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Expeditious Sonochemical Synthesis of New 1,8-Naphthyridine Derivatives and their Inhibitory Activity on HepG2 Cell Line

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Abstract

A new series of 2-phenyl-1,8-naphthyridine derivatives were synthesized via traditional heating and under ultrasonic irradiation to run out comparative study and confirm the utility of the green chemistry in organic synthesis. An improvement in the rates and yields were observed upon carrying out the reactions under environmentally benign protocol. The newly produced compounds were scanned *in vitro* for their adverse activity on HepG2 (Human liver) carcinoma cell lines. Results revealed that the tested compounds possess an inhibitory effect on the growth of HepG2 carcinoma cells. The naphthyridinyl pyridine derivatives **4c** and **5c** showed significant cytotoxic activity. The oxo-pyridine derivative **4c** was more potent than the reference drug doxorubicin (DOX), while the imino-pyridine derivative **5c** showed slight reduction in the potency. On the other hand, Mannich bases **(2a,c,d,e)** showed good activity and the styryl derivatives (**6b-d)** showed moderate activity when compared to (DOX).

Keywords: ultrasound irradiations; 1,8-naphthyridine; Mannich bases; styryl compounds; pyridine; cytotoxicity.

1. Introduction

Despite the tremendous efforts made in discovering and developing innovative anticancer drugs, it still holds the record for being one of the most common leading causes of death worldwide. 1,8-Naphthyridine derivatives' anticancer properties earned exceptional recognition by researchers, a number of their derivatives were explored as antitumor agents [1-7]. The highly significant and best explored derivative of 1,8-naphthyridine as anticancer is voreloxin, SNS-595(+)-1,4-dihydro-7-(trans-3-methoxy-4 methylamino-1-pyrrolidinyl)-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acid Fig. 1 [8]. Voreloxin (SNS-595) has shown *in vitro* potent adverse actions on a wide variety of cancer cell lines as well as *in vivo* tumor models. The drug's effect on ovarian cancer and acute myelogenous leukemia (AML) is currently being tested under phase III clinical trial investigations. Voreloxin's main target is DNA damage which is carried out by DNA intercalation, in turn leading to inhibition of topoisomerase II. Impressive results were reported for 2-phenyl-1,8-naphthyridin-4-ones as potent cytotoxic compounds [7]. Moreover, the potent cytotoxic activity against HepG-2 cell line was clearly stated in our previous article for 1,8-naphthyridines incorporated into N-β glycosides, Mannich bases, Schiff's bases and 5-membered heterocyclic ring system [9]. However, most of the chemical modifications made were at C-4 positions in 1,8-naphthyridines. C-3 and C-7 positions have not been well exploited. Herein modifications at C-3 and C-7 were done aiming to produce more active products and study the structure activity relationship in cancer treatment. The newly discovered technique entitled sonochemistry has provided a pathway that is rather versatile for a vast variety of chemical syntheses. In turn, numerous reactions can be carried out using ultrasound irradiation with more favorable mild reaction conditions in a shorter time with higher yields [10-15]. In continuity of our efforts towards

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developing suitable synthetic approaches for building heterocyclic compounds of biological value and our increasing concern in sonochemistry [16-21] , our plan is to develop a facile sonochemical method of synthesis and high yield method in the preparing of some novel 1,8-napthyridines linked with pyridine derivatives, Mannich bases, styryl and/or enamine side chains which were chosen upon their known biological values [22-26]. Pyridine and Mannich bases have been introduced at C-3 position and styryl or enamine groups at C-7 position of 1,8-naphthyridine ring. Cytotoxic evaluations of some of the new products in suppressing the growth of HepG-2 cancer cells were tested.

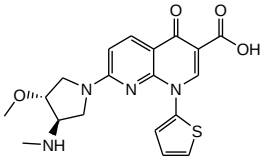


Figure 1; **SNS-595 (Voreloxin)**

2. Experimental section

General Information

All melting points were uncorrected and determined by the Barnstead international 1002 melting point apparatus. Thin layer chromatography (TLC) was performed on aluminium silica gel 60 F254 (E-Merk). The spots were detected by iodine and UV light absorption. IR spectra were recorded for the compounds in Thermo-Nicolet-6700 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C spectra were recorded on Burker WM 400 and 850 MHz spectrometer using TMS as a reference (0.00 ppm). Chemical shifts (δ) are given in ppm relative to the signal for TMS as standard, and coupling constants in Hz. The reactions that carried out by US irradiation was done using Daihan Wiseclean sonicator, (with a frequency of 40 kHz and a nominal power 180 W). Microanalysis was performed by Perkin Elmer elemental analyse at the Faculty of Science, King Abdul Aziz University. Biological activity tests were performed at Al-Azhar University the Regional Center for Mycology & Biotechnology.

2.1. *Chemistry*

3-((Substituted amino) methyl)-7-methyl-2-phenyl-1,8-naphthyridin-4(1H)-one (2a-e)

Method I; thermally (Δ)

A mixture of paraformaldehyde (0.08 g, 0.85 mmol), and the appropriate secondary amine (8.5 mmol) namely, piperidine, piperazine, morpholine, diethylamine and sulphanilimide was refluxed in 10 ml absolute ethanol till complete solubility of the paraformaldehyde. A solution of 7-methyl-2-phenyl-1,8-naphthyridin-4-ol (0.6 g, 2.5mmol) naphthyridine derivative **1** in 3 ml ethanol was added to the reaction mixture while stirring. The mixture was refluxed till completion of the reaction (monitored by TLC). After cooling, the formed precipitate was filtered and recrystallized from ethanol to yield the products **2a-e.** (Table **1**)

Method II; ultrasonic reaction (US)

The above reactions were repeated under ultrasound irradiation at 50-60ºC till completion of the reaction (was monitored by TLC). The reaction mixture was cooled and the formed precipitate was filtered and recrystallized from ethanol to yield the products **2a-e.** (Table **1**).

