Research Article

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Novel 1,8-Naphthyridine Derivatives: Design, Synthesis and *in vitro* screening of their cytotoxic activity against MCF7 cell line

https://doi.org/10.1515/chem-2019-0097
received December 6, 2018; accepted March 11, 2019.

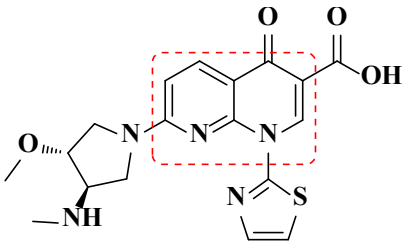
Abstract: A series of new 2-phenyl-7-methyl-1,8-naphthyridine derivatives with variable substituents at C3 were synthesized for an *in vitro* evaluation of their anticancer activity against human breast cancer cell line (MCF7). On one hand, compounds **3f**, **6f**, **8c**, and **10b** showed IC_{50} values (6.53, 7.88, 7.89, 7.79 μM, respectively) compared to that of the mentioned drug staurosparine (IC_{50} = 4.51 μM). On the other hand, derivatives **10c**, **8d**, **4d**, **10f** and **8b** displayed better activity than staurosporin with IC_{50} values (1.47, 1.62, 1.68, 2.30, 3.19 μM, respectively).

Keywords: 1,8- naphthyridine; pyrazole; pyrimidine; pyridine; cytotoxic; breast cancer.

1 Introduction

Cancer is one of the deadliest diseases affecting public health around the world. The occurrence and death statistics represent that it is on the rise in the developed and economically developing countries [1,2]. Cancer related deaths in females is caused primarily by breast cancer. It constitutes 23% of the total cancer cases, out of which 14% lead to death, the incidence that rises significantly with age [1,2]. On a cellular level, cancer develops as a result of failed cell division and deregulation of signaling cascades. What follows is an uncontrolled cell division, increased cell survival and the development of resistance to anticancer therapies [3].

Even though an early discovery of the disease improves the survival rates through recognized therapeutic routes, metastatic breast malignancy is still the leading cause of breast cancer-related deaths [4]. Noticeably, about 30% of the primarily detected localized malignant masses will progress to an advanced or metastatic disease in 5 years [5,6]. Naphthyridine derivatives are broadly spread in naturally occurring products, principally as tricyclic benzo[f] [1,7] naphthyridines and benzo[c] naphthyridines [2,7]. These derivatives play a vital role in therapeutic products [7-9]. Naphthyridines derivatives have a wide spectrum of biological effects, for example anti-inflammatory, antimalarial, antifungal, and antibacterial [10-12]. Moreover, naphthyridine derivatives displayed good HIV-1 integrase inhibitor profiles and cytotoxicity [13,14]. Certain 1,8-naphthyridine derivatives are considered good DNA intercalators. They bind with double stranded DNA (ds-DNA) by intercalating between adjacent base pairs, thus changing the DNA conformation and inhibit DNA duplication or transcription leading to suppression of cancer cell growth [15-17]. Due to their wide range of biological effects, 1,8-naphthyridine derivatives have been considered promising antitumor agents [18-20]. Voreloxin (AG-7352, SPC-595, SNS 595, voreloxin) is a naphthyridine analog. It intercalates DNA in the presence of topoisomerase II, resulting in selective, replication-dependent DNA damage, irreversible G2 arrest and rapid apoptosis [21] **(Figure 1)**.



Voreloxin

Figure 1: The chemical structure of Voreloxin.

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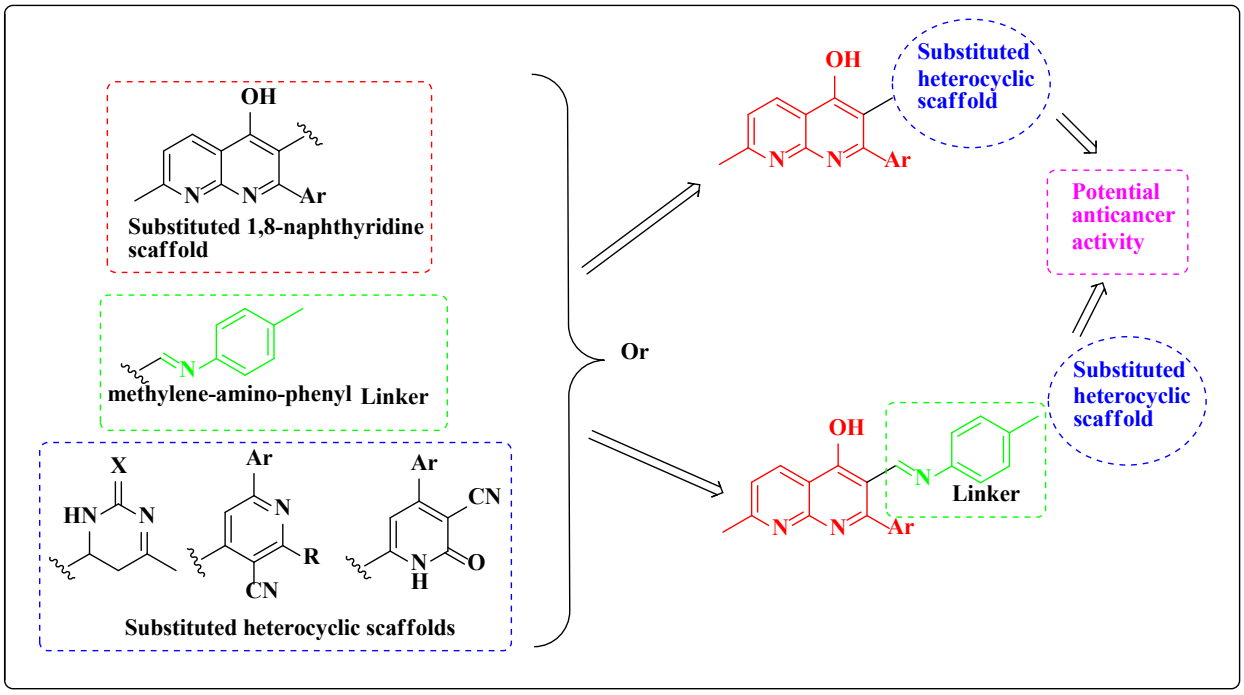


Figure 2: Design of 1,8-naphtheridine hybrid compounds as anticancer agents.

In addition, a novel class of 1,8-naphthyridine-3-carboxamide carrying cyclic and open chain amino acids that were linked at C-3 position had been synthesized. These chemicals were tested for anticancer activity, and the results obtained revealed a promising *in vitro* cytotoxic activity profile against different human cancer cell lines such as breast, colon, oral [22-26]. As a continuation of our previous efforts [27-29], herein, we described the synthetic preparation of a new class of compounds bearing 4-hydroxy-7-methyl-2-phenyl-1,8-naphthyridine scaffold. This new class was hybridized with different heterocylic ring systems known to exhibit anticancer activity such as substituted pyrazole, pyridine and pyrimidine rings [30,31] **(Figure 2)**. The new compounds were evaluated as cytotoxic agents against breast cancer cell lines (MCF7). Some of these compounds displayed a noticeable effect on cancer cells, with IC_{50} values comparable with the used reference drug.

2 Materials and Methods

Informed consent has been obtained from all individuals included in this study.

Ethical approval: The conducted research is not related to either human or animals use.

2.1 General Information

Commercially available solvents and reagents were purified according to the standard procedures. All melting points were measured on a Barnstead international 1002 melting point apparatus and were calibrated. Thin layer chromatography (TLC) was performed on aluminum silica gel 60 F_{254} (E-Merk). The spots were detected by iodine and UV light absorption. IR spectra were recorded for the compounds on a FTIR, Perkin Elmer SP 100 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker WM 300 and 850 MHz spectrometers using TMS (0.00 ppm) or the signal of the deuterated solvent were used as internal standard. Chemical shift (δ) is given in ppm relative to the signal for TMS as standard, and coupling constant in expressed in Hz. Microanalysis was performed by Perkin Elmer elemental analyzer at the Faculty of Science, King Abdul Aziz University [32].

2.1.1 Synthesis of 4-Hydroxy-7-methyl-2-phenyl-1,8-naphthyridine-3-carbaldehyde (2)

$POCl_3$ (1.86mL, 20 mmol) was added dropwise with stirring to an ice-cooled flask containing *N, N*-dimethylformamide (20 mL). After a complete addition, the solution was stirred at room temperature for 90 min. Then the flask was cooled again in ice-bath and compound **1** (2.36 g,

10 mmol) was added by portions. The reaction mixture was warmed to at kept at 75°C for 5 h then cooled to room temperature and poured onto an ice water, basified (sat. aqua. K_2CO_3 solution) and extracted with $CHCl_3$ (120 mL), washed, dried with ($MgSO_4$) and evaporated under *vacuo* to attained compound **2 (**2.55g**,** 97% yield**)**, yellowish crystals, m.p.114°C. FTIR, cm^{-1}: 3420 (OH); 1661 (C=O). ^{1}H NMR (DMSO-d6) δ_H: 3.05 (3H, s, CH_3), 5.4 (1H, br. s, OH), 7.5 -8.4 (5H, m, Ar-H), 8.6 (1H,d,C_6-H, *J* = 9.2 Hz), 8.8 (1H,d,C_5-H, *J* = 9.2 Hz), 9.5 (1H, s, CHO). MS (*m/z*) 264 M^+ Found: C, 72.75; H, 4.23; N, 10.27. $C_{16}H_{12}N_2O_2$ requires 72.72; H, 4.58; N, 10.60%.

