Scientific paper

Novel 5,6,7,8-tetrahydrobenzo[*b*]pyran Derivatives: Synthesis and Anticancer Activity

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Abstract

Many new cyclized pyran systems with a potential anti-cancer activity were designed and prepared. Pyran systems showed high reactivity to various chemical reagents. 24 products of the prepared compounds were chosen and tested in (mM) as respectable anticancer factors. The findings revealed that compounds **3b**, **6b**, and **8** were the widely effective compounds against the three cancer cell lines including A-549 (lung carcinoma), HC-29 (colorectal adenocarcinoma), and MKN-45 (gastric cancer) compared to the standard reference control foretinib.

Keywords: Tetrahydrobenzo[*b*]pyran, thiophene, pyridine, anti-proliferative activity.

1. Introduction

Pyran as a six-membered heterocyclic ring system was considered one of the most important rings in the synthesis of numerous bioactive fused systems with carbocyclic or heterocyclic ring systems. Figure 1 displays some important pyran-based synthetic marketed drugs. Moreover, Figure 2 shows some natural product compounds which have the pyran ring in their structures and are found in different food sources such as fruits, trees, and olive oil in addition to pigments in leaves.[1]

Due to the continuous need to prepare novel polyfunctionalized heterocyclic compounds used in a variety of applications in industry and medicine, terahydrobenzo[*b*]pyrans were selected as important bioactive scaffolds widely utilized in such fields. For drug and pharmaceutical applications, tetrahydrobenzo[*b*]pyran derivatives were used as antiviral,[2,3] antioxidant,[4] anticancer,[5–7] anticoagulant,[8] diuretic,[9,10] antimicrobial,[11–13] anti-inflammatory,[14] and anti-anaphylactic agents.[15] In the area of industrial application, they were used as a raw material in laser dyes, food additives, hand soaps, detergents, lotions and perfume.

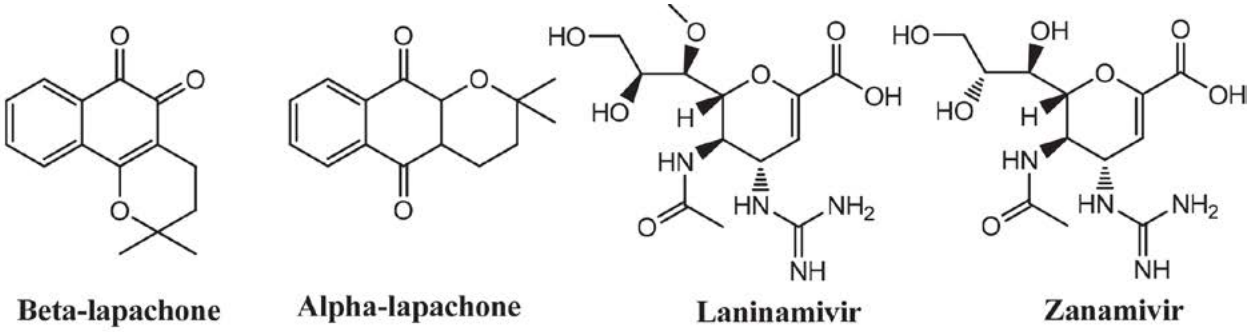


Figure 1. Some important pyran-based synthetic marketed drugs.

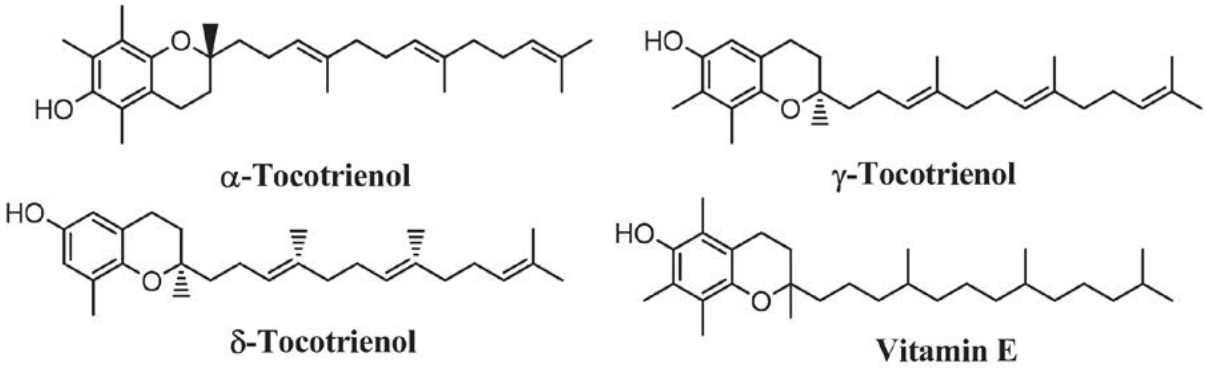


Figure 2. Pyran-containing natural product compounds.

Multi-component reactions were widely used to prepare tetrahydrobenzo[*b*]pyran systems in the presence of different catalysts,[16–20] and reaction conditions such as microwave, ultrasonic, or electrochemical synthesis conditions in deep eutectic solvents.[21–25] These methods mostly introduce green synthesis for the production of tetrahydrobenzo[*b*]pyrans obtained in pure form with good yields and shorter reaction times than the other traditional methods.

The current study constitutes a further thread in green organic chemistry and one-pot multi-component reactions (MCRs); it aims to improve the synthetic procedures of various prepared compounds.[26–30] Herein preparations of many 5,6,7,8-tetrahydrobenzo[*b*]pyrans are described through multi-component reactions and via other simple methods. The obtained products were tested on three human tumor cell lines, including A-549 (lung carcinoma), HC-29 (colorectal adenocarcinoma), and MKN-45 (gastric cancer).

2. Experimental Section

On a digital thermoelectric melting point instrument, the melting points were measured and are not calibrated. By using Pye Unicam SP-1000 spectrophotometer, the infrared spectra (KBr disc) were determined. The Varian Gemini-300 (300 MHz) (Cairo University) instrument was utilized in the measurement of the ^{1}H NMR spectra by using DMSO-d_6 as solvent and TMS as the internal standard; chemical shifts (δ) are given in ppm. The mass spectrometry was carried out using a GCMS-QP2010 Shimadzu instrument. The analytical data were recorded at Cairo University, by using a Vario El III Elemental CHNS analyzer.

2. 1. Synthetic Procedures

2. 1. 1. General Method for the Preparation of 2-Amino-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile Derivatives 1a,b

To a solution of compound cyclohexanone (0.98 g, 0.01 mol) in absolute ethanol (25 mL), either benzaldehyde (1.06 g, 0.01 mol) or *para*-methoxybenzaldehyde (1.08 g, 0.01 mol) was added with malononitrile (0.66 g, 0.01 mol) in triethylamine (0.50 mL). Under reflux, the reaction was heated for 1 h. The resultant products were treated by adding them onto ice/water mixture with a few drops of HCl added. The precipitated product was collected by filtration, and recrystallize from ethanol.

2-Amino-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1a). Brown crystals, yield: 1.66 g (66%), m.p. 257–260 °C. IR (ν, cm^{-1}): 3417, 3340 (NH_2), 3031 (CH-aromatic), 2932–2831 (CH_2), 2209 (CN), 1645, 1599 (C=C). ^{1}H NMR (δ, ppm): 1.66–1.71 (m, 4H, 2CH_2), 2.16–2.81 (m, 4H, 2CH_2), 5.73 (s, 1H, CH pyran), 7.14–7.89 (m, 7H, C_6H_5, NH_2). ^{13}C NMR (δ, ppm): 21.0, 24.9, 27.0, 42.9, 112.4, 116.2, 126.9, 128.6, 128.8, 128.9, 129.3, 132.4, 134.6, 143.5. Anal. Calcd for $C_{16}H_{16}N_2O$ (252.31): C, 76.16; H, 6.39; N, 11.10. Found: C, 76.21; H, 6.40; N, 11.12

2-Amino-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile (1b). Pale brown crystals, yield: 1.98 g (70%), m.p. 269–272 °C. IR (ν, cm^{-1}): 3419, 3340 (NH_2), 3013 (CH aromatic), 2943–2836 (CH_2, CH_3), 2211 (CN), 1645, 1602 (C=C). ^{1}H NMR (δ, ppm): 1.46–1.48 (m, 4H, 2CH_2), 2.16–2.51 (m, 4H, 2CH_2), 3.87 (s, 3H, OCH_3), 5.72 (s, 1H, CH pyran), 6.99–7.89 (m, 6H, C_6H_4, NH_2). Anal. Calcd for $C_{17}H_{18}N_2O_2$ (282.34): C, 72.32; H, 6.43; N, 9.92. Found: C, 72.55; H, 6.65; N, 10.29.

2. 1. 2. General Method for the Preparation of Ethyl *N*-(3-cyano-4-phenyl-5,6,7,8,-tetrahydro-4*H*-chromen-2-yl)formimidate Derivatives 2a,b

To form a mixture of an equimolar amount of **1a** (2.52 g, 0.01 mol) or **1b** (2.82 g, 0.01 mol) in acetic acid (20 mL), triethyl orthoformate (1.45 g, 0.01 mol) was added. The reaction was refluxed for 2 h and then added to a mixture of ice/water with a few drops of HCl added. The obtained products were filtered and recrystallized by using acetic acid.

Ethyl *N*-(3-cyano-4-phenyl-5,6,7,8,-tetrahydro-4*H*-chromen-2-yl)formimidate (2a). Green crystals, yield: 2.00 g (65%), m.p. 212–215 °C. IR (ν, cm^{-1}): 3061 (CH-aromatic), 2937, 2868 (CH_2, CH_3), 2191 (CN), 1639, 1491 (C=C), 1580 (C=N). ^{1}H NMR (δ, ppm): 1.20 (t, 3H, CH_3), 1.56–1.91 (m, 4H, $2CH_2$), 2.49–2.51 (m, 4H, $2CH_2$), 4.25 (q, 2H, CH_2), 6.35 (s, 1H, CH-pyran), 6.95 (s, 1H, CH), 7.19–7.52 (m, 5H, C_6H_5). MS *m/z* (%): 310 [M^+ + 2] (1.51), 309 [M^+ + 1] (1.51), 308 [M^+] (1.28), 275 (100.00), 77 [C_6H_5]$^+$ (14.10). Anal. Calcd for $C_{19}H_{20}N_2O_2$ (308.37): C, 74.00; H, 6.54; N, 9.08. Found: C, 74.29; H, 6.67; N, 9.40.

