International Research Journal of Natural and Applied Sciences
ISSN: (2349-4077)
Impact Factor- 5.46, Volume 5, Issue 3, March 2018
Website- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

SYNTHESIS OF SOME NEW PYRIMIDO[4,5-B][1,6]NAPHTHYRIDINE AND THEIR BIOLOGICAL EVALUATION AS ANTICANCER AND ANTIVIRAL AGENTS

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ABSTRACT

The pyrimido[4,5-b][1,6]naphthyridin-4(3H)-ones ***1-4*** *and their 2-methyl analogs* ***5–8*** *as well as the hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thiones* ***9–12*** *and the targeted tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-amines* ***13–16*** *was successfully achieved from the reaction of the 2 aminonaphthyridine derivatives with the appropriate reagent. The cytotoxicity and antiviral activity of some of the prepared derivatives was also investigated.*

Keywords: Pyrimidonaphthyridine, Anticancer, Antiviral

1.Introduction

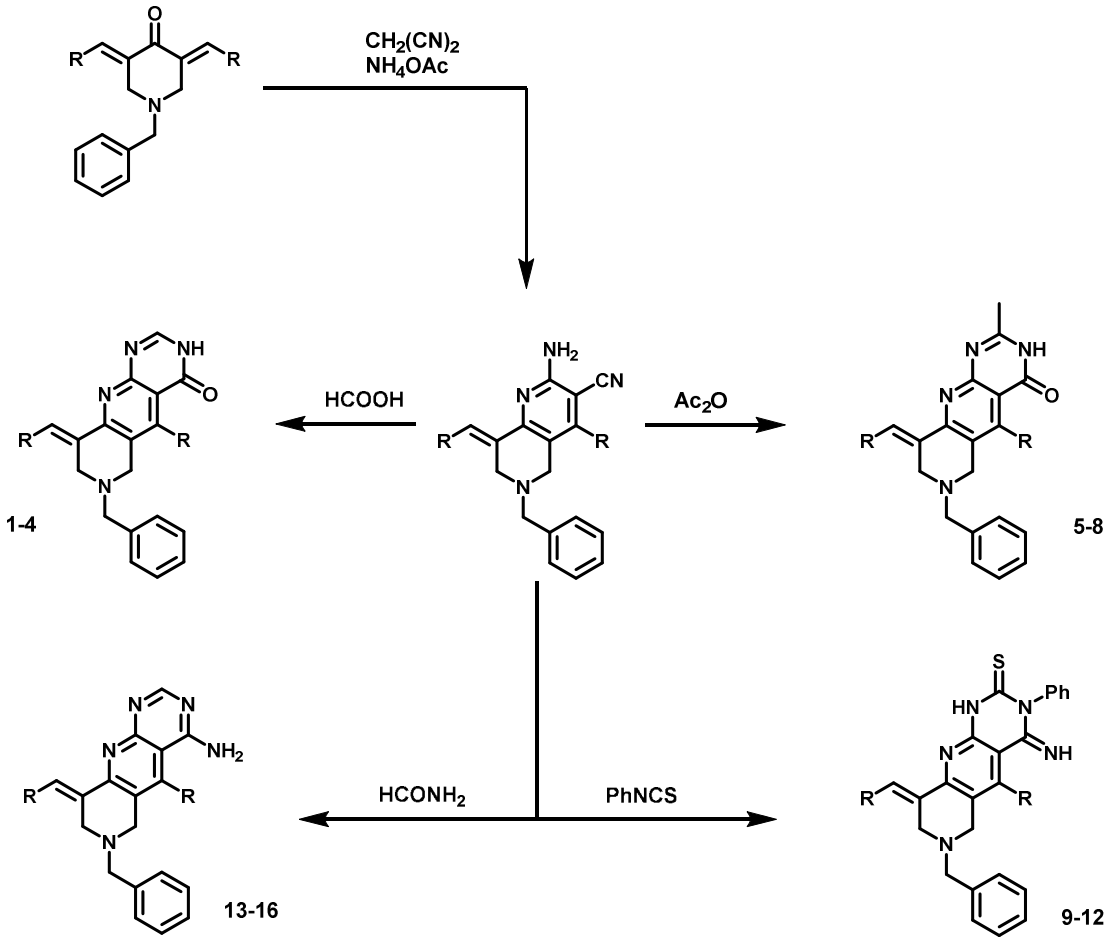
Curcumin which is a natural active ingredient separated from the rhizome of *Curcuma longa* [1]. Studies have proven its anti-cancer[2,3], anti-inflammatory[4], and anti-bacterial activities [5]. However, its use is limited because its low anticancer activity and poor bioabsorption,[6] Because of the low anticancer activity of Curcumin, researchers were looking for a new Curcumin analogs with better biological effects specially its cytotoxic activity. N-substituted-3,5-bis(arylidene)piperidin-4-one is a cyclic α, β-unsaturated ketones (chalcone) which is being structurally related to curcumin and its derivatives have been

