MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Synthesis, Anticancer Evaluation and SAR-Study of Novel Sulfapyridine Derivatives

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Received:22/7/2024
Accepted: 28/7/2024

Abstract: This study focuses on developing and evaluating a novel series of sulfapyridine derivatives for their potential as anticancer agents. The strategic starting material, 2-chloro-*N*-(4-(*N*-(pyridine-2-yl)sulfamoyl)phenyl)acetamide, served as the foundation for the synthesis of various compounds. Clarifying the synthetic compounds' structures was accomplished through the analysis of IR, ^{1}H NMR, ^{13}C NMR, and MS spectral data.

In vitro screening assays were conducted to assess the freshly generated compounds' anticancer activity in opposition to a panel of human cancer cell lines, which included (HePG-2), (PC-3), (HCT-116), and breast cancer cell lines from the mammary gland (MCF-7). Interestingly, compounds with a thiophene moiety compounds 4 and 8 in particular showed notable activity. Compound 8 demonstrated encouraging results against each of the four cancer cell lines, suggesting its potential as a lead candidate for further expansion as an anti-tumor agent.

This study contributes to understanding the structure-activity relationship of sulfapyridine derivatives and highlights the potential of compound 8 as a valuable applicant for future anticancer drug development.

keywords: Sulfapyridine derivatives, Anticancer agents, Structure-activity relationship, Efficacy assessment, Thiophene moiety.

1.Introduction

Cancer is a serious global health issue that is regarded as the second originating cause of death globally [1, 2]. Therefore, Previous investigations aimed to establish new antitumor agents with a wide spectrum of activity that target various cell lines to efficiently control the expansion and dissemination of cancer cells [3-5]. Researching a variety of chemical fragments that might be used as a framework for novel antitumor agents, it was discovered that the sulfonamide moiety was shown to be a bioactive core present in numerous biologically captivating agents [6, 7]. The sulfonamide derivatives were found to be active for biosynthetic reactions and biomedical applications including the treatment of epilepsy [8], glaucoma [9,10], and the most important moiety that is clinically used as an anticancer for several cell lines [11-13]. It was noted that heterocyclic compounds containing pyridine ring acted as bases of numerous drug classes and have been shown to exhibit strong anti-carcinogenic properties [14], antioxidant [15], antimicrobial agents [16, 17], anti-virus [18], anti-HIV [19, 20] and antimutagenic activity and cytotoxicity toward tumor cells [21-23]. fortunately, it is known that aryl/hetero-aryl sulfonamides can function as antitumor agents through an array of mechanisms, including impairment of microtubule assembly, angioplasty inhibition, cell cycle perturbation [24]. The researcher chose to study acetylated derivatives because they are widely used in medicinal chemistry to create pro-drugs and advance biopharmaceutical, physicochemical, and pharmacokinetic properties [25, 26]. The

combination between pyridine nucleus and sulfonamide moieties has recently drawn attention in searching for new anticancer agents [27]. Moreover, previous studies proved that the addition of indole [28, 29], thiazole [30, 31], quinoxaline [32], pyrimidine [33, 34], and thiophene [35,36] moieties to any compounds turned out the structure-activity to be more successful and increased their activity for the treatment various anticancer cell lines. Based on these findings, this work aimed to synthesize some new sulfapyridine derivatives incorporated into biologically active scaffolds to evaluate their anticancer activity. Therefore, a new series of sulfapyridine derivatives were produced, and investigated for their antineoplastic activity toward various cell lines such as breast (MCF-7), HCT116, PC3, and HGP2 cell lines, and study their structure-activity relationship (SAR)

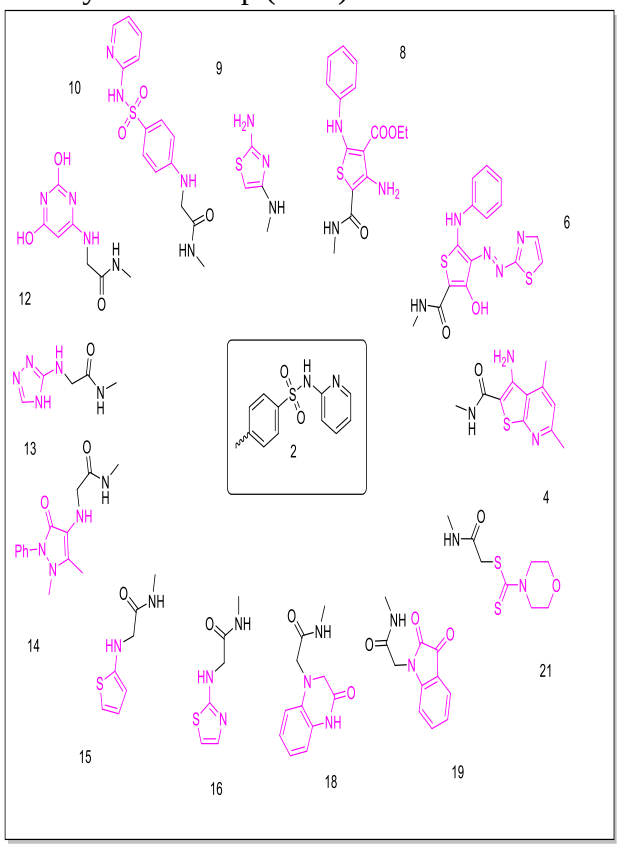


Figure 1 Synthesis of compounds from 2 –

212. Materials and methods

Instruments

Melting point determination was performed using a Gallenkamp electric melting point apparatus, and the value obtained was uncorrected. The Thermo Fisher Scientific Nicolet IS 10 FTIR spectrometer (USA), frequency analyzes unit Mansoura university, was used to capture the IR spectra ν /cm^{-1} (KBr). Using (DMSO-d6, DMF) as an internal reference and a Bruker WP spectrometer, the ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, correspondingly. Azhar University's regional center for mycology is that and biological technology, Thermo Scientific Focus/DSQII (Waltham, MA), offered the mass spectra (EI) at 70 eV.

Human cancer cell lines representing (PC-3), (HEPG-2), (MCF-7), and (HCT-116) cancer were employed in this study. These cell lines were procured from the American Type Culture Collection (ATCC) through VACSERA (Holding Company for Biological Products and Vaccines), Cairo, Egypt.

Synthesis of 2-chloro-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl) phenyl) acetamide (2)

formerly, compound 2 was prepared by adding chloroacetyl chloride dropwise slowly to an agitated solution of 4-amino-N-(pyridin-2-yl) benzenesulfonamide in DMF or sodium hydroxide [37] for two hours at the ambient temperature [38]. Nevertheless, a combination of 4-amino-N-(pyridin-2-yl) benzenesulfonamide was used in this investigation (0.3g, 0.001mole) and chloroacetyl chloride (0.15ml, 0.001mole) in acetone (10ml) containing potassium carbonate (0.165g, 0.001mole) was stirring for 24 hours at room temperature. The precipitate that formed was filtrated off, dried and recrystallized from ethanol to afford compound 2 in good yield; yield (0.3g, 92%); m.p.190-192^{0}C; Mol.Formula:$C_{13}H_{12}ClN_3O_3S$; M.Wt.: 325.77. IR (KBr): *ν*/cm^{-1}= 3322, 3203 (2NH), 2815 (CH aliphatic), 1677 (CO, amide), 1631(C=N). ^{1}H NMR (DMSO-d6, 500MHz, *δ* ppm): 4.26 (s, 2H, CH_2), 6.85 (t, 1H, *J*=5.1Hz, Ar-H), 7.10 (d, 1H, *J*=7.1Hz, Ar-H), 7.69 (d, 3H, *J*=7.7Hz, Ar-H), 7.81 (d, 2H, *J*=7.8Hz, Ar-H), 7.99 (d, 1H, *J*= 7.9Hz, Ar-H), 10.62 (s, 1H, NH), 12.00 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 43.50(1C), 113.57(1C), 115.71(1C), 118.97(1C), 119.16(1C), 127.86(1C), 136.42(1C), 140.30(1C), 141.76(1C), 141.88(1C), 153.02(1C), 165.25(1C), 171.59 (CON).