Ethyl *N*-(3-cyano-4-(4-methoxyphenyl)-5,6,7,8,-tetrahydro-4*H*-chromen-2-yl)formimidate (2b). Redish brown crystals, yield: 2.40 g (71%), m.p. 82–85 °C. IR (ν, cm^{-1}): 3010 (CH-aromatic), 2935 (CH, CH_2, CH_3), 2200 (CN), 1637, 1510 (C=C), 1602 (C=N). ^{1}H NMR (δ, ppm): 1.10 (t, 3H, CH_3), 1.66–1.91 (m, 4H, $2CH_2$), 2.49–2.50 (m, 4H, $2CH_2$), 3.81 (s, 3H, OCH_3), 4.30 (q, 2H, CH_2), 5.43 (s, 1H, CH-pyran), 6.85 (s, 1H, CH), 6.88–7.89 (m, 4H, C_6H_4). ^{13}C NMR (δ, ppm): 21.4, 22.0, 22.4, 24.9, 26.6, 55.1, 55.4, 113.8, 114.1, 141.5, 118.0, 129.3, 129.7, 131.8, 146.7, 149.2, 158.6, 159.2, 159.7. Anal. Calcd for $C_{20}H_{22}N_2O_3$ (338.40): C, 70.99; H, 6.55; N, 8.28. Found: C, 71.32; H, 6.90; N, 8.49.

2. 1. 3. General Method for the Preparation of *N″*-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)formimidohydrazide derivatives 3a–d

To an equimolar amount of **2a** (3.08 g, 0.01 mol) or **2b** (3.38 g, 0.01 mol) in absolute ethanol (25 mL), hydrazine hydrate (0.50 g, 0.01 mol) or phenyl hydrazine (1.08 g, 0.01 mol) were added. By using the reflux heating, the reaction lasted for 3 h. The resultant products were treated by adding them to ice/water mixture with a few HC1 drops added. The resultant products were collected and filtered; then ethanol was used to recrystallize them.

***N″*-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)formimidohydrazide (3a).** Yellow crystals, yield: 2.79 g (95%), m.p. 157–160 °C. IR (ν, cm^{-1}): 3444, 3355 (NH_2), 3243 (NH), 3070 (CH-aromatic), 2934, 2865 (CH_2), 2197 (CN), 1639, 1448 (C=C), 1596 (C=N). ^{1}H NMR (δ, ppm): 1.58–1.74 (m, 4H, $2CH_2$), 2.13–2.15 (m, 4H, $2CH_2$), 6.40 (s, 1H, CH-pyran), 6.96 (s, 1H, CH), 7.20–7.54 (m, 7H, C_6H_5, NH_2), 10.80 (s, 1H, NH). ^{13}C NMR (δ, ppm): 22.0, 22.3, 24.9, 26.7, 45.7, 95.4, 115.4, 115.7, 124.6, 127.6, 128.2, 128.5, 128.6, 143.6, 146.8, 150.1, 150.7. Anal. Calcd for $C_{17}H_{18}N_4O$ (294.35): C, 69.37; H, 6.16; N, 19.03. Found: C, 69.71; H, 6.30; N, 19.12. MS *m/z* (%): 295 [M^+ + 1] (27.04), 294 [M^+] (66.09), 293 [M^+ – 1] (100.00).

***N‴*-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-*N*'-phenylformimidohydrazide (3b).** Dark brown crystals, yield: 2.82 g (75%), m.p. 107–110 °C. IR (ν, cm^{-1}): 3442–3244 (2NH), 3075 (CH-aromatic), 2936, 2865 (CH_2), 2211(CN), 1638, 1492 (C=C), 1598 (C=N). ^{1}H NMR (δ, ppm): 1.56–1.74 (m, 4H, $2CH_2$), 2.13–2.17 (m, 4H, $2CH_2$), 6.37 (s, 1H, CH-pyran), 6.60 (s, 1H, CH), 7.19–7.86 (m, 10H, $2C_6H_5$), 10.30 (s, 1H, NH), 10.90 (s, 1H, NH). MS *m/z* (%): 378 [M^+ + 2] (4.24), 377 [M^+ + 1] (6.47), 376 [M^+] (8.13), 273 (100.00), 77 [C_6H_5]$^+$ (20.56). Anal. Calcd for $C_{23}H_{28}N_4O$ (376.49): C, 69.37; H, 6.16; N, 19.03. Found: C, 69.39; H, 6.20; N, 19.04.

***N″*-(3-Cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)formimidohydrazide (3c).** Redish brown crystals, yield: 2.76 g (85%), m.p. 97–100 °C. IR (ν, cm^{-1}): 3437, 3348 (NH_2), 3225 (NH), 3080 (CH-aromatic), 2934, 2862 (CH_2), 2197 (CN), 1639, 1511 (C=C), 1605 (C=N). ^{1}H NMR (δ, ppm): 1.63–1.70 (m, 4H, $2CH_2$), 2.16–2.20 (m, 4H, $2CH_2$), 3.60 (s, 3H, OCH_3), 6.30 (s, 1H, CH-pyran), 6.86 (s, 1H, CH), 6.89–7.32 (m, 6H, C_6H_4, NH_2), 10.90 (s, 1H, NH). ^{13}C NMR (δ, ppm): 22.3, 24.9, 25.6, 26.6, 44.7, 55.1, 113.9, 114.1, 115.9, 119.0, 129.6, 129.7, 130.5, 149.2, 158.6. MS *m/z* (%): 322 [M^+ – 2] (6.18), 305 (100.00), 76 [C_6H_4]$^+$ (4.89). Anal. Calcd for $C_{18}H_{20}N_4O_2$ (324.38): C, 66.65; H, 6.21; N, 17.27. Found: C, 70.01; H, 6.30; N, 17.29.

***N″*-(3-Cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)formimidohydrazide (3d).** Dark brown crystals, yield: 4.04 g (99%), m.p. 82–85 °C. IR (ν, cm^{-1}): 3435–3225 (NH), 3005 (CH-aromatic), 2861, 2838 (CH, CH_2, CH_3), 2207 (CN), 1639, 1510 (C=C), 1602 (C=N). ^{1}H NMR (δ, ppm): 1.56–1.71 (m, 4H, $2CH_2$), 2.16–2.20 (m, 4H, $2CH_2$), 3.86 (s, 3H, OCH_3), 6.31 (s, 1H, CH-pyran), 6.96 (s, 1H, CH), 7.03–7.88 (m, 9H, C_6H_4, C_6H_5), 9.87 (s, 1H, NH), 10.10 (s, 1H, NH). MS *m/z* (%): 408 [M^+ + 2] (38.22), 407 [M^+ + 1] (35.80), 406 [M^+] (26.39), 405 [M^+ – 1] (12.85), 404 [M^+ – 2] (7.92), 303 (100.00), 77 [C_6H_5]$^+$ (35.65), 76 [C_6H_4]$^+$ (5.20). Anal. Calcd for $C_{24}H_{30}N_4O_2$ (406.52): C, 71.98; H, 6.04; N, 13.99. Found: C, 72.12; H, 6.30; N, 13.99.

2. 1. 4. Synthesis of *N*'-(3-Cyano-4-phenyl -5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-*N*-phenylformimidamide (4).

For an equimolar amount of **2a** (3.08 g, 0.01 mol) in absolute ethanol (25 mL), aniline (0.93 g, 0.01 mol) was added. The reaction was refluxed for 3 h and then the mixture was added to an ice/water mixture with a few HC1 drops added. The obtained product was filtered and recrystallized by using ethanol.

Brown crystals, yield: 2.84 g (80%), m.p. 97–100 °C. IR (ν, cm^{-1}): 3417–3242 (NH), 3058 (CH-aromatic), 2935, 2862 (CH, CH_2), 2210 (CN), 1640, 1495 (C=C), 1596 (C=N). ^{1}H NMR (δ, ppm): 1.54–1.79 (m, 4H, CH_2), 2.13–2.39 (m, 4H, CH_2), 5.73 (s, 1H, CH-pyran), 6.80–7.58 (m,

11H, $2C_6H_5$, CH), 10.01 (s, 1H, NH). ^{13}C NMR (δ, ppm): 21.4, 22.0, 24.9, 26.9, 42.9, 66.4, 112.6, 115.4, 115.8, 116.2, 124.3, 128.0, 128.2, 128.3, 128.5, 128.6, 129.3, 129.4, 137.3, 143.6, 146.9, 150.1, 150.7. MS *m/z* (%): 357 [M^+ + 2] (31.33), 356 [M^+ + 1] (40.77), 355 [M^+] (23.18), 300 (100.00), 77 [C_6H_5]$^+$ (36.91). Anal. Calcd for $C_{23}H_{21}N_3O$ (355.43): C, 77.72; H, 5.96; N, 11.82. Found: C, 77.94; H, 6.17; N, 12.20.

2. 1. 5. General Method for the Preparation of 2-(((3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)imino)methyl) malononitrile derivatives 5a–d

To compound **2a** (3.08 g, 0.01 mol) or **2b** (3.38 g, 0.01 mol) in absolute ethanol (25 mL), malononitrile (0.66 g, 0.01 mol) and ethyl cyanoacetate (1.13 g, 0.01 mol) were added. On the reflux system, the reaction was heated for 3 h. The resultant products were poured onto the ice/water mixture with a few drops of HCI added. The precipitated products were collect by filtration and then recrystallized from ethanol.