proved for having antitumor, multidrugresistance-reverting, and antimycobacterial, and Antioxidant activities [7-11]. Based on the above-mentioned findings, the present study deals with the synthesis of a series of pyrimido[4,5-b][1,6]naphthyridine ring systems carrying some biologically active rings such as thienyl, 1,3-Benzodioxolyl and fluorinated aryl groups. The aim of the study was to evaluate their anticancer and/or antiviral activities .

2. Results and Discussion

2.1. Chemistry

Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in **Scheme 1**. The key intermediate 2-aminonaphthyridine derivatives was prepared via reactions of N-benzyl-3,5-bis(arylidene)piperidin-4-one, malononitrile and ammonium acetate. 2-aminonaphthyridine derivatives which was utilized as a kay intermediate when 2-aminonaphthyridine were heated with either formic acid or acetic anhydride, the targeted substituted pyrimido[4,5-b][1,6]naphthyridin-4(3H)-one **1-4** and their 2-methyl analogs **5–8,** respectively, were successfully obtained. The IR spectra of the latter compounds showed the absence of the CN group absorption and the appearance of new sharp absorption bands at 1650–1661 cm^{-1} attributed to the newly formed C=O groups at position 4, beside the NH absorption bands at 3265–3279 cm^{-1}. Meanwhile, the^{1} H-NMR spectra of compounds **5–8** showed new singlets at δ 2.50–2.67 ppm due to the newly introduced CH3 group, whereas their ^{13}C NMR spectral data exhibited new singlets at δ 21.71–22.54 ppm due to the new CH3 group and the CO signals at δ 164.85– 165.60 ppm. Reacting compounds 2-aminonaphthyridine with the phenyl isothiocyanate in pyridine medium led to the formation of the substituted hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thione **9–12**. Their IR spectra showed new bands at 1619–1628 cm−1 and at 1195–1233 cm^{-1} corresponding to the C=N moiety and to the C=S group, respectively. Their ^{13}C NMR spectral data were characterized by the presence of two signals at δ 175.12– 176.60 and δ 164.70–165.19 ppm for the C=S and C=NH groups, respectively. Finally, the synthesis of the targeted substituted tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-amine **13–16** was successfully achieved via reaction of 2-aminonaphthyridine with formamide. The IR spectra of these tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-amine **13-16** derivatives were characterized by the disappearance of the CN group absorptions and the appearance of two broad absorption bands at 3354-3366 and 3415-3432 cm^{-1} due to the amino group.



Scheme 1

2.2 In vitro MTT cytotoxicity assay of the prepared compounds

Some synthesized compounds namely **1, 4, 5, 8, 9, 10, 12, 13, 15** and **16** were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [12,13] against a panel of three human tumor cell lines namely; Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HePG2 and colon carcinoma HT29. The results are presented in Table **1** as LC_{50} (µg/mL) which is the lethal concentration of the compound which cause death of 50% of the cells in 24h. The obtained data revealed that, the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards fourteen of the tested compounds namely; **9, 11, 12, 13** and **16**.