2.1.2 Synthesis of 3-(4-hydroxy-7-methyl-2-phenyl-1,8-naphthyridin-3-yl)-1-arylprop-2-en-1-one (3a-g).

In an Erlenmeyer flask, a solution of aldehyde **2** (0.5 g, 2 mmol) in ethanol (10 mL), acetyl compounds (2 mmol) and Mg-Al- hydrotalcite (0.3g) [33] were refluxed together for appropriate time as examined by TLC. The reaction mixture was filtered to isolate the HT. The filtrate was concentrated under *vacuo*. The residual solid was crystallized by ethanol to give the title product.

1-Furan-2-yl-3-(4-hydroxy-2-phenyl-[1,8]-naphthyridin-3-yl)-propenone (3a):

Light brown crystals, (89.55%), m.p. 189-190°C. IR: ν_{max}/cm^{-1} 3350 (OH), 1656 (CO).1H NMR (DMSO-d6) δH: 2.08 (3H, s, CH_3), 5.79 (1H, s, -OH) , 6.68-7.57 (3H, m, C-H of furan), 7.85 (1H, d, C_6-H, *J* = 9.8 Hz), 8.03 (1H, d, C_5-H, *J* = 9.8 Hz), 8.30-8.45 (5H, m, Ar-H) 8.38 (1H, d, CH=CHCO, *J* = 8.4 Hz), 8.58 (1H, d, CH=CHCO, *J* = 8.4 Hz). ^{13}C-NMR (DMSO-d6) δC: 22.41, 106.10, 113.26, 116.62, 122.53, 123.19, 128.19, 129.39, 130.11, 130.16, 131.06, 137.65, 142.48, 147.50, 152.65, 156.01, 158.48, 159.29, 167.89, 175.29. MS (*m/z*) 356 M^+ Found: C, 74.29; H, 4.42; N, 7.51 $C_{22}H_{16}N_2O_3$ requires C, 74.15; H, 4.53; N, 7.86%.

3-(4-Hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-1-thiophen-2-yl-propenone (3b):

Brown crystals, (93.36%), m.p.249-251°C. IR: ν_{max}/cm^{-1} 3470 (OH), 1690 (CO).1H NMR (DMSO-d6) δH: 2.09 (3H, s, CH_3), 5.78 (1H, s, -OH), 6.80 (1H, d, C_6-H, *J* = 8.5 Hz), 7.50 (1H, d, C_3H of thiophene), 7.60 (1H, dd, C_4H of thiophene,), 7.74 (1H, d, C_5H of thiophene), 8.09 (1H, d, C_5-H, *J* = 8.5 Hz), 8.31-8.36 (5H, m, Ar-H), 8.37 (1H, d, CH=CHCO, *J* = 8.4 Hz), 8.78 (1H, d, CH=CHCO, *J* = 8.4 Hz). ^{13}C NMR (DMSO-d6) δC: 22.15, 107.11, 116.89, 117.87, 125.19, 127.96, 128.08 ,128.22, 129.43 130.12, 133.49, 134.47, 137.84, 143.25, 146.42,150.58, 156.18, 158.62, 162.82,186.93. MS (*m/z*) 372 M^+ Found: C, 71.29; H, 4.02; N, 7.13. $C_{22}H_{16}N_2O_2S$ requires C, 70.95; H, 4.33; N, 7.52%.

3-(4-Hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-1-phenyl-propenon (3c):

Orange crystals, (96.02%), m.p. above 320°C. IR: ν_{max}/cm^{-1} 3170 (OH), 1696 (CO). 1H NMR (DMSO-d6) δH:2.73 (3H, s, CH_3), 5.79 (1H, s, -OH), 7.75, 8.29 (10H, 2m, Ar-H), 7.62 (1H, d, C_6-H, *J* = 7.7 Hz), 8.30 (1H, d, C_5-H, *J* = 7.7 Hz), 8.10 (1H, d, CH=CHCO, *J* = 8.3 Hz), 8.56 (1H, d, CH=CHCO, *J* = 8.3 Hz). ^{13}C NMR (DMSO-d6) δC: 22.19, 107.27, 116.89, 119.09, 123.19, 125.96, 127.96, 128.23, 129.12, 130.11, 133.49, 134.47, 137.86, 143.25, 146.42, 150.95, 156.18, 158.76, 162.82 , 168.93. MS (*m/z*) 366 M^+ Found: C, 78.30; H, 4.43; N, 7.26. $C_{24}H_{18}N_2O_2$ requires C, 78.67; H, 4.95; N, 7.65%.

3-(4-Hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-1-p-tolyl-propenone (3d):

Yellow crystals, (91.84%), m.p. 228-230°C. IR: ν_{max}/cm^{-1} 3457 (OH), 1677 (CO). 1H NMR (DMSO-d6) δH: 1.50 (3H, s, CH3), 2.08 (3H, s, CH_3), 5.80 (1H, s, -OH) ,7.50 (1H, d, C_6-H, *J* = 8.3 Hz), 8.00 (1H, d, C_5-H, *J* = 8.3 Hz), 8.17 (1H, d, CH=CHCO, *J* = 7.2 Hz) , 7.51, 8.30 (9H, 2m, Ar-H), 8.34 (1H, d, CH=CHCO, *J* = 7.2 Hz). ^{13}C NMR (DMSO-d6) δC: 14.79, 22.69, 107.99, 117.91, 124.62, 128.10, 129.18, 129.39, 130.12, 133.72, 135.74, 140.00, 142.84, 145.21, 150.91, 157.42, 158.44, 162.89, 189.03. MS (*m/z*) 380 M^+ Found: C, 78.49; H, 4.92; N, 7.11. $C_{25}H_{20}N_2O_2$ requires C, 78.93; H, 5.30; N, 7.36%.

3-(4-Hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-1-(4-methoxy-phenyl)-propenone (3e):

Dark brown crystals, (86.60%), m.p. 160-162°C. IR: ν_{max}/cm^{-1} 3480 (OH), 1690 (CO). 1H NMR (DMSO-d6) δH: 2.73 (3H, s, CH3), 2.89 (3H, s, -OCH_3), 5.78 (1H, s, -OH), 7.55, 8.30 (9H, 2m, Ar-H)), 8.10 (1H, d, C_6-H, *J* = 8.4 Hz), 8.25 (1H, d, C_5-H, *J* = 8.4 Hz), 8.38 (1H, d, CH=CHCO, *J* = 7.4 Hz), 8.54 (1H, d, CH=CHCO, *J* = 7.4 Hz).^{13}C-NMR (DMSO-d6) δC: 14.65, 55.92, 106.31, 113.16, 116.38, 125.06 , 127.91, 128.24, 128.68, 129.37, 139.01, 140.81, 143.94, 150.14, 158.03, 159.57, 162.76, 169.99, 186.83. MS (*m/z*) 396 M^+ Found: C, 75.46; H, 4.72; N, 6.71. $C_{25}H_{20}N_2O_2$ requires C, 75.74; H, 5.08; N, 7.07%.

1-(4-Fluoro-phenyl)-3-(4-hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-propenone (3f):

Yellow crystals, (94.23%), m.p. 207-210°C. IR: ν_{max}/cm^{-1} 3456 (OH), 1697 (CO). 1H NMR (DMSO-d6) δH: 2.89 (3H, s, CH_3), 5.78 (1H, s, OH), 7.49, 8.37 (9H, 2m, Ar-H), 7.80 (1H, d, C_6-H, *J* = 9.9 Hz), 8.90 (1H, d, C_5-H, *J* = 9.8 Hz), 7.93 (1H, d, CH=CHCO, *J* = 8.4 Hz), 8.45 (1H, d, CH=CHCO, *J* = 8.4Hz).^{13}C-NMR (DMSO-d6) δC: 22.49, 106.57, 116.30, 117.51, 122.34, 127.14, 128.27, 130.12, 131.69, 135.34, 140.20, 142.03, 150.13, 157.63, 158.84, 162.10, 163.57, 186.22. . MS (*m/z*) 384 M^+ Found: C, 75.36; H, 4.17; N, 6.91. $C_{24}H_{17}FN_2O_2$ requires C, 74.99; H, 4.46; N, 7.29%.