2-(((3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)imino)methyl)malononitrile (5a). Yellow crystals, yield: 3.12 g (95%), m.p. 95–98 °C. IR (ν, cm^{-1}): 3100 (CH-aromatic), 2936, 2865 (CH, CH_2), 2260, 2220, 2208 (3CN), 1639, 1448 (C=C), 1597 (C=N). ^{1}H NMR (δ, ppm): 1.49–1.72 (m, 4H, CH_2), 2.13–2.18 (m, 4H, CH_2), 5.80 (s, 1H, CH-pyran), 6.37, 6.93, (2d, 2H, 2CH), 7.10–7.53 (m, 5H, C_6H_5). ^{13}C NMR (δ, ppm): 21.1, 22.4, 24.4, 24.9, 26.9, 66.4, 112.4, 115.4, 115.8, 116.2, 124.3, 127.9, 128.2, 128.5, 128.6, 137.3, 143.6, 146.7, 150.1, 150.7. MS *m/z* (%): 330 [M^+ + 2] (17.26), 329 [M^+ + 1] (13.72), 328 [M^+] (17.92), 327 [M^+ – 1] (12.17), 300 (100.00), 77 [C_6H_5]$^+$ (19.91). Anal. Calcd for $C_{20}H_{16}N_4O$ (328.42): C, 73.15; H, 4.91; N, 17.06. Found: C, 73.34; H, 4.95; N, 17.28.

Ethyl-2-cyano-3-((3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)imino)propanoate (5b). Brown crystals, yield: 2.48 g (66%), m.p. 147–150 °C. IR (ν, cm^{-1}): 3061 (CH-aromatic), 2937, 2865 (CH, CH_2, CH_3), 2260, 2213 (2CN), 1697 (C=O), 1644, 1448 (C=C), 1595 (C=N). ^{1}H NMR (δ, ppm): 1.19–1.21 (t, 3H, CH_3), 1.54–1.74 (m, 4H, $2CH_2$), 2.13–2.17 (m, 4H, $2CH_2$), 4.19–4.21 (q, 2H, CH_2), 5.75 (s, 1H, CH-pyran), 6.37, 6.80 (2d, 2H, 2CH), 7.20–7.53 (m, 5H, C_6H_5). MS *m/z* (%): 377 [M^+ + 2] (4.15), 375 [M^+] (2.60), 273 (100.00), 77 [C_6H_5]$^+$ (9.84). Anal. Calcd for $C_{22}H_{21}N_3O_3$ (375.42): C, 70.38; H, 5.64; N, 11.19. Found: C, 70.58; H, 5.67; N, 11.29.

2-(((3-Cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)imino)methyl)malononitrile (5c). Red crystals, yield: 1.97 g (55%), m.p. 82–85 °C. IR (ν, cm^{-1}): 3006 (CH-aromatic), 2936 (CH, CH_2, CH_3), 2260, 2203, 2190 (3CN), 1639, 1450 (C=C), 1604 (C=N). ^{1}H NMR (δ, ppm): 1.46–1.73 (m, 4H, $2CH_2$), 2.16–2.25 (m, 4H, $2CH_2$), 3.84 (s, 3H, OCH_3), 5.03 (s, 1H, CH-pyran), 6.32–6.70 (2d, 2H, 2CH), 6.85–7.99 (m, 4H, C_6H_4). Anal. Calcd for $C_{21}H_{18}N_4O_2$ (358.39): C, 70.38; H, 5.06; N, 15.63. Found: C, 70.39; H, 5.17; N, 15.70.

Ethyl-2-cyano-3-(3-cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)imino)propanoate (5d). Yellow crystals, yield: 2.03 g (50%), m.p. 102–105 °C. IR (ν, cm^{-1}): 3100 (CH-aromatic), 2935, 2862 (CH, CH_2, CH_3), 2260, 2202 (2CN), 1701 (C=O), 1639, 1451 (C=C), 1600 (C=N). ^{1}H NMR (δ, ppm): 1.06–1.08 (t, 3H, CH_3), 1.67–1.70 (m, 4H, $2CH_2$), 2.18–2.22 (m, 4H, $2CH_2$), 3.84 (s, 3H, OCH_3), 4.22–4.24 (q, 2H, CH_2), 5.70 (s, 1H, CH-pyran), 6.30, 6.67 (2d, 2H, 2CH), 6.86–7.32 (m, 4H, C_6H_4). MS *m/z* (%): 407 [M^+ + 2] (4.56), 406 [M^+ + 1] (4.27), 405 [M^+] (2.99), 404 [M^+ – 1] (2.63), 305 (100.00), 76 [C_6H_4]$^+$ (6.13). Anal. Calcd for $C_{23}H_{23}N_3O_4$ (405.45): C, 68.13; H, 5.72; N, 10.36. Found: C, 68.33; H, 5.78; N, 10.69.

2. 1. 6. General Method for the Preparation of 2-Cyano-*N*-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)acetamide derivatives 6a,b

To form a mixture of equimolar amounts of **1a** (2.52 g, 0.01 mol) or **1b** (2.82 g, 0.01 mol) in *N*,*N*-dimethylformamide (15 mL), ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The chemical reaction was refluxed for 3 h and then added into a beaker containing a mixture of ice and water. The precipitated products were collected by filtration and recrystallized from *N*,*N*-dimethylformamide.

2-Cyano-*N*-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)acetamide (6a). Pale brown crystals, yield: 3.18 g (99%), m.p. 250–253 °C. IR (ν, cm^{-1}): 3417–3227 (NH), 3033 (CH-aromatic), 2932–2861 (CH_2), 2260, 2209 (2CN), 1647 (C=O), 1600, 1448 (C=C). ^{1}H NMR (δ, ppm): 1.45–1.49 (m, 4H, $2CH_2$), 2.16–2.20 (m, 4H, $2CH_2$), 3.80 (s, 2H, CH_2), 5.73 (s, 1H, CH-pyran), 7.33–7.43 (m, 5H, C_6H_5), 10.01 (s, 1H, NH). ^{13}C NMR (δ, ppm): 21.0, 24.8, 27.0, 42.9, 81.5, 112.3, 116.2, 126.9, 128.6, 128.8, 128.9, 129.3, 132.4, 134.6, 143.6. MS *m/z* (%): 317 [M^+ – 2] (0.31), 300 (100.00), 77 [C_6H_5]$^+$ (2.82). Anal. Calcd for $C_{19}H_{17}N_3O_2$ (319.36): C, 71.46; H, 5.37; N, 13.16. Found: C, 71.83; H, 5.38; N, 13.19.

2-Cyano-*N*-(3-cyano-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)acetamide (6b). Reddish brown crystals, yield: 1.74 g (50%), m.p. 122–125 °C. IR (ν, cm^{-1}): 3426–3242 (NH), 3100 (CH-aromatic), 2936 (CH_2, CH_3), 2260, 2208 (2CN), 1714 (C=O), 1593, 1439 (C=C). ^{1}H NMR (δ, ppm): 1.66–1.71 (m, 4H, $2CH_2$), 2.10–2.20 (m, 4H, $2CH_2$), 3.73 (s, 3H, OCH_3), 4.34 (s, 2H, CH_2), 5.70 (s, 1H, CH-pyran), 6.91–7.90 (m, 4H, C_6H_4), 8.31 (s, 1H,

NH). MS *m/z* (%): 347 [M^+ – 2] (15.34), 330 (100.00). Anal.Calcd for $C_{20}H_{19}N_3O_3$ (349.38): C, 68.75; H, 5.48; N, 12.03. Found: C, 69.03; H, 5.49; N, 12.27

2. 1. 7. General Method for the Preparation of 3, 5-Diamino-4-cyano-*N*-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl) thiophene-2-carboxamide derivatives 7a,b

To an equimolar amounts of **6a** (3.19 g, 0.01 mol) in absolute ethanol (25 mL) and triethylamine (0.50 mL), and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added with elemental sulfur (0.32 g, 0.01 mol). The reaction was refluxed for 3 h and then added onto the mixture of ice, water and HCl (a few drops). The precipitated products were collected by filtration and then recrystallized from ethanol.

3,5-Diamino-4-cyano-*N*-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)thiophene-2-carboxamide (7a). Reddish brown crystals, yield: 2.97 g (71%), m.p. 201–204 °C. IR (ν, cm^{-1}): 3417, 3340 ($2NH_2$), 3244 (NH), 3034 (CH-aromatic), 2932, 2864 (CH_2), 2260, 2209 (2CN), 1644 (C=O), 1601, 1448 (C=C). ^{1}H NMR (*δ*, ppm): 1.45–1.71 (m, 4H, $2CH_2$), 2.01–2.24 (m, 4H, $2CH_2$), 5.73 (s, 1H, CH-pyran), 7.28–7.62 (m, 9H, C_6H_5, $2NH_2$), 8.50 (s, 1H, NH). ^{13}C NMR (*δ*, ppm): 21.0, 22.0, 24.8, 26.5, 42.8, 81.5, 112.3, 113.7, 116.2, 124.2, 128.2, 128.6, 128.7, 128.8, 132.3, 133.4, 134.6, 143.5. MS *m/z* (%): 418 [M^+ + 1] (4.38), 417 [M^+] (6.65), 300 (100.00). Anal. Calcd for $C_{22}H_{19}N_5O_2S$ (417.48): C, 63.29; H, 4.59; N, 16.78; S, 7.68. Found: C, 63.30; H, 4.70; N, 16.98; S, 7.86.

