

Evaluation of N-Sulfanoamide Pyridines from Chalchon with Anticancer Effect

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ABSTRACT: Reaction of 1-(2,5-dichlorothiophene-3-yl)ethanone **1** with p-chlorobenzaldehyde **2** was afforded 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** which used as starting material for synthesis of different heterocyclic compounds such as pyridine derivatives, thieno[2,3-b]pyridine **10**, **11** and N-sulfonyl aryl compounds **15a-c** by two methods traditional and grinding methods and compare time and percentage of yield in each method and the obtained N-sulfonyl aryl compounds were evaluated for their in vitro antitumor activity against Human hepatocarcinoma cell lines (HepG2).

KEYWORDS: 2,5-dichlorothiophene, chalchon, pyridine derivatives, thienopyridines, anticancer and Human hepatocarcinoma cell lines.

I. INTRODUCTION

Chalcones and their derivatives are very useful intermediates in synthesis of organic compounds [1-3]. They used as starting material for the synthesis of variety of heterocyclic compounds which are of physiological importance. Due to the presence of ketonic group in chalcone moiety confers biological activity upon it, like antioxidant [4], antitumor [5], analgesic [6], antifungal [7], antituberculosis [8], antimalarial [9], anti HIV [10,11] and anti-inflammatory [12] activities. In recent years, Green chemistry became one of the most important branches of chemistry due to Many organic solvents are hazardous and can be deleterious to human health. They are volatile and cause an environmental threat by polluting the atmosphere [13,14] The replacement of volatile organic solvents in organic reaction processes is an important green chemistry goal. Furthermore, the solvent-free or solid-state reactions have numerous advantages: reduced pollution, low costs, high yields and purities of products, simplicity in process and handling [15,16]. On the other hand the sulfonamide derivatives widely used in clinical medicine as pharmacological agents with a wide variety of biological actions [17] Sulfonamides are well renowned for their antibacterial [18-22] antitumor [23] diuretic [24], carbonic anhydrase inhibitory [25,26], insulin releasing [21] and anti-inflammatory [27] activities. As a continuation of our program, we report facile synthesis for different heterocyclic compounds from chalcones by two different method and compare between results in each case and synthesis of N-Sulfanoamide Pyridines derivatives and measure anticancer effect and calculate IC₅₀.

II. EXPERIMENTAL

Instrumentation

All melting points are uncorrected. IR spectra were obtained (KBr disk) by using a Perkin Elmer 11650 FT-IR spectrophotometer. ¹H NMR and ¹³C NMR spectra were recorded in CDCl₃ on a Varian mercury VXR-300 (300 MHz for ¹H and 75 MHz for ¹³C). Mass spectra were recorded on a Varian MAT 112 spectrometer. Analytical data were carried out at Micro analytical Centre at Cairo University and Beni-Suef University.

Synthesis of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one (**3**)

Method (A):- Traditional method.

A solution of (0.01mol.) potassium hydroxide in 30 ml methanol, stirring for completely soluble then add aqua molar of (0.01mol.) 1-(2,5-dichlorothiophene-3-yl)ethanone **1** and p-chlorobenzaldehyde **2** was Left stirring until completely solidification, the mixture poured on ice/water, The solid product was filtered off and recrystallized from ethanol.

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Method (B):- Grinding method.

Amixture of 1-(2,5-dichlorothiophene-3-yl)ethanone **1** (0.01 mol) and p-chlorobenzaldehyde **2** (0.01 mol), potassium hydroxide (0.01 mol) and few drops of water was ground in an open mortar at room temperature for 2-3 min. and mixed well by a pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from ethanol.

brown (yield 84%), m. p. 86-88°C, $\nu_{\max} / \text{cm}^{-1}$ (KBr) 3041 (CH aromatic), 1719 (C=O), 1653 (C=C), 1605, 1433 (C=C aromatic). $^1\text{H NMR}$ (DMSO) $\delta = 6.17$ (d, 1H, C-CH=CH), $\delta = 6.54$ (s, 1H, CH thiophene proton), $\delta = 7.08$ (d, 1H, CO-CH=CH), $\delta = 7.34-7.56$ (m, 4H, aromatic protons). $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , δ , ppm): 115.3, 123.1, 124.5, 125.0-134.6 (6C, aromatic carbons), 135.1, 143.7, 146.9, 185.8. m/z 316 (Calcd for $\text{C}_{13}\text{H}_7\text{Cl}_3\text{OS}$: C, 49.16; H, 2.22; S, 10.10%. Found: C, 49.33; H, 2.35; S, 10.02%.

General procedure for the synthesis of compounds 6 and 7:-

Method (A):- Traditional method.

A mixture of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one (**3**) (0.01 mol) and 2-Cyanoethanethioamide (**5**), 2-cyanoethaneamide (**4**) (0.01 mol) and sodium acetate (0.01 mmol) was heated in ethanol (20 mL) under reflux for 4 hrs. The solid formed was filtered off and crystallized from ethanol.