Synthesis of 3-amino-4,6-dimethyl-*N*-(4-(*N*-(pyridin-2-yl) sulfamoyl)phenyl)thieno[2,3-b]pyridine-2-carboxamide (4)

Refluxing compound 2 (0.3g, 0.001mole) with 2-mercapto-4,6-dimethylnicotinonitrile [39] (3) (0.164g, 0.001mole), in ethanol (10ml) and sodium metal (0.046g, 0.002mole) for 6 hours. To obtain the desired compound 4, the crystalline form that proposed was cleared off, dried out, along with recrystallized from methanol.; yield (0.45g, 99%); m.p.295-297^{0}C; Mol.Formula:$C_{21}H_{19}N_5O_3S_2$; M.Wt.: 453.54. IR (KBr): ν/cm^{-1}= 3451, 3474, 3228, 3115 (NH_2, 2NH), 2924 (CH aliphatic), 1663(CO, amide), 1634 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 2.47 (s, 3H, CH_3), 2.71 (s, 3H, CH_3), 6.40 (t, 1H, *J*=4.8Hz), 6.64 (d, 1H, *J*=6.6Hz, H_{Ar}), 6.99 (br. s, 2H, NH_2), 7.04 (br. s, 1H, NH), 7.22 (t, 1H, *J*= 5.4Hz, Ar-H), 7.67 (q, 5H, *J*=5.1Hz, Ar-H), 7.87 (dd, 1H, *J*=7.8Hz, Ar-H), 9.52 (s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 19.99 (CH_3), 23.97, CH_3), 96.54(1C), 112.41(1C), 113.89(1C), 120.25 (2C), 122.14, 122.92, 127.08 (2C), 136.54(1C), 140.17(1C), 141.27(1C), 145.01(1C), 147.36(1C), 149.62(1C), 159.19(1C), 159.35(1C), 160.71(1C), 164.45 (CON). MS (EI): m/z (%): 454.58 (M^{+1}, 3.72), 427.24 (55.52), 367.34 (100.00), 366.30 (63.61), 335.25 (43.73), 324.23 (38.13), 243.61 (36.08), 153.12 (23.55), 69.07 (37.53).

Synthesis of 3-hydroxy-5-(phenylamino)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-4-(thiazol-2-yldiazenyl)thiophene-2-carboxamide (6)

A mixture of compound 2 (0.3g, 0.001mole) with ethyl -3-mercapto-3-(phenylamino)-2-(thiazol-2-yldiazenyl) propanoate (5) (0.336g, 0.001mole), in ethanol (10ml) and sodium metal was refluxed for 4 hours. To produce the intended compound 6, the layer of precipitate that developed was passed through a filter off, evaporated, and reconstituted from methanol as gray powder; yield (0.5g, 86%); m.p.225-227^{0}C; Mol.Formula:$C_{25}H_{19}N_7O_4S_3$; M.Wt.:577.65. IR (KBr): ν/cm^{-1}= 3402 (OH), 3326, 3305, 3254 (3NH), 2934, 2852 (CH aliphatic), 1687(CONH) 1635(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 6.85 (t, 2H, *J*=5Hz, Ar-H), 7.10 (d, 2H, *J*=7Hz, Ar-H), 7.17 (d, 1H, *J*=7Hz, C^5H_{thiazole}), 7.47 (d, 1H, *J*=7.5Hz, C^4H_{thiazole}), 7.67 (d, 4H, *J*=7.6Hz, Ar-H), 7.79 (dd, 3H, *J*= 7.7Hz, Ar-H), 8.00 (d, 2H, *J*=8Hz, Ar-H), 9.98 (s, 1H, NH), 10.27 (br. s, 1H, NH), 10.45 (br. s, 1H, NH), 11.82 (br. s, 1H, OH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 111.62 (1C), 113.43 (1C), 113.48 (1C), 115.83 (1C), 117.88 (1C), 118.69 (3C), 118.95 (2C), 125.85 (1C), 127.74 (1C), 127.83 (3C), 127.91 (2C), 132.07 (1C), 138.83 (1C), 140.29 (1C), 142.23 (1C), 153.00 (1C), 155.71 (1C), 157.46 (1C), 164.87 (CON). MS (EI): m/z (%): 577.32 (M^+, 10.65), 516.29 (17.88), 368.59 (83.15), 327.43 (21.53), 295.54 (18.09), 219.65 (20.60), 171.48 (15.71), 97.26 (31.24), 69.18 (77.96), 55.34 (100.00).

Synthesis of ethyl 4-amino-2-(phenylamino)-5-((4-(*N*-(pyridin-2-yl) sulfamoyl) phenyl) carbamoyl)thiophene-3-carboxylate (8)

A solution of 2-chloro-N-(4-(N-(pyridin-2-yl)sulfamoyl) phenyl) acetamide (2) (0.3 g, 0.001 mole) and ethyl-2-cyano-3-mercapto-3-(phenylamino)acrylate [40] (7) (0.001mole), in ethanol with sodium metal (0.046g, 0.001mole) was refluxed for 6 hours . For the purpose of getting the ideal compound 8, the solid substance that formed had been passed off, air-dried, and reconstructed from ethanol.

as buff residue; produce (0.48g, 89%); m.p.>300^{o}C; Mol.Formula:$C_{25}H_{23}N_5O_5S_2$; M.Wt.: 537.61. IR (KBr): ν/cm^{-1}= 3487, 3418, 3242 (NH_2, 3NH), 2992, 2940 (CH aliphatic), 1724 (ester group) 1637 (CO, amide), 1619 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 1.16 (t, 3H, *J*=1.1Hz, CH_3), 4.02 (q, 2H, *J*=2.6Hz, CH_2), 5.57 (br. s, 2H, NH_2), 6.37 (br. s, 1H), 6.45 (d, 1H, *J*=6.4Hz, Ar-H), 6.58 (dd, 1H, *J*=6.5Hz, Ar-H), 7.00 (t, 1H, *J*=5.3Hz, Ar-H), 7.23 (t, 2H, *J*=5.4Hz, Ar-H), 7.60 (t, 2H, *J*= 5.7Hz, Ar-H), 7.66 (dd, 1H, *J*= 7.6Hz, Ar-H), 7.73 (d, 2H, *J*= 7.7Hz, Ar-H), 7.85 (d, 1H, *J*= 7.8Hz, Ar-H), 10.01 (s, 1H, NH), 12.13 (br. s, 1H, NH), 12.32 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 14.91 (CH_3), 58.24 (CH_2), 112.41(1C), 118.15 (2C), 122.58 (2C), 123.43 (2C), 127.25 (2C), 128.14 (2C), 128.30 (2C), 136.52 (2C), 147.52(1C), 147.64(1C), 147.78(1C), 155.24(1C),

158.03(1C), 158.78(1C), 160.95(1C), 163.19 (CON). MS (EI): m/z (%): 537.33 (M^+, 42.72), 522.60 (49.89), 489.85 (38.32), 475.65 (54.16), 461.81 (44.15), 378.52 (84.28), 353.61 (44.88), 246.84 (79.18), 178.80 (100.00).