Ethyl 2,4-diamino-5-((3-cyano-4-phenyl--5,6,7,8-tetrahydro-4*H*-chromen-2-yl)carbamyle)thiophene-3-carboxylate (7b). Brown crystals, yield: 2.78 g (60%), m.p. 232–235 °C. IR (ν, cm^{-1}): 3418, 3340 ($2NH_2$), 3251 (NH), 3035 (CH-aromatic), 2933–2831 (CH_2, CH_3), 2209 (CN), 1709 (C=O), 1645, 1450 (C=C). ^{1}H NMR (*δ*, ppm): 1.29–1.31 (t, 3H, CH_3), 1.66–1.71 (m, 4H, $2CH_2$), 2.15–2.20 (m, 4H, $2CH_2$), 4.31–4.34 (q, 2H, CH_2), 5.73 (s, 1H, CH-pyran), 7.03–7.63 (m, 9H, C_6H_5, $2NH_2$), 8.41 (s, 1H, NH). Anal. Calcd for $C_{24}H_{24}N_4O_4S$ (464.54): C, 62.05; H, 5.21; N, 12.06; S, 6.90. Found: C, 62.16; H, 5.23; N, 12.25; S, 7.11.

2. 1. 8. Synthesis of *N*-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-2-oxo-2*H*-chromene-3-carboxamide (8)

A solution of compound **6a** (3.19 g, 0.01 mol) is made by adding absolute ethanol (25 mL) and piperidine (0.50 mL) with salicylaldehyde (1.22 g, 0.01 mol). The chemical reaction was refluxed for 3 h and then added into a beaker containing a mixture of ice and water. The precipitated product was filtered and then recrystallized from ethanol.

Brown crystals, yield: 2.13 g (50%), m.p. 91–94 °C. IR (ν, cm^{-1}): 3434, 3245 (NH), 3054 (CH-aromatic), 2932, 2855 (CH_2), 2215 (CN), 1738, 1696 (2C=O), 1598, 1441 (C=C). ^{1}H NMR (*δ*, ppm): 1.69–1.71 (m, 4H, $2CH_2$), 2.10–2.20 (m, 4H, $2CH_2$), 6.30 (s, 1H, CH-pyran), 6.89 (s, 1H, CH-coumarin), 6.92–7.37 (m, 9H, C_6H_4, C_6H_5), 8.25 (s, 1H, NH). ^{13}C NMR (*δ*, ppm): 22.1, 22.3, 23.6, 25.6, 43.8, 87.9, 113.6, 114.6, 116.4, 118.2, 119.2, 125.2, 125.9, 127.2, 127.7, 128.1, 128.3, 128.6, 128.7, 129.1, 146.9, 148.3, 150.8, 153.2, 158.7, 163.2. MS *m/z* (%): 426 [M^+ + 2] (0.45), 425 [M^+ + 1] (1.54), 424 [M^+] (4.77), 423 [M^+ – 1] (17.73), 422 [M^+ – 2] (31.09), 77 $[C_6H_5]^+$ (1.97), 76 $[C_6H_4]^+$ (0.83). Anal. Calcd for $C_{26}H_{20}N_2O_4$ (424.45): C, 73.57; H, 4.75; N, 6.60. Found: C, 73.60; H, 4.89; N, 6.98.

2. 1. 9. General Method for the Preparation of 1-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine -3-carbonitrile derivatives 9a,b

To a solution of compound **6a** (3.19 g, 0.01 mol) in absolute ethanol (25 mL) and piperidine (0.50 mL), either acetylacetone (1.00 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added. The reaction was carried out for 3 h. Thereafter, the reaction mixture was poured onto the mixture of ice/water with a few drops of HC1 added. The precipitated products were collected by filtration and then recrystallized from ethanol.

1-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-4,6-dimethyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (9a). Reddish brown crystals, yield: 1.91 g (50 %), m.p. 71–74 °C. IR (ν, cm^{-1}): 3034 (CH-aromatic), 2943, 2866 (CH_2, CH_3), 2258, 2218 (2CN), 1751 (C=O), 1636, 1447 (C=C). ^{1}H NMR (*δ*, ppm): 1.02–1.06 (s, 3H, CH_3), 1.08–1.29 (s, 3H, CH_3), 1.60–1.70 (m, 4H, $2CH_2$), 2.10–2.20 (m, 4H, $2CH_2$), 5.70 (s, 1H, CH-pyran), 6.30 (s, 1H, CH-pyridine), 6.96–7.53 (m, 5H, C_6H_5). ^{13}C NMR (*δ*, ppm): 21.5, 22.1, 22.3, 26.9, 27.1, 28.2, 40.5, 53.3, 113.6, 115.1, 115.5, 115.8, 120.6, 125.9, 127.0, 127.7, 128.6, 128.7, 143.5, 146.9, 150.1, 150.7, 158.7, 166.0. Anal. Calcd for $C_{24}H_{21}N_3O_2$ (383.44): C, 75.18; H, 5.52; N, 10.96. Found: C, 75.19; H, 5.81; N, 11.15.

1-(3-Cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-6-hydroxy-4-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (9b). Brown crystals, yield: 2.76 g (72%), m.p. 56–58 °C. IR (ν, cm^{-1}): 3442–3245 (OH), 3100 (CH-aromatic), 2940, 2869 (CH_2), 2260, 2219 (2CN), 1745 (C=O), 1635, 1447 (C=C). ^{1}H NMR (*δ*, ppm): 1.24–1.29 (s, 3H, CH_3), 1.60–1.70 (m, 4H, $2CH_2$), 2.10–2.27 (m, 4H, $2CH_2$), 5.71 (s, 1H, CH-pyran), 6.26 (s, 1H, CH-pyridine), 7.26–7.53 (m, 5H, C_6H_5), 8.40 (s, 1H, OH). MS *m/z* (%): 387 [M^+ + 2] (32.13), 386 [M^+ + 1] (26.38), 385 [M^+] (15.59), 384 [M^+ – 1] (13.19), 383 [M^+ – 2] (19.66), 346

(100.00). Anal. Calcd for $C_{23}H_{19}N_3O_3$ (385.42): C, 71.67; H, 4.97; N, 10.90. Found: C, 71.76; H, 5.24; N, 11.02.

2. 1. 10. Synthesis of 2-Cyano-*N*-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-3-ethoxyacrylamide (10)

To a mixture of equimolar amounts of **6a** (3.19 g. 0.01 mol) in acetic acid (25 mL), triethyl orthoformate (1.45 g, 0.01 mol) was added. The chemical reaction was refluxed for 1 h and then added into a beaker containing a mixture of ice and water. The resultant product was filtered and then recrystallized from acetic acid.

Yellow powder, yield: 2.25 g (60%), m.p. 172–175 °C. IR (ν, cm^{-1}): 3427–3245 (NH), 3100 (CH-aromatic), 2934, 2864 (CH_2, CH_3), 2260–2199 (2CN), 1701 (C=O), 1638–1490 (C=C). ^{1}H NMR (δ, ppm): 1.05–1.10 (t, 3H, CH_3), 1.50–1.99 (m, 4H, $2CH_2$), 2.06–2.27 (m, 4H, $2CH_2$), 4.22–4.24 (q, 2H, CH_2), 5.80 (s, 1H, CH-pyran), 6.80–7.53 (m, 6H, C_6H_5, CH), 11.10 (s, 1H, NH). MS *m/z* (%): 377 [M^+ + 2] (10.63), 376 [M^+ + 1] (7.20), 375 [M^+] (12.60), 374 [M^+ – 1] (6.39), 373 [M^+ – 2] (7.67), 329 (100.00), 77 [C_6H_5]$^+$ (21.72). Anal. Calcd for $C_{22}H_{21}N_3O_3$ (375.42): C, 70.38; H, 5.64; N, 12.79. Found: C, 70.62; H, 5.82; N, 12.96.

2. 1. 11. Synthesis of 2-Cyano-*N*-(3-cyano-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-3-(phenyl amino) acrylamide (11)

To a solution of compound **10** (3.75 g. 0.01 mol) in absolute ethanol (25 mL), aniline (0.93 g, 0.01 mol) was added. The reaction was refluxed for 3 h and then poured onto an ice/water mixture with a few drops of HC1 added. The obtained product was filtered and recrystallized from ethanol.

Yellow crystals, yield: 2.78 g (65%), m.p. 210–213 °C. IR (ν, cm^{-1}): 3422–3244 (2NH), 3061 (CH-aromatic), 2934–2864 (CH, CH_2), 2260, 2211 (2CN), 1696 (C=O), 1638, 1490 (C=C). ^{1}H NMR (δ, ppm): 1.56–1.74 (m, 4H, $2CH_2$), 2.13–2.17 (m, 4H, $2CH_2$), 5.80 (s, 1H, CH-pyran), 6.96–7.50 (m, 11H, $2C_6H_5$, CH), 8.50, 10.10 (2s, 2H, 2NH). MS *m/z* (%): 424 [M^+ + 2] (33.57), 423 [M^+ + 1] (20.37), 422 [M^+] (14.63), 273 (100.00), 77 [C_6H_5]$^+$ (42.32). Anal. Calcd for $C_{26}H_{22}N_4O_2$ (422.48): C, 73.92; H, 5.25; N, 13.26. Found: C, 74.09; H, 5.29; N, 13.28.

2. 1. 12. General Method for the Preparation of 6-Amino-1-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile derivatives 12a,b

To a solution of **10** (3.75 g, 0.01 mol) in absolute ethanol (25 mL), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. On the reflux system, the reaction was heated for 3 h. The resultant product was poured onto the ice/water mixture with a few drops of HCl added. The precipitated products were collected by filtration and then recrystallized from ethanol.

6-Amino-1-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (12a). Brown crystals, yield: 3.00 g (76%), m.p. 112–115 °C. IR (ν, cm^{-1}): 3442, 3351 (NH_2), 3242 (NH), 3100 (CH-aromatic), 2935, 2865 (CH_2), 2260, 2220, 2199 (3CN), 1699 (C=O), 1638, 1449 (C=C). ^{1}H NMR (δ, ppm): 1.54–1.74 (m, 4H, $2CH_2$), 2.13–2.17 (m, 4H, $2CH_2$), 5.80 (s, 1H, CH-pyran), 6.37 (s, 1H, CH-pyridine), 6.95–7.53 (m, 7H, C_6H_5, NH_2). ^{13}C NMR (δ, ppm): 21.4, 22.0, 22.4, 26.9, 42.9, 95.7, 115.4, 115.8, 124.2, 127.6, 127.9, 128.5, 128.7, 137.3, 146.9, 150.1, 150.7. MS *m/z* (%): 397 [M^+ + 2] (2.57), 396 [M^+ + 1] (2.71), 395 [M^+] (3.46), 394 [M^+ – 1] (4.29), 393 [M^+ – 2] (10.61), 391 (100.00), 77 [C_6H_5]$^+$ (5.32). Anal. Calcd for $C_{23}H_{17}N_5O_2$ (395.41): C, 69.86; H, 4.33; N, 17.71. Found: C, 70.01; H, 4.49; N, 17.98.