7-Methyl -2-phenyl-3-(piperidin-1-ylmethyl)-1,8-naphthyridin-4(1H)-one (2a)

The m.p.; 97-100ºC, IR (υ_{max} / cm^{-1}); 3416 (NH), 1600 (C=O),1596 (C=N).^{1}H-NMR($CDCl_3$)δ; 1.2 (1H, s, NH, D_2O exchangeable), 1.4-1.7 (6H, m, $3CH_2$ of piperdine ring), 2.5(3H,s,CH_3), 2.9(4H, m, $2CH_2$,C_2-H,C_6-H of piperdine ring),4.1(2H, s, CH_2-N), 7.2 (1H,d, *J* = 8.4, C_6-H),7.5-7.6 (5H, m, Ar-H), 8.5(1H,d, *J* = 8.4, C_5-H), ^{13}C-NMR ($CDCl_3$)δ; 21.4 (CH_3), 25.1(C_4 of piperidine), 30.9 (C_3, C_5 of piperidine),45.9 (CH_2-N), 55.4 (C_2 ,C_6 of piperidine),112.4, 114.4, 116.3, 118.5, 119.8, 125.7, 128.9, 135.8, 150.3, 161.6, 161.7, (Ar-C) , 178.1 (C=O), MS (m/z) 333 M^{+}. Anal. Calcd. For $C_{21}H_{23}N_3O$ (333.43); C, 75.65, H, 6.95, N, 12.60 %, Found; C, 75.83, H, 6.69, N, 12.48%.

7-Methyl-2-phenyl-3-(piperazin-1-ylmethyl)-1,8-naphthyridin-4(1H)-one (2b)

The m.p.; 145-147ºC,IR (υ_{max} / cm^{-1}); 3214,3150 (2NH),1664(C=O), 1596 (C=N); ^{1}H-NMR($CDCl_3$) δ;1.1, 1.5 (2H, 2s, 2NH, D_2O exchangeable), 2.9 (3H,s,CH_3), 3.7 (4H, m,$2CH_2$ of piperazine), 4.2 (4H,m, $2CH_2$ of piperazine), 7.4 (1H,d, *J* =8.4, C_6-H), 7.5-7.8 (5H, m, Ar-H),8.92 (1H,d, *J* =8.4, C_5-H), ^{13}C-NMR ($CDCl_3$)δ; 22.4(CH_3), 25.1, 29.7, 45.9 (C-piperazine and CH_2-N), 112.1, 112.4, 113.8, 116.3, 118.5, 119.8, 134.2, 135.8, 144.9, 151.6, 161.7 (Ar-C) , 168.9(C=O), MS (m/z) 334 M^{+}. Anal. Calcd. For $C_{20}H_{22}N_4O$ (334.41); C, 71.83, H, 6.63, N, 16.75 % Found; C, 71.64, H, 6.38, N, 16.42 %.

7-Methyl-3-(morpholinomethyl)-2-phenyl-1,8-naphthyridine-4(1H)-one (2c)

m.p.; 130-132ºC,IR (υ_{max} / cm^{-1}); 3365 (NH), 1600(C=O), 1590 (C=N); ^{1}H-NMR($CDCl_3$) δ;2.8 (3H, s, CH_3), 3.85, 3.90 (4H, 2t, J= 6.1, $2CH_2$, C_2-H,C_6-Hof morpholine), 4.05 (2H, s, CH_2-N), 4.30(4H,t, J= 6.1, $2CH_2$, C_3-H,C_5-H of morpholine), 5.4 (1H, br.s, NH, D_2O exchangeable),7.4 (1H,d, *J* =7.6, C_6-H),7.5-7.8 (5H, m, Ar-H)8.9 (1H, d, *J* =7.6, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 25.6 (CH_3), 45.5 (CH_2-N), 47.8 (C_2, C_6 of morpholine), 65.4 (C_3, C_5 of morpholine), 116.7, 119.3, 127.7, 128.1, 128.3, 130.4, 136.2, 136.3,146.5, 147.6, 153.1, (Ar-C), 206.3 (C=O), MS (m/z) 335 M^+. Anal. Calcd. For $C_{20}H_{21}N_3O_2$ (335.40): C, 71.62; H, 6.31; N, 12.53 % Found: C, 71.36; H, 6.08; N, 12.28 %.

3-((Diethyl amino) methyl)-7-methyl-2-phenyl-1, 8--naphthyridin-4(1H)-one (2d)

The m.p; 159-161ºC, IR (υ_{max} / cm^{-1}); 3455 (NH),1630 (C=O), 1605 (C=N); ^{1}H-NMR($CDCl_3$)δ; 0.8 (1H, br.s, NH, D_2O exchangeable), 1.2 (6H, t, *J*=7.2, $2CH_3$, -N-$(CH_2CH_3)_2$, 2.8(3H, s, CH_3), 3.9 (2H, s, CH_2-N), 4.1 (4H, q, *J*=7.2, $2CH_2$, of –N-$(CH_2CH_3)_2$), 7(1H,d, *J* = 8.4, C_6-H),7.2-7.7 (5H, m, Ar-H), 8.8 (1H, d, *J* =8.4, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 11.1(-N-$(CH_2CH_3)_2$), 24.3 (CH_3), 47.3 (-CH_2N), 47.9 (-N$(CH_2CH_3)_2$), 110.1, 117.3, 128.7, 128.8, 128.9, 131.2, 132.3, 136.8, 141.9, 148.8, 153.9 (Ar-C), 207.2 (C=O) MS (m/z) 321 M^+.Anal.Calcd. For $C_{20}H_{23}N_3O$ (321.42): C, 74.74; H, 7.21; N, 13.07% Found: C, 74.51, H, 6.89, N, 12.88%.

4-(((7-Methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)methyl)amino)benzene sulfonamide (2e)

The m.p.; 155-152ºC,IR (υ_{max} / cm^{-1}); 3382, 3249, 3150 (NH_2,NH), 1600(C=O),1593 (C=N).^{1}H-NMR($CDCl_3$)δ; 1.6 (1H, br.s, NH, D_2O exchangeable); 2.7 (3H, s, CH_3), 4.3 (2H, s, CH_2-NH), 4.6 (1H,br.s., NH, D_2O exchangeable), 5.3 (1H,br.s,NH_2, D_2O exchangeable), 6.3(1H,d, *J* =8, C_6-H), 7.2-7.8 (9H, m, Ar-H), 8.5 (1H, d, *J* =8, C_5-H),^{13}C-NMR ($CDCl_3$) δ; 24.0 (CH_3), 40.1 (CH_2-NH), 106.8, 112.2, 117.1, 121.0, 127.1, 128.3, 128.4, 129.4, 130.9, 136.5, 141.2, 146.5, 147.3, 152.6, 160.4, 166.5 (Ar-C,C=O), MS (m/z) 420 M^+. Anal. Calcd. For $C_{22}H_{20}N_4O_3S$ (420.48): C, 62.84; H, 4.79; N, 13.32% Found: C, 62.98; H, 4.43; N, 13.14%.