Synthesis of 4-((2-amino-2,3-dihydrothiazol-5-yl)amino)-*N*-(pyridin-2-yl)benzenesulfonamide (9)

Refluxing compound 2 (0.3g, 0.001mole) with thiourea (0.076g, 0.001mole), in ethanol and 3 drops of triethyl amine for 6 hours. For the purpose of getting the wanted compound 9, the solid material that produced was squeezed eliminated, dried, and reorganized from methanol as brown precipitate; give up (0.3g, 85%); m.p.250-252^{0}C; Mol.Formula:$C_{14}H_{13}N_5O_2S_2$; M.Wt.: 347.41. IR (KBr): ν/cm^{-1}= 3352, 3318, 3191 (NH_2, 2NH), 2939 (CH aliphatic). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 5.94 (br. s, 2H, NH_2), 6.52 (d, 3H, *J*=6.5Hz, Ar-H), 6.87 (t, 1H, *J*=5Hz, Ar-H), 7.05 (d, 2H, *J*=7Hz, Ar-H), 7.63 (t, 1H, *J*=7.5Hz, Ar-H), 8.07 (d, 1H, *J*=5.7Hz, Ar-H) 10.76 (s, 1H, NH), 10.98 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 112.14(1C), 112.14 (3C), 117.10(1C), 125.67(1C), 128.92 (3C), 138.75(1C), 146.33(1C), 152.32 (2C), 152.76 (CS). MS (EI): m/z (%): 347.39 (M^+, 28.01), 335.12 (69.72), 325.99 (42.91), 298.06 (67.91), 267.15 (56.57), 257.74 (100.00), 196.23 (56.04), 168.18 (53.37), 96.16 (45.47).

Reaction of chloroacetamide derivative 2 with primary amines: Synthesis of compounds 10-16.

General procedure:

A mixture of 2-chloro-N-(4-(N-(pyridin-2-yl)sulfamoyl) phenyl) acetamide (2) (0.3g, 0.001 mole) in ethanol (10 ml), and amines (0.001mole) (namely, 4-amino-N-(pyridin-2-yl)benzenesulfonamide, 6-aminopyrimidine-2,4-diol [41], 4H-1,2,4-triazol-3-amine, 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one, thiophen-2-amine, and or thiazol-2-amine) with few drops of triethyl amine was refluxed for 6 hours . For the production of the planned compound 10, 12-16, the particles that set up was purified off, dehydrated, and reconstructed from ethanol, in order that.

***N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)-2-((4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)amino)acetamide (10)**

As buff powder; yield (0.48g, 89%); m.p.>300^{0}C; Mol.Formula:$C_{24}H_{22}N_6O_5S_2$; M.Wt.: 538.60. IR (KBr): ν/cm^{-1}= 3403, 3328, 3303, 3115 (4NH), 2962 (CH aliphatic), 1687(CO, amide) 1635(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 4.81 (s, 2H, CH_2), 6.73 (t, 2H, *J*=5.5Hz, Ar-H), 7.18 (d, 4H, *J*=7Hz, Ar-H), 7.49 (d, 4H, *J*= 7.5Hz, Ar-H), 7.68 (d, 2H, *J*=7.8Hz, Ar-H), 7.80 (t, 2H, *J*= 7.9Hz, Ar-H), 7.93 (br. s, 2H, 2NH), 8.02 (d, 2H, *J*=7.8Hz), 10.30 (br. s, 2H, 2NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 56.92(1C), 109.79(1C), 110.16(1C), 115.30(1C), 116.32(1C), 116.75(1C), 117.26(1C), 117.98(1C), 125.33(1C), 125.79(1C), 125.93(1C), 134.99(1C), 139.05(1C), 140.63(1C), 141.39(1C), 141.83(1C), 142.89(1C), 143.57(1C), 154.01(1C), 156.78(1C), 162.22 (3C), 165.04 (CON). MS (EI): m/z (%): 538.26 (M^+, 20.24), 487.60 (45.76), 367.40 (69.90), 312.53 (24.97), 287.28 (35.39), 222.08 (61.13), 215.65 (100.00), 211.01 (58.79), 177.46 (54.63).

2-((2,6-dihydroxypyrimidin-4-yl)amino)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide (12)

As buff precipitate; convey (0.4g, 96%); m.p.>300^{0}C; Mol.Formula:$C_{17}H_{16}N_6O_5S$; M.Wt.: 416.41. IR (KBr): ν/cm^{-1}= 3409, 3334, 3249, 3181, 3142 (2OH, 3NH), 2935, 2831 (CH aliphatic), 1699 (CO, amide), 1635(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 3.07 (s, 2H, CH_2), 4.39 (s, 1H, CH), 6.18 (br. s, 1H, NH), 6.85 (t, 1H, *J*=5.1Hz, Ar-H), 7.10 (d, 1H, *J*=7.1Hz, Ar-H), 7.68 (t, 1H, *J*=5.7Hz, Ar-H), 7.79 (q, 4H, *J*=5.2Hz, Ar-H), 8.00 (d, 1H, *J*= 7.9Hz, Ar-H), 9.99 (s, 1H, NH), 10.08 (br. s, 2H, NH, OH), 11.71 (br. s, 1H, OH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 54.02 (1C, CH_2), 74.20 (1C, CH), 113.58(1C), 115.83(1C), 116.73(1C), 119.03(1C), 125.98(1C), 127.80(1C), 135.90(1C), 140.29(1C),

141.92(1C), 143.79(1C), 151.09(1C), 153.04(1C), 155.24(1C), 164.44(CON), 169.14 (1C). MS (EI): m/z (%): 416.07 (M^+, 20.35), 390.79 (82.76), 365.20 (100.00), 337.02 (18.61), 316.54 (41.33), 291.07 (49.92), 230.19 (40.90), 175.38 (72.88), 141.82 (33.66).

2-((4*H*-1,2,4-triazol-3-yl)amino)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide (13)

As white powder; give away (0.3g, 80%); m.p.>300^{0}C; Mol.Formula:$C_{15}H_{15}N_7O_3S$; M.Wt.: 373.39. IR (KBr): ν/cm^{-1}= 3449, 3404, 3329, 3117 (4NH), 1688 (CONH), 1635 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 3.07 (br. s, 2H, CH_2), 5.03 (br. s, 1H, NH), 6.85 (t, 1H, *J*=5Hz, Ar-H), 7.10 (d, 1H, *J*=7Hz, Ar-H), 7.68 (dd, 2H, *J*=7.6Hz, Ar-H), 7.77 (q, 4H, *J*= 5Hz, Ar-H), 8.00 (d, 1H, *J*=8Hz, Ar-H), 9.99 (br. s, 1H, NH), 10.84 (s, 1H, NH), 11.77 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 54.02 (1C, CH_2), 113.52(1C), 115.84(1C), 118.98 (3C), 127.77 (3C), 135.82(1C), 140.19(1C), 141.93(1C), 143.81(1C), 153.00(1C), 169.25(CON). MS (EI): m/z (%): 373.77 (M^+, 13.09), 353.30 (36.15), 314.13 (100.00), 298.97 (20.20), 254.80 (35.10), 208.08 (32.50), 180.19 (21.03), 158.83 (19.36), 93.98 (9.86).