Moreover, a remarkable cytotoxic potential was displayed by compounds **9**, **11** and **12** against the human colon carcinoma HT29 cell line (20.2, 25.4 and 22.3µg/mL, respectively). Moreover, compounds **13 and 16** were able to exhibit moderate activity against the same cell line with LC_{50} values range of 44.5-55.8 µg/mL. Furthermore, the growth of the human hepatocellular carcinoma HePG2 cell line was found to be moderately inhibited by some of the active compounds namely; **9, 11** and **12** with LC_{50} values range of 25.6-28.4 µg/mL. Among these, the highest cytotoxic activity was displayed by compound **11** (LC_{50} values 25.6, µg/mL, respectively). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only some of the test compounds. However, an outstanding growth inhibition potential was shown by compound **9** as evidenced from their LC_{50} value (8.5 µg/mL,). The rest active compounds namely **11** and **12** showed moderate to mild activity against the same cell line with LC_{50} values of 20.8 and 22.8 µg/mL, respectively (Table 1). Further interpretation of the results revealed that, compounds **9, 11** and **12** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. A close examination of the structure of the active compounds showed that the 4-bromophenyl, 4-fluorophenyl and 2-Theinyl counterpart at position-4 or -5 of the pyrimido-naphthyridine skeletons respectively are the most favourable substituents when compared with other analogs.

Table 1. Cytotoxic effects (LC_{50}; μg/mL)[a] of the prepared compounds on some human tumor cell lines using the MTT assay

compound no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HePG2	Human breast cancer MCF 7
9	20.2	28.4	8.5
11	25.4	25.6	20.8
12	22.3	26.8	28.8
13	44.5	-[b]	-[b]
16	55.8	-[b]	-[b]
Doxorubicin [c]	12.1	1.69	2.14

[a]LC50: Lethal concentration of the compound which causes death of 50% of cells in 24h (μg/mL).

[b]Totally inactive against this cell line.

[c]: positive control cytotoxic agent.

2.3 In vitro effect on the replication of hepatitis-C virus in HCV-infected HepG2 hepatocellular carcinoma cell line

Eleven compounds were selected and tested by the Genetic Engineering and Biotechnology Research Institute (GEBRI), Mubarak City for Science and Technology Applications, Alexandria, Egypt.

Cell Culture and RNA Extraction

HepG2 cells were washed twice in EMEM media supplemented with 200 □M L - Glutamine, 100U Penicillin, 100 □g streptomycin and 25 □M HEPES buffer; N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (Bio Whittaker, USA). The cells were suspended in EMEM Culture media and then were left to adhere on polystyrene 6-well plates for 24 h in 37 ºC, 5% CO_2, 95% humidity incubator. The cells were washed twice from debris and dead cells using EMEM media and then infected with 2% HCV-

infected serum in EMEM culture medium with 8% FBS. Each of the tested compounds was added at concentrations of 10, 25, 50, and 100 μg/mL. Positive and negative control cultures were included. After 96 h incubation another dose of the test compound was added and the cells were further incubated for another 96 h. The RNA was extracted following a method reported by El-Awady et al. [14]. The positive strand and its replicating form (negative strand) of HCV were detected by RT-PCR using specific primers to the 5'-untranslated region of the virus.

Antiviral activity

Compounds **1, 4, 6, 9, 10, 12, 13** and **15** were investigated for their *in vitro* effect on the replication of hepatitis-C virus in HepG2 hepatocellular carcinoma cell line infected with the virus. Out of these compounds only two derivatives **10** and **12** were able to inhibit the hepatitis-C virus RNA (+) and (–) strands at 10-100 μg/mL concentration range. The rest of the series were either inactive or exhibited insignificant activity.

3. Experimental

3.1. Chemistry.

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on Shimadzu FT-IR 8400S infrared spectrophotometer using the KBr pellet technique. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-600 FT NMR spectrometer using tetramethylsilane (TMS) as the internal standard and DMSO-d_6 as a solvent (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: *s*: singlet; *d*: doublet; *m*: multiplet; *q*: quartet. Elemental analyses were performed on a 2400 Perkin Elmer Series 2 analyzer and the found values were within ±0.4% of the theoretical values. Follow up of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminum sheets (Type 60 F254, Merck) and the spots were detected by exposure to UV-lamp at λ 254.

7-benzyl-9-(aryllidene)-5-(aryl)-6,7,8,9-tetrahydropyrimido[4,5-b][1,6]naphthyridin-4(3H)-one 1-4

A solution of appropriate 2-aminonaphthyridine**s** (10 mmol) in formic acid (10 ml) was heated under reflux for 3h. The reaction mixture was poured on crushed ice (15 g) and the separated

solid product was filtered, washed with water, dried and recrystallized from the appropriate solvent.