3-(4-Hydroxy-7-methyl-2-phenyl-[1,8]-naphthyridin-3-yl)-1-(4-nitro-phenyl)-propenone (3g):

Reddish brown crystals, (96.40%), m.p. > 320°C. IR: ν_{max}/cm^{-1} 3345 (OH), 1630 (CO). 1H NMR (DMSO-d6) δH: 2.09 (3H, s, CH_3), 5.32 (1H, s, -OH), 7.52 (1H, d, C_6-H, *J* = 7.5 Hz), 7.56 – 7.90 (9H, 2m, Ar-H) 8.33 (1H, d, C_5-H, *J* = 7.5 Hz), 7.76 (1H, d, CH=CHCO, *J* = 8.3 Hz), 8.42 (1H, d, CH=CHCO, *J* = 8.3 Hz). ^{13}C-NMR (DMSO-d6) δC: 21.10, 109.24, 115.62, 118.05, 122.68, 125.12, 127.26, 129.37, 130.84, 132.13, 136.48, 140.59, 143.78, 150.65, 153.27, 156.18, 158.04, 164.93, 189.20. MS (*m/z*) 411 M$^+$ Found: C, 70.37; H, 3.87; N, 9.94. $C_{24}H_{17}FN_2O_2$ requires C, 70.07; H, 4.16; N, 10.21%.

2.1.3 Synthesis of 3-(4,5-dihydro-1-phenyl-3-aryl-1*H*-pyrazol-5-yl)-7-methyl-2-phenyl-1,8-naphthyridine-4-ol (4d,f, and g).

A mixture from chalcons **4** (10 mmol) and phenylhydrazine (1.2 mL, 12mmol) in 10ml acetic acid were refluxed for 8-10h. The reaction mixture was cooled, poured onto ice water (50mL), and neutralized with cold NH_4OH solution. The formed solid product was collected by filtration and crystalized from ethanol to give the title products.

3-(4,5-dihydro-1-phenyl-3-*p*-tolyl-1*H*-pyrazol-5-yl)-7-methyl-2-phenyl-1,8-naphthyridine-4-ol (4d):

Dark brown solid, (63%), m.p. 108-110°C. IR: ν_{max}/cm^{-1} 3158 (OH), 1596, 1504 (C=C, C=N). 1H NMR (DMSO-d6) δH: 2.00 (2H, dd, C_4-H, *J* = 9.3 Hz), 2.74, 2.90 (6H, 2s, $2CH_3$), 5.32 (1H, t, C_5-H, *J* = 9.3 Hz), 5.64 (1H, s, -OH), 6.41-8.51 (16H, m, Ar-H). MS (*m/z*) 470 M$^+$ Found: C, 79.16; H, 5.23; N, 11.46. $C_{31}H_{26}N_4O$ requires C, 79.12; H, 5.57; N, 11.91%.

3-[5-(4-Fluoro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-7-methyl-2-phenyl-1,8-naphthyridin-4-ol (4f):

Yellow solid, (62%), m.p.104-106°C. IR: ν_{max}/cm^{-1} 3210 (OH), 1596, 1560 (C=C, C=N).

1H NMR (DMSO-d6) δH: 1.98 (2H, dd, C_4-H, *J* = 9.3 Hz), 2.58 (3H, s, CH_3), 5.32 (1H, t, C_5-H, *J* = 9.3 Hz), 5.85 (1H, s, -OH), 6.38-8.57 (16H, m, Ar-H). MS (*m/z*) 474 M$^+$ Found: C, 75.46; H, 4.41; N, 11.39. $C_{30}H_{23}FN_4O$ requires C, 75.93; H, 4.89; N, 11.81%.

7-Methyl-3-[5-(4-nitro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl]-2-phenyl-1,8-naphthyridin-4-ol (4g):

Orange solid, (78%), m.p. 160-162°C. IR: ν_{max}/cm^{-1} 3130 (OH), 1620,1597 (C=C, C=N).

1H NMR ($CDCl_3$) δH: 2.02 (2H, dd, C_4-H, *J* = 9.3 Hz), 2.70 (3H, s, CH_3), 5.35 (1H, t, C_5-H, *J* = 9.3 Hz), 5.85 (1H, s, -OH), 6.70-8.37 (16H, m, Ar-H). ^{13}C NMR ($CDCl_3$) δC: 22.49, 38.10, 43.24, 107.14, 111.43, 116.90, 125.58, 126.56, 127.44, 128.45, 129.62, 130.41, 135.11, 137.37, 140.14, 140.46, 150.14, 154.43, 157.63, 168.65, 165.11. MS (*m/z*) 501 M$^+$ Found: C, 72.06; H, 4.23; N, 13.49. $C_{30}H_{23}N_5O_3$ requires C, C, 71.84; H, 4.62; N, 13.96%.

2.1.4 Synthsis of 6-(4-Hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-4-aryl-5,6-dihydro-1H-pyrimidin-2-one (5d,f, and g)

Chalcons **4** (10 mmol) were added to a mixture made from urea (1g, 15mmol) in 20 mL of ethanol and few drops of conc. HCl. The mixture was refluxed for 10h. The reaction was concentrated, cooled, poured onto ice water (50mL), and neutralized with cold NH_4OH solution. The solid product formed was collected by filtration and then crystalized from ethanol to give the products **5d,g, and f**.

6-(4-Hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-4-p-tolyl-5,6-dihydro-1H-pyrimidin-2-one (5d):

White crystals, (51%), m.p. 290-292°C. IR: ν_{max}/cm^{-1} 3230 (OH), 1711 (C=O).1H NMR (DMSO-d6) δH: 1.97 (2H, dd, C_5-H, *J* = 12.7 Hz), 2.73, 2.90 (6H, 2s, $2CH_3$), 5.31 (1H, t, C_6-H, *J* =12.7 Hz), 5.78 (1H, s, -OH), 6.92-8.62 (12H, m, Ar-H , NH). ^{13}C NMR (DMSO-d6) δC: 22.49, 38.10, 43.24, 107.14, 111.43, 116.90, 125.58, 126.56, 127.44, 128.45, 129.62, 130.41, 135.11, 137.37, 150.30, 158.19, 159.40, 163.04, 164.44, 168.28. MS (*m/z*) 422 M$^+$ Found: C, 73.06; H, 4.93; N, 13.03. $C_{25}H_{22}N_4O_2$ requires C, 73.92; H, 5.25; N, 13.26%.

4-(4-Fluoro-phenyl)-6-(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-yl)-5,6-dihydro-1H-pyrimidin-2-one (5f):

Yellow crystals, (53%), m.p.115-117°C. IR: ν_{max}/cm^{-1} 3119 (OH), 1764 (C=O).1H NMR (DMSO-d6) δH: 1.98 (2H, dd, C_5-H, *J* = 11.9 Hz), 2.71 (3H, s, CH_3), 5.32 (1H, t, C_6-H, *J* = 11.9 Hz), 5.43 (1H, s, -OH), 6.67-8.35 (12H, m, Ar-H , NH). ^{13}C NMR (DMSO-d6) δC: 13.26, 22.45, 29.10, 101.21, 115.30, 116.31, 121.19, 125.01, 127.03, 128.11, 129.25, 129.33, 135.32, 139.57, 150.44, 151.57, 154.36, 161.64, 164.45, 166.07. MS (*m/z*) 426 M$^+$ Found: C, 70.96; H, 4.21; N, 12.99. $C_{25}H_{19}FN_4O_2$ requires C, 70.41; H, 4.49; N, 13.14%.

6-(4-Hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-4-(4-nitro-phenyl)-5,6-dihydro-1H-pyrimidin-2-one (5g):

Brown solid, (67%), m.p. > 300°C. IR: ν_{max}/cm^{-1} 3162 (OH), 1718 (C=O). 1H NMR (DMSO-d6) δH: 2.00 (2H, dd, C_5-H, *J* =10.2 Hz), 2.74 (3H, s, CH_3), 5.33 (1H, t, C_6-H, *J* =10.2 Hz), 5.77 (1H, s, -OH), 7.53-8.82 (12H, m, Ar-H, NH). ^{13}C NMR (DMSO-d6) δC: 22.45, 29.52, 42.48, 107.82, 123.38, 124.20,

125.01, 126.05, 128.16, 129,67, 135.64, 138.34, 140.55, 150.92, 152.52, 158.33, 159.40, 163.21, 164.68, 167.60. MS (*m/z*) 453 M^+ Found: C, 66.61; H, 4.01; N, 15.01.

$C_{25}H_{19}N_5O_4$ requires C, C, 66.22; H, 4.22; N, 15.44%.

2.1.5 Synthesis of 5,6-dihydro-6-(4-Hydroxy-7-methyl-2-phenyl-1,8-naphthyridin-3-yl)-4-aryl-2-(1H)-thione (6d,f, and g)

A mixture of chalcons **4** (10 mmol), thiourea (1.1 mL, 14mmol) and NaOH (0.1 g) in 25 mL ethanol were refluxed for 10h. The reaction mixture was concentrated, cooled, and the formed solid product was collected by filtration, then crystalized from ethanol to give the title products **5d,g, and f.**

5,6-dihydro-6-(4-Hydroxy-7-methyl-2-phenyl-1,8-naphthyridin-3-yl)-4-p-tolylpyrimidine-2-(1H)-thione (6d).

