2-carbaldehyde or thiophene-2-carbaldehyde. 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(4H)-one derivatives 5a–

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The structures of the latter products were established 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 \(\delta 2.62–2.78\) ppm for the two \(\text{CH}_2\) groups, a singlet at \(\delta 4.73\) ppm (\(\text{D}_2\text{O}\) exchangeable) indicating the presence of the \(\text{NH}_2\) group, a singlet at \(\delta 6.84\) for the pyran \(-4\) and a multiplet at \(\delta 6.82–7.86\) ppm for the furan protons. In addition, the \(^{13}\)C NMR spectrum revealed three signals at \(\delta 16.6, 36.3\) and 39.5 equivalent to the three \(\text{CH}_2\) groups, two signals at \(\delta 112.6\) and 158.4 for the \(\text{C} = \text{CH}\) group, a signal at \(\delta 116.8\) for the \(\text{CN}\) group, eight signals at \(\delta 135.4, 140.6, 141.4, 142.2, 142.7, 144.8, 145.6, 146.5\) for the thiophene and furan carbons and a signal at \(\delta 179.3\) indicating the \(\text{CO}\) group. The reaction of either compound 3a or 3b with either of malononitrile (4a) or ethyl cyanoacetate (4b) in ethanol containing a catalytic amount of triethylamine gave the \(2H\)-chromen-5-one derivatives 6a–d, respectively (Scheme 1).

![Scheme 1](image-url)

Table 3. The inhibitory 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            |
| 17c      | 92                        | 0.23            |
| 17f      | 90                        | 0.25            |
| 19c      | 18                        | > 10            |
| SGI-1776 | –                         | 0.048           |

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The reactivity of compounds 3a and 3b toward thi-azole synthesis was studied. Thus, the reaction of either compound 3a or 3b with elemental sulfur and phenyl isothiocyanate (7) 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-2H-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(5H)-one oxime derivatives 12a and 12b, respectively (Scheme 2).

Next, we moved toward studying the use of 2-(ethoxymethylene)cyclohexane-1,3-dione (14), obtained according to the reported work via the reaction of cyclohexane-1,3-dione (1) 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. 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 cyclohexane-1,3-dione (1) 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$_2$O exchangeable) for the NH$_2$ group, a multiplet at δ 7.28–7.40 ppm for the phenyl protons and a singlet at δ 8.38 ppm (D$_2$O 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$_6$H$_5$ and thiophene carbons and two signals at δ 164.3, 179.6 confirming the presence of two CO groups.

Compounds 19a–c were ready to form amide derivatives through their reactions with cyanomethylene esters. Thus, the reaction of either compounds 19a–c with ethyl cyanoacetate in N,N-dimethylformamide solution

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took place 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 phenyl isothiocyanate 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).

4. Conclusion

Forty novel heterocyclic compounds bearing cyclohexanone moiety 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 are good candidates as anti-cancer agents that will encourage further work in the future.

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.

Human and Animal Rights

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

5. References

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Povzetek

V prispevku opisujemo serijo heterociklizacijskih reakcij, ki smo jih izvedli na cikloheksan-1,3-dionu s furan-2-karbaldehidom ali tiofen-2-karbaldehidom pri čemer sta nastala ustrezena ilidenska derivata 3a,b; ti dve spojini sta bili izhodišče za nadaljnje heterociklizacijske reakcije, ki so vodile do tiofenskih in piranskih derivatov 5a–d oz. 6a–d. Ob reakciji spojin 3a,b z elementarnim žveplom in fenil izotiocianatom sta nastala pripojena tiazolska derivata 8a,b. Pri reakciji s hidrazin hidratom ali fenilhidrazinom pa so nastali 4-hidrazono-4,5,6,7-tetrahidro-2H-indazolski derivati 10a–d. Podobno sta pri reakciji med 3a ali 3b s hidroksilamin hidrokloridom nastala 6,7-dihidrobenzo[c]izoksazol-4(5H)-on oksima 12a in 12b. Pri pravili smo še več drugih heterocikličnih spojin in določili njihove strukturo. Pripravljenim spojinam smo določili citotoksične aktivnosti na izbrane celične linije raka. Za najbolj aktivne spojine smo v nadaljevanju določili še inhibitorne lastnosti proti tirozin kinazam in Pim-1 kinazi.