2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)amino)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide (14)

As buff powder; relinquish (0.48g, 97%); m.p.>300^{0}C; Mol.Formula:$C_{24}H_{24}N_6O_4S$; M.Wt.: 492.55. IR (KBr): ν/cm^{-1}= 3248, 3139, 3114 (3NH), 2934, 2816 (CH aliphatic), 1696 (CONH), 1636 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 2.49 (s, 3H, CH_3), 4.78 (s, 3H, CH_3), 5.02 (s, 2H, CH_2), 6.73 (t, 1H, *J*=5 Hz, Ar-H), 7.15 (d, 3H, *J*=7Hz, Ar-H), 7.47 (d, 3H, *J*=7.5Hz, Ar-H), 7.65 (d, 2H, *J*=7.6Hz, Ar-H), 7.81 (d, 2H, *J*= 5.8Hz, Ar-H), 8.00 (d, 2H, *J*= 7.9Hz, Ar-H), 10.28 (s, 1H, NH), 10.71, 10.82 (br. s, 2H, 2NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 18.51(1C), 28.04(1C), 56.73(1C), 110.12 (2C), 116.64(1C), 117.86 (3C), 125.80 (3C), 138.83 (2C), 140.39 (2C), 141.36 (2C), 141.72 (2C), 153.47 (2C), 164.79, 167.63 (CON). MS (EI): m/z (%): 492.90 (M^+, 20.91), 459.28 (54.91), 447.64 (59.53), 393.83 (76.66), 369.51 (79.83), 242.10 (52.24), 163.58 (88.23), 138.90 (68.42), 77.05 (100.00).

***N*-(4-(*N*-(pyridin-2-yl) sulfamoyl)phenyl)-2-(thiophen-2-ylamino)acetamide (15)**

As buff powder; yield (0.48g, 89%); m.p.>300^{0}C; Mol.Formula:$C_{17}H_{16}N_4O_3S_2$; M.Wt.: 388.46. IR (KBr): ν/cm^{-1}= 3250, 3139, 3113 (3NH), 2935, 2818 (CH aliphatic), 1696 (CO, amide), 1637 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 3.07 (s, 2H, CH_2), 6.85 (t, 1H, *J*=5Hz, Ar-H), 7.11 (d, 2H, *J*=7Hz, Ar-H), 7.68 (m, 2H), 7.79 (q, 5H, *J*=7.8Hz, Ar-H), 7.99 (d, 1H, *J*= 7.9Hz), 10.00 (s, 2H, NH), 11.75 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 53.97(1C), 113.47 (2C), 113.71(1C), 115.85(1C), 118.94 (3C), 127.72 (3C), 135.80(1C), 141.88 (2C), 143.80(1C), 152.92(1C), 169.07 (CON). MS (EI): m/z (%): 388.67 (M^+, 15.11), 387.63 (26.43), 378.41 (100.00), 332.20 (33.49), 295.85 (55.37), 257.22 (76.66), 225.30 (62.86), 201.36 (34.69), 151.98 (24.85).

***N*-(4-(*N*-(pyridin-2-yl) sulfamoyl)phenyl)-2-(thiazol-2-ylamino)acetamide (16)**

As buff residue; part with (0.35g, 90%); m.p.>300^{0}C; Mol.Formula:$C_{16}H_{15}N_5O_3S_2$; M.Wt.:389.45. IR (KBr): ν/cm^{-1}= 3404, 3328, 3116 (3NH), 1688 (CO, amide), 1635(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 4.78 (br. s, 2H, CH_2), 5.03 (br. s, 1H, NH), 6.74 (t, 2H, *J*=5Hz, Ar-H), 7.15 (d, 2H, *J*=7Hz, Ar-H), 7.47 (d, 2H, *J*=7.5Hz, Ar-H), 7.66 (d, 1H, *J*= 7.6Hz, Ar-H), 7.81 (t, 2H, *J*=5.8Hz, Ar-H), 8.01 (d, 1H, *J*=8Hz, Ar-H), 10.29 (br. s, 1H, NH), 10.72 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 56.79 (1C, CH_2), 110.20 (2C), 116.13(1C), 116.68(1C), 117.90(1C), 125.86 (2C), 140.45(1C), 141.46 (2C), 141.79(1C), 153.49 (2C), 164.87 (CON). MS (EI): m/z (%): 389.27 (M^+, 26.52), 350.93 (53.53), 326.76 (58.65), 289.15 (81.43), 251.73 (35.77), 197.30 (61.45), 146.35 (69.81), 125.69 (47.32), 88.61 (100.00).

Synthesis of 2-(3-oxo-3,4-dihydroquinoxalin-1(2H)-yl)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide (18)

Refluxing compound 2 (0.3g, 0.001mole) with 3,4-dihydroquinoxalin-2(1H)-one [42] (17) (0.336g, 0.001mole), in ethanol (10ml) and 3drops of triethyl amine for 4 hours. For the extraction of the required compound 18, the solid that formed was separated off, drained and reformed from methanol.

As brown powder; yield (0.36g, 82%); m.p.>300^{0}C; Mol.Formula:$C_{21}H_{19}N_5O_4S$; M.Wt.: 437.47. IR (KBr): ν/cm^{-1}= 3403, 3327, 3262 (3NH), 2957 (CH aliphatic), 1688 (CO, amide) 1635(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 3.92 (s, 2H, CH_2), 4.78 (s, 2H, CH_2), 6.74 (t, 1H, *J*=6.8Hz), 7.17 (d, 3H, *J*=7Hz, Ar-H), 7.49 (d, 1H, *J*=7.5Hz, Ar-H), 7.65-7.74 (m, 3H, Ar-H), 7.81 (d, 2H, *J*= 7.8Hz, Ar-H), 8.01 (d, 2H, *J*= 8Hz, Ar-H), 9.77 (br. s, 1H, NH), 10.32 (br. s, 1H, NH), 10.88 (br. s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 62.85(1C), 68.51(1C), 85.97(1C), 96.95(1C), 107.76(1C), 108.23(1C), 110.03(1C), 111.49(1C), 115.68(1C), 116.01(1C), 116.82(1C), 117.36(1C), 118.01 (2C), 125.96(1C), 134.27(1C), 138.82(1C), 141.23(1C), 145.19(1C), 149.04 (CON), 153.83 (CON). MS (EI): m/z (%): 437.12 (M$^+$, 19.39), 436.21 (22.11), 335.86 (5.74), 321.16 (15.63), 264.28 (100.00), 206.35 (6.19), 165.06 (3.81), 103.77 (15.39), 55.16 (16.67).

Synthesis of 2-(2,3-dioxoindolin-1-yl)-*N*-(4-(*N*-(pyridin-2-yl)sulfamoyl)phenyl)acetamide (19)

Refluxing compound 2 (0.3g, 0.001mole) with indoline-2,3-dione (0.147g, 0.001mole), in acetone (10ml) and potassium carbonate (0.138g, 0.001mole) for 4 hours. In order to generate the expected compound 19, the solid that formed was picked off, evaporated, and detached from methanol as buff powder; yield (0.41g, 94%); m.p.>300^{0}C; Mol.Formula:$C_{21}H_{16}N_4O_5S$; M.Wt.: 436.44. IR (KBr): ν/cm^{-1}= 3402, 3328 (2NH), 1687(CO, amide) 1636(C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 4.76 (s, 2H, CH_2), 6.73 (t, 2H, *J*=5Hz, Ar-H), 7.18 (d, 2H, *J*=7.1Hz, Ar-H), 7.49 (d, 2H, *J*=7.4Hz, Ar-H), 7.57 (d, 1H, *J*=7.5Hz, Ar-H), 7.65 (d, 2H, *J*= 7.6Hz, Ar-H), 7.74 (d, 1H, *J*=7.7Hz, Ar-H), 7.81 (t, 1H, *J*=5.8Hz, Ar-H), 8.01 (d, 1H, *J*=8Hz, Ar-H), 10.32 (s, 1H, NH), 10.75 (s, 1H, NH). ^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 56.66(1C), 110.77(1C), 115.87(1C), 116.53(1C), 117.15 (2C), 117.85(1C), 118.42(1C), 125.82 (2C), 126.73 (2C), 126.96(1C), 138.79(1C), 141.36(1C), 141.69(1C), 153.52(1C), 155.12(1C), 159.30 (C=O), 164.46 (C=O), 164.88 (CON). MS (EI): m/z (%): 436.33 (M$^+$, 25.25), 403.53 (26.10), 372.81 (40.80), 357.15 (100.00), 346.74 (99.48), 339.61 (60.74), 304.55 (45.96), 231.71 (58.50), 141.32 (80.58).