Yellow crystals (64 %), m.p. > 300°C. IR: ν_{max}/cm^{-1} 3342 (OH).1H NMR (DMSO-d6) δH: 1.97 (2H, dd, C_5-H, *J* = 9.3 Hz), 2.08 (1H, s, -NH), 2.69 (6H, s, $2CH_3$), 5.31 (1H, t, C_6-H, *J* = 9.3 Hz), 5.45 (1H, s, -OH), 6.45-8.74 (11H, m, Ar-H). ^{13}C NMR (DMSO-d6) δC: 22.35, 34.93, 41.06, 106.42, 122.75, 124.45, 126.65, 129.15, 130.12, 135.12, 139.37, 150.29, 158.33, 159.19, 162.54, 165.66, 187.51. MS (*m/z*) 438 M^+ Found: C, 71.46; H, 4.83; N, 12.36. $C_{26}H_{22}N_4O_2S$ requires C, 71.21; H, 5.06; N, 12.78%.

4-(4-Fluoro-phenyl)-5,6-dihydro-6-(4-hydroxy-7-methyl-2-phenyl-1,8-naphthyridin-3-yl) pyrimidine-2(1H)-thione (6f):

White solid (37 %), m.p. 286- 288 °C. IR: ν_{max}/cm^{-1} 3452 (OH).1H NMR (DMSO-d6) δH: 1.98 (2H, dd, C_5-H, *J* = 9.1 Hz), 2.09 (1H, s, -NH), 2.71 (3H, s, CH_3), 5.31 (1H, t, C_6-H, *J* = 9.1 Hz), 5.40 (1H, s, -OH), 6.89-8.71 (11H, m, Ar-H) . MS (*m/z*) 442 M^+ Found: C, 68.26; H, 4.01; N, 12.32. $C_{25}H_{19}FN_4O$ requires: C, 67.86; H, 4.33; N, 12.66%.

5,6-dihydro- 6-(4-Hydroxy-7-methyl-2-phenyl-1,8-naphthyridin-3-yl)-4-(4-nitro-phenyl) pyrimidine-2-(1H)-thione (6g):

Yellow crystal (46%), m.p. 243-245°C. IR: ν_{max}/cm^{-1} 3343 (OH).1H NMR (DMSO-d6) δH: 2.00 (2H, dd, C_5-H, *J* = 8.5 Hz), 2.10 (1H, s, -NH), 2.74 (3H, s, CH_3), 5.33 (1H, t, C_6-H, *J* = 8.5 Hz), 5.77 (1H, s, -OH), 7.53-8.52 (11H, m, Ar-H). ^{13}C NMR (DMSO-d6) δC: 22.94, 35.40, 40.47, 107.82, 116.14, 122.23, 124.20, 125.01, 127.43, 128.74, 130.06, 135.93, 137.26, 141.01, 150.92, 151.91, 156.57, 157.00, 160.66, 161.08, 187.34. MS (*m/z*) 469 M^+ Found: C, 63.48; H, 3.98; N, 14.59. $C_{25}H_{19}N_5O_3S$ requires: C, 63.95; H, 4.08; N, 14.92%.

2.1.6 Synthesis of 1-{4-[(4-Hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-ethanone (7).

A solution of aldehyde **2** (5 g, 20 mmol) and p-aminoacetophenone (2.7g, 20mmol) in ethanol (30 mL) were refluxed together for 2h. The reaction was concentrated, cooled, and the formed preciptate was recrystallized from dilute ethanol to give the title compound as orange crystal in 84% yield, m.p.137-139°C. IR: ν_{max}/cm^{-1} 3463 (OH), 1659 (C=O), 1621 (C=N).1H NMR ($CDCl_3$) δH: 2.19, 2.52 (6H, 2s, $2CH_3$), 5.50 (1H, s, -OH), 7.53-8.52 (11H, m, Ar-H), 7.55 (1H, s, CH=N). ^{13}C NMR ($CDCl_3$) δC: 22.70, 26.11, 108.35, 113.74, 117.88, 121.29, 124.46, 128.75, 129.62, 130.46, 130.67, 135.95, 138.56, 141.05, 151.11, 158.60, 161.07, 164.48, 166.92, 196.57. MS (*m/z*) 381 M^+ Found: C, 75.91; H, 4.88; N, 10.86. $C_{24}H_{19}N_3O_2$ requires: C, 75.57; H, 5.02; N, 11.02%.

2.1.7 Synthesis of 4-(aryl)-6-{4-[(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-2-oxo-1,2-dihydro-pyridine-3-carbonitrile 8a-d

A mixture made from **7** (0.8g, 2mmol), aldehyde (2 mmol), ethylcyanoacetate (0.21mL, 2 mmol) were refluxed for 2-4h. in a 10 mL absolute ethanol. The reaction mixture was allowed to cool and the resulting solid precipitate was filtered and recrystallized from ethanol to obtain the title product **8a-d**.

4-(4-Chloro-phenyl)-6-{4-[(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (8a):

Brownish crystal (83%), m.p. 209-211°C. IR: ν_{max}/cm^{-1} 3159 (OH), 2226 (CN), 1712 (C=O). 1H NMR (DMSO-d6) δH: 2.60 (3H, s, CH_3), 5.61 (1H, s, -OH), 6.80 (1H, s, C_5-H of pyridone), 7.40-8.60 (16H, m, Ar-H, NH), 7.54 (1H, s, CH=N). ^{13}C NMR (DMSO-d6) δC: 22.93, 96.08, 101.31, 107.41, 111.05, 117.39, 121.37, 122.11, 123.19, 127.41, 129.26, 129.69, 131.01, 131.60, 134.72, 135.52, 136.01, 137.95, 148.75, 154.51, 158.05, 162.63, 163.44, 167.41, 169.71. MS (*m/z*) 567 M^+, 569 M^++2 Found: C, 71.43; H, 3.68; N, 11.99. $C_{34}H_{22}ClN_5O_2$ requires: C, 71.89; H, 3.90; N, 12.33%.

6-{4-[(4-Hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-ylmethylene)-amino]-phenyl}-4-(4-nitro-phenyl)-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (8b):

Reddish crystal (88%), m.p. 179-181°C. IR: ν_{max}/cm^{-1} 3315 (OH), 2220 (CN), 1695 (C=O). 1H NMR (DMSO-d6) δH: 2.37 (3H, s, CH_3), 5.62 (1H, s, -OH), 7.01 (1H, s, C_5-H

of pyridone), 6.72-8.59 (16H, m, Ar-H, NH), 7.60 (1H, s, CH=N). MS (*m/z*) 578 M^+ Found: C, 70.79; H, 3.38; N, 14.09. $C_{34}H_{22}N_6O_4$ requires: C, 70.58; H, 3.83; N, 14.53%.

4-(4-Fluoro-phenyl)-6-{4-[(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (8c):

Green crystal (78%), m.p. 188-190°C. IR: ν_{max}/cm^{-1} 3150 (OH), 2140 (CN), 1736 (C=O). 1H NMR (DMSO-d6) δH: 2.60 (3H, s, CH_3), 5.60 (1H, s, -OH), 6.76 (1H, s, C_5-H of pyridone), 7.19-8.62 (16H, m, Ar-H, NH), 7.30 (1H, s, CH=N). ^{13}C NMR (DMSO-d6) δC: 22.65, 95.41, 100.33, 107.41, 112.47, 115.70, 119.12, 122.17, 123.19, 128.11, 128.42, 129.65, 129.86, 131.02, 139.30, 139.44, 137.55, 140.18, 148.13, 158.80, 161.62, 162.83, 166.45, 167.08, 169.91. MS (*m/z*) 551 M^+ Found: C, 74.48; H, 3.63; N, 12.41. $C_{34}H_{22}FN_5O_2$ requires: C, 74.04; H, 4.02; N, 12.70%.

4-(4-Bromo-phenyl)-6-{4-[(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-2-oxo-1,2-dihydro-pyridine-3-carbonitrile (8d):

Light brown crystal (76%), m.p. 151-153°C. IR: ν_{max}/cm^{-1} 3256 (OH), 2132 (CN), 1736 (C=O). 1H NMR (DMSO-d6) δH: 2.30 (3H, s, CH_3), 5.59 (1H, s, -OH), 6.81 (1H, s, C_5-H of pyridone), 7.33-8.42 (16H, m, Ar-H, NH), 7.59 (1H, s, CH=N). ^{13}C NMR (DMSO-d6) δC: 22.69, 95.08, 100.63, 108.21, 112.21, 112.61, 120.96, 122.17, 123.94, 128.09, 128.24, 129.62, 129.66, 130.11, 133.01, 135.27, 136.51, 139.55, 140.54, 149.24, 158.95, 162.61, 164.05,165.06 167.01, 178.11. MS (*m/z*) 612 M^+, 614 M^++2 Found: C, 66.93; H, 3.18; N, 11.22. $C_{34}H_{22}BrN_5O_2$ requires: C, 66.67; H, 3.62; N, 11.43%.