Method (B):- Grinding method.

Amixture of 2,5-dichlorothiophene **1** (0.01 mol) and p-chlorobenzaldehyde **2** (0.01 mol), and cyanothioacetamide **5**, cyanoacetamide **4** (0.01 mol), and ammonium acetate (0.02 mol) was ground in an open mortar at room temperature for 2-3 min. and mixed well by pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from ethanol.

4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3- carbonitrile (**6**):-

Yellow (yield 79%), m. p. 146-148°C, $\nu_{\max} / \text{cm}^{-1}$ (KBr) 3367 (NH), 3033 (CH aromatic), 2343 (CN), 1721 (C=O), 1645 (C=N), 1605, 1433 (C=C aromatic). $^1\text{H NMR}$ (DMSO) $\delta = 6.54$ (s, 1H, CH thiophene proton), $\delta = 7.08$ (s, 1H, CH pyridine), $\delta = 7.50-7.59$ (m, 4H, aromatic protons), $\delta = 9.21$ (s, 1H, NH pyridine). $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , δ , ppm): 105.1, 109.8, 111.3, 117.4, 120.5, 123.9, 126.3, 127.2-137.6 (6C, aromatic carbons), 153.3, 160.1, 177.5. m/z 380 (Calcd for $\text{C}_{16}\text{H}_7\text{Cl}_3\text{N}_2\text{OS}$: C, 50.35; H, 1.85; N, 7.34; S, 8.40%. Found: C, 50.49; H, 1.75; N, 7.42; S, 8.29%.

4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine -3-carbonitrile (**7**):-

Orange (yield 80%), m. p. 298-300°C, $\nu_{\max} / \text{cm}^{-1}$ (KBr) 3041 (CH aromatic), 2234 (CN), 1633 (C=N), 1605, 1433 (C=C aromatic), 1252 (SH, tautomer). $^1\text{H NMR}$ (DMSO) $\delta = 6.54$ (s, 1H, CH thiophene proton), $\delta = 7.15$ (s, 1H, CH pyridine), $\delta = 7.30-7.81$ (m, 4H, aromatic protons), 8.8 (s, 1H, NH exchangeable, tautomerization). $^{13}\text{C NMR}$ (75 MHz, CDCl_3 , δ , ppm): 93.2, 95.6, 100.7, 118.4, 120.3, 123.2, 126.9, 128.05-139.4 (6C, aromatic carbons), 154.4, 159.8, 162.5. m/z 396 (Calcd for $\text{C}_{16}\text{H}_7\text{Cl}_3\text{N}_2\text{S}_2$: C, 48.32; H, 1.77; N, 7.04; S, 16.12%. Found: C, 48.45; H, 1.62; N, 7.32; S, 16.25%.

General procedure for the synthesis of compounds 8 and 9:-

Method (A):- Traditional method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3- carbonitrile **7** (0.01mol) and potassium hydroxide (0.01 mol) in N,N-dimethylformamide (20 mL) was stirred for 2 hrs at room temperature. The appropriate of iodomethane, iodoethane, ethylchloroacetate or chloroacetone (0.01mol) was added while stirring and was continued for 2 hrs. The resulting solid was collected and crystallized from N,N-dimethylformamide.

Method (B):- Grinding method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3- carbonitrile **7** (0.01mol) and potassium hydroxide (0.01 mol) was ground in an open mortar followed by iodomethane, iodoethane, ethyl chloroacetate or chloroacetone (0.01 mol) at room temperature for 2-3 min and mixed well by pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from N,N-dimethylformamide.

4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-2-(methylthio)pyridine-3- carbonitrile (**8a**):-

Yellow crystals (yield 82%), m. p. 256-258°C, $\nu_{\max} / \text{cm}^{-1}$ (KBr) 3045 (CH aromatic), 2441 (CN), 1632 (C=N), 1612, 1421 (C=C aromatic), 1322 (CH_3). $^1\text{H NMR}$ (DMSO) $\delta = 3.04$ (s, 3H, SCH_3), $\delta = 6.21$ (s, 1H, CH thiophene proton), δ

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= 7.25 (s, 1H, CH pyridine), δ = 7.44-7.65 (m, 4H, aromatic protons). m/z 410 (Calcd for $C_{17}H_9Cl_3N_2S_2$: C, 49.59; H, 2.20; N, 6.80; S, 15.57%. Found: C, 49.76; H, 2.32; N, 6.62; S, 15.44%.