Synthesis of 2-oxo-2-((4-(*N*-(pyridin-2-yl)sulfamoyl) phenyl) amino)ethyl morpholine-4-carbodithioate (21)

Refluxing compound 2 (0.3g, 0.001mole) with morpholine-4-carbodithioic acid [43] (20) (0.163g, 0.001mole), in ethanol and 3drops of triethyl amine for 4 hours. For the purpose of getting the preferred compound 21, the solid product that formed underwent filtering off, was completely dry, and reconstituted from methanol as white powder; deliver (0.42g, 93%); m.p.250-252^{0}C; Mol.Formula:$C_{18}H_{20}N_4O_4S_3$; M.Wt.: 452.56. IR (KBr): ν/cm^{-1}= 3316, 3278 (2NH), 2916, 2850 (CH aliphatic), 1699 (CO, amide), 1631 (C=N). ^{1}H NMR (DMSO-d6, 500MHz, δ ppm): 3.66 (t, 4H, *J*=2.7Hz, CH_2), 3.94 (br. s, 2H, CH_2), 4.23 (t, 4H, *J*=3Hz, CH_2), 6.70 (t, 1H, *J*=5Hz, Ar-H), 6.94 (d, 1H, *J*=6.9Hz, Ar-H), 7.53 (t, 1H, *J*= 5.6Hz, Ar-H), 7.63 (d, 1H, *J*=7.6Hz, Ar-H), 7.75 (d, 3H, *J*=7.7Hz, Ar-H), 7.94 (d, 1H, *J*=7.9Hz, Ar-H), 10.60 (br. s, 2H, NH).^{13}C NMR (DMSO-d6, 125MHz, δ ppm): 41.05 (1C), 41.27 (1C), 65.60 (1C), 65.69 (1C), 67.14 (1C), 113.66 (1C), 114.79 (1C), 118.48 (1C), 127.70 (1C), 137.32 (1C), 139.17(1C), 141.68 (1C), 144.59 (1C), 154.91 (1C), 165.87 (CON), 192.99 (1C), 194.96 (1C), 196.30 (C=S). MS (EI): m/z (%): 453.95 (M^{+1}, 40.73), 411.59 (73.52), 384.41 (45.35), 311.24 (94.99), 280.21 (43.94), 177.00 (34.85), 118.48 (89.17), 42.46 (100.00).

Cytotoxicity Assay

To assess the anticancer potential of the synthesized compounds, their effects on a panel of human cancer cell lines representing diverse

tumor types were evaluated. (PC3), liver (HEPG-2), (MCF-7), and (HCT-116) cancer cell lines, procured from the American Type Culture Collection (ATCC) through VACSERA, Egypt's distributor for biological products and vaccines, were employed. Doxorubicin, a clinically established anticancer agent, served as a positive control to benchmark the efficacy of the novel compounds.

Materials and Methods

Cell Culture: Human cancer cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (GIBCO, UK), 100 U/mL penicillin, and 100 µg/mL streptomycin (GIBCO, UK) in a humidified incubator at 37°C with 5% CO2.

MTT Assay:

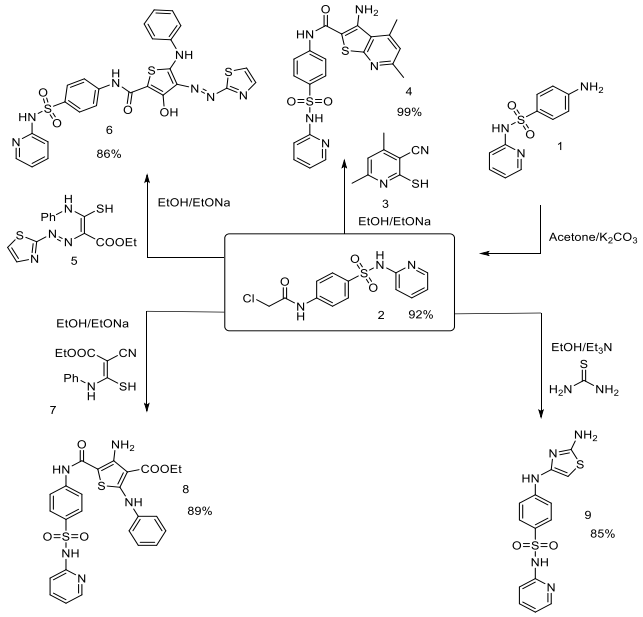
The cytotoxicity of compounds was determined using the MTT assay. Briefly, cells were seeded at a density of 1.0×10^4 cells/well in 96-well plates and incubated for 48 hours. Subsequently, cells were exposed to various concentrations of the test compounds for 24 hours. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 hours. The formazan product formed was solubilized with 100 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a plate reader (EXL 800, USA). Cell viability was expressed as a percentage of control (untreated) cells, calculated using the formula: (A570 treated/A570 control) × 100. [44, 45].

3. Results and Discussion

3.1. Chemistry

The synthetic pathways for the target compounds are outlined in Schemes 1-3 and Figure 1. Acylation of 4-amino-N-(pyridin-2-yl)benzene sulfonamide with chloroacetyl chloride in acetone, catalyzed by potassium carbonate, afforded 2-chloro-N-(4-(N-pyridine-2-yl)sulfamoyl)phenyl)acetamide (2). Structural elucidation of compound 2 was supported by spectroscopic data. Infrared spectroscopy exhibited characteristic bands at 3322, 3203, and 1677 cm^{-1} attributable to two NH functionalities and an amide carbonyl group, respectively. The ^1H NMR spectrum displayed signals at δ 4.26, 10.62, and 12.00 ppm corresponding to the aliphatic protons and two NH protons. The ^{13}C NMR spectrum revealed resonances at δ 43.50 and 171.59 ppm assigned to the aliphatic carbon and amide carbonyl carbon, respectively.