***7-benzyl-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4(3H)-one 1**:*

Recrystallized from ethanol/H_2O(5:1) as needles. (Yield 72%), m.p. 125-127°C . $\nu_{max.}$ (cm^{-1}, KBr): 3359(NH), 1662(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 3.20 (s, 2H, C_6-*H*), 3.74 (s, 2H, C_8-*H*), 3.86 (s, 2H, CH_2), 7.19-8.92 (m,15H, Ar + olefinic *H* + C_2-*H*) 9.44 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 49.74 (C-6), 60.31 (C-8), 75.44 (CH_2), 115.33, 115.45, 116.06, 119.54, 127.23, 128.02, 128.12, 128.45, 128.82, 129.52, 130.42, 130.82, 132.62, 133.34, 136.90, 145.72, 151.23, 157.21, 161.21, 162.12 (ArC), 163.12(CO). Anal.% Calcdfor $C_{30}H_{22}F_2N_4O$: C, 73.16; H, 4.50; N,11.38. Found: C, 73.23; H, 4.59; N, 11.26.

***7-benzyl-9-(4-bromobenzylidene)-5-(4-bromophenyl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4(3H)-one 2**:*

Recrystallized from ethanol (Yield 66%), m.p. 138-140°C . $\nu_{max.}$ (cm^{-1}, KBr): 3411(NH), 1659(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 3.29 (s, 2H, C_6-*H*), 3.66 (s, 2H, C_8-*H*), 3.79 (s, 2H, CH_2), 7.37-8.86 (m,15H, Ar + olefinic *H* + C_2-*H*) 9.37 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 48.32 (C-6), 59.58 (C-8), 76.11 (CH_2), 114.08, 114.74, 115.81, 117.19, 125.08, 127.88, 127.64, 128.41, 128.53, 129.17, 130.14, 130.68, 132.66, 133.70, 136.49, 145.47, 152.32, 157.29, 161.46, 162.60 (ArC), 164.23 (CO). Anal.% Calcdfor $C_{30}H_{22}Br_2N_4O$: C, 58.65; H, 3.61; N,9.12. Found: C, 58.27; H, 4.11; N, 9.30.

***7-benzyl-9-(benzo[d][1,3]dioxol-5-ylmethylene)-5-(benzo[d][1,3]dioxol-5-yl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4(3H)-one 3**:*

Recrystallized from methanol/DMF(3:2) as needles. (76%), m.p. 211-213°C . $\nu_{max.}$ (cm^{-1}, KBr): 3419(NH), 1660(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 3.36 (s, 2H, C_6-*H*), 3.69 (s, 2H, C_8-*H*), 3.83 (s, 2H, CH_2), 6.15 (s, 2H, CH_2), 7.22-9.13 (m,13H, Ar + olefinic *H* + C_2-*H*) 9.41 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 48.11 (C-6), 60.01 (C-8), 76.29 (CH_2), 101.09 (CH_2), 109.21, 111.75, 114.82, 117.34, 125.24, 127.77, 128.80, 129.45, 129.87, 130.38, 130.76, 131.08, 132.60, 133.97, 136.18, 145.55, 151.38, 157.25, 161.11, 162.52 (ArC), 164.50(CO). Anal.% Calcdfor $C_{32}H_{24}N_4O_5$: C, 70.58; H, 4.44; N,10.29. Found: C, 70.18; H, 4.87; N, 10.96.

7-benzyl-9-(thiophen-2-ylmethylene)-5-(thiophen-2-yl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4(3H)-one 4*:*

Recrystallized from DMF. (Yield 70%), m.p. >360°C . ν $_{max.}$ (cm^{-1}, KBr): 3372(NH), 1647(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 3.30 (s, 2H, C_6-*H*), 3.74 (s, 2H, C_8-*H*), 3.74 (s, 2H, CH_2), 7.50-8.73 (m,13H, Ar + olefinic *H* + C_2-*H*) 9.29 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 49.40 (C-6), 59.17 (C-8), 74.71 (CH_2), 111.41, 118.30, 120.52, 123.43, 127.08, 128.91, 129.40, 130.37, 130.88, 131.17, 131.56, 132.73, 133.08, 136.13, 145.23, 151.92, 157.51, 161.42, 162.79 (ArC), 163.96(CO). Anal.% Calcdfor $C_{26}H_{20}N_4OS_2$: C, 66.64; H, 4.30; N,11.96. Found: C, 66.18; H, 4.78; N, 12.39.