7-Methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridine-3-carbaldehyde (3)

To an ice cooled flask containing *N,N*-dimethylformamide (20 ml), $POCl_3$ (1.86ml,20 mmol) was added dropwise, while stirring. After complete addition, the mixture was stirred at room temperature for 90 minutes and cooled again in ice-bath. Naphthyridine derivative **1**(2.36 g,10 mmol) was added to the reaction mixture and warmed at 75 °C for 5 h.The mixture was cooled at room temperature and poured onto ice water, basified (sat.aqu.K_2CO_3 solution) and extracted with $CHCl_3$ (120 ml), dried with anhydrous ($MgSO_4$) and the solution was evaporated under vacuum. After cooling, compound **3** was obtained in **(**97 % yield**).** The m.p.; 114-116ºC, IR (υ_{max} / cm^{-1}); 3420 (NH), 1661 (C=O), 1590 (C=N); ^{1}H-NMR ($CDCl_3$) δ; 3.05 (3H, s, CH_3),5.4 (1H, br.s, NH, D_2O exchangeable), 7.5 -8.4 (5H, m, Ar-H), 8.6 (1H,d, J = 9.2, C_6-H);8.8 (1H,d, J =9.2, C_5-H); 9.5 (1H, s, CHO), ^{13}C-NMR ($CDCl_3$) δ; 25.1(CH_3),118.4, 119.8, 127.25. 127.5, 128.3, 135.8, 142.7, 144.8, 150.4, 151.7, 161.8 (Ar-C), 169.4, 178.1 (2C=O), MS (m/z) 264 M^+..Anal.Calcd. For $C_{16}H_{12}N_2O_2$ (264.28): C, 72.72; H, 4.58; N, 10.60 % Found: C, 72.53; H, 4.23; N, 10.27%.

General procedure for the preparation of 6-(aryl)-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4a-d)or 6-(aryl)-4-(7-methyl-4-oxo-2-phenyl-1,8-naphthyridin-3-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile derivatives (5a-d)

Method I; thermally (Δ)

A mixture of naphthyridine carboaldehyde **3** (0.52g, 2mmol), the appropriate acetyl compounds namely acetophenone, *p*-floroacetophenone, 2-acetylthiophene, 2-acetylfurane (2 mmol), ammonium acetate (1.23g, 16 mmol), ethyl cyanoacetate or malonitirile (2mmol) in 20 ml absolute ethanol were refluxed till completion of the reaction (was monitored by TLC).The reaction mixture was cooled, the formed precipitate was filtered and recrystallized from ethanol to afford the title compounds **4a-d** and **5a-d** respectively(Table **2**)

Method II; ultrasonic reaction (US)

The above reactions were repeated using ultrasound irradiation and the temperature was kept at 60-70ºC till completion of the reaction. The mixture was cooled and the formed precipitate was filtered and recrystallized from ethanol to afford the products **4 a-d** and **5 a-d** respectively (Table **2**)

4-(7-Methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile (4a)

The m.p.; 218-220ºC, IR (υ_{max} / cm^{-1}); 3384, 3210 (2NH), 2195 (C≡N), 1610 (C=O),1590 (C=N), ^{1}H-NMR ($CDCl_3$) δ; 1.6 (1H, br.s, NH, D_2O exchangeable), 2.6 (3H, s, CH_3), 7.0 (1H, br.s, NH, D_2O exchangeable), 7.4-7.6 (5H, m, Ar-H), 8.0 (1H,s, C_5-H of 3760yridine ring), 8.1-8.2 (5H, m, Ar-H), 8.5 (1H, d, *J* =8.5, C_5-H), 8.4 (1H, d, *J*=8.5, C_6-H);^{13}C-NMR ($CDCl_3$) δ; 24.2 (CH_3), 106.6, 108.8, 116.7, 117.9, 118.8, 119.2, 127.2, 127.7, 127.8, 128.8, 129.0, 129.2, 130.3, 131.6, 132.8, 138.2, 143.2, 152.0, 154.9, 160.1, 160.9, 161.1 (Ar-C, CN and C=O), MS (m/z) 430 M^+. Anal. Calcd. For $C_{27}H_{18}N_4O_2$ (430.46): C, 75.34; H, 4.21; N, 13.02 % Found: C, 75.67; H, 3.97; N, 12.89 %.

6-(4-Fluorophenyl)-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4b)

The m.p.; 228-230ºC, IR (υ_{max} /cm^{-1}); 3241, 3210 (2NH), 2257(C≡N), 1659 (C=O), 1619 (C=N).^{1}H-NMR ($CDCl_3$) δ; 2.1(3H**,** s, CH_3), 7.1 (1H, br.s, NH, D_2O exchangeable), 7.5-7.8 (10H, m, Ar-H and NH), 8.0(1H, s, CH of 3760yridine ring),8.5 (1H,d, *J* = 9.3, C_6-H), 8.6 (1H, d, *J* = 9.3, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 24.2(CH_3), 104.3, 106.6, 110.9, 115.0, 116.2, 116.7, 119.2, 120.6, 127.1, 127.8, 128.5, 129.2,130.2, 131.6, 136.0, 136.8, 137.2143.4, 146.9, 154.5, 154.9,161.1,161.9, 162.7, 163.3(Ar-C, CN and C=O), MS (m/z) 448 M^+. Anal.Calcd. For $C_{27}H_{17}FN_4O_2$ (448.45): C, 72.31; H, 3.82; F, 4.24; N, 12.49 % Found C, 72.51; H, 3.59; N, 12.17%.

4-(7-Methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (4c)

The m.p.; 205-207ºC, IR (υ_{max} /cm^{-1}); 3280,3242 (2NH), 2228 (C≡N), 1674(C=O), 1591 (C=N).^{1}H-NMR ($CDCl_3$) δ; 1.1 (1H, br.s, NH, D_2O exchangeable), 3.1 (3H, s, CH_3),7.3(1H, s, NH, D_2O exchangeable),7.54-9.08 (9H, m, Ar-H), 8.4 (1H, br.s, C_5–H of pyridine ring), 8.6 (1H, d, *J* =8.7, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 24.9 (CH_3), 103.9, 114.5, 116.6, 119.1, 119.4, 119.5, 126.2, 127.0, 127.9, 128.1, 129.1, 129.4, 131.0, 131.2, 135.0, 137.4,142.8,143.1, 147.4, 155.8, 156.3,160.1, 160.4(Ar-C, CN and C=O), MS (m/z) 436 M^+. Anal. Calcd. For $C_{25}H_{16}N_4O_2S$ (436.49) C,68.79; H,3.69; N,12.84% Found; C,68.46; H,3.42; N,12.65%.