4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-2-(ethylthio)pyridine-3- carbonitrile (8b):-

Yellow (yield 76%), m. p. 201-203°C, ν_{max} / cm^{-1} (KBr) 3021 (CH aromatic), 2322 (CN), 1633 (C=N), 1609, 1411 (C=C aromatic), 1442 (CH₂), 1336 (CH₃). ¹HNMR (DMSO) δ = 1.11 (t, 3H, CH₃CH₂), δ = 3.52 (q, 2H, SCH₂CH₃), δ = 6.13 (s, 1H, CH thiophene proton), δ = 7.11 (s, 1H, CH pyridine), δ = 7.36-7.52 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ , ppm): 12.2, 20.3, 98.6, 113.06, 116.9, 117.5, 120.4, 124.7, 129.5, 130.1-139.7 (6C, aromatic carbons), 147.4, 151.6, 156.2. m/z 424 (Calcd for $C_{18}H_{11}Cl_3N_2S_2$: C, 50.78; H, 2.60; N, 6.58; S, 15.06%. Found: C, 50.63; H, 2.71; N, 6.67; S, 15.11%.

Ethyl-2-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-3-cyanopyridine-2-ylthio) acetate (9a):-

Yellow crystal (yield 74%), m. p. 211-213°C, ν_{max} / cm^{-1} (KBr) 3086 (CH aromatic), 2247 (CN), 1739 (C=O), 1632 (C=N), 1622, 1410 (C=C aromatic), 1464 (CH₂), 1362 (CH₃), 1165 (C-O). ¹HNMR (DMSO) δ = 1.23 (t, 3H, CH₃CH₂), δ = 3.42 (s, 2H, SCH₂CO), δ = 4.32 (q, 2H, OCH₂), δ = 6.32 (s, 1H, CH thiophene proton), δ = 7.27 (s, 1H, CH pyridine), δ = 7.62-7.81 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ , ppm): 15.2, 29.7, 55.07, 96.1, 113.3, 115.6, 116.04, 120.3, 124.6, 129.9, 130.5-138.3 (6C, aromatic carbons), 148.5, 153.4, 156.2, 172.1. m/z 482 (Calcd for $C_{20}H_{13}Cl_3N_2O_2S_2$: C, 49.65; H, 2.71; N, 5.79; S, 13.25%. Found: C, 49.52; H, 2.86; N, 5.65; S, 13.17%.

2-(2-oxopropylthio)-4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)pyridine-3-carbonitrile (9b):-

Yellow crystal (yield 71%), m. p. 132-134°C, ν_{max} / cm^{-1} (KBr) 3011 (CH aromatic), 2265 (CN), 1719 (C=O), 1634 (C=N), 1621, 1412 (C=C aromatic), 1442 (CH₂), 1322 (CH₃). ¹HNMR (DMSO) δ = 2.52 (s, 3H, CH₃CO), δ = 3.78 (s, 2H, SCH₂CO), δ = 6.23 (s, 1H, CH thiophene proton), δ = 7.15 (s, 1H, CH pyridine), δ = 7.44-7.64 (m, 4H, aromatic protons). m/z 452 (Calcd for $C_{19}H_{11}Cl_3N_2OS_2$: C, 50.29; H, 2.44; N, 6.17; S, 14.13%. Found: C, 50.35; H, 2.53; N, 6.32; S, 14.27%.

General procedure for the synthesis of compounds 10 and 11:-

Method (A):- Traditional method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3- carbonitrile7 (0.01mol) and potassium hydroxide (0.01 mol) in N,N-dimethylformamide (20 mL) was stirred for 2 hrs at room temperature. The appropriate of phenacyl bromide or chloroacetonitrile (0.01mol) was added while stirring and was continued for 2 hrs. The resulting solid was collected and crystallized from N,N-dimethylformamide.

Method (B):- Grinding method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3- carbonitrile7 (0.01mol) and potassium hydroxide (0.01 mol) was ground in an open mortar followed by phenacyl bromide or chloroacetonitrile (0.01mol) at room temperature for 2-3 min and mixed well by pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from N,N-dimethylformamide.

(3-amino-4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)thieno[2,3-b] pyridine-2-yl)(Phenyl)methanone (10):-

Yellow (yield 69%), m. p. 205-207°C, ν_{max} / cm^{-1} (KBr) 3567, 3314 (NH₂), 3045 (CH aromatic), 1719 (C=O), 1623(C=N), 1612, 1421 (C=C aromatic). ¹HNMR (DMSO) δ = 5.27 (s, 2H, NH₂), δ = 6.19 (s, 1H, CH thiophene proton), δ = 7.23 (s, 1H, CH pyridine), δ = 7.34-7.79 (m, 9H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ , ppm): 93.1, 115.3, 119.6, 121.5, 125.7, 129.3, 133.01, 130.2-141.6 (12C, aromatic carbons), 140.9, 152.6, 154.8, 156.09, 187.6. m/z 514 (Calcd for $C_{24}H_{13}Cl_3N_2OS_2$: C, 55.88; H, 2.54; N, 5.43; S, 12.43%. Found: C, 55.76; H, 2.44; N, 5.37; S, 12.62%.