Compound 4 was synthesized by reacting compound 2 with 2-mercapto-4,6-dimethylnicotinonitrile under reflux conditions in ethanol using sodium metal as a catalyst. Similarly, thiophene derivative 6 was obtained through the reaction of compound 2 with ethyl-3-mercapto-3-(phenylamino)-2-(thiazol-2-yldiazenyl) propanoate under the same conditions. Furthermore, compound 8 was prepared by the condensation of compound 2 with ethyl-2-cyano-3-mercapto-3-(phenylamino) acrylate. Finally, compound 9 was synthesized via cyclocondensation of compound 2 with thiourea in refluxing ethanol



Scheme 1: Synthesis of thiophene and thiazole derivatives

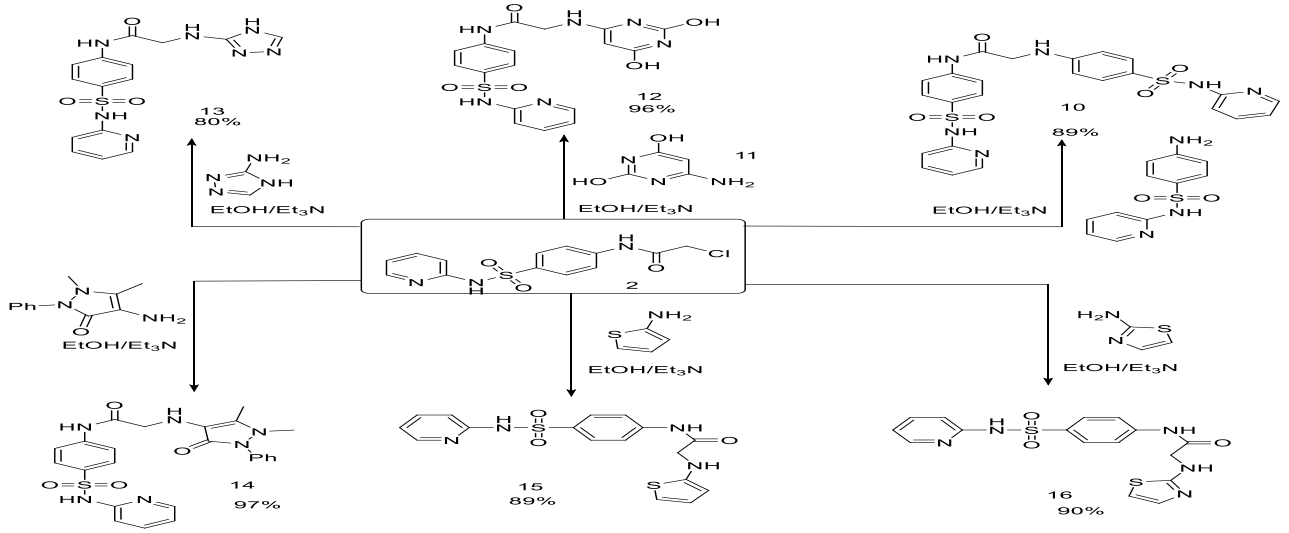
The structural elucidation of the synthesized thiophene and thiazole derivatives (4-9) was achieved through spectroscopic analysis. The absence of proton resonances attributable to active methylene groups at δ 4.26 ppm in the ^1H NMR spectra provided compelling evidence for the cyclization of precursors into the

thiophene and thiazole products (4-9), as depicted in Scheme 1. Specifically, the structural assignment of compound 4 was corroborated by its infrared spectrum, exhibiting absorption bands at 3474, 3451, 3228, 3115, 2924, and 1663 cm^{-1} corresponding to NH_2, NH functionalities, aliphatic moieties, and an amide carbonyl group, respectively. The ^1H NMR spectrum displayed signals at δ 2.47, 2.71, 6.99, 7.04, and 9.52 ppm assignable to six protons from two methyl groups, NH_2, and two NH groups. Furthermore, the ^{13}C NMR spectrum exhibited characteristic resonances at δ 19.99, 23.97, and 164.45 ppm corresponding to two CH_3 groups and the amide carbonyl group.

For the thiophene derivative 6, the infrared spectrum displayed absorption bands at 3402, 3326, 3305, 3254, and 1687 cm^{-1} attributable to OH, NH, and amide carbonyl functionalities. In the ^1H NMR spectrum, aromatic protons were observed within the δ 6.85-8.00 ppm range, while three NH groups and an OH group appeared at δ 9.98, 10.27, 10.45, and 11.82 ppm, respectively. The ^{13}C NMR spectrum exhibited a characteristic resonance at δ 164.87 ppm for the amide carbonyl group. Mass spectrometric analysis confirmed the proposed structure with a molecular ion peak at m/z = 577.32, consistent with the molecular formula $C_{25}H_{19}N_7O_4S_3$.

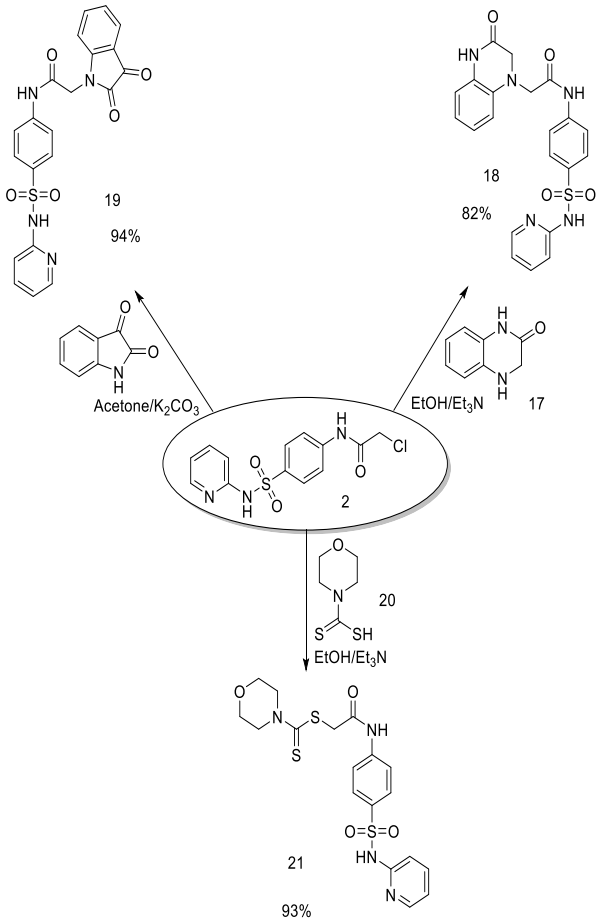
Regarding the thiophene derivative 8, the infrared spectrum displayed absorption bands at 3487, 3418, 3242, and 2992 cm^{-1} corresponding to NH_2 and three NH groups, and at 1724 and 1637 cm^{-1} corresponding to the ester and amide carbonyl groups, respectively. The ^1H NMR spectrum exhibited signals at δ 1.16 and 4.02 ppm for the ester protons, while aromatic protons of the phenyl ring were observed within the δ 6.37-7.85 ppm range. Additionally, NH_2 and three NH protons were observed at δ 5.57, 10.01, 12.13, and 12.32 ppm, respectively. The ^{13}C NMR spectrum displayed characteristic peaks at δ 14.91 and 58.24 ppm corresponding to the CH_3 and CH_2 groups of the ester, and at δ 163.19 ppm for the amide carbonyl group. Mass spectrometric analysis confirmed the proposed structure with a molecular ion peak at m/z = 537.33, consistent with the molecular formula $C_{25}H_{23}N_5O_5S_2$.

The infrared spectrum of compound 9 exhibited absorption bands at 3352, 3318, and 3191 cm^{-1} attributable to NH_2 and two NH groups. The ^1H NMR spectrum displayed a signal at δ 5.94 ppm corresponding to the protons of the amino groups, while the absence of active methylene protons at δ 4.26 ppm and the presence of aromatic protons within the δ 6.52-8.07 ppm range were noted. Additionally, two NH groups appeared at δ 10.76 and 10.98 ppm. The ^{13}C NMR spectrum exhibited a characteristic resonance at δ 152.76 ppm for the CS group. Mass spectrometric analysis confirmed the proposed structure with a molecular ion peak at m/z = 347.39, consistent with the molecular formula $C_{14}H_{13}N_5O_2S_2$.