6-(Furan-2-yl)-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4d)

The m.p.;220-223ºC,IR (υ_{max} /cm^{-1});3222, 3203 (2NH), 2228 (C≡N), 1676 (C=O), 1589 (C=N).^{1}H-NMR ($CDCl_3$) δ;1.1(1H, br.s, NH, D_2O exchangeable),3.1(3H, s, CH_3),7.3 (1H, br.s, NH, D_2O exchangeable), 7.5-9.0(9H, m, Ar-H),8.4 (1H, br.s, C_5-H of 3760yridine ring), 8.6 (1H,d, *J* = 8.8, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 24.9 (CH_3), 103.8, 104.3,114.4, 115.2, 117.2, 119.0, 119.3, 119.5, 127.9, 128.1, 129.1, 131.2, 134.9, 136.2,143.1,148.1, 154.1,155.7, 157.2, 159.7, 160.4, 160.5 (Ar-C, CN and C=O), MS (m/z) 420 M^+. Anal.Calcd. For $C_{25}H_{16}N_4O_3$ (420.42): C, 71.42; H, 3.84; N, 13.33 % Found: C, 71.08; H, 3.61; N, 13.18%.

2-Imino-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-6-phenyl-1,2-dihydropyridine-3-carbonitrile (5a)

The m.p.; 155-157 ºC, IR (υ_{max} /cm^{-1}); 3341, 3270 (br, 3NH),2188 (C≡N); 1660 (C=O), 1619 (C=N).^{1}H-NMR ($CDCl_3$) δ;1.2 **(**1H, s, NH, D_2O exchangeable), 1.4 (1H, br.s, NH, D_2O exchangeable), 2.1 (1H, s, NH, D_2O exchangeable),2.6 (3H, s, CH_3), 7.5-7.52 (5H, m, Ar-H), 8.0 (1H,s, C_5-Hof pyridine ring), 8.1-8.2 (5H, m, Ar-H), 8.47 (1H, d, *J* =9.3, C_6-H),8.5(1H, d, *J* =9.3, C_5-H),^{13}C-NMR ($CDCl_3$) δ; 24.2(CH_3), 97.5(C5 of pyridine ring), 108.0,115.0, 117.2, 119.2, 120.0, 125.9, 127.8,128.3, 129.2, 129.4, 132.2, 135.5,136.1, 143.4, 144.0,149.9, 150.7, 161.2, 162.3, 163.8.(Ar-C, CN and C=O), MS (m/z) 429 M^+. Anal. Calcd. For $C_{27}H_{19}N_5O$ (429.47): C, 75.51; H, 4.46; N, 16.31% Found: C, 75.21; H, 4.19; N, 16.15%.

6-(4-Fluorophenyl)- 2-imino-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)- 1,2-dihydropyridine-3-carbonitrile (5b)

The m.p.; 205-207ºC, IR (υ_{max} /cm^{-1}); 3257, 3242(3NH); 2202 (C≡N), 1661(C=O). ^{1}H-NMR ($CDCl_3$) δ;1.2 (1H, s, NH, D_2O exchangeable), 2.1 (1H, s, NH, D_2O exchangeable), 2.6 (3H, s, CH_3), 7.56-7.60 (5H, m, Ar-H), 8.0 (1H, br.s, C_5-H of pyridine ring), 8.01-8.3 (5H, m, Ar,-H and NH),8.4 (1H,d, *J* = 8.5, C_6-H), 8.50 (1H, d, *J* = 8.5, C_5-H),^{13}C-NMR ($CDCl_3$) δ; 24.2(CH_3), 106.6 (C5 of pyridine ring), 112.6, 116.4, 116.7,119.2, 120.0, 123.2, 127.2, 127.8, 129.2, 131.5, 136.8, 143.4, 146.9, 154.5, 157.4, 159.1, 160.6, 161.1, 162.7 (Ar-C, CN and C=O), MS (m/z) 447 M^+. Anal. Calcd. For $C_{27}H_{18}FN_5O$ (447.46): C, 72.47; H, 4.05; N, 15.65 % .Found: C, 72.23; H, 3.91; N, 15.35%.

2-Imino-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (5c)

The m.p.; 181-183ºC, IR (υ_{max} /cm^{-1}); 3476, 3289 (3NH), 2219 (C≡N), 1630 (C=O). ^{1}H-NMR (DMSO-d6) δ; 1.9, 2.7 (2H, 2s, 2NH, D_2O exchangeable),3.1(3H, s, CH_3), 5.2 (1H, br.s, NH, D_2O exchangeable), 7.5-8.1 (5H, m, Ar-H), 8.3 (1H, d, *J* = 8.4, C_6-H),8.4 (1H, br.s, C_5-H of pyridine ring), 8.6 (1H,d, *J* = 8.4, C_5-H), 8.80-8.87 (3H, m, Ar-H), ^{13}C-NMR (DMSO-d6) δ; 25.1(CH_3), 108.4(C5 of pyridine ring), 111.0, 111.1, 117.8, 119.5, 119.6, 123.2, 123.3, 128.4, 135.1, 143.8, 146.7, 147.9, 148.0, 149.0, 150.1, 153.0, 161.3, 168.6, 170.2, 171.9, 176.1 (Ar-C, CN and C=O), MS (m/z) 435 M^+. Anal. Calcd. For $C_{25}H_{17}N_5OS$ (435.50): C, 68.95; H, 3.93; N, 16.08% Found: C, 69.09; H, 3.57; N, 15.79%.