3-amino-4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)thieno[2,3-b] pyridine-2-carbonitrile (11):-

Yellow (yield 82%), m. p. 289-291°C, ν_{max} / cm^{-1} (KBr) 3566, 3342 (NH₂), 3029 (CH aromatic), 2249 (CN), 1635 (C=N), 1621, 1416 (C=C aromatic). ¹HNMR (DMSO) δ = 5.33 (s, 2H, NH₂), δ = 6.12 (s, 1H, CH thiophene proton), δ = 7.19 (s, 1H, CH pyridine), δ = 7.33-7.56 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ , ppm): 80.7, 109.3, 117.6, 119.8, 121.3, 125.4, 127.9, 132.4, 128.03-137.6 (6C, aromatic carbons), 142.7, 149.3, 154.6, 158.9. m/z 435 (Calcd for $C_{18}H_8Cl_3N_3S_2$: C, 49.50; H, 1.85; N, 9.62; S, 14.68%. Found: C, 49.67; H, 1.92; N, 9.59; S, 14.37%.

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Synthesis of 1-amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (14):-

Method (A):- Traditional method.

A mixture of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one (**3**) (0.01 mol) and 2-cyanoacetohydrazide **12** (0.01 mol) and sodium acetate (0.01 mol) was heated in ethanol (20 mL) under reflux for 4 hrs. The solid formed was filtered off and crystallized from ethanol.

Method (B):- Grinding method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile **7** (0.01 mol) and potassium hydroxide (0.01 mol) was ground in an open mortar followed by 2-cyanoacetohydrazide **12** (0.01 mol) at room temperature for 2-3 min and mixed well by pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from ethanol. Pale yellow (yield 71%), m. p. 235-237°C, ν_{\max} / cm^{-1} (KBr) 3310, 3287 (NH₂), 3056 (CH aromatic), 2267 (CN), 1717 (C=O), 1608, 1421 (C=C aromatic). ¹HNMR (DMSO) δ = 4.91 (s, 2H, NH₂), δ = 6.32 (s, 1H, CH thiophene proton), δ = 7.16 (s, 1H, CH pyridine), δ = 7.34-7.49 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ , ppm): 107.5, 110.9, 113.4, 117.3, 119.7, 123.5, 126.4, 127.4-135.6 (6C, aromatic carbons), 137.1, 152.7, 159.4. m/z 395 (Calcd for C₁₆H₈Cl₃N₃OS: C, 48.45; H, 2.03; N, 10.59; S, 8.08%. Found: C, 48.63; H, 2.13; N, 10.35; S, 8.01%.

Synthesis of N-substitutedamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (15a-c):-

Method (A):- Traditional method.

Method (1):- A mixture of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** (0.01 mol) and N-sulfonylaryl-2-cyanoacetohydrazide **13** (0.01 mol) and sodium acetate (0.01 mmol) was heated in ethanol (20 mL) under reflux for 4 hrs. The solid formed was filtered off and crystallized from ethanol.

Method (2):- Condensation of compound 1-amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **14** (0.01 mol) with aldehydes and Arylsulfonyl chlorides (0.01 mol) in refluxing ethanol in presence of catalytic amount of piperidine for 1h. The solid product was collected at the pump after adding drops of hydrochloric acid to the solution and recrystallized from ethanol.

Method (B):- Grinding method.

A mixture of 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile **7** (0.01 mol) and potassium hydroxide (0.01 mol) was ground in an open mortar followed by and N-sulfonylaryl-2-cyanoacetohydrazide **13** (0.01 mol) at room temperature for 2-3 min and mixed well by pestle till mixture was melted. The reaction mixture solidified within 3-5 min. Grinding was continued for 5-10 min. The solid was washed with water and recrystallized from ethanol.

N-benzenesulfonylamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (15a):-

White (yield 80%), m. p. 286-288°C, ν_{\max} / cm^{-1} (KBr) 3343 (NH), 3025 (CH aromatic), 2267 (CN), 1718 (C=O), 1605, 1421 (C=C aromatic) 1365, 1170 (S=O sulfonyl). ¹HNMR (DMSO) δ = 6.13 (s, 1H, CH thiophene proton), δ = 7.12 (s, 1H, CH pyridine), δ = 7.29-7.60 (m, 9H, aromatic protons), δ = 8.87 (s, 1H, NH). m/z 535 (Calcd for C₂₂H₁₂Cl₃N₃O₃S₂: C, 49.22; H, 2.25; N, 7.83; S, 11.95%. Found: C, 49.40; H, 2.15; N, 7.98; S, 11.82%.

N-(4-chlorobenzenesulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (15b):-

White crystal (yield 70%), m. p. 252-254°C, ν_{\max} / cm^{-1} (KBr) 3376 (NH), 3025 (CH aromatic), 2278 (CN), 1719 (C=O), 1614, 1435 (C=C aromatic) 1371, 1179 (S=O sulfonyl). ¹HNMR (DMSO) δ = 6.21 (s, 1H, CH thiophene proton), δ = 7.10 (s, 1H, CH pyridine), δ = 7.23-7.55 (m, 8H, aromatic protons), δ = 9.05 (s, 1H, NH). m/z 569 (Calcd for C₂₂H₁₁Cl₄N₃O₃S₂: C, 46.25; H, 1.94; N, 7.36; S, 11.23%. Found C, 46.37; H, 1.82; N, 7.56; S, 11.13%.