6-Amino-1-(3-cyano-4-phenyl-5,6,7,8-tetrahydro-4*H*-chromen-2-yl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile (12b). Yellowish brown crystals, yield: 3.75 g (95%), m.p. 87–90 °C. IR (ν, cm^{-1}): 3419–3240 (OH), 3100 (CH-aromatic), 2935, 2864 (CH_2), 2260, 2240, 2209 (3CN), 1696 (C=O), 1640, 1448 (C=C). ^{1}H NMR (δ, ppm): 1.54–1.73 (m, 4H, $2CH_2$), 2.13–2.17 (m, 4H, $2CH_2$), 5.70 (s, 1H, CH-pyran), 6.36 (s, 1H, CH-pyridine), 7.20–7.52 (m, 5H, C_6H_5), 8.40 (s, 1H, OH). MS *m/z* (%): 397 [M^+ + 1] (4.82), 396 [M^+] (8.26), 300 (100.00), 77 [C_6H_5]$^+$ (13.12). Anal. Calcd for $C_{23}H_{16}N_4O_3$ (396.40): C, 69.69; H, 4.07; N, 14.13. Found: C, 69.93; H, 4.29; N, 14.39.

2. 2. Biological Evaluations

2. 2. 1. Materials and Methods

- Gibco Invitrogen Company (Scotland, UK): Provide fetal bovine serum (FBS) and L-glutamine.
- Cambrex (New Jersey, USA): Provide RPMI-1640 medium
- Sigma Chemical Company. (Saint Louis, MO, USA): Provide dimethyl sulfoxide (DMSO), foretinib, penicillin, streptomycin, and sulforhodamine B (SRB).

2. 2. 2. Samples

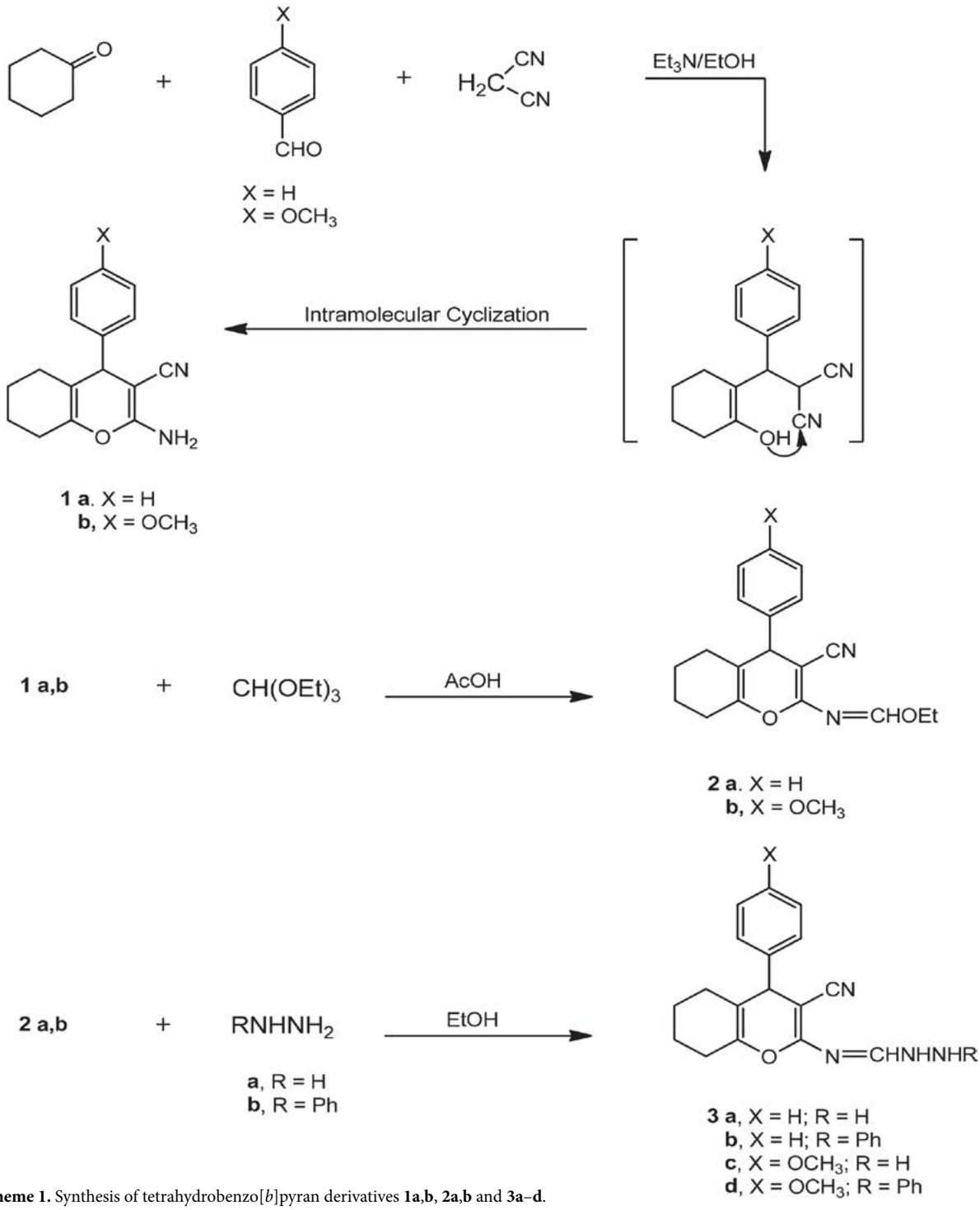
Tumor Cell Proliferation Assay: The effects of **1a**,**b** to **12a**,**b** on the *in vitro* proliferation of human cancer cell lines were tested. The method were obtained from the National Cancer Institute (NCI, USA) in the *In vitro Anticancer Drug Discovery Screen* using the protein-binding dye sulforhodamine B to assess cell proliferation.

2. 2. 3. Cell Cultures

The three human cancer cell lines were A-549 (lung carcinoma), HC-29 (colorectal adenocarcinoma),

and MKN-45 (gastric cancer). The later cells were obtained from the National Cancer Institute (NCI), Cairo, Egypt.

The cell cultures were prepared as the following: They were grown as monolayers and plated in RPMI 1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL and streptomycin 100 μg/mL) in a humidified atmosphere at 37 °C. Permanently maintained at 5% CO_2, exponentially growing cells were plated at 0.75 × 104 cells/mL followed by 1.5 × 105 cells/mL for MCF-7 and SF-268 and 0.75 × 104 cells/mL for three-cell line and maintained the incubation for 48 h. The effect of carrier solvent (DMSO) on the growth of these cell lines was examined in all experiments by exposing untreated control cells to the highest concentration of DMSO used in each assay (0.5%).



Scheme 1. Synthesis of tetrahydrobenzo[*b*]pyran derivatives **1a**,**b**, **2a**,**b** and **3a**–**d**.