6-(Furan-2-yl)-2-imino-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-1,2-dihydropyridine-3-carbonitrile (5d)

The m.p.; 185-187ºC,IR (υ_{max} /cm^{-1}); 3301, 3063 (3NH),2215 (C≡N), 1594 (C=O). ^{1}H-NMR ($CDCl_3$) δ; 1.6, 2.6 (2H, 2s, 2NH, D_2O exchangeable), 3.1 (3H, s, CH_3), 5.0 (1H, br.s, NH, D_2O exchangeable),7.4-8.1 (6H,m, Ar-H), 8.3 (1H,br.s, C_5-H of pyridine ring), 8.4 (1H,d, *J* =8.9, C_5-H), 8.5-8.7 (3H, m, Ar-H), ^{13}C-NMR ($CDCl_3$) δ; 25.1(CH_3), 108.6(C5 of pyridine ring), 110.6, 110.8, 112.4, 118.5, 119.8, 122.3, 124.0, 126.0, 127.2, 135.8, 145.1, 149.3, 149.4, 150.4, 151.4,151.7, 151.8, 161.2, 161.7, 169.0, 178.1 (Ar-C, CN and C=O), MS (m/z) 419 M^+. Anal. Calcd. For $C_{25}H_{17}N_5O_2$ (419.43): C, 71.59; H, 4.09; N, 16.70 % Found: C, 71.31; H, 3.96; N, 16.54%.

Synthesis of 7-(styryl derivatives)-2-phenyl-1, 8-naphthyridin-4(1H)-one (6 a-d)

Method I; thermally (Δ)

A mixture of naphthyridine derivative **1** (2.36 g, 10 mmol), different aromatic aldehydes, namely, *p*-nitrobenzaldehyde, *P*-fluorobenzaldehyde, *p*-chlorobenzaldehyde and thiophenaldhyde (20 mmol) in xylene(20 ml) was refluxed for 5 h., in the presence of catalytic amount of piperidine(0.5 ml). After completion of the reaction and cooling, the precipitate was filtered, recrystallized from ethanol to give the styryl products **6a-d** (Table 3)

Method II; ultrasonic reaction (US)

The above reactions were repeated using ultrasound irradiation at 70-80ºC, till completion of the reaction. After cooling the solid product was filtered, dried and recrystallized from ethanol to afford the corresponding 7- styryl-1,8-naphthyridines **6a-d;** (Table 3)

7-(4-Nitrostyryl)-2-phenyl-1, 8-naphthyridin-4(1H)-one (6a)

The m.p.; 140-142ºC, IR (υ_{max} / cm^{-1}); 3337 (NH), 1671 (C=O), 1623 (C=N). ^{1}H-NMR ($CDCl_3$) δ;7 (1H, s, C_3-H), 7.3 (8H, m, Ar- H and CH olefinic,NH), 7.6 (2H, d, *J* =8.8, (C_3-H,C_5-H), 8.0 (1H, d, *J* =8.0, C_6-H), 8.3 (2H, d, *J* =8.8, C_2-H,C_6-H), 8.41 (1H, d, *J* =8.0, C_5-H), ^{13}C-NMR (DMSO-d6) δ; 111.6, 117.8, 117.9, 119.6, 120.0, 135.1, 147.8, 149.6, 150.0, 152.8, 153.3, 161.4, 161.8, 166.2, 166.6, 176.2, 176.3, MS (m/z) 369 M^+. Anal.Calcd. For $C_{22}H_{15}N_3O_3$ (369.37): C, 71.54; H, 4.09; N, 11.38 % Found: C, 71.23; H, 3.87; N, 11.16%.

7-(4-Fluorostyryl)-2-phenyl-1, 8-naphthyridin-4(1H)-one (6b)

The m.p.; 106-108ºC, IR (υ_{max} /cm^{-1}); 3499 (NH), 1610 (C=O), 1592 (C=N). ^{1}H-NMR ($CDCl_3$) δ; 6.9 (1H, s, C_3- H), 7.11 (1H, d, *J* = 8.0, Ar-CH=CH), 7.2 (1H, d, *J* = 8.0, Ar-CH=CH), 7.4-7.8 (10H, m, Ar-H, NH), 8.8 (1H, d, *J* =8.0, C_6-H), 9.1 (1H, d, *J* = 8.0, C_5-H), ^{13}C-NMR (DMSO-d6) δ; 111.2, 111.4, 111.9, 117.8, 119.6, 119.7, 120.6, 120.7, 122.0, 126.4, 129.9, 135.1, 135.2, 139.9, 149.9, 152.9, 156.9, 157.7, 161.5, 166.9, 168.7, 169.3, MS (m/z) 342M^+. Anal. Calcd. For $C_{22}H_{15}FN_2O$ (342.12): C, 77.18; H, 4.42; N, 8.18 % Found: C, 76.88; H, 4.36; N, 7.87%.

7-(4-Chlorostyryl)-2-phenyl-1,8-naphthyridin-4(1H)-one (6c)

The m.p.; 145-1147ºC, IR (υ_{max} / cm^{-1}); 3375 (NH), 1700 (C=O), 1600 (C=N). ^{1}H-NMR ($CDCl_3$) δ; 6.3 (1H,s, C_3-H), 7.0-7.9 (12H, m, Ar-H and CH olefinic, NH), 8.3 (1H, d, *J* = 8.0, C_6-H), 8.4 (1H, d, *J* =8.0, C_5-H), ^{13}C-NMR ($CDCl_3$) δ; 120.6, 128.0, 128.1, 128.8, 128.9, 129.3, 129.4, 129.5, 130.6, 130.9, 131.2, 132.1, 135.1, 136.6, 139.9, 148.1, 163.1., MS (m/z) 358, 360 M^+, M^++2 . Anal.Calcd. For $C_{22}H_{15}ClN_2O$ (358.82): C, 73.64; H, 4.21; N, 7.81 % Found: C, 73.32; H, 4.09; N, 7.63%.

2-Phenyl-7-(2-(thiophen-2-yl) vinyl)-1,8-naphthridin-4(1H)-one (6d)

The m.p.; 85-87 ºC IR (υ_{max} /cm^{-1}); 3234 (NH), 1605 (C=O), 1559 (C=N). ^{1}H-NMR ($CDCl_3$) δ; 6.5 (1H, s, C_3-H), 6.9 (1H, d, J=8.0, Ar-H), 7-7.2 (5H, m, Ar-H, NH), 7.31 (1H, d, J=8.5, Ar-CH=CH), 7.4 (1H, d, J=8.5, Ar-CH=CH), 7.9-8.1 (4H, m, Ar-H), 8.67 (1H, br.s, NH).^{13}C-NMR ($CDCl_3$)δ; 109.4,

115.3, 122.5, 126.1, 126.5, 126.8, 127.2, 127.6, 133.7, 137.8, 138.4, 140.9, 143.4, 144.7, 149.3, 160.7, 163.4, MS (m/z) 330 M^+. Anal.Calcd. For $C_{20}H_{14}N_2OS$ (330.40): C, 72.70; H, 4.27; N, 8.48% Found; C, 72.56; H, 4.09; N, 8.13%.