N-(4-methylbenzenesulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile (15c):-

Colorless crystals (yield 83%), m. p. \geq 300°C, ν_{\max} / cm^{-1} (KBr) 3317 (NH), 3045 (CH aromatic), 2228 (CN), 1714 (C=O), 1632, 1412 (C=C aromatic), 1390 (CH₃), 1371, 1179 (S=O sulfonyl). ¹HNMR (DMSO) δ = 1.78 (s, 3H, CH₃), δ = 6.19 (s, 1H, CH thiophene proton), δ = 7.21 (s, 1H, CH pyridine), δ = 7.28-7.61 (m, 8H, aromatic protons), δ =

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9.21 (s, 1H, NH). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 102.6, 106.3, 110.2, 114.6, 116.9, 119.6, 122.8, 126.5-134.7 (12C, aromatic carbons), 138.6, 157.4, 160.7. m/z 549 (Calcd for C₂₃H₁₄Cl₃N₃O₃S₂: C, 50.15; H, 2.56; N, 7.63; S, 11.64%. Found: C, 50.35; H, 2.41; N, 7.79; S, 11.52%.

III. PHARMACOLOGY

Human tumor cell lines

Human hepatocarcinoma cell lines (HepG2) used in this study were obtained from the American Type Culture Collection (ATCC, Minisota, U.S.A.). The tumor cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

Cell line Propagation

The cells were propagated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub cultured two times a week.

Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x10⁴ cells per well in 100 µL of growth medium. Fresh medium containing different concentrations of the test sample was added after 24h of seeding. Serial two-fold dilutions of the tested chemical compounds were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method. After the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gentle shaking on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated [28,29]. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells and their IC₅₀ in µM/L which is the concentration of the compound that inhibits the tumor cell growth by 50%.

IV. RESULT AND DISCUSSION

3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** was synthesized by two method traditional method which 1-(2,5-dichlorothiophene-3-yl)ethanone **1** and p-chlorobenzaldehyde **2** were stirred in methanol in presence of potassium hydroxide and other method grinding method which compound **1** and compound **2** and moist potassium hydroxide were ground with a pestle in an open mortar. Structure of the product **3** was confirmed on the basis of its correct elemental analysis and spectral data Thus compound **3** is supported by its mass spectrum, which showed a molecular formula C₁₃H₇Cl₃OS (M⁺ 316) ¹H NMR (DMSO) δ = 6.17 (d, 1H, C-CH=CH), δ = 6.54 (s, 1H, CH thiophene proton), δ = 7.08 (d, 1H, CO-CH=CH), δ = 7.34-7.56 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 115.3, 123.1, 124.5, 125.0-134.6 (6C, aromatic carbons), 135.1, 143.7, 146.9, 185.8. Treatment of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** with 2-cyanoethaneamide **4** and 2-Cyanoethanethioamide **5** by traditional and grinding methods afforded 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3- carbonitrile **6** and 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine -3-carbonitrile **7** respectively. Structure of the product **7** was confirmed on the basis of its correct elemental analysis and spectral data Thus compound **7** is supported by its mass spectrum, which showed a molecular formula C₁₆H₇Cl₃N₂S₂ (M⁺ 396) ¹H NMR (DMSO) δ = δ = 6.54 (s, 1H, CH thiophene proton), δ = 7.15 (s, 1H, CH pyridine), δ = 7.30-7.81 (m, 4H, aromatic protons), 8.8 (s, 1H, NH exchangeable, tautomerization). ¹³C NMR (75