Scheme 2: Reactions of chloroacetamide derivative 2 with primary aromatic and heterocyclic amines

A series of derivatives 10, 12-16 were synthesized through the reaction of compound 2 with various amines, including 4-amino-N-(pyridin-2-yl)benzenesulfonamide, 6-aminopyrimidine-2,4-diol, 4H-1,2,4-triazol-3-amine, 4-amino-1,5-dimethyl-2-phenylpyrazolone, and thiophen-2-amine or thiazol-2-amine, in the presence of triethylamine in ethanol (Scheme 2). Structural elucidation of compounds 10-16 was achieved through spectroscopic analysis. For instance, the structure of compound 10 was confirmed by infrared spectroscopy, exhibiting absorption bands at 3403, 3328, 3303, 3115, 2962, and 1687 cm^{-1} corresponding to four NH functionalities, an aliphatic group, and an amide carbonyl group, respectively. Proton NMR spectroscopy displayed signals at δ 4.81, δ 7.93, and δ 10.30 ppm attributable to two protons of an active methylene group and four NH protons. Carbon-13 NMR spectroscopy revealed a resonance at δ 165.04 ppm corresponding to the amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 538.26, consistent with the molecular formula $C_{24}H_{22}N_6O_5S_2$. The infrared spectrum of compound 12 exhibited absorption bands at 3409, 3334, 3249, 3181, and 3142 cm^{-1} indicative of two hydroxyl groups and three NH functionalities, along with a band at 1699 cm^{-1} assigned to the amide carbonyl group. Proton NMR spectroscopy revealed a signal at δ 3.07 ppm for methylene protons, aromatic proton resonances within the δ 6.85-8.00 ppm range, and signals for three NH and two OH groups at δ 6.18, δ 9.99, δ 10.08, and δ 11.71 ppm, respectively. Carbon-13 NMR spectroscopy displayed a characteristic resonance at δ 164.44 ppm corresponding to the amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 416.07, consistent with the molecular formula $C_{17}H_{16}N_6O_5S$. The infrared spectrum of compound 13 exhibited absorption bands at 3449, 3404, 3329, 3117, and 1688 cm^{-1} attributable to four NH functionalities and an amide carbonyl group. Proton NMR spectroscopy displayed aromatic proton resonances within the δ 6.85-8.00 ppm range and signals for four NH protons at δ 5.03, δ 9.99, δ 10.84, and δ 11.77 ppm. Carbon-13 NMR spectroscopy revealed a resonance at δ 169.25 ppm corresponding to the amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 373.77, consistent with the molecular formula $C_{15}H_{15}N_7O_3S$. The infrared spectrum of compound 14 exhibited absorption bands at 3248, 3139, 3114, 2934, and 1696 cm^{-1} indicative of three NH functionalities, aliphatic groups, and an amide carbonyl group. Proton NMR spectroscopy displayed signals at δ 2.49 and 4.78 ppm for methyl protons, aromatic proton resonances within the δ 6.73-8.00 ppm range, and signals for three NH protons at δ 10.28, 10.71, and 10.82 ppm. Carbon-13 NMR spectroscopy revealed characteristic resonances at δ 18.51, 28.04, 164.79, and 167.63 ppm corresponding to two methyl groups, a carbonyl group, and an amide carbonyl group. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 492.90, consistent with the molecular formula $C_{24}H_{24}N_6O_4S$. The infrared spectrum of compound 15 exhibited absorption bands at 3250, 3139, and 3113 cm^{-1} indicative of three NH functionalities, and a band at 1696 cm^{-1} assigned to the amide carbonyl group. Proton NMR spectroscopy displayed signals for three NH groups at δ 10.00 and 11.75 ppm. Carbon-13 NMR spectroscopy revealed a resonance at δ 169.07 ppm corresponding to the amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 388.67, consistent with the molecular formula $C_{17}H_{16}N_4O_3S_2$. The infrared spectrum of compound 16 exhibited absorption bands at 3404, 3328, 3116, and 1688 cm^{-1} attributable to three NH functionalities and an amide carbonyl group. Proton NMR spectroscopy displayed a signal at δ 4.78 ppm for methylene protons, aromatic proton resonances within the δ 6.74-8.01 ppm range, and signals for three NH groups at δ 5.03, 10.29, and 10.72 ppm. Carbon-13 NMR spectroscopy revealed a resonance at δ 164.87 ppm corresponding to the amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 389.27, consistent with the molecular formula $C_{16}H_{15}N_5O_3S_2$



Scheme 3: Reactions of chloroacetamide derivative 2 with secondary heterocyclic amines

Novel derivatives (18-21) were synthesized by reacting compound 2 with various secondary amines (Scheme 3). Specifically, treatment of compound 2 with 3,4-dihydroquinoxalin-2(1H)-one or morpholine-4-carbodithioic acid in ethanol, catalyzed by triethylamine, afforded compounds 18 and 21, respectively. Compound 19 was obtained in a 94% yield through the reaction of compound 2 with indoline-2,3-dione in acetone using potassium carbonate. Structural elucidation of compounds 18-21 was achieved through spectroscopic analysis.

The infrared spectrum of compound 18 exhibited absorption bands at 3403, 3327, 3262, and 1688 cm^{-1} corresponding to three NH functionalities and an amide carbonyl group. Proton NMR spectroscopy displayed signals at δ 3.92, 4.78, 9.77, 10.32, and 10.88 ppm attributable to four protons of two active methylene groups and three NH protons. Carbon-13 NMR spectroscopy revealed characteristic resonances at δ 149.04 and 153.83 ppm indicative of two amide carbonyl carbons. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 437.12, consistent with the molecular formula $C_{21}H_{19}N_5O_4S$. The infrared spectrum of compound 19 exhibited absorption bands at 3402, 3328, and 1687 cm^{-1} corresponding to two NH functionalities and an amide carbonyl group. Proton NMR spectroscopy displayed a signal at δ 4.76 ppm for the two protons of the active methylene group, aromatic proton resonances within the δ 6.73-8.01 ppm range, and signals for two NH protons at δ 10.32 and 10.75 ppm. Carbon-13 NMR spectroscopy revealed characteristic resonances at δ 159.30, 164.46, and 164.88 ppm corresponding to two carbonyl carbons and an amide carbonyl carbon. Mass spectrometry confirmed the proposed structure with a molecular ion peak at m/z = 436.33, consistent with the molecular formula $C_{21}H_{16}N_4O_5S$. The infrared spectrum of compound 21 exhibited absorption bands at 3316 and 3278 cm^{-1} corresponding to two NH functionalities and a band at 1699 cm^{-1} assigned to the amide carbonyl group. Proton NMR spectroscopy displayed signals at δ 3.94, 3.66, and 4.23 ppm for the protons of the active methylene group and the aliphatic protons of the morpholine moiety, along with aromatic proton resonances within the δ 6.70-7.94 ppm range and signals for two NH protons at δ 10.60 ppm. Carbon-13 NMR spectroscopy revealed characteristic resonances at δ 165.87 and 196.30 ppm corresponding to the amide carbonyl carbon and C=S, respectively.

3.2. Anticancer Activity

The anticancer properties of the synthesized compounds were evaluated through a cytotoxicity assay against a panel of human cancer cell lines. Inhibitory activities were assessed, and the results are summarized in Figure 1 and Table 1. Compounds demonstrating promising or comparable inhibitory effects were subjected to dose-response studies to determine their IC50 values. The human cancer cell lines employed in this study included PC-3 (prostate adenocarcinoma), HEPG-2 (hepatocellular carcinoma), MCF-7 (mammary gland adenocarcinoma), and HCT-116 (colorectal

carcinoma). Doxorubicin served as a positive control for comparative analysis. The MTT assay was utilized to quantify the compounds' effects on cell growth within these cancer cell lines.