7-(2-(Dimethyl amino) vinyl)-2-phenyl-1,8-naphthyridin-4(1H)-one (7)

A mixture of napthyridine **1** (2.36 g, 10 mmol) and dimethylformamide/dimethylacetal (DMF/DMA) (2 ml, 16.7 mmol) was refluxed for 3 h. The residue obtained was cooled at room temperature, filtered, washed with petroleum ether and recrystallized from toluene to give the product **7** (72 %) yield

The m.p.; above 300ºC, IR (υ_{max} / cm^{-1}); 3408 (NH), 1609 (C=O), 1597 (C=N).^{1}H-NMR ($CDCl_3$) δ; 0.8 (1H, br.s, NH, D_2O exchangeable), 2.7, 2.8 (6H, 2s, $2CH_3$), 4.12 (1H, d, *J* = 6.8, $(CH_3)_2$ N-CH=CH), 7-8.1 (9H, m, Ar-H and CH olefinic)**.**^{13}C-NMR ($CDCl_3$) δ; 31.9(N-$(CH_3)_2$), 84.4 $(CH_3)_2$ N-CH=CH), 104.3, 116.1, 119.8, 127.1, 127.5, 129.1, 131.9, 132.7, 141.9, 147.5, 152.5, 158.1, 165.9 (Ar-C, CN and C=O), MS (m/z) 291 M^+. Anal.Calcd. For $C_{18}H_{17}N_3O$ (291.35): C, 74.20; H, 5.88; N, 14.42 % Found: C, 74.03; H, 5.64; N, 14.19%.

Synthesis of hydrazinovinyl -2-phenyl-1,8-naphthyridin-4(1H)-one (8a-c)

A mixture of the iminoenamine**7**(0.58 g, 2 mmol) and hydrazine derivatives namely, hydrazine hydrate, methyl hydrazine and phenyl hydrazine (2 mmol) was fused together at 130-140ºC for 4h. After cooling the solid residue was recrystallized from toluene to yield the required products 8a-c, (85-92%) yield.

7-(2-Hydrazinylvinyl)-2-phenyl-1,8-naphthyridin-4(1H)-one (8a)

The m.p.; 215-217ºC,IR (υ_{max} /cm^{-1}); 3343, 3226; 3071 (NH_2, 2NH), 1600(C=O), 1596 (C=N). ^{1}H-NMR ($CDCl_3$) δ; 0.8 (1H, br.s, NH, D_2O exchangeable),1.4 (3H, br.s, NH-NH_2, D_2O exchangeable),4.5 (1H,d, *J* = 5.5, NH_2-NHCH=CH), 5.4 (1H, br.s, NH),6.3 (1H, s, C_3-H), 7.2-7.9 (6H, m, Ar-H and CH olefinic), 8.6 (1H,d, *J*= 8.0 C_5-H), 8.7 (1H, d, *J* =8.0, C_6-H).^{13}C-NMR ($CDCl_3$) δ; 81.9 (NH_2-NHCH=CH),104.3, 116.5, 118.8, 128.3, 128.7, 129.0, 132.7, 133.7,142.4, 149.1, 150.2,153.5, 166.9(Ar-C, CN and C=O), MS (m/z) 278 M^+..Anal.Calcd. For $C_{16}H_{14}N_4O$ (278.31): C, 69.05; H, 5.07; N, 20.13 % Found: C, 69.25; H, 4.97; N, 19.93%**.**

7-(2-(2-Methylhydrazinyl) vinyl)-2-phenyl-1, 8-naphthyridin-4(1H)-one (8b)

The m.p.; 195-197ºC,IR (υ_{max} /cm^{-1});3218, 3059 (3NH), 1600(C=O), 1597 (C=N).^{1}H-NMR ($CDCl_3$) δ; 0.8 (1H, br.s, NH, D_2O exchangeable), 1.4 (1H, br.s, NH, D_2O exchangeable), 2.4 (3H, s, CH_3),4.5 (1H, d, *J*=5.8, CH_3NH-NHCH=CH), 5.3 (1H, br.s, NH, D_2O exchangeable), 6.2 (1H, s, C_3-H), 7-7.8 (6H, m, Ar-H and CH olefinic) 8.6 (1H, d, *J* = 8, C_6-H),8.7 (1H,d, *J* = 8, C_5-H).^{13}C-NMR ($CDCl_3$) δ; 30.2(CH_3NH),81.1(CH_3NH-NHCH=CH),105.0, 113.1, 119.8, 126.4, 128.3, 129.0, 132.1, 135.7, 140.1, 141.5, 152.4, 153.6, 159.8 (Ar-C, CN and C=O), MS (m/z) 292 M^+. Anal.Calcd. For $C_{17}H_{16}N_4O$ (292.34): C, 69.85; H, 5.52; N, 19.17 % Found: C, 69.76; H, 5.23; N, 18.96%.

2-Phenyl-7-(2-(2-phenylhydrazinyl) vinyl)-1,8-naphthyridin-4(1H)-one (8c)

The m.p.; 240-242ºC, IR (υ_{max} /cm^{-1}); 3341, 3198 (3NH), 1600 (C=O), 1596 (C=N).^{1}H-NMR ($CDCl_3$) δ; 0.8 (1H, br.s, NH, D_2O exchangeable), 1.4 (1H, br.s, NH, D_2O exchangeable); 4.5 (1H, d, *J* = 5.2, -NHCH=CH), 5.4 (1H, br.s, NH, D_2O exchangeable), 6.4(1H, s, C_3-H), 6.8-7.8 (11H, m, Ar-H), 8.1 (1H, d, *J* = 7.84, C_6-H), 8.9 (1H, d, *J*=7.84, C_5-H). ^{13}C-NMR ($CDCl_3$) δ; 82.1 (PhNH-NHCH=CH),103.3(C3), 113.8, 114.9, 118.1, 125.3, 127.5, 128.2, 128.5, 129.0, 131.3, 133.7, 141.9, 144.2, 145.9, 147.2, 152.2, 161.2, MS (m/z) 354 M^+. Anal. Calcd. For $C_{22}H_{18}N_4O$ (354.40): C, 74.56; H, 5.12; N, 15.81 % Found, 74.43; H, 4.87; N, 15.52%.