<u>7-benzyl-9-(aryllidene)-5-(aryl)-2-methyl-6,7,8,9-tetrahydropyrimido[4,5-b][1,6] naphthyridin-4(3H)-one (5-8)</u>

A mixture of the start 2-aminonaphthyridine**s** (10 mmol), acetic anhydride (10 mL) and conc. H_2SO_4 (1 mL) was heated in a boiling water bath for 10 min. The reaction mixture was cooled, poured carefully onto icecold water, treated with 20% NaOH solution till alkaline. The precipitated crude solid product was filtered, washed with water, dried and recrystallized from ethanol.

7-benzyl-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-2-methyl-6,7,8,9-tetrahydro pyrimido[4,5-b][1,6]naphthyridin-4(3H)-one 5:

(yield 72%), m.p. 258-260°C . ν $_{max.}$ (cm^{-1}, KBr): 2371(NH), 1654(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 2.64 (s, 3H, CH_3), 3.47 (s, 2H, C_6-*H*), 3.61 (s, 2H, C_8-*H*), 3.70 (s, 2H, CH_2), 6.87-7.64 (m,14H, Ar + olefinic *H*) 9.33 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.84 (CH3), 45.60 (C-6), 60.41 (C-8), 77.22 (CH_2), 111.41, 114.74, 115.64, 117.08, 121.43, 124.81, 126.70, 127.16, 128.33, 129.21, 129.69, 130.22, 131.06, 132.48, 138.33, 145.81, 151.61, 157.73, 161.51, 162.47 (ArC), 164.96(CO). Anal.% Calcdfor $C_{31}H_{24}F_2N_4O$: C, 73.50; H, 4.78; N,11.06. Found: C, 73.21; H, 4.90; N, 10.77.

7-benzyl-9-(4-bromobenzylidene)-5-(4-bromophenyl)-2-methyl-6,7,8,9-tetrahydro pyrimido[4,5-b][1,6]naphthyridin-4(3H)-one 6:

(yield 75%), m.p. 264-266°C . ν $_{max.}$ (cm^{-1}, KBr): 3265(NH), 1650(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 2.67 (s, 3H, CH_3), 3.41 (s, 2H, C_6-*H*), 3.59 (s, 2H, C_8-*H*), 3.82 (s, 2H, CH_2),

7.10-8.22 (m,14H, Ar + olefinic *H*) 9.38 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 22.08 (CH3), 45.91 (C-6), 60.22 (C-8), 76.95 (CH_2), 117.63, 119.11, 121.63, 121.97, 125.46, 125.80, 127.19, 127.35, 128.66, 129.28, 129.43, 130.05, 130.66, 131.86, 131.65, 131.81, 132.68, 149.70, 157.37, 161.95 (ArC), 165.35(CO). Anal.% Calcdfor $C_{31}H_{24}Br_2N_4O$: C, 59.26; H, 3.85; N,8.92. Found: C, 58.76; H, 4.12; N, 9.25.

7-benzyl-9-(benzo[d][1,3]dioxol-5-ylmethylene)-5-(benzo[d][1,3]dioxol-5-yl)-2-methyl-6,7,8,9-tetrahydro pyrimido[4,5-b][1,6]naphthyridin-4(3H)-one 7:

(yield 67%), m.p. > 360°C . ν $_{max.}$ (cm^{-1}, KBr): 3279(NH), 1659(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 2.59 (s, 3H, C*H*$_3$), 3.41 (s, 2H, C$_6$-*H*), 3.66 (s, 2H, C$_8$-*H*), 3.84 (s, 2H, C*H*$_2$), 6.17 (s, 2H, C*H*$_2$),7.21-8.33 (m,12H, Ar + olefinic *H*) 9.41 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.71 (CH3), 49.74 (C-6), 60.31 (C-8), 75.44 (CH_2), 101.21 (CH3), 115.33, 115.45, 116.06, 119.54, 127.23, 128.02, 128.12, 128.45, 128.82, 129.52, 130.42, 130.82, 132.62, 133.34, 136.90, 145.72, 151.23, 157.21 161.21, 162.12 (ArC), 163.12(CO). Anal.% Calcdfor $C_{33}H_{26}N_4O_5$: C, 70.96; H, 4.69; N,10.03. Found: C, 70.66; H, 4.97; N, 10.28.