The assay results indicated that the majority of synthesized compounds exhibited inhibitory activity against all four cancer cell lines, albeit with varying potencies. Notably, several compounds demonstrated significant antitumor efficacy, with IC50 values in the nanomolar range across all tested cell lines. MCF-7 and HepG2 cell lines generally displayed heightened sensitivity compared to the other cell lines. Among the compound classes, thiophene derivatives exhibited the most pronounced activity, followed by pyrazoles, quinoxalines, thiazoles, and pyrimidines.

Compound 8, a thiophene derivative, emerged as the most potent compound within the series, demonstrating IC50 values of 5.73 ± 0.3, 9.86 ± 0.7, 6.95 ± 0.4, and 16.42 ± 1.3 µM against HGP2, HCT-116, MCF-7, and PC-3 cell lines, respectively. Notably, the potency of compound 8 was comparable to that of the reference drug, doxorubicin. The thiophene-containing thienopyridine carboxamide derivative (compound 4) exhibited the second highest potency, with IC50 values of 10.19 ± 0.9, 9.21 ± 0.6, 13.56 ± 1.0, and 17.80 ± 1.3 µM against HGP2, HCT-116, MCF-7, and PC-3 cell lines, respectively. Incorporation of a phenyl pyrazolone ring into compound 2 (resulting in compound 14) enhanced inhibitory activity against HGP2, HCT-116, and MCF-7 cell lines, with IC50 values of 10.69 ± 0.8, 15.72 ± 1.2, and 12.91 ± 0.9 µM, respectively, while demonstrating moderate activity against the PC-3 cell line (IC50 23.77 ± 1.7 µM). In contrast, compound 18, containing a quinoxaline moiety, displayed potent activity against HGP2 and MCF-7 (IC50 values of 14.28 ± 1.2 and 19.83 ± 1.4 µM, respectively), moderate activity against HCT-116 (IC50 28.94 ± 2.1 µM), and weak activity against PC-3 (IC50 57.52 ± 3.3 µM). Interestingly, compound 6, incorporating both thiophene and thiazole moieties, exhibited potent activity against HGP2 (IC50 18.75 ± 1.4 µM) but significantly reduced activity against the other cell lines. Compound 13, bearing a triazole moiety, demonstrated moderate activity across all cell lines. Compound 12, containing a hydroxy pyrimidine ring, displayed potent activity specifically against HGP2 (IC50 19.14 ± 1.5 µM), with moderate activity against HCT-116 and MCF-7 and weak activity against PC-3. Conversely, compounds 2, 9, 16, 15, 19, and 21 did not exhibit significant improvements in activity. These findings suggest that the presence of a thiophene moiety, a free ester group, or a methyl group may contribute to enhanced inhibitory activity.

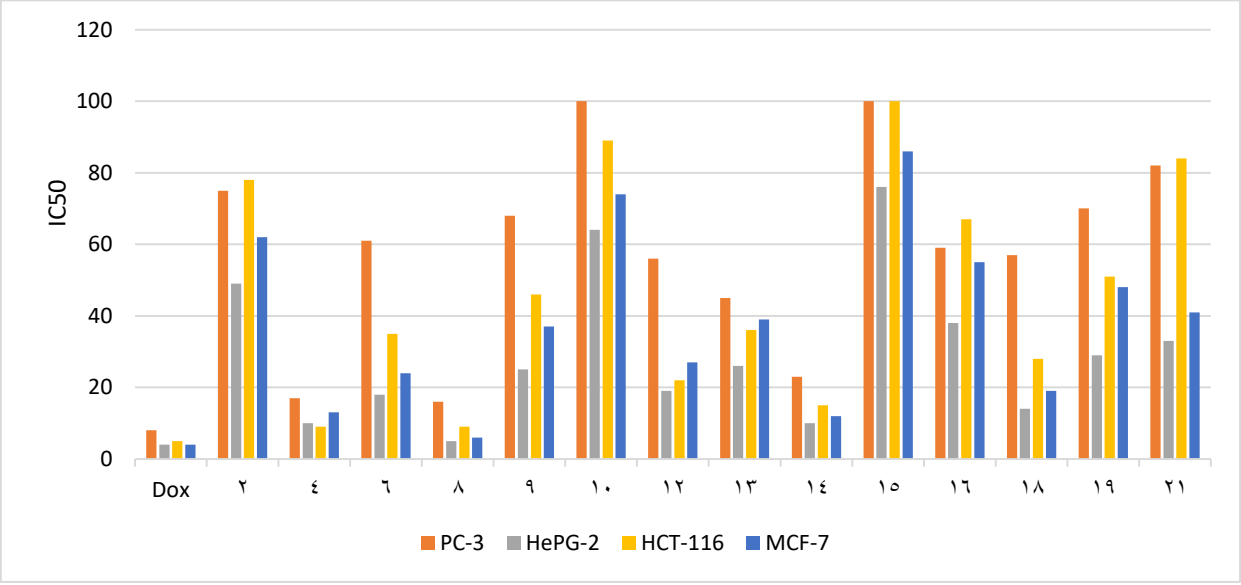


Figure 2 Anticancer activity for some sulfapyridine derivatives against four cell lines.

Table 1 Cytotoxic activity of some compounds against tumor cell lines.

Compd.No	In vitro Cytotoxicity IC50 (µM)*			
	PC-3	HePG-2	HCT-116	MCF-7
DOX	8.87±0.6	4.50±0.2	5.23±0.3	4.17±0.2
2	75.75±4.2	49.52±2.8	78.70±4.1	62.23±3.6
4	17.80±1.3	10.19±0.9	9.21±0.6	13.56±1.0
6	61.43±3.6	18.75±1.4	35.88±2.3	24.72±1.9
8	16.42±1.3	5.73±0.3	9.86±0.7	6.95±0.4
9	68.29±3.8	25.71±1.8	46.43±2.6	37.94±2.2
10	>100	64.27±3.4	89.63±4.7	74.40±3.9
12	56.25±3.1	19.14±1.5	22.78±1.6	27.06±1.8
13	45.92±2.5	26.39±1.7	36.12±2.2	39.83±2.1
14	23.77±1.7	10.69±0.8	15.72±1.2	12.91±0.9
15	>100	76.32±3.8	>100	86.03±4.3
16	59.13±3.3	38.89±2.3	67.71±3.5	55.29±2.9
18	57.52±3.3	14.28±1.2	28.94±2.1	19.83±1.4
19	70.40±3.9	29.79±1.9	51.76±2.9	48.14±2.7
21	82.02±4.4	33.83±2.2	84.63±4.3	41.70±2.4

* IC50 (µM): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)

** DOX: Doxorubicin

Conclusion

The activity of sulfapyridine derivatives was tested by incorporated with thiazole, indole, quinoxaline, and thiophene scaffold. According to the above results reported in this study, the role of our compounds as inhibitors of cancer cell growth was measured. Therefore, the effect of compounds 2-21 on cell cycle distribution for four cancer cell lines was investigated. The thiophene moiety of several of the compounds under test showed notable activity; compounds 4, and 8 most notably showed favorable activity contrary to four cancer cell lines for all cell lines followed by compounds 14,18 they revealed significant activity against several tumor cell lines related to the reference drug doxorubicin and may be promising as potential antitumor agents in preclinical scholarships. therefore, further investigate is desirable to establish their efficacy and safety in human scientific prosecutions.

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