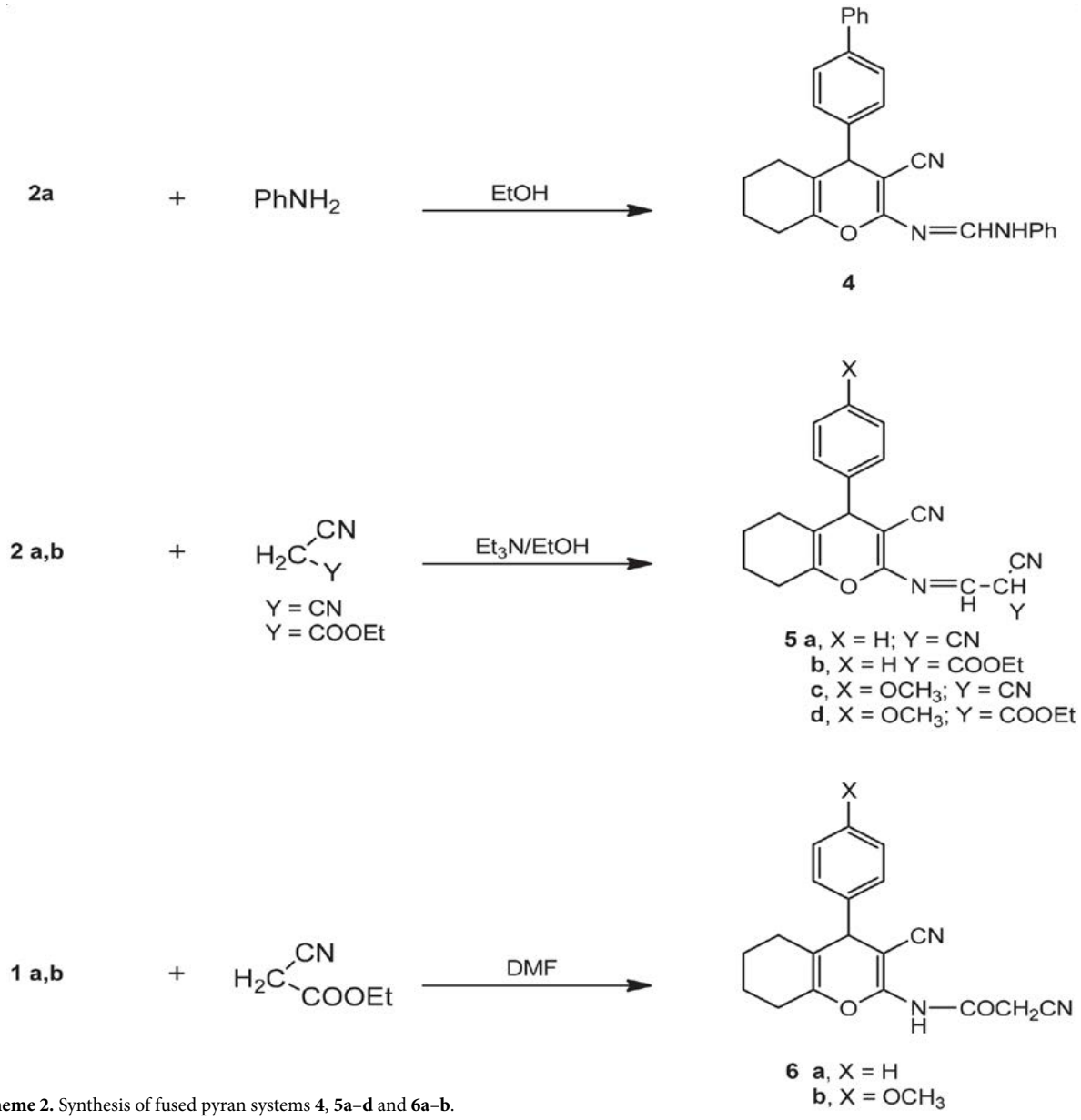
3. Results and Discussion

3. 1. Chemistry

The reaction of cyclohexanone with malononitrile and either of benzaldehyde or 4-methoxybenzaldehyde gave the pyran derivatives **1a** and **1b**,[31] respectively (Scheme 1). According to the data obtained from the spectroscopic analysis methods, the structures of the resultant products were indicated. The obtained structures were confirmed by ^{1}H NMR and IR spectroscopy. Thus, for ^{1}H NMR spectrum of the compound **1a**, a multiplet at δ 1.66–1.71 ppm for $2CH_2$ cyclohexene, a multiplet at 2.16–2.81 ppm for the other $2CH_2$ of cyclohexene ring and a singlet at 5.73 ppm for CH pyran were observed. Moreover, the presence of a multiplet at δ 7.14–7.89 ppm for phenyl moiety and NH_2 group and cyano group in the IR spectrum in the range of 2209 cm^{-1} supported the proposed structure. Besides, for compound **1b**, the presence of the methoxy group in the ^{1}H NMR in the range of 3.87 ppm confirmed its structure.

The reaction of compound **1a** or **1b** with triethylorthoformate in acetic acid gave the 2-*N*-ethoxymethino derivatives **2a** and **2b**, respectively (Scheme 1). The disappearance of the NH_2 group signal in the ^{1}H NMR and IR spectrum of the compounds **2a** and **2b**, confirmed the structures. The appearance of the ethoxy group in the range between 1.10–1.20 ppm for the CH_3 group and 4.25–4.30 ppm for the CH_2 confirmed the structures.

Previously obtained products **2a** or **2b** were reacted with either of hydrazine hydrate or phenylhydrazine to give hydrazino derivatives **3a–d**, respectively (Scheme 1). The ^{1}H NMR spectrum of **3a** indicated a multiplet at δ

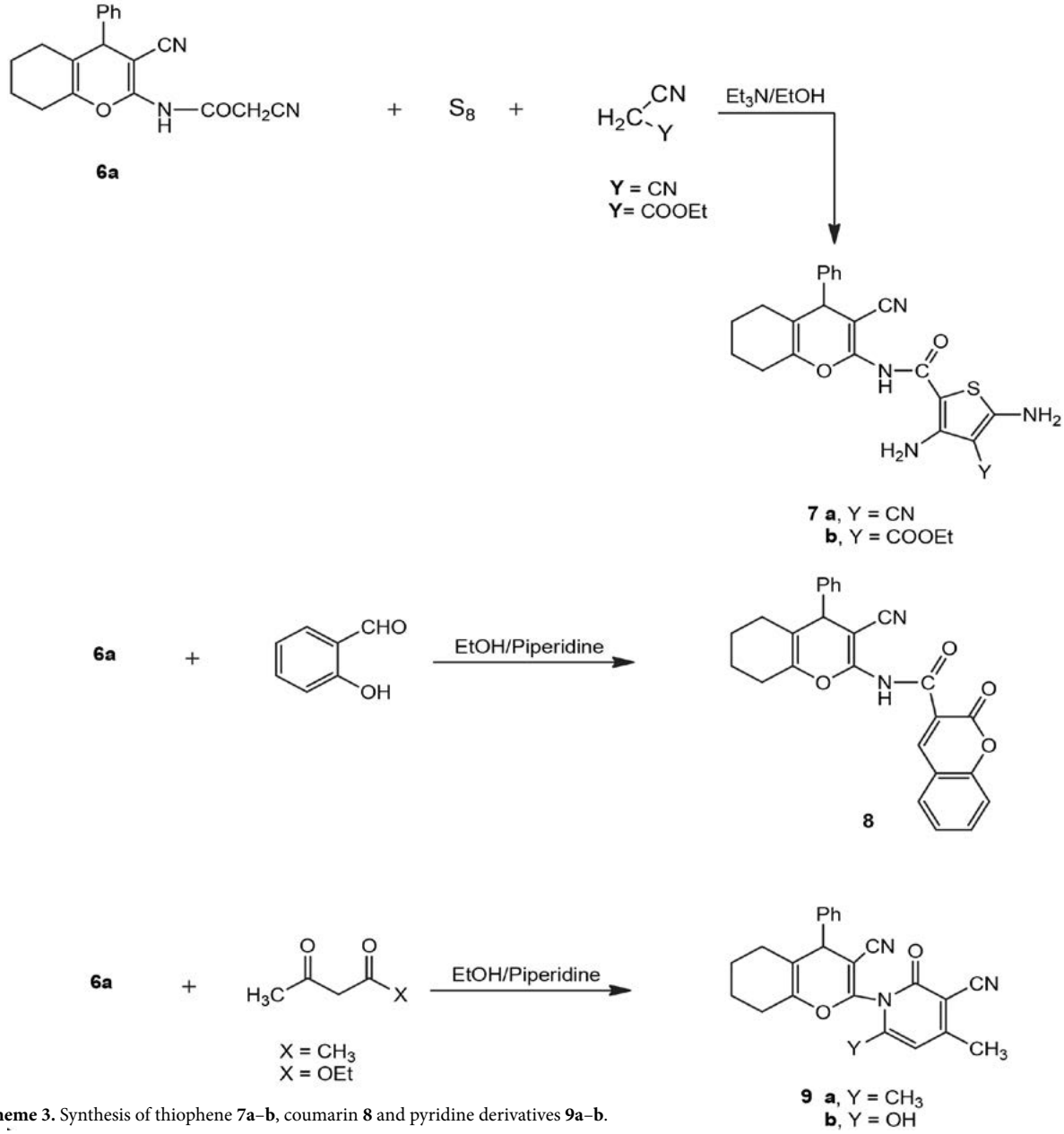


Scheme 2. Synthesis of fused pyran systems **4**, **5a–d** and **6a–b**.

1.58–1.74 ppm for $2CH_2$ cyclohexene moiety, a multiplet at δ 2.13–2.15 ppm for the other $2CH_2$, a singlet at δ 6.40 ppm for CH-pyran ring, a singlet at δ 6.96 ppm for CH group, a multiplet at δ 7.20–7.54 ppm for NH_2 group and phenyl ring. Moreover, the appearance of the singlet at δ 10.80 ppm for NH group elucidated the chemical structure of compound **3a**.

Compound **2a** upon reaction with aniline in ethanol gave the aniline derivative **4** (Scheme 2). The reaction of compound **2a** or **2b** with either of malononitrile or ethyl cyanoacetate gave 2-*N*-alkyl products **5a–d**, respectively (Scheme 2). The structures of these products were confirmed by the presence of the ethoxy groups in the ^{1}H NMR spectra for compounds **5b** and **5d** in the range at δ 1.06–1.21 ppm for CH_3 group and 4.19–4.24 ppm for CH_2 group. On the other hand, the appearance in the IR spectra of compounds **5a** and **5c** of the three cyano moieties in the range at ν 2190–2260 cm^{-1} elucidated the proposed structures.

Compounds **1a** and **1b** showed interesting reactivity towards amide formation. Thus, the reaction of either of compound **1a** or **1b** with ethyl cyanoacetate gave the cyanoacetamide derivatives **6a** and **6b**, respectively (Scheme 2). The analytical and spectral data of **6a** and **6b** elucidated their structures. Thus, the ^{1}H NMR of **6a** contains a multiplet at δ 1.45–1.49 ppm for $2CH_2$ cyclohexene ring, a multiplet at δ 2.16–2.20 ppm for the second $2CH_2$ cyclohexene moiety, a singlet at δ 5.73 ppm CH-pyran ring, a multiplet at δ 7.33–7.43 ppm for phenyl ring and a singlet at δ 10.01 ppm for NH group; also spectrum of **6b** revealed a multi-