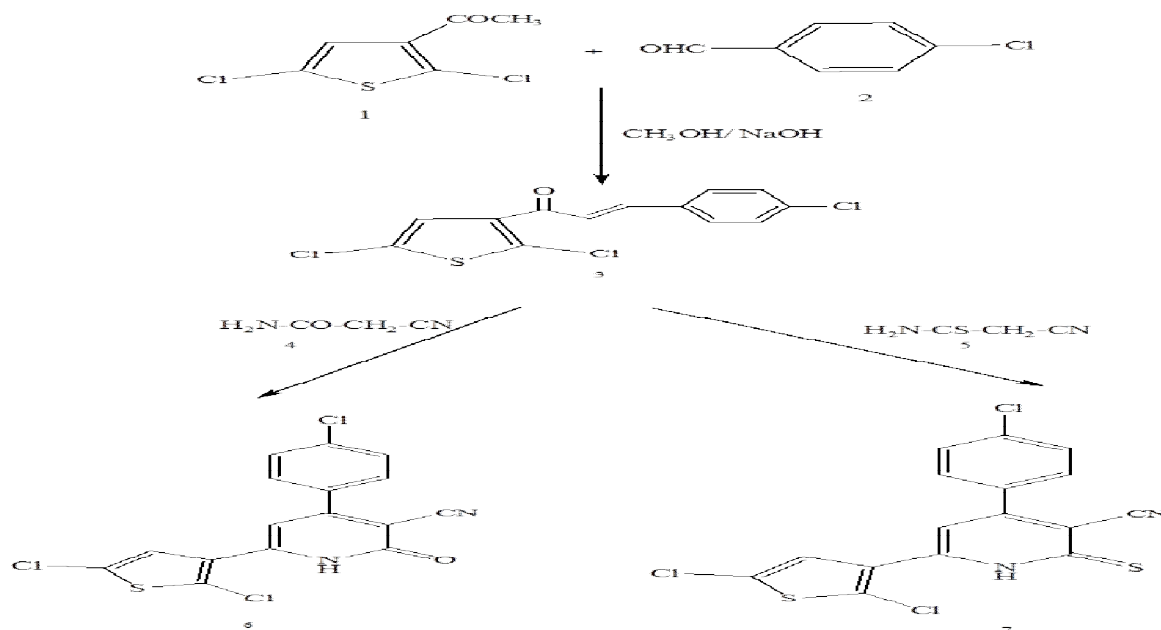
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MHz, CDCl₃, δ, ppm): 93.2, 95.6, 100.7, 118.4, 120.3, 123.2, 126.9, 128.05-139.4 (6C, aromatic carbons), 154.4, 159.8, 162.5. (Scheme 1).



Scheme (1)

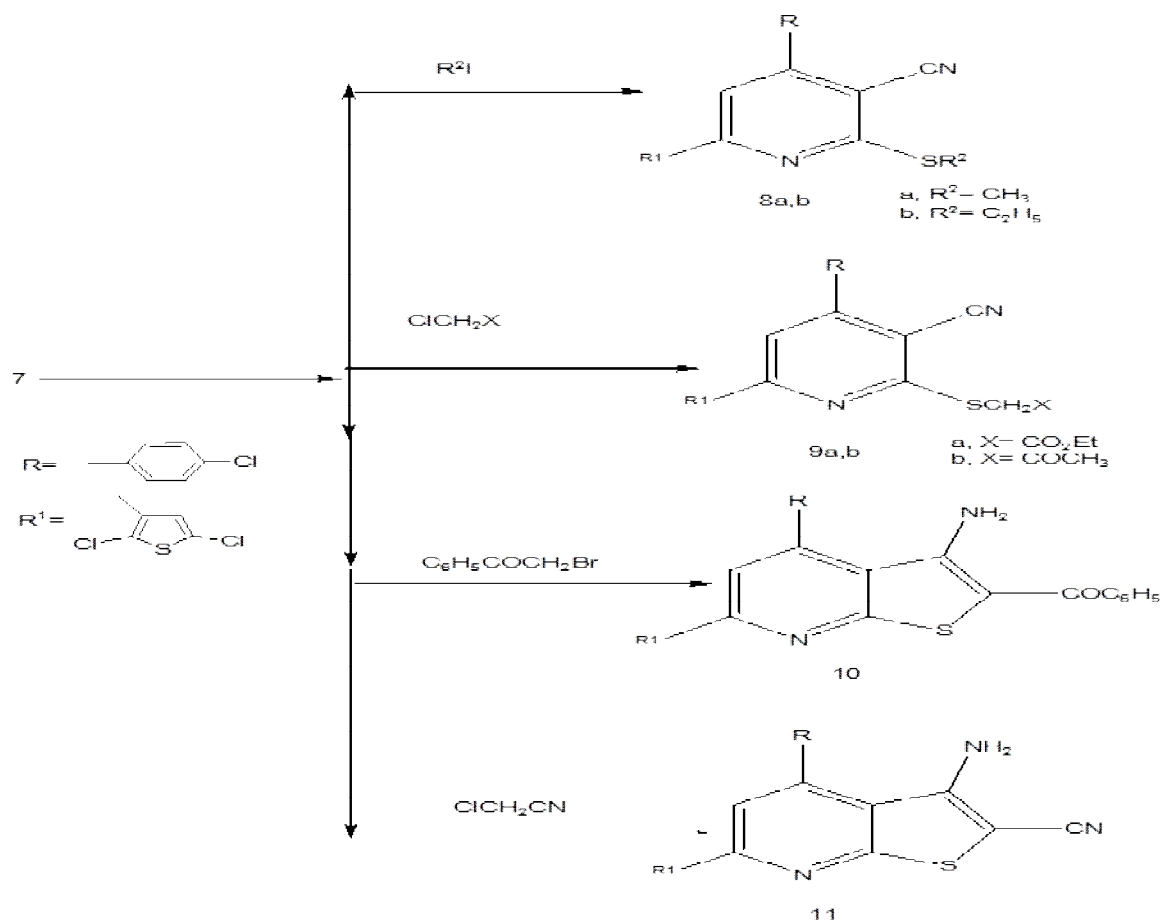
Also by traditional and grinding method compound 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile **7** was reacted with iodomethane, iodoethane, ethylchloroacetate and chloroacetylpyridine derivatives **8a,b** and **9a,b**. Structure of the compounds **8, 9** were confirmed on the basis of its correct elemental analysis and spectral data. Thus compound **9a** is supported by its mass spectrum, which showed a molecular formula C₂₀H₁₃Cl₃N₂O₂S₂ (M⁺ 482). ¹HNMR (DMSO) δ = 1.23 (t, 3H, CH₃CH₂), δ = 3.42 (s, 2H, SCH₂CO), δ = 4.32 (q, 2H, OCH₂), δ = 6.32 (s, 1H, CH thiophene proton), δ = 7.27 (s, 1H, CH pyridine), δ = 7.62-7.81 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 15.2, 29.7, 55.07, 96.1, 113.3, 115.6, 116.04, 120.3, 124.6, 129.9, 130.5-138.3 (6C, aromatic carbons), 148.5, 153.4, 156.2, 172.1. and 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-thioxopyridine-3-carbonitrile **7** was reacted with phenacyl bromide and chloroacetonitrile by stirring in N,N-dimethylformamide in presence of potassium hydroxide as traditional method or potassium hydroxide was ground with a pestle in an open mortar at room temperature for 2-3 min. until the mixture turned into a melt as grinding method to yield (3-amino-4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)thieno[2,3-b]pyridine-2-yl)(Phenyl)methanone **10** and (3-amino-4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)thieno[2,3-b]pyridine)-2-carbonitrile **11** respectively. Structure of the compounds **10, 11** were confirmed on the basis of its correct elemental analysis and spectral data. Thus compound **11** is supported by its mass spectrum, which showed a molecular formula C₁₈H₈Cl₃N₃S₂ (M⁺ 435). ¹HNMR (DMSO) δ = 5.33 (s, 2H, NH₂), δ = 6.12 (s, 1H, CH thiophene proton), δ = 7.19 (s, 1H, CH pyridine), δ = 7.33-7.56 (m, 4H, aromatic protons). ¹³C NMR (75 MHz, CDCl₃, δ, ppm): 80.7, 109.3, 117.6, 119.8, 121.3, 125.4, 127.9, 132.4, 128.03-137.6 (6C, aromatic carbons), 142.7, 149.3, 154.6, 158.9. (Scheme 2).