7-benzyl-9-(thiophen-2-ylmethylene)-5-(thiophen-2-yl)-2-methyl-6,7,8,9-tetrahydro pyrimido[4,5-b][1,6]naphthyridin-4(3H)-one 8:

(yield 72%), m.p. 228-230°C . ν $_{max.}$ (cm^{-1}, KBr): 3275(NH), 1661(C=O). ^{1}H NMR (δ/ppm, DMSO-d_6): 2.50 (s, 3H, C*H*$_3$), 3.48 (s, 2H, C$_6$-*H*), 3.56 (s, 2H, C$_8$-*H*), 3.78 (s, 2H, C*H*$_2$), 7.27-8.68 (m,12H, Ar + olefinic *H*) 9.30 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 21.71 (CH3), 45.73 (C-6), 60.09 (C-8), 75.84 (CH_2), 114.31, 115.63, 119.10, 125.38, 128.39, 128.67, 128.96, 129.30, 130.44, 130.88, 132.16, 133.47, 136.76, 145.16, 151.09, 157.35, 161.64, 162.56 (ArC), 164.85(CO). Anal.% Calcdfor $C_{27}H_{22}N_4OS_2$: C, 67.20; H, 4.59; N,11.61. Found: C, 67.51; H, 4.81; N, 11.30.

7-benzyl-9-(aryllidene)-5-(aryl)-4-imino-3-phenyl-3,4,6,7,8,9-hexahydropyrimido [4,5-b][1,6]naphthyridine-2(1H)-thione (9-12)

A mixture of the start 2-aminonaphthyridine**s** (10 mmol), phenyl isothiocyanate (1.35 g, 10 mmol) in pyridine (15 ml) was refluxed for 2-3 h. After cooling, the solid product was filtered off, washed thoroughly with water, dried and recrystallized from acetic acid.

7-benzyl-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-4-imino-3-phenyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thione 9:

(yield 78%), m.p. 135-137°C . $\nu_{max.}$ (cm^{-1}, KBr): 3192 (NH), 1619 (C=N) 1195 (C=S). ^1H NMR (δ/ppm, DMSO-d_6): 3.49 (s, 2H, C_6-*H*), 3.60 (s, 2H, C_8-*H*), 3.79 (s, 2H, CH_2), 4.90 (s,1H,N*H*), 6.85-8.13 (m,19H, Ar + olefinic *H*) 9.42 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 45.87 (C-6), 60.11 (C-8), 77.71 (CH_2), 111.41, 112.73, 116.07, 117.18, 121.78, 122.19, 125.00, 125.31, 125.71, 126.11, 126.51, 126.77, 127.25, 128.38, 128.50, 130.01, 130.38, 130.67, 131.55, 131.96, 132.40, 132.71, 135.92, 136.68, 139.34, 141.81, 145.77, 151.69, 157.20, 161.37, 163.68 (ArC), 164.70 (C=NH), 175.82 (CS). Anal.% Calcdfor $C_{36}H_{27}F_2N_5S$: C, 72.10; H, 4.54; N,11.68. Found: C, 71.73; H, 4.80; N, 11.99.

7-benzyl-9-(4-bromobenzylidene)-5-(4-bromophenyl)-4-imino-3-phenyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thione 10:

(yield 70%), m.p. 128-130°C . $\nu_{max.}$ (cm^{-1}, KBr): 3180 (NH), 1628 (C=N) 1219 (C=S). ^1H NMR (δ/ppm, DMSO-d_6): 3.43 (s, 2H, C_6-*H*), 3.66 (s, 2H, C_8-*H*), 3.80 (s, 2H, CH_2), 5.22 (s,1H,N*H*), 6.71-8.20 (m,19H, Ar + olefinic *H*) 9.30 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 45.08 (C-6), 60.63 (C-8), 77.15 (CH_2), 110.10, 111.36, 115.37, 115.91, 119.22, 120.38, 123.40, 124.76, 125.19, 125.65, 126.46, 126.91, 127.20, 128.68, 129.59, 130.20, 130.65, 131.44, 131.69, 131.96, 132.36, 133.50, 134.19, 135.32, 138.70, 141.99, 144.69, 151.56, 156.31, 162.19, 163.79 (ArC), 164.93 (C=NH), 175.12 (CS). Anal.% Calcdfor $C_{36}H_{27}Br_2N_5S$: C, 59.93; H, 3.77; N,9.71. Found: C, 59.66; H, 3.96; N, 9.64.