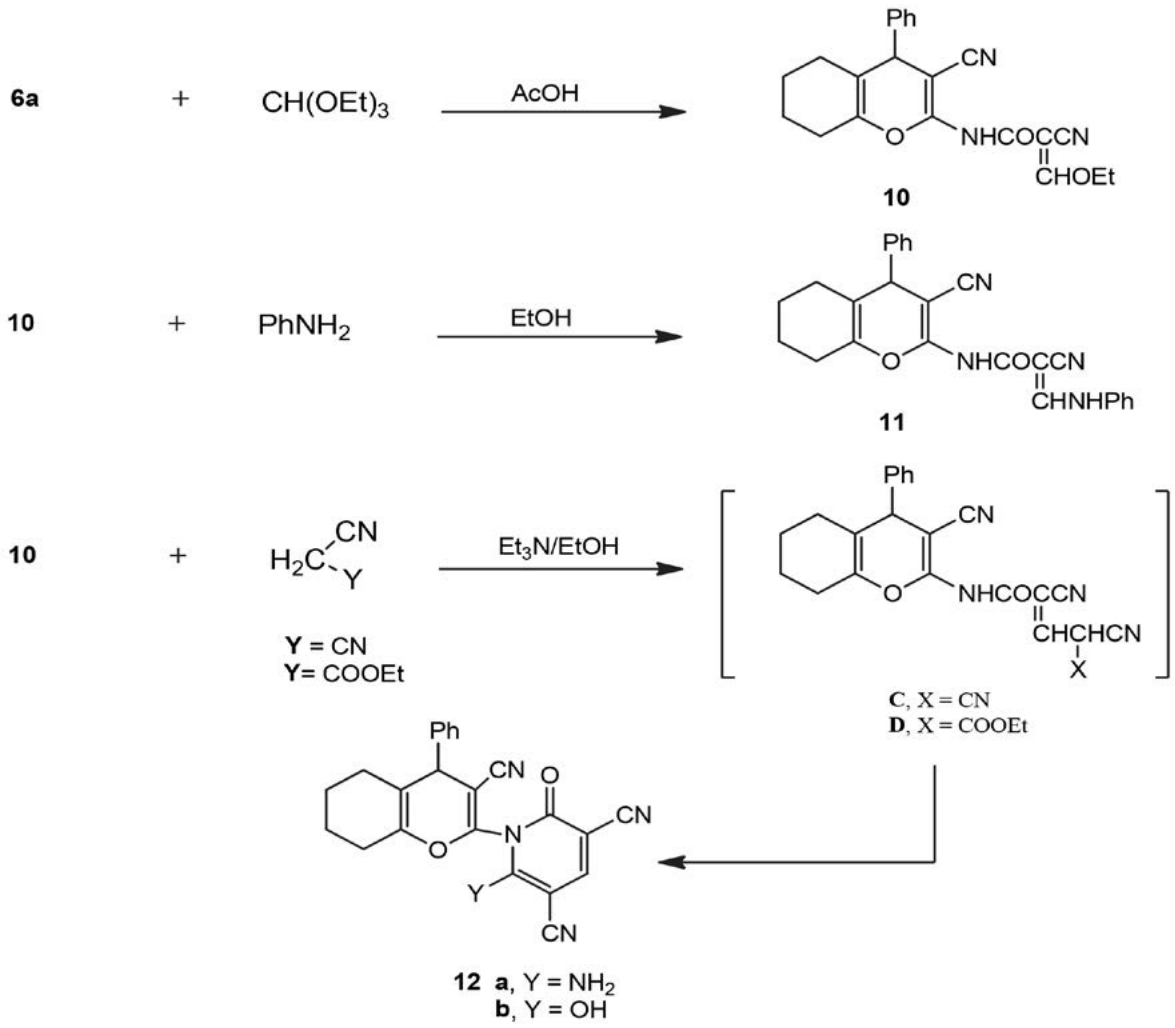


Scheme 3. Synthesis of thiophene **7a–b**, coumarin **8** and pyridine derivatives **9a–b**.

plet at δ 1.66–1.71 ppm for $2CH_2$ groups, a multiplet at δ 2.10–2.20 ppm for the other $2CH_2$ cyclohexene moiety, a singlet at δ 3.73 ppm for OCH_3 group, a singlet at δ 4.34 ppm for CH_2 group, a singlet at δ 5.70 ppm for CH-pyran ring, a multiplet at δ 6.91–7.90 ppm for C_6H_4 moiety and a singlet at δ 8.31 ppm for NH group.

Compound **6a** underwent the Gewald's thiophene synthesis[32–34] by the reaction of either of malononitrile or ethyl cyanoacetate with elemental sulfur in ethanol and triethylamine to give the thiophene derivatives **7a** and **7b**, respectively (Scheme 3). The formation of the latter products was confirmed by the ^{1}H NMR spectrum via the presence of the two NH_2 moieties in the range between δ 7.19–7.63 ppm with the phenyl groups. In addition, the IR spectrum of compounds **7a** and **7b** showed two bands in the range between ν 3417–3340 cm^{-1} due to the presence of the two NH_2 groups. Moreover, compound **6a** upon the reaction with salicylaldehyde in ethanol and piperidine gave the coumarin derivative **8** (Scheme 3). Mass spectrum of **8** exhibited molecular ion at *m/z* 424 corresponding to the molecular formula $C_{26}H_{20}N_2O_4$, which confirmed the assignment for coumarin structure **8**. The other resulting peaks which confirmed the molecular ion peak were observed at *m/z* 426, 425, 423, 422, 77 and 76 which correspond to $[M^+ + 2]$, $[M^+ + 1]$, $[M^+ - 1]$, $[M^+ - 2]$, $[C_6H_5]^+$ and $[C_6H_4]^+$, respectively. In addition, the structure of **8** was elucidated via the ^{13}C NMR which confirmed the presence of two carbonyl groups at δ 158.7 and 163.2 ppm.

The reactivity of compound **6a** towards 1,3-dicarbonyl compounds was studied to give bioactive pyridine derivatives. Compound **6a** reacted with either of acetylacetone or ethyl acetoacetate to afford the pyridine derivatives **9a** and **9b**, respectively (Scheme 3). The structures of the latter products were confirmed according to the results of the spectral data. Thus, the ^{13}C NMR spectrum of **9a** showed the carbonyl carbon signal at δ 166.00 ppm. Moreover, the mass spectrum of **9b** exhibited a molecular ion peak $[M^+]$ at *m/z* 385 corresponding to the molecular formula $C_{23}H_{19}N_3O_3$. Many other peaks were observed to confirm the final chemical structure of **9b**, such as the peak at *m/z* 387, 386, 384 and 383 which corresponds to $[M^+ + 2]$, $[M^+ + 1]$, $[M^+ - 1]$ and $[M^+ - 2]$, respectively.



Scheme 4. Synthesis of tetrahydrobenzo[*b*]pyran derivatives **10**, **11** and pyridines **12a**,**b**.

Compound **6a** upon the reaction with ethyl orthoformate gave ethoxyvinyl product **10** which reacted with aniline to give the aniline derivative **11** (Scheme 4). The structure of **10** was confirmed based on analytical and spectral data. Thus, the IR spectrum revealed absorption bands at ν 1701 cm^{-1} corresponding to C=O. ^{1}H NMR showed a triplet in the range at δ 1.05–1.10 ppm for the CH_3 group and quartet in the range at δ 4.22–4.24 ppm for the CH_2 moiety which confirmed the presence of the ethyl group in compound **10**. Mass spectra of compounds **10** and **11** revealed molecular ion peaks at *m/z* 375 and 422, respectively, corresponding to the respective molecular formulas $C_{22}H_{21}N_3O_3$ and $C_{26}H_{22}N_4O_2$. Compound **10** was reacted with either of malononitrile or ethyl cyanoacetate in ethanol under reflux to give pyridine derivatives **12a** and **12b**, respectively (Scheme 4). The latter products were formed through the intermediate acyclic products **C** and **D**, respectively. Compounds **12a**,**b** were confirmed; thus, the ^{1}H NMR spectrum of **12a** showed a multiplet at δ 1.54–1.74 ppm for $2CH_2$, a multiplet at δ 2.13–2.17 ppm for the other $2CH_2$ groups, a singlet at δ 6.37 ppm for CH-pyridine ring, a singlet at δ 5.80 ppm for CH-pyran moiety and a multiplet at δ 6.95–7.53 ppm for NH_2 group and C_6H_5 moiety. In addition, the mass spectrum of **14b** showed molecular ion peak [M^+] 396 corresponding to its molecular formula $C_{23}H_{16}N_4O_3$.

3. 2. Biological Activity Evaluations

3. 2. 1. Structure Activity Relationship

Table **1** demonstrates the cytotoxicity of the prepared products on the three cancer cell lines comparing compounds **1a** and **1b**, where compound **1b** has more potency than compound **1a** due to the 4-methoxyphenyl group present in compound **1b**. The same also appears in compound **2b** which has higher cytotoxicity than **2a**. By comparing compound **3a**–**d**, it can be noticed that compound **3b** has the highest cytotoxic effect among the other compounds **3**. Moreover, in the case of the pyran compounds **5a**–**d**, compound **5b** with the ethoxy carbonyl group has the highest potency within the four compounds; reaction of compounds **1a** and **1b** with ethyl cyanoacetate gave compounds **6a** and **6b**. Compound **6b** with the 4-methoxyaryl group demonstrated higher potency than compound **6a**. Reaction of compound **6a** with ethyl orthoformate gave the ethoxy metheno derivative **10** possessing moderate cytotoxicity. Besides, compound **11** obtained from the reaction of **10** with aniline has shown the same moderate cytotoxicity.

Comparing compounds **7a** and **7b** explains that compound **7a** with the electronegative cyano group exhibited higher potency than **7b** with the ester group. The coumarine derivative **8** shows a high potency. The pyridine derivatives **9a** and **9b** showed similar cytotoxicity. The cytotoxic effect for the compounds **12a** and **12b** represents moderate activity, but compound **12b** showed a higher effect than **12a** especially for the A-549 and MKN-45 cell lines. The latter activity is attributed to the presence of the hydroxyl group in compound **12b**.

Finally, the presence of the two phenyl rings, methoxy group and coumarin moiety in the compounds **3b**, **6b** and **8**, respectively, were responsible for the highest effect of these compounds among all the other tested compounds.