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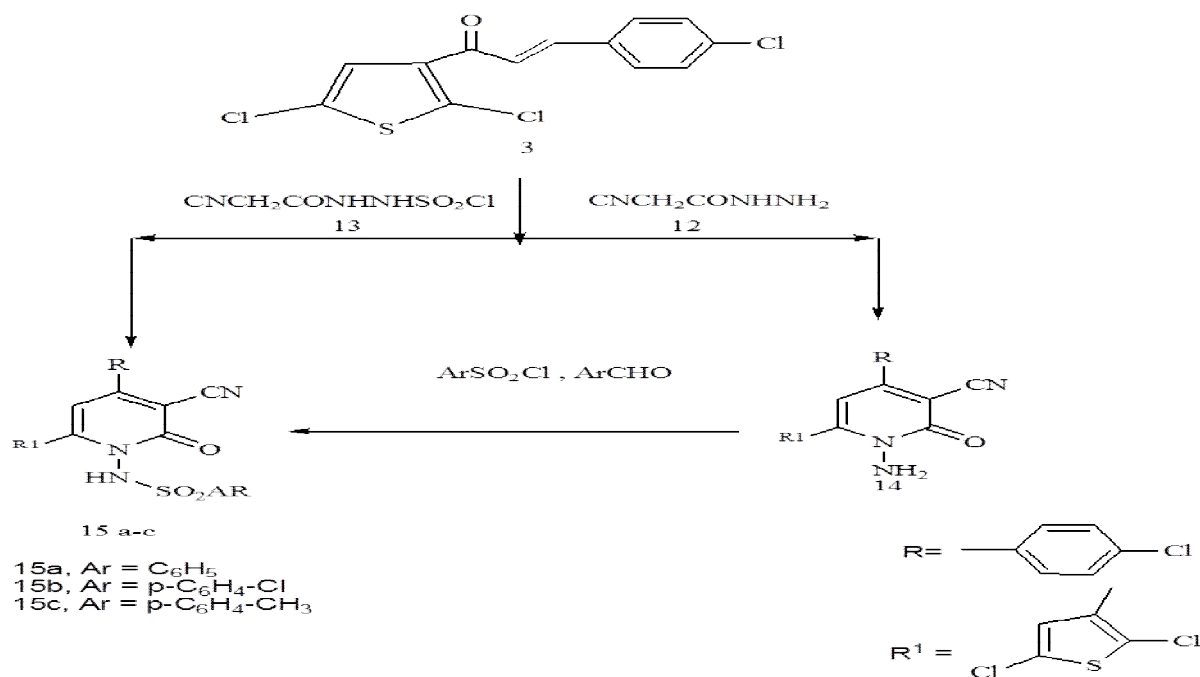
N-substitutedamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3- carbonitrile **15a-c** can be prepared directly by the reaction of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** with N-sulfonylaryl-2-cyanoacetohydrazide **13** in ethanol in presence of sodium acetate or compounds **15a-c** were prepared by reaction of 3-(4-chlorophenyl)-1-(2,5-dichlorothiophen-3-yl)prop-2-ene-1-one **3** with 2-cyanoacetohydrazide (**12**) to form 1-amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3- carbonitrile **14** which was reacted with Arylsulfonyl chlorides and aldehydes and refluxed in ethanol and small amount of catalytic piperidine to produce N-substitutedamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3- carbonitrile (**15a-c**), Structure of the compounds **15a-c** were confirmed on the basis of its correct elemental analysis and spectral data Thus compound **15a** is supported by its mass spectrum, which showed a molecular formula $C_{22}H_{12}Cl_3N_3O_3S_2$ (M^+ 535). 1H NMR (DMSO) $\delta = 6.13$ (s, 1H, CH thiophene proton), $\delta = 7.12$ (s, 1H, CH pyridine), $\delta = 7.29-7.60$ (m, 9H, aromatic protons), $\delta = 8.87$ (s, 1H, NH) (Scheme 3).