2.1.8 Synthesis of 2-(4-Hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-ylmethylene)-malononitrile 9.

A mixture of aldehyde **2** (2.5g, 10 mmol) and malononitrile (0.66g, 10 mmol) were stirred together at room temperature for 24h in a 20mL ethanol in the presence of few drops of piperidine. The formed precipitate was filtered, dried, and recrystallized from n-butanol to give **9** as a light green crystal in 89%, m.p. 253-255°C. IR: ν_{max}/cm^{-1} 3345 (OH), 2166 (CN). 1H NMR ($CDCl_3$) δH: 2.19 (3H, s, CH_3), 5.37 (1H, s,-OH), 7.59 (5H, m, -Ph), 8.03 (1H, s, -CH=C), 8.52 (1H, d, C_6-H, *J* = 8.5), 9.62 (1H, d, C_5-H, *J* = 8.5). ^{13}C-NMR ($CDCl_3$) δC: 22.79, 84.64, 116.80, 119.24, 127.85, 129.24, 143.19, 146.95, 150.57, 154.53, 158.82, 161.20, 163.99. MS (*m/z*) 312 M^+ Found: C, 72.84; H, 3.41; N, 17.56. $C_{19}H_{12}N_4O$ requires: C, 73.07; H, 3.87; N, 17.94%.

2.1.9 Synthesis of 2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-yl)-6-aryl-2-yl-nicotinonitrile (10 a-f).

A mixture of equimolar amounts of **9** (0.3 g, 10 mmol) and methyl aryl ketones (10 mmol) was stirred at room temperature (25–30°C) for the proper time controlled by Thin-layer chromatography (TLC), in the alcohol (20 mL) containing sodium (0.46 g, 20 mmol). The separated solid was collected, washed with water and crystallized from n-butanol to obtain the title compounds **10a–f.**

2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-6-thiophen-2-yl-nicotinonitrile (10a):

Yellow crystal (82%), m.p. 227-229°C. IR: ν_{max}/cm^{-1} 3256 (OH), 2182 (CN).1H NMR ($CDCl_3$) δH: 0.88 (3H, t, -CH_2CH_3, *J* = 7.6), 2.17 (3H, s, CH_3), 4.03 (2H, q, -CH_2CH_3, *J* = 7.6), 5.26 (1H, s, -OH), 7.26 (1H, s, C_5-H of pyridine), 7.38-8.89 (10H, m, Ar-H). ^{13}C NMR ($CDCl_3$) δC: 14.12, 22.40, 65.24, 97.05, 107.29, 109.39, 114.32, 120.11, 122.91, 124.20, 126.62, 127.02, 129.42, 135.94, 140.05, 142.63, 150.74, 152.14, 156.62, 158.23, 159.25, 165.32, 166.16. MS (*m/z*) 464 M^+ Found: C, 69.45; H, 3.88; N, 11.79. $C_{27}H_{20}N_4O_2S$ requires: C, 69.81; H, 4.34; N, 12.06%.

2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-6-phenyl-nicotinonitrile (10b):

Brown crystal (85%), m.p. 279-281°C. IR: ν_{max}/cm^{-1} 3332 (OH), 2132 (CN).1H NMR (DMSO-d_6) δH: 0.86 (3H, t, -CH_2CH_3 *J* = 6.8), 2.58 (3H, s, CH_3), 4.07 (2H, q, -CH_2CH_3, *J* = 7.6), 5.42 (1H, s, -OH), 7.28 (1H, s, C_5-H of pyridine), 7.52-8.34 (12H, m, Ar-H). ^{13}C NMR (DMSO-d_6) δC: 14.36, 22.40, 64.73, 97.20, 107.00, 109.02, 117.21, 119.11, 119.51, 121.01, 127.58, 128.35, 129.30, 129.81, 130.11, 131.94, 142.50, 150.93, 151.81, 156.56, 158.11, 159.19, 160.46, 164.05, 169.21. MS (*m/z*) 458 M^+ Found: C, 75.42; H, 4.36; N, 11.79. $C_{29}H_{22}N_4O_2$ requires: C, 75.97; H, 4.84; N, 12.22%.

2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-6-p-tolyl-nicotinonitrile (10c):

Yellow crystal (79%), m.p. 293-295°C. IR: ν_{max}/cm^{-1} 3343 (OH), 2199 (CN).1H NMR (DMSO-d_6) δH: 1.05 (3H, t, -CH_2CH_3, *J* = 7.6), 2.54, 2.75 (6H, 2s, $2CH_3$), 5.43 (2H, q, -CH_2CH_3, *J* = 7.6), 5.83 (1H, s, -OH), 7.56-8.57 (12H, m, Ar-H). ^{13}C NMR (DMSO-d_6) δC: 14.36, 17.45, 22.65, 64.21, 99.10, 107.21, 108.22, 116.65, 118.51, 119.65, 119.82, 120.62, 127.93, 128.03, 129.41, 135.91, 136.34, 137.61, 139.37, 150.29, 151.20, 158.37, 159.29, 160.61, 163.38, 169.91. MS (*m/z*) 472 M^+ Found: C, 67.69; H, 4.83; N, 11.59. $C_{30}H_{29}N_4O_2$ requires: C, 76.25; H, 5.12; N, 11.86%.

2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-6-(4-methoxy-phenyl)-nicotinonitrile (10d):

Brown crystal (68%), m.p. 269-271°C. IR: ν_{max}/cm^{-1} 3326 (OH), 2183 (CN).1H NMR ($CDCl_3$) δH: 0.89 (3H, t, -CH_2CH_3, *J* = 7.6), 2.56 (3H, s, CH_3), 3.87(3H, s, -OCH_3), 4.06 (2H, q, -CH_2CH_3, *J* = 7.6), 5.27 (1H, s, -OH), 6.91-8.51 (12H, m, Ar-H). ^{13}C NMR ($CDCl_3$) δC: 14.52, 22.68, 46.47, 63.42, 97.45, 107.38, 108.60, 113.48, 119.24, 127.42, 129.48, 129.72, 129.77, 130.35, 130.60, 131.60, 132.48, 139.77, 150.94, 151.91, 156.92, 158.82, 161.27, 162.29.163.48, 171.46. MS (*m/z*) 488 M^+ Found: C, 73.26; H, 4.66; N, 11.19. $C_{30}H_{24}N_4O_3$ requires: C, 73.76; H, 4.95; N, 11.47%.

2-Ethoxy-6-(4-fluoro-phenyl)-4-(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-yl)-nicotinonitrile; compound with methane (10e):

Brown crystal (76%), m.p. 278-280°C. IR: ν_{max}/cm^{-1} 3333 (OH), 2213 (CN).1H NMR ($CDCl_3$) δH: 1.44 (3H, t, -CH_2CH_3, *J* = 6.8), 2.55 (3H, s, CH_3), 4.09 (2H, q, -CH_2CH_3, *J* = 6.8), 5.25 (1H, s, -OH), 7.96-8.21 (12H, m, Ar-H). ^{13}C NMR ($CDCl_3$) δC: 14.69, 22.63, 63.74, 96.03, 107.94, 108.80, 114.01, 115.01, 119.25, 123.34, 127.02, 129.23, 129.92, 130.26, 130.60, 131.60, 135.65, 136.76, 139.85, 150.03, 152.56, 158.41, 159.29, 162.91, 170.39. MS (*m/z*) 492 M^+ Found: C, 73.48; H, 4.83; N, 11.06. $C_{30}H_{25}FN_4O_2$ requires: C, 73.16; H, 5.12; N, 11.37%.

2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8] naphthyridin-3-yl)-6-(4-nitro-phenyl)-nicotinonitrile (10f):

brown crystal (93%), m.p. 244-246°C. IR: ν_{max}/cm^{-1} 3331 (OH), 2229 (CN). 1H NMR ($CDCl_3$) δH: 0.87 (3H, t, -CH_2CH_3, *J* = 7.6), 2.68 (3H, s, CH_3), 4.08 (2H, q, -CH_2CH_3, *J* = 7.6), 5.24 (1H, s, -OH), 7.26-8.51 (12H, m, Ar-H). ^{13}C NMR ($CDCl_3$) δC: 14.12, 22.68, 63.62, 97.05, 107.18, 108.39, 114.07, 115.24, 123.89, 127.82, 128.23, 129.32, 131.65, 136.76, 140.62, 145.27, 147.70, 150.23, 157.14, 158.23, 162.18, 164.72, 170.59. MS (*m/z*) 503 M^+ Found: C, 68.78; H, 3.97; N, 13.48. $C_{29}H_{21}N_5O_4$ requires: C, 69.18; H, 4.20; N, 13.91%.