Table 1. The cytotoxic effect of the prepared products against three cancer cell lines

Compd. Number	[GI_{50}(mM)] A-549	HC-29	MKN-45
1a	29.48±5.43	40.69±4.61	48.90±12.53
1b	18.48±1.84	19.54±2.80	11.85±4.75
2a	49.11±10.42	52.2±10.32	36.59±4.80
2b	**0.26±0.08**	1.69±0.59	0.86±0.04
3a	45.24±6.55	70.2±10.50	64.21±10.33
3b	**0.08±0.002**	**0.09±0.09**	**0.1±0.01**
3c	48.29±6.81	73.2±12.53	69.31±12.59
3d	14.23±1.80	15.80±2.79	12.64±2.55
4	14.70±1.83	18.11±2.82	20.12±4.15
5a	40.63±8.62	45.60 ± 3.51	37.39± 4.21
5b	4.70±1.93	**0.1±0.02**	**0.02±0.005**
5c	28.19±6.73	19.26±2.60	22.80±4.76
5d	38.41±6.80	22.59±6.90	29.30±5.70
6a	4.73±1.69	5.80±0.98	2.66±0.39
6b	**0.03±0.002**	**0.06±0.09**	**0.2±0.01**
7a	8.09±2.70	10.39±4.62	8.39±3.77
7b	12.37±2.75	6.19±1.65	8.62±2.63
8	**0.08±0.003**	**1.20±0.22**	**0.07±0.01**
9a	10.69±2.73	12.70±2.84	12.61±3.74
9b	12.69±2.59	14.72±2.80	8.91±3.76
10	8.33±1.75	6.29±1.39	4.28±1.30
11	10.50±2.65	6.08±1.27	14.59±1.19
12a	4.82±0.27	3.79±0.92	10.55±1.76
12b	4.73±1.69	5.80±0.98	2.66±0.39
Foretinib (mM)	0.18±0.09	0.24±0.023	0.021±0.0016

4. Conclusions

The current research describes a practical synthesis method for 24 novel pyran derivatives. The variety of the final products prepared can be attributed to the various ways of possible attacks of chosen reagents on the reactive points in the pyran system. Moreover, anti-cancer activities of all the compounds were examined on three human cancer cell lines. Some of the tested products were shown to be favorable as anti-proliferative agents. The most promising compounds were **3b**, **6b**, and **8** against the three tumor cell lines such A-549 (lung carcinoma), HC-29 (colorectal adenocarcinoma), and MKN-45 (gastric cancer).

Conflict of Interests

The authors do not report any conflicts of interest in this work.

Compliance with Ethical Standards

Any of the author's experiments involving animals or human subjects are not included in this article.

5. References

1. E. Middleton, C. Kandaswami, T. C. Theoharides, *Pharmacol Rev*. **2000**, *52*, 673–751.
2. H. F. Roaiah, S. S. El-Nakkady, W. S. El-Serwy, M. A. A. Ali, A. H. Abd El-Rahman, Z. El-Bazzad, *Nat Sci.* **2010**, *8*, 20–29. **DOI:**10.7537/marsnsj080710.04
3. V. R. Rao, P. V. Kumar, V. R. Reddy, K. M. Reddy, *Heterocycl. Commun.* **2005**, *11*, 273–284. **DOI:**10.1515/HC.2005.11.3-4.273
4. M. Grażul, A. Kufelnicki, M. Wozniczka, I.-P. Lorenz, P. Mayer, A. Jóźwiak, M. Czyz, E. Budzisz, *Polyhedron*. **2012**, *31*, 150–158. **DOI:**10.1016/j.poly.2011.09.003
5. D. A. Vasselin, A. D. Westwell, C. S. Matthews, T. D. Bradshaw, M. F. Stevens, *J. Med. Chem.* **2006**, *49*, 3973–3981. **DOI:**10.1021/jm060359j
6. M. M. F. Ismail, H. S. Rateb, M. M. M. Hussein, *Eur. J. Med. Chem.* **2010**, *45*, 3950–3959. **DOI:**10.1016/j.ejmech.2010.05.050
7. Z.-F. Wang, X.-L. Nai, Y. Xu, F.-H. Pan, F.-S. Tang, Q.-P. Qin, L. Yang, S.-H. Zhang, *Dalton Trans.* **2022**, *51*, 12866–12875. **DOI:**10.1039/D2DT01929A
8. D. Ashok, K. Pallavi, *Heterocycl. Commun.* **2006**, *12*, 103–106. **DOI:**10.1111/j.1355-0691.2006.01241_1.x
9. N. Artizzu, L. Bonsignore, G. Loy, A. Calignano, *Farmaco* **1995**, *50*, 853–856.
10. L. Bonsignore, G. Loy, D. Secci, A. Calignano, *Eur. J. Med. Chem.* **1993**, *28*, 517–520. **DOI:**10.1016/0223-5234(93)90020-F
11. A. Dutta, N. Rahman, J. E. Kumar, J. Rabha, T. Phukan, R. Nongkhlaw, *Synth. Commun.* **2021**, *51*, 263–278. **DOI:**10.1080/00397911.2020.1825741
12. M. R. P. Heravi, P. Aghamohammadi, E. Vessally, *J. Mol. Struct*. **2022**, *1249*, 131534. **DOI:**10.1016/j.molstruc.2021.131534
13. J. Li, C.-W. Lv, X.-J. Li; D. Qu, Z. Hou, M. Jia, X.-X. Luo, X. Li, M.-K. Li, *Molecules* **2015**, *20*, 17469–17482. **DOI:**10.3390/molecules200917469
14. I. E. Bylov, M. V. Vasylyev, Y. V. Bilokin, *Eur. J. Med. Chem.* **1999**, *34*, 997–1001. **DOI:**10.1016/S0223-5234(99)00119-1
15. A. Nohara, T. Umetani, Y. Sanno, *Tetrahedron* **1974**, *30*, 3553–3561. **DOI:**10.1016/S0040-4020(01)97034-6
16. M. Biglari, F. Shirini, N. O. Mahmoodi, M. Zabihzadeh, M. Mashhadinezhad, *J. Mol. Struct.* **2020**, *1205*, 127652. **DOI:**10.1016/j.molstruc.2019.127652
17. H. R. Saadati-Moshtaghin, F. M. Zonoz, *Mater Chem. Phys.* **2017**, *199*, 159–165. **DOI:**10.1016/j.matchemphys.2017.06.066
18. A. Thongni, P. T. Phanrang, A. Dutta, R. Nongkhlaw, *Synth. Commun.* **2022**, *52*, 43–62. **DOI:**10.1080/00397911.2021.1998535
19. J. Yang, S. Liu, H. Hu, S. Ren, A. Ying, *Chin. J. Chem. Eng.* **2015**, *23*, 1416–1420. **DOI:**10.1016/j.cjche.2015.04.020
20. F. Sepehr, S. Allameh, A. Davoodnia, *Ann. Romanian Soc. Cell Biol.* **2021**, *25*, 19067–19072.
21. F. Mohamadpour, *J. Taiwan Inst. Chem. Eng.* **2021**, *129*, 52–63. **DOI:**10.1016/j.jtice.2021.09.017
22. F. Mohamadpour, *Polycycl. Aromat. Compd.* **2021**, *42*, 7607–7615. **DOI:**10.1080/10406638.2021.2006244
23. I. Devi, P. J. Bhuyan, *Tetrahedron Lett.* **2004**, *45*, 8625–8627. **DOI:**10.1016/j.tetlet.2004.09.158
24. J.-T. Li, W.-Z. Xu, L.-C. Yang, T.-S. Li, *Synth. Commun*. **2004**, *34*, 4565–4571. **DOI:**10.1081/SCC-200043233
25. D. Elhamifar, Z. Ramazani, M. Norouzi, R. Mirbagheri, *J. Colloid Interface Sci*. **2018**, *511*, 392–401. **DOI:**10.1016/j.jcis.2017.10.013
26. A. E. M. Abdallah, R. M. Mohareb. *Pigm. Resin Technol.* **2019**, *48*, 89–103. **DOI:**10.1108/PRT-11-2017-0085
27. A. E. M. Abdallah, R. M. Mohareb, E. A. Ahmed, *J. Heterocycl. Chem.* **2019**, *56*, 3017–3029. **DOI:**10.1002/jhet.3697
28. R. M. Mohareb, E. M. Khalil, A. E. Mayhoub, A. E. M. Abdallah, *J. Heterocycl. Chem.* **2020**, *57*, 1330–1343. **DOI:**10.1002/jhet.3870
29. A. E. M. Abdallah, R. M. Mohareb, M. H. E. Helal, G. J. Mofeed. *Acta Chim. Slov.* **2021**, *68*, 604–616. **DOI:**10.17344/acsi.2020.6446
30. R. M. Mohareb, M. H. E. Helal, S. S. Mohamed, A. E. M. Abdallah, *Anti-Cancer Agents in Med. Chem*. **2022**, *22*, 2327–2339. **DOI:**10.2174/1871520622666211224102301
31. F. Javadi, R. Tayebee, *Micropor. Mesopor. Mat.* **2016**, *231*, 100–109. **DOI:**10.1016/j.micromeso.2016.05.025
32. D. Wang, J. Wu, Q. Cui, *Chin. Chem. Lett.* **2014**, *25*, 1591–1594. **DOI:**10.1016/j.cclet.2014.07.007
33. Y. Han, W.-Q. Tang, C.-G. Yan, *Tetrahedron Lett.* **2014**, *55*, 1441–1443. **DOI:**10.1016/j.tetlet.2014.01.043
34. V. D. Dyachenko, A. N. Chernega, *Russ. J. Gen. Chem.* **2005**, *75*, 952–960. **DOI:**10.1007/s11176-005-0351-6

Povzetek

Doslej so bili načrtovani in pripravljeni že mnogi novi ciklični piranski sistemi s potencialnimi delovanjem proti raku. Poleg tega piranski sistemi kažejo tudi visoko reaktivnost do mnogih kemijskih reagentov. Pripravili smo 24 produktov in jih preizkusili kot morebitne protirakave učinkovine (v mM območju). Rezultati kažejo, da so spojine **3b**, **6b** in **8** široko učinkovite proti trem rakavim celičnim linijam in sicer A-549 (pljučni karcinom), HC-29 (kolorektalni adenokarcinom) in MKN-45 (rak želodca) in kažejo primerljivo aktivnost glede na standarndo referenčno kontrolno spojino foretinib.