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Scheme (3)

Finally, a comparison between two methods of synthesis according to time of reaction and percentage of yield which was found grinding method is better than traditional method which no hazardous, no effect on human health, less time and more percentage of yield (table 1).

Table 11 Comparison between traditional and grinding method for synthesized compounds

Compound NO.	Traditional method		Grinding method	
	Time	Yield	Time	Yield
6	3hrs	79%	7min	90%
7	4hrs	80%	10min	92%
8a	3hrs	82%	7min	95%
8b	5hrs	76%	13-15min	89%
9a	4hrs	74%	10min	88%
9b	4hrs	71%	10min	85%
10	3hrs	69%	7min	82%
11	3hrs	82%	7min	95%
14	4hrs	71%	10min	85%
15a	5hrs	80%	13-15min	92%
15b	3hrs	70%	7min	84%
15c	4hrs	83%	10min	96%
15d	4hrs	82%	10min	95%

Cytotoxicity against different human cancer cell lines in vitro

4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxypyridine-3-carbonitrile **6**, 1-amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxypyridine-3-carbonitrile **14**, N-benzene sulfonylamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxypyridine-3-carbonitrile **15a**, N-(4-chlorobenzenesulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxypyridine-3-

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carbonitrile **15b** and N-(4-methylbenzenesulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **15c** were screened for their in vitro cytotoxic activity against human hepatocellular liver carcinoma (HepG2). (Table 2). 4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **6** is a mild anticancer activity and IC₅₀ value is 62.50. Introducing amino group to the compound **6** increase activity of 1-amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **14** against human hepatocellular liver carcinoma (HepG2) and IC₅₀ value is 39.66. formation of N-sulfanoamide pyridines increase anticancer activity gradually from N-(4-methylbenzenesulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **15c** and IC₅₀ value is 27.77 and replacing methyl group by hydrogen atom increase anticancer activity for N-benzene sulfonylamino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **15a** and IC₅₀ value is 22.90 and introducing chlorine atom to the compound produce N-(4-chlorobenzene sulfonyl)amino-(4-(4-chlorophenyl)-6-(2,5-dichlorothiophen-3-yl)-1,2-dihydro-2-oxopyridine-3-carbonitrile **15b** which is the potent anticancer activity against human hepatocellular liver carcinoma (HepG2) and IC₅₀ value is 10.33.

Table 2 Growth inhibition percent and IC₅₀ values of the tested compounds against HepG2 cell line.

Compound NO.	Growth inhibition %						IC ₅₀ (μM/L)
	1.6	3.13	6.25	12.5	25	50	
6	2.18	10.81	24.62	37.31	53.28	69.47	62.50
14	6.38	15.95	21.84	48.13	67.37	79.09	39.66
15a	6.86	18.38	35.5	74.38	86.21	89.59	22.90
15b	42.3	48.68	70.02	84.36	89.17	93.09	10.33
15c	8.58	19.07	37.84	56.17	80.54	88.62	27.77

V. CONCLUSION

Grinding method is better than traditional method for synthesis of different heterocyclic compounds from chalcones due to its rapid, high yield, simple, practical, economic, no hazardous and no effect on human health method and N-Sulfanoamide pyridines are potent anticancer activity against HepG2 cell line.

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