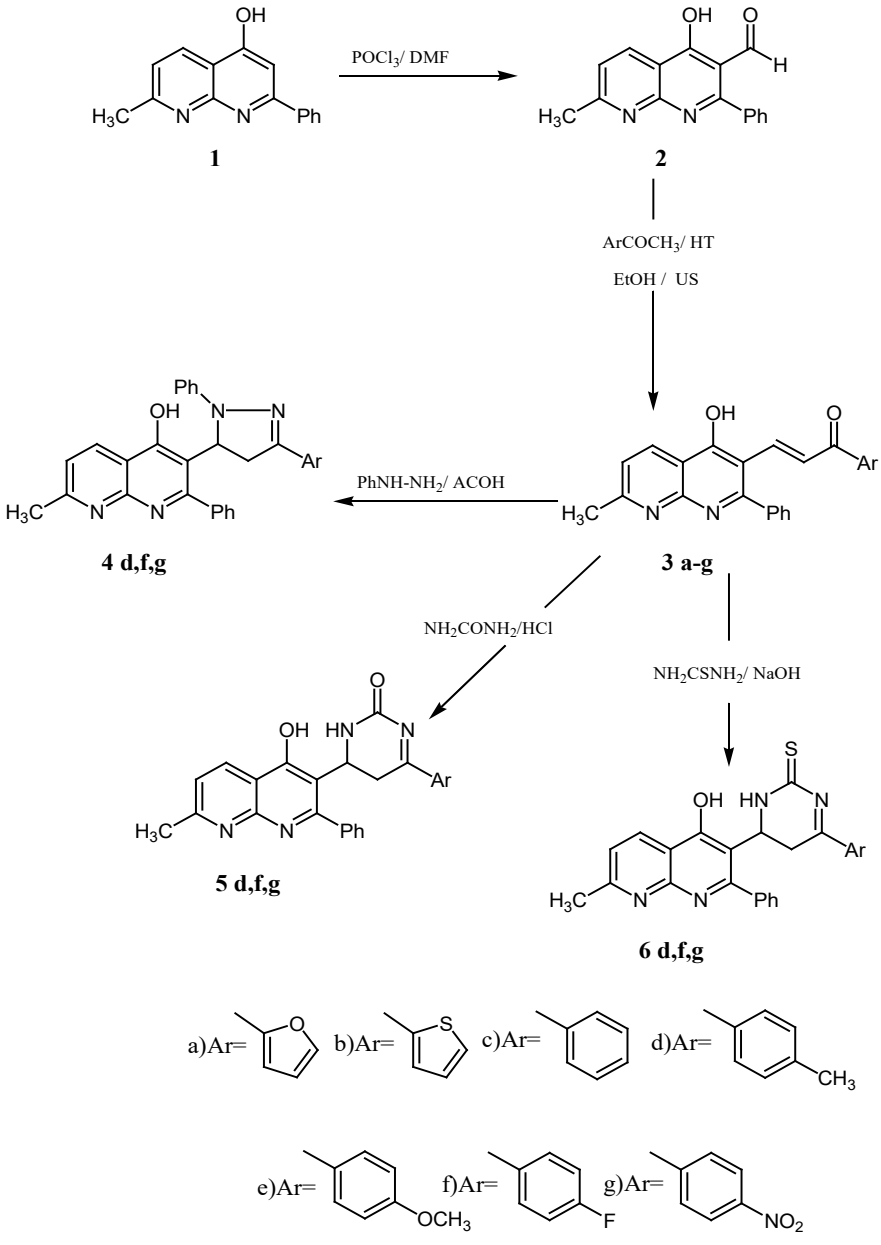
Antitumor activity: To evaluate the antitumor efficacy of the new compounds' contrary to MCF7 cell line, a standard MTT technique was applied. The numerical assess rest on the capacity of the mitochondrial dehydrogenase of feasible cells to slash the tetrazolium ring of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). The fashioned purple dye is measured spectrophotometrically and therefore the intensification or reduction in cell amount can specify the antitumor action of verified compounds. The antitumor efficacy was determined as the concentration of the compound that produced 50% growing embarrassment (IC_{50}, mean ±SEM) in contrast to the development of raw cells. Cells for Cell Line were gotten from American Type Culture Collection, and cultivated by DMEM (Invitrogen) complemented by 10% FBS (Hyclone,), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. 96-well dish was utilized for the test. Cells were preserved with sequential concentrations of test compounds and hatched for 48 hours at 37°C. The dish was then studied utilizing an overturned microscope earlier the MTT assess. The cultures stayed detached from incubator to a laminar stream top and MTT was added as a 10% of culture medium volume formerly incubated for 2-4 hours. Afterwards elimination from incubator, the fashioned Formazan crystals were dissolved by means of MTT solubilizing solution. The absorbance was measured at a wavelength of 570 nm. [33,34].

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

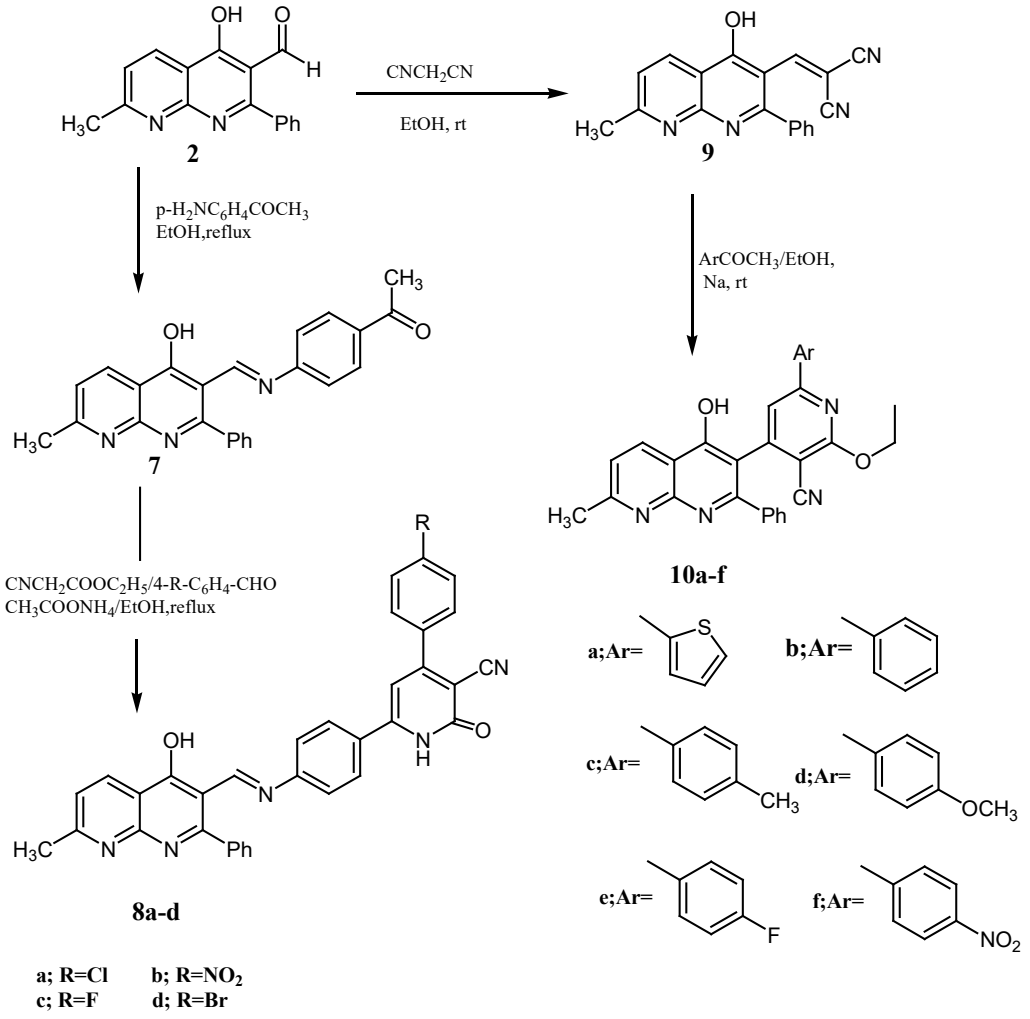
3.1 Chemistry

Synthetic methodology to attain target compounds is clarified in Scheme 1 and 2. Formylation of 7-methyl-2-phenyl-1,8-naphthyridin-4-ol **1** via Vilsmeier–Haack reaction, afforded the vital starting compound in our study: the 4-hydroxy-7-methyl-2-phenyl-1,8-naphthyridine-3-carbaldehyde **2** [35]. The structure of aldehyde **2** was identified based on microanalysis and spectral data. The IR spectra showed the absorption band at ν = 1661 cm^{-1}due to aldehydic carbonyl group. Its ^{1}H NMR spectrum showed the disappearance of C_3-H at δ = 6.30 ppm and a characteristic singlet signal at δ = 9.56 ppm for –CHO group proton. ^{13}C NMR spectrum for **2** displayed the presence of carbon of a carbonyl group at δ = 190.05 ppm. The formed aldehyde derivative **2** was treated with different acetyl compounds in the presence of MgAl-hydrotalcite (HT) [36] in refluxing ethanol and it produced only one isolable product (as examined by TLC) identified as the chalcones derivatives **3a-g** (Scheme **1**). As a descriptive example, the ^{1}H NMR spectrum of **3a** displays a characteristic signal at δ = 8.38 and 8.58 ppm as a pair of doublet referring to the two olefinic protons. ^{13}C NMR spectrum of **3e** shows the existence of the carbon of methoxide group at δ = 55.9 ppm. The signals for the two olefinic carbons appeared at δ = 128 and 143 ppm, respectively. Treatment of chalcone **3** with different binuclophilic agents, namely phenylhydrazine, urea, and thiourea afforded in each case one isolable product (examined by TLC) corresponding to pyrazoline **4d,f,g**, dihydropyrimidinone **5d,f,g**, and dihydropyrimidinethione **6d,f,g** derivatives (Scheme 1).