7-benzyl-9-(benzo[d][1,3]dioxol-5-ylmethylene)-5-(benzo[d][1,3]dioxol-5-yl)-4-imino-3-phenyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thione 11:

(yield 79%), m.p. 132-134°C . $\nu_{max.}$ (cm^{-1}, KBr): 3266 (NH), 1620 (C=N) 1228 (C=S). ^1H NMR (δ/ppm, DMSO-d_6): 3.41 (s, 2H, C_6-*H*), 3.63 (s, 2H, C_8-*H*), 3.77 (s, 2H, CH_2), 4.87 (s,1H,N*H*), 6.21 (s, 2H, CH_2), 7.12-8.22 (m,17H, Ar + olefinic *H*) 9.51 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 44.21 (C-6), 60.84 (C-8), 78.09 (CH_2), 101.13 (CH_2), 114.21, 115.98, 116.10, 116.27, 118.70, 119.39, 124.53, 125.31, 125.86, 126.00, 126.43, 126.86, 127.21, 128.66, 128.84, 130.16, 130.53, 130.70, 131.48, 131.65, 132.14, 132.66, 134.56, 135.47, 137.33, 140.80, 141.77, 146.70, 149.12, 155.46, 164.83 (ArC), 165.19 (C=NH), 176.60 (CS).

Anal.% Calcdfor $C_{38}H_{29}N_5O_4S$: C, 70.03; H, 4.49; N,10.75. Found: C, 69.86; H, 4.81; N, 10.57.

7-benzyl-9-(thiophen-2-ylmethylene)-5-(thiophen-2-yl)-4-imino-3-phenyl-3,4,6,7,8,9-hexahydropyrimido[4,5-b][1,6]naphthyridine-2(1H)-thione 12:

(yield 82%), m.p. 108-110°C . $\nu_{max.}$ (cm^{-1}, KBr): 3249 (NH), 1624 (C=N) 1228 (C=S). ^1H NMR (δ/ppm, DMSO-d_6): 3.47 (s, 2H, C_6-*H*), 3.59 (s, 2H, C_8-*H*), 3.82 (s, 2H, CH_2), 5.19 (s,1H,N*H*), 6.76-8.31 (m,17H, Ar + olefinic *H*) 9.39 (s,1H,N*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 44.54 (C-6), 60.30 (C-8), 78.27 (CH_2), 109.30, 111.63, 116.26, 120.99, 123.46, 125.63, 125.54, 125.47, 126.68, 126.96, 127.34, 128.30, 128.59, 130.15, 130.66, 131.76, 132.39, 132.68, 135.88, 136.51, 139.27, 141.90, 145.49, 151.77, 157.10, 161.31, 163.53 (ArC), 165.11 (C=NH), 176.44 (CS). Anal.% Calcdfor $C_{32}H_{25}F_2N_5S_3$: C, 66.75; H, 4.38; N,12.16. Found: C, 66.30; H, 4.69; N, 11.81.

<u>7-benzyl-9-(aryllidene)-5-(aryl)-6,7,8,9-tetrahydropyrimido[4,5-b][1,6]naphthyridin-4-amine (13-16)</u>

A mixture of the start 2-aminonaphthyridine**s** (10 mmol) and formamide (10 mL) was heated under reflux for 2-3 h. The reaction mixture was allowed to cool and the precipitated solid product was collected, washed with cold ethanol and recrystallized from acetic acid containing few drops of water.

7-benzyl-9-(4-fluorobenzylidene)-5-(4-fluorophenyl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4-amine 13:

(yield 63%), m.p. 179-181°C . $\nu_{max.}$ (cm^{-1}, KBr): 3354, 3409 (NH_2). ^1H NMR (δ/ppm, DMSO-d_6): 3.42 (s, 2H, C_6-*H*), 3.69 (s, 2H, C_8-*H*), 3.77 (s, 2H, CH_2), 6.37 (s,2H,NH_2), 7.14-8.31 (m,15H, Ar + olefinic *H* + C_2-*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 45.91 (C-6), 60.24 (C-8), 76.11 (CH_2), 119.14, 120.73, 120.15, 123.41, 127.90, 128.09, 128.36, 128.77, 129.28, 129.67, 130.47, 130.88, 131.65, 132.04, 136.61, 145.16, 151.50, 157.38, 161.21, 162.23 (ArC). Anal.% Calcdfor $C_{30}H_{23}F_2N_5$: C, 73.31; H, 4.72; N, 7.73. Found: C, 73.04; H, 4.94; N, 7.44.