2.2. Cytotoxicity evaluation using viability assay

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h 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 37ºC in a humidified incubator with 5% CO_2 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 at 37°C, various concentrations of sample were added, and the incubation was continued for 24 h and viable cells

yield was determined by a colorimetric method. In brief, 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 being gently shaken 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. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cytotoxic effect of each tested compound was calculated**.** The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graph pad Prism software (San Diego, CA. USA).

3. Results and Discussion

3.1. Chemistry

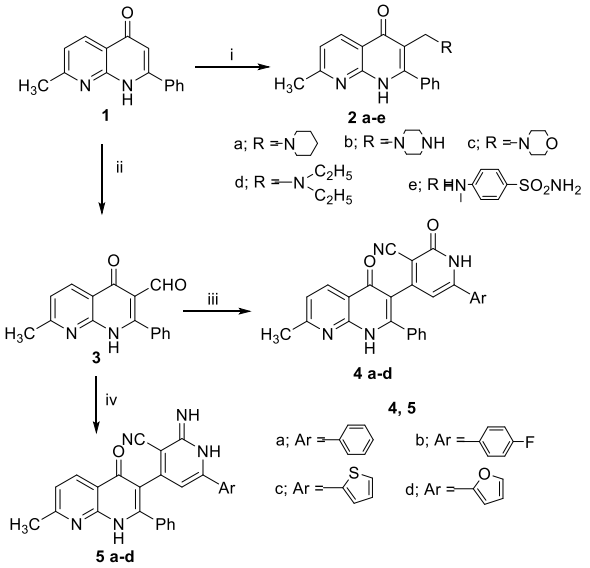
The assumed synthetic approach to obtain the target compounds was achieved by using the key intermediate 7-methyl-2-phenyl-1,8-naphthyridin-4(1*H*)-one (**1)** [27]. A series of 3-((substituted amino) methyl)-7-methyl -2-phenyl-1,8-naphthyridin-4-one (**2a-e**) was obtained by the reaction of 7-methyl-2-phenyl-1,8-naphthyridinone **(1**) with paraformaldehyde and the appropriate secondary amine namely, piperidine, piperazine, morpholine, diethylamine and sulphanilimide in absolute ethanol, under either traditional heating or ultrasound irradiation through Mannich reaction to give the products **2a-e** (Scheme **1**). The ^{1}HNMR spectra of compounds **2a-e** indicated the disappearance of C_3-H of 1,8-naphthyridine at δ 6.3 ppm, and new additional signals appeared at δ 3.9-4.3 ppm as singlet signals for $–CH_2N$- of Mannich base side chain. The data cited in (Table **1**) shows a comparison in the reaction time and the yield of the products of the two methodologies, results revealed that the ultrasonic irradiation had a vital role in the improvement of the rapid synthesis of Mannich bases.

Table1: Synthesis of compounds **2**a-e under both conventional method and ultrasonic irradiation.

Compound No.	Ultrasonic irradiation		*Conventional method*	
	Time (min)	Yield (%)	Time (h)	Yield (%)
2a	10	84	8	60
2b	25	82	12	59
2c	30	94	15	62
2d	35	92	17	57
2e	40	95	20	70

Formylation of compound **1** via Vilsmeier–Haack reaction method [28] afforded the corresponding 7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridine-3-carbaldehyde (**3)**. The structure of the naphthyridine carbaldehyde **3** was established on the bases of its spectral data and elemental analysis. One pot cyclocondensation reaction of naphthyridine carboldehyde **3** with acetyl derivatives namely, acetophenone, *p*-floroacetophenone, 2-acetylthiophene, 2-acetylfurane and excess ammonium acetate in the presence of, ethylcyanoacetate or malononitrile in ethanol, under either conventional heating or ultrasound irradiation, afforded the derivatives 6-(aryl)-4-(7-methyl-4-oxo-2-phenyl-1,4-dihydro-1,8-naphthyridin-3-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**4a-d**) or 6-(aryl)-4-(7-methyl-4-oxo-2-phenyl-1,8-naphthyridin-3-yl)-2-imino-1,2-dihydropyridine-3-carbonitrile derivatives (**5a-d)**respectively (Scheme **1**). IR spectra of **4a-d** and **5a-d** products demonstrated bands at ~ 3210-3270 cm^{-1} attributed to the N–H bond and stretching bands at 2195-2257cm^{-1} for CN group. 3-Cyano-2-pyridone derivatives **4a-d** exhibited extra band at 1610-1676 cm^{-1} which indicated the presence of carbonyl group in the form imide. Table **2** shows the beneficial effect of the ultrasound in the reaction enhancement.

Compound No.	**Ultrasonic irradiation**		**Conventional method**	
	Time (min)	Yield (%)	Time (min)	Yield (%)
4a	10	88	4	53
4b	15	84	6	51
4c	30	90	8	57
4d	45	96	14	64
5a	10	87	5	58
5b	15	80	10	55
5c	35	92	14	52
5d	40	95	17	73



Scheme 1: Reagents and conditions: (i) Amines, p-formaldehyde, ethanol US or heat (ii) $POCl_3$/DMF (iii) $RCOCH_3$,$CNCH_2COOC_2H_5$,CH_3COONH_4,C_2H_5OH; US or heat (iv) $RCOCH_3$,$CH_2(CN)_2$, CH_3COONH_4, C_2H_5OH; US or heat

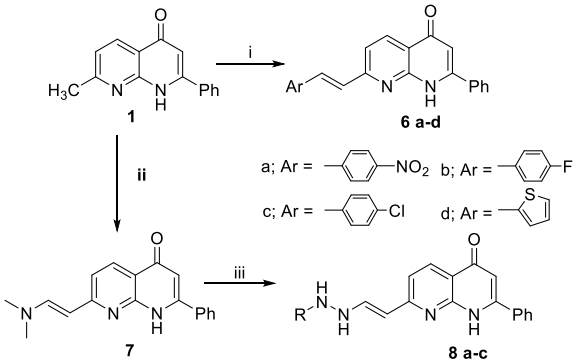
Furthermore, reaction of 7-methyl-2-phenyl-1,8-naphthyridin-4-one (**1)** with different aromatic aldehydes namely, *p*-nitrobenzaldehyde, *p*-flourobenzaldehyde, *p*-chlorobenzaldehyde, thiophene-2-carboxaldehyde in xylene with catalytic amount of piperidine under reflux or ultrasound irradiation afforded the corresponding 7- styryl-1,8-naphthyridinone **6a-d (**Scheme **2**). The ^{1}HNMR of **6a-d** showed the disappearance of CH_3 protons and the appearance of the two olefinic CH protons. The ^{13}C NMR of **6c** as an example showed the disappearance of CH_3 band at δ 24.0 ppm and the appearance of olefinic carbons at 132.1 and 135.1 ppm. The Mass spectrum (EI) of **6c** reveals the molecular ion peak at m/z 358 as base peak and M+2 at 360 (33%) signifying its high stability. The data .