Scheme 1

The established structures of all new chemical entities **4-6** were verified by their spectral data and microanalyses. The IR spectra of pyrazoline derivatives showed the disappearance of the carbonyl group of chalcones. The ^{1}H NMR spectrum of **4d** displayed two signals, at δ = 2.00 ppm as a doublet of doublet and a triplet signal at δ = 5.32 ppm for the protons at C4 and C5 of pyrazoline ring, respectively. ^{1}H NMR spectrum of **5f** revealed multiplet signal at δ = 1.98 and triplet signal at 5.32 ppm for protons at C5 and C6 of hydropyrimidinone derivative. Reaction of aldehyde **2** with p-aminoacetophenone in ethanol under reflux gave 1-{4-[(4-Hydroxy-7-methyl-2-phenyl-[1, 8] naphthyridin-3-ylmethylene)-amino]-phenyl}-ethanone **7** (Scheme 2). Its structure was confirmed through microanalysis and spectral data. The IR spectra of **7** revealed a strong absorption peak at ν = 1659 cm^{-1} for C=O group, ^{1}H NMR spectrum of **7** displayed two singlet signals at δ = 2.19 and 7.55 ppm due to $COCH_3$ and CH=N- respectively, in addition to multiplet signals due to aromatic protons. Furthermore, treatment of **7** with different aromatic aldehyde, ethylcyanoacetate, and excess ammonium acetate in EtOH under reflux (Scheme 2), afforded only one isolable product (examined by TLC) identified as 4-(substituted)-6-{4-[(4-hydroxy-7-methyl-2-phenyl-[1,8]



Scheme 2

naphthyridin-3-ylmethylene)-amino]-phenyl}-2-oxo-1,2-dihydro-pyridine-3-carbonitrile **8a-d**. The structures of **8a-d** were established based on elemental analysis and spectral data. The IR spectra revealed the absorption band at ν = 2132- 2226 cm^{-1}due to CN group, the ^1H NMR spectrum of **8a** displays a singlet signal at δ = 6.80 ppm referring to C_5-H of pyridone. The carbaldehyde **2** which reacted with malononitrile in refluxing ethanol, gave 1-{4-[(4-Hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-ylmethylene)-amino]-phenyl}-ethanone **9**. According to the cited procedure [37], the required 2-Ethoxy-4-(4-hydroxy-7-methyl-2-phenyl-[1,8]naphthyridin-3-yl)-6-aryl-2-yl-nicotinonitrile **10a-f** were obtained from the reaction of ylidenemalononitrile **9** with different ketones catalyzed by sodium ethoxide (Scheme 2). The structure of compounds **10a-f** was confirmed in the same way as the previous compounds. An IR spectrum of compound **10a** shows a characteristic stretching vibration band at ν = 2182 cm^{-1} for CN group. Its ^1H NMR spectrum displays two signals at δ = 0.88 and 4.03 ppm characteristic for the ethoxide group, while a singlet peak at δ = 7.26 ppm for pyridinyl H-5 appears. ^{13}C NMR spectrum of **10a** shows the presence of ethoxide carbons at δ = 14.12 and 65.24 ppm, C-3, C-5 of pyridine and the carbon of nitrile group appears at δ = 97.05, 107.92, and 114.32 ppm, respectively. The mass spectrum (EI) of **10a** displays a peak at 482.27 with relative intensity value 30.7% for the molecular ion.

3.2 Biological Evaluation

The antitumor activity of compounds **3b, f, g**, **4d, g**, **5f**, **6f**, **7**, **8a-d**, and **10a-f** was examined and compared with staurosporin as a standardized medicine antagonizing human breast cancer cell lines (MCF-7) using standard MTT assay process [35]. Cancer cells were either isolated

Table 1: The cytotoxic activity of several newly synthesized compounds against human breast cancer cell lines (MCF7).

Compounds	MW	IC50 μM
3c	466	27.19 ±1.13
3f	384	7.88 ±0.33
3g	393	9.10 ±0.34
4d	470	1.68 ±0.05
4g	501	8.17 ±0.29
5f	426	35.33 ±1.75
6f	442	6.53 ±0.25
7	381	10.13 ±0.41
8a	567.5	16.15 ±0.77
8b	578	3.19 ±0.12
8c	551	7.89 ±0.33
8d	612	1.62 ±0.05
10a	464	136.82 ±6.22
10b	458	7.97 ±0.24
10c	472	1.47 ±0.04
10d	488	8.80 ±0.28
10e	476	21.41 ±0.92
10f	503	2.30 ± 1.17
Staurosporin	466.53	4.51 ±0.16

separately as a control or with a range of concentrations of the compounds tested (100, 25, 6.25, 1.56, 0.39 μM). Results obtained were presented as IC50 (μM) values, which is the average of minimally three independent trials shown in Table 1. Results showed that compounds **10c, 8d, 4d, 10f** and **8b** produced strongest cytotoxic effects at IC_{50} ranges from 1.47 to 3.19 μM vs staurosporin of IC_{50}; 4.51 μM. The changes in the kinds of aryl substituents in the heterocyclic rings attached to the parent 1,8-naphthyridine scaffold significantly impacted the antitumor activity of the tested compounds. The conjugation of 4-tolyl and 4-bromophenylcyanopyridine entity to the 1,8-naphthyridine ring in **10c, 8d** and 3-tolylpyrazoline **4d**, resulted in an approximately 3-fold development in the growth inhibition of the cancer cell lines of IC_{50}; 1.47, 1.62, and 1.68 μM. The increase was larger in comparison with staurosporin. On the other hand, cytotoxic potency was completely eliminated due the replacement of the 4-tolyl moiety in compound **10c** with thiophenyl ring as compound **10a**, IC_{50}; 136.82 μM. On the contrary, the replacement of 3-tolyl moiety in pyrazoline conjugated with 1,8-naphthyridine scaffold **4d** with p-nitrophenyl in **4f** reduced the cytotoxicity from 1.68 to 8.17 μM.

In summary, breast malignant tumors MCF-7 cell lines were viciously inhibited by 1,8-naphthyridine scaffold showing its significant antitumor activity. Variation in the types of heterocyclic rings conjugated with it considerably affected the observed activity. In general, 1,8-napthyridine conjugated with pyridine and pyrazoline, specially 4-bromophenyl pyridine, 4-tolyl pyridine and 3-tolylpyrazoline, introduced novel candidates which could serve as key templates to fight against antitumor effects on breast cancer cells.

4 Conclusions

In this article, a novel series of 2-phenyl-7-methyl-1,8-naphthyridine derivatives with variable substituents at C-3 were synthesized. Most of the synthesized derivatives showed potential as anticancer agents against breast cancer cell line MCF7. Compounds 10c, 8d, 4d, 10f and 8b displayed better activity with an IC_{50} values (1.47, 1.62, 1.68, 2.30, 3.19 μM, respectively) compared with the reference drug staurosporin (IC_{50} = 4.51 μM).

Supplementary Materials: Supplementary materials are available online.

Acknowledgments: The project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. J-739-247-38. The authors, therefore, acknowledge with thanks DSR for technical and financial support.

Author Contributions: The listed authors contributed to this work as described in the following. Nesreen S. Ahmed and Thoraya S. J carried out the synthesis work, interpreted the results and Abeer N.Al-romaizan and Nesreen S. Ahmed interpreted the results and cooperated in the preparation of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflicts of interest

References

[1] Liu J., Ming B., Gong G.H., Wang D., Bao G.L., Yu L.J., Current research on anti-breast cancer synthetic compounds, RSC Adv., 2018, 8, 4386-4416.

[2] Li X., Wu C., Lin X., Cai X., Liu L., Luo G., et al., Synthesis and biological evaluation of 3-aryl-quinolin derivatives as anti-breast cancer agents targeting ERα and VEGFR-2, Eur. J. Med. Chem., 2019, 161, 445-455.

[3] Sunil Kumar A., Kudva J., Bharath B.R., Ananda K., Sadashiva R., Madan Kumar S., et al., Synthesis, structural, biological and in silico studies of new 5-arylidene-4-thiazolidinone derivatives as possible anticancer, antimicrobial and antitubercular agents, New J. Chem., 2019, 43, 1597- 1610.

[4] Pavelic S.K., Sedic M., Bosnjak H., Spaventi S., Pavelic K., Metastasis: new perspectives on an old problem, Mol. Cancer, 2011, 10, 22–35.

[5] O'Shaughnessy J., Extending survival with chemotherapy in metastatic breast cancer, Oncologist, 2005, 10, 20–29.

[6] Cardoso F., Harbeck N., Fallowfield L., Kyriakides S., Senkus E., E.G.W. Group, Locally recurrent or metastatic breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up, Ann. Oncol., 2012, 23, vii11–vii19.

[7] Plodek A., Raeder S., Bracher F., Regioselective hemolytic substitution of benzo[c][2,7]naphthyridines, Tetrahedron, 2012, 68, 4693-4700.

[8] Li X. L., Xu M. J., Zhao Y. L., Xu J., A Novel Benzo[f][1,7] Naphthyridine Produced by Streptomyces Albogriseolus from Mangrove Sediments, Molecules, 2010, 15, 9298- 9307.

[9] Xu D. B., Ye W. W., Han Y., Deng Z. X., Hong K., Natural Products from Mangrove Actinomycetes, Mar. Drugs, 2014, 12, 2590-2613.

[10] Bonocorso H. G., Andrighetto R., Kruger N., Zanatta N., Martins M. A. P., General Pathway for a Convenient One-Pot Synthesis of Trifluoromethyl-Containing 2-amino-7-alkyl(aryl/heteroaryl)-1,8-naphthyridines and Fused Cycloalkane Analogues, Molecules, 2011, 16, 2817-2832.

[11] Mogilaiah K., Sudhakar G. R., Synthesis of pyrazoline, pyrimidine, and 1,5-benzodiazepine derivatives of 1,8-naphthyridine and evaluation of antimicrobial activity, Indian J. Chem., 2003, 42B, 636-640.

[12] Mohammed, A. A. M., Suaifan, g.A.R.Y., Shehadeh, M. B., Okechukwu, P. N., Design, synthesis and biological evaluation of 1,8-naphthyridine glucosamine conjugates as antimicrobial agents, Drug Dev Res., 2019, 80,179-186

[13] Yamuna E., Zeller M., Prasad K. J. R., Microwave assisted synthesis of indolo[2,3-b]dibenzo[b,g][1,8]naphthyridines, Tetrahendron Lett., 2012, 53, 1514-1517.

[14] Bernardino A. M. R., Azevedo A. R., Pinheiro L. C. S., Borges J. C., Paixao I. C. P., Mesquita M. T., et al., Synthesis and anti- HSV-1 evaluation of new 3H- benzo[b]pyrazolo[3,4-H]-1,6-naphthyridines and 3H-pyrido[2,3-b]pyrazolo[3,4-H]-1,6-naphthyridines, Org. Med. Chem. Lett., 2012, 2, 1-7.

[15] Tomczyk M.D., Walczak K.Z., l,8-Naphthalimide based DNA intercalators and anticancer agents. A systematic review from 2007 to 2017, Eur. J. Med. Chem., 2018, 159, 393-422.

[16] Madaan A., Verma R., Kumar V., Singh A.T., Jain S.K., Jaggi M., 1,8-Naphthyridine Derivatives: A Review of Multiple Biological Activities, Arch. Pharm. (Weinheim, Ger.), 2015, 348, 837-860.

[17] Eweas A.F., Khalifa N.M., Ismail N.S., Al-Omar M.A., Soliman A.M., Synthesis, molecular docking of novel 1,8-naphthyridine derivatives and their cytotoxic activity against HepG2 cell lines, Med. Chem. Res., 2014, 23, 76–86.

[18] Atanasova M., Ilieva S., Galabov B., QSAR analysis of 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridines with anticancer activity, Eur. J. Med. Chem., 2007, 42, 1184–1192.

[19] Willemann C., Grünert R., Bednarski P. J., Troschütz R., Synthesis and cytotoxic activity of 5,6-heteroaromatically annulated pyridine-2,4-diamines, Bioorg. Med. Chem., 2009, 17, 4406–4419.

[20] Banti I., Nencetti S., Orlandini E., Lapucci A., Breschi M. C., Fogli S., Synthesis and in-vitro antitumour activity of new naphthyridine derivatives on human pancreatic cancer cells, J. Pharm. Pharmacol., 2009, 61, 1057–1066.

[21] Makhanya T. R., Gengan R. M., Pandian P., Chuturgoon A. A., Tiloke C., Atar A., Phosphotungstic Acid Catalyzed One Pot Synthesis of 4,8,8-Trimethyl-5-phenyl-5,5a,8,9-tetrahydrobenzo[b] [1,8]Naphthyridin-6(7H)-one Derivatives and Their Biological Evaluation Against A549 Lung Cancer Cells, J. Heterocycl. Chem., 2018, 55, 1193-1204.

[22] Srivastava S. K., Jaggi M., Singh A. T., Madan A., Rani N., Vishnoi M., et al., Anticancer and anti-inflammatory activities of 1,8-naphthyridine-3-carboxamide derivatives, Bioorg. Med. Chem. Lett., 2007, 17, 6660–6664.

[23] Kumar V., Jaggi M., Singh A. T., Madaan A., Sanna V., Singh P., et al., 1,8-Naphthyridine-3-carboxamide derivatives with anticancer and anti-inflammatory activity, Eur. J. Med. Chem., 2009, 44, 3356–3362.

[24] Kumar V., Madaan A., Sanna V. K., Vishnoi M., Joshi N., Singh A. T., et al., Anticancer and immunomodulatory activities of novel 1,8-naphthyridine derivatives, J. Enzyme Inhib. Med. Chem., 2009, 24, 1169–1178.

[25] Hwang H. J., Kang Y. J., Hossain M. A., Kim D. H., Jang J. Y., Lee S. H., et al., Novel dihydrobenzofuro[4,5-b][1,8]naphthyridin-6-one derivative, MHY-449, induces apoptosis and cell cycle arrest in HCT116 human colon cancer cells, Int. J. Oncol., 2012, 41, 2057–2064.

[26] Hwang Y. J., Chung M. L., Sohn U. D., Im C., Cytotoxicity and structure-activity relationships of naphthyridine derivatives in human cervical cancer, leukemia, and prostate cancer, Korean J. Physiol. Pharmacol., 2013, 17, 517–523.

[27] Ahmed N. S., AlFooty K. O., Khalifah S. S., Synthesis of 1,8-naphthyridine derivatives under ultrasound irradiation and cytotoxic activity against HepG2 cell lines, J. Chem., 2014, 2014, 1–8.

[28] Ahmed N. S., Badahdah K.O., Qassar H. M., Novel quinoline bearing sulfonamide derivatives and their cytotoxic activity against MCF7 cell line, Med. Chem. Res., 2017, 26, 1201-1212.

[29] Ahmed N. S., Alfooty K. O., Khalifah S. S., An efficient sonochemical synthesis of novel Schiff's bases, thiazolidine and pyrazolidine incorporating 1,8-naphthyridine moiety and their cytotoxic activity against HePG2 cell lines, Sci. World J., 2014, 25, 1-10.

[30] El-Naggar M., Almahli H., Ibrahim H.S., Eldehna W.M., Abdel-Aziz H.A., Pyridine-Ureas as Potential Anticancer Agents: Synthesis and In Vitro Biological Evaluation, Molecules, 2018, 23, 1459-1474.

[31] Amin K.M., Syam Y.M., Anwar M.M., Ali H.I., Abdel-Ghani T.M., Serry A.M., Synthesis and molecular docking study of new

benzofuran and furo[3,2-g]chromone-based cytotoxic agents against breast cancer and p38a MAP kinase inhibitors, Bioorg. Chem., 2018, 76, 487–500.
[32] Badahdah K.O., Abdel Hamid H.M., Noureddin S.A., Functionalized 2-Hydrazinobenzothiazole with Isatin and Some Carbohydrates under Conventional and Ultrasound Methods and Their Biological Activities, 2015,52 (1), 67-74.
[33] Berridge M. V., Herst P. M., Tan A. S., Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction, Biotechnol. Annu. Rev., 2005, 11, 127-152.
[34] Shoemaker R. H., The NCI60 human tumor cell line anticancer drug screen, Nat. Rev. Cancer, 2006, 6, 813- 823.
[35] El-Shishtawy R. M., Borbone F., Al-Amshany Z. M., Tuzi A., Barsella A., Asiri A. M., Thiazole azo dyes with lateral donor branch: Synthesis, structure and second order NLO properties, Dyes Pigm., 2013, 96, 45-51.
[36] Basahel S.N., Al-Thabaiti S.A., Narasimharao K., Ahmed N.S., Mokhtar M., Nanostructured Mg-Al hydrotalcite: A benign efficient alternative to the homogeneous catalysts in the synthesis of fine chemicals, J. Nanosci. Nanotechnol., 2014, 14, 1931-1945.
[37] Kalifa N.M., Srour A.M., Abd El-Karim S.S., Saleh D.O., Al-Omar M.A., Synthesis and 2D-QSAR Study of Active Benzofuran-Based Vasodilators, Molecules, 2017, 22, 1820-1835.