Table3: Synthesis of 7- styryl-1,8-naphthyridines derivative **(6a-d)** under both ultrasonic irradiation and conventional method.

Compound No.	Ultrasonic irradiation		*Conventional method*	
	Time (min)	Yield (%)	Time (h)	Yield (%)
6a	10	89	4	60
6b	15	82	6	55
6c	30	90	8	70
6d	40	96	14	64

presented in (Table **3)** shows a drop in the reaction time and a rise in the yield of the products as a result of using ultrasonic irradiation. These results show the crucial role of ultrasonic irradiation in improvement of the rapid synthesis of styryl compounds.

Moreover, 7-methyl-2-phenyl-1,8-naphthyridin-4-one (**1)** was refluxed with dimethylformamide/dimethylacetal (DMF/DMA) to yield the corresponding iminoenamine**7**. The ^{1}H-NMR spectrum of **7** showed the disappearance of CH_3 protons at C-7, the appearance of two singlet signals at δ 2.7 and 2.8 ppm for $-N(CH_3)_2$, two doublet signals at δ 4.1 for α CH and the β CH appeared with the aromatic protons in the range 7.0-8.1 ppm. Fusion of the iminoenamine**7** with hydrazine derivatives namely, hydrazine hydrate, methyl hydrazine and phenyl hydrazine at 130-140^{o}C, afforded the products **8a-c**. The structures of these compounds were assigned based on their microanalysis and spectral data. However, carrying out this reaction under ultrasound irradiation failed. This may be attributed to the absence of the solvent; it is well known that sono-chemistry deals with studying and understanding the effect of ultrasound irradiation in creating acoustic cavitation in liquids that initiate and enhance the chemical activity in the solution. For this reason, the chemical effects of ultrasound waves do not come from direct interaction of this wave with the molecules in the solution [29,30] Sonochemistry comes from acoustic cavitation, the construction, growing, and implosive breakdown of bubbles in a liquid [30]. The implosive breakdown of these bubbles causes tremendously high pressures and temperatures in a microscopic district of the sonicated liquid, these extreme conditions of pressure and temperature result in the chemical excitation of the reactant that was inside the bubbles, or in the close surroundings of the bubble as it quickly collapsed.



Scheme 2: Reagents and conditions: (i) ArCHO/xylene, US and heat (ii) DMF/DMA ? (iii) $RNHNH_2$, heat

3.2. Biological Evaluation

In the work presented, the new compounds **2a-e**, **4c**, **5c,d** and **6b-d**, were selected to test their effects on human cultured hepatic cancer cell lines (HePG2) regarding their *in-vitro* growth inhibitory activities, comparing it to Doxorubicin, a highly effective anticancer agent, using standard MTT assay method [31]. According to cultivated results (Table **4**), remarkably, all tested derivatives demonstrated growth inhibitory actions against the hepatic cancer cells at IC_{50} slightly more or less than that presented by the reference drug. Noticeably, the 2-oxo-6-thiophenyl pyridine derivative **4c** proved to have the highest potency (IC_{50}: 0.015 μM). A minute decrease in the potency was seen when the carbonyl group in pyridine is replaced by imino group **5c** (IC_{50}: 0.017 μM). On the other hand, when the thiophenyl group in **5c** is replaced by furayl group **5d**, its activity sharply decreases (IC_{50}: 0.244 μM), this reflects the importance of the presence of Sulphur atom in the ring system. The Mannich bases **2a, c, d, e** showed also good activity at (IC_{50}: 0.03- 0.08μM) which may be attributed due to the presence of the basic side chain. On the other hand, the styryl derivatives (**6b-d)** showed moderate activities at (IC_{50}: 0.18 and 0.25 μM). According to literature survey the compounds bearing 1,8-Naphthyridine scaffold can produce their anticancer potency via different molecular mechanisms such as; protein kinase inhibition like topoisomerase II, c-Met, VEGFR-2, EGFRPDGFR-β, apoptosis inducing effect and via enhancing the activity of different proapoptotic proteins such as caspases, p53, Bax/BCl-2 [32,33]. In addition, various 1,8-naphthyridine derivatives intercalate the adjacent base pairs of DNA resulting in inhibition of DNA duplication or transcription and suppression the growth of cancer cells [34].

4. Conclusion

The main target of the work presented is to synthesize new 1,8-naphthyridine derivatives through the application of changes at C-3 and C-7 positions. In turn, we have created a variety of novel Mannich base, pyridone, iminopyridone, and styryl side chain incorporated into 1,8-naphthyridine nucleus. The goal of the work done has been reached by examining the cytotoxicity of these compounds against HePG2 (Liver hepatocellular carcinoma) cell line, compared to the outdated anticancer drug DOX. Results declared that, reasonable antitumor activity was seen with all the tested compounds, **5c** and **4c** in particular showed the most significant inhibition of HePG2 cell line with value IC_{50} values (0.017, 0.015μM) comparing with DOX, with IC_{50} (0.016 μM).

Table 4: The influence of some newly synthesized compounds against human liver carcinoma cell line (HePG2).

Compounds No.	IC_{50} (μM)
2 a	0.030 ± 0.50
2 b	0.160 ± 0.90
2 c	0.040 ±0.40
2 d	0.080 ± 0.90
2 e	0.029 ± 0.4
4 c	0.015 ± 0.12
5 c	0.017 ± 0.30
5 d	0.244 ± 6.20
6 b	0.230 ± 1.10
6 c	0.250 ± 2.50
6 d	0.180 ± 3.80
DOX	0.016 ± 0.08

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