7-benzyl-9-(4-bromobenzylidene)-5-(4-bromophenyl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4-amine 14:

(yield 59%), m.p. 246-248°C . $\nu_{max.}$ (cm^{-1}, KBr): 3366, 3415 (NH_2). ^1H NMR (δ/ppm, DMSO-d_6): 3.39 (s, 2H, C_6-*H*), 3.63 (s, 2H, C_8-*H*), 3.79 (s, 2H, C*H*$_2$), 6.22 (s,2H,N*H*$_2$), 7.25-8.27 (m,15H, Ar + olefinic *H* + C_2-*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 46.30 (C-6), 60.83 (C-8), 76.28 (CH_2), 117.26, 119.64, 120.22, 121.65, 125.86, 128.30, 128.58, 128.68, 129.67, 129.81, 130.70, 130.95, 131.11, 132.14, 136.63, 145.72, 151.44, 157.93, 161.71, 162.65 (ArC). Anal.% Calcdfor $C_{30}H_{23}Br_2N_5$: C, 58.75; H, 3.78; N, 11.42. Found: C, 58.53; H, 4.11; N, 11.64.

7-benzyl-9-(benzo[d][1,3]dioxol-5-ylmethylene)-5-(benzo[d][1,3]dioxol-5-yl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4-amine 15:

(yield 64%), m.p. > 360°C . $\nu_{max.}$ (cm^{-1}, KBr): 3358, 3429 (NH_2). ^1H NMR (δ/ppm, DMSO-d_6): 3.43 (s, 2H, C_6-*H*), 3.67 (s, 2H, C_8-*H*), 3.80 (s, 2H, C*H*$_2$), 6.19 (s, 2H, C*H*$_2$), 6.49 (s,2H,N*H*$_2$), 6.84-8.22 (m,13H, Ar + olefinic *H* + C_2-*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 46.49 (C-6), 61.34 (C-8), 75.17 (CH_2), 101.22 (CH_2), 118.30, 119.36, 120.05, 120.56, 125.90, 127.11, 128.27, 128.69, 129.35, 130.44, 130.71, 130.93, 131.37, 132.47, 136.60, 145.28, 151.61, 157.79, 161.77, 162.60 (ArC). Anal.% Calcdfor $C_{32}H_{25}N_5O_4$: C, 70.71; H, 4.64; N, 12.88. Found: C, 70.38; H, 4.89; N, 13.19.

7-benzyl-9-(thiophen-2-ylmethylene)-5-(thiophen-2-yl)-6,7,8,9-tetrahydropyrimido [4,5-b][1,6]naphthyridin-4-amine 16:

(yield 55%), m.p. 231-233°C . $\nu_{max.}$ (cm^{-1}, KBr): 3360, 3432 (NH_2). ^1H NMR (δ/ppm, DMSO-d_6): 3.40 (s, 2H, C_6-*H*), 3.60 (s, 2H, C_8-*H*), 3.73 (s, 2H, C*H*$_2$), 6.40 (s,2H,N*H*$_2$), 7.13-8.29 (m,15H, Ar + olefinic *H* + C_2-*H*). ^{13}C NMR (δ/ppm, DMSO-d_6): 45.71 (C-6), 60.20 (C-8), 75.61 (CH_2), 108.21, 119.61, 120.22, 121.61, 127.18, 128.00, 128.37, 128.96, 129.19, 129.46, 130.54, 131.93, 132.44, 136.38, 145.31, 151.39, 157.97, 161.24, 162.82 (ArC). Anal.% Calcdfor $C_{26}H_{21}N_5S_2$: C, 66.78; H, 4.53; N, 14.53. Found: C, 66.54; H, 4.83; N, 14.80.

3.2. In vitro MTT cytotoxicity assay

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete

growth medium in 96-well microtiter plastic plates at 37^{o}C for 24h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 – 50 – 25 – 12.5 – 6.25 – 3.125 – 1.56 – 0.78 μg/mL). DMSO was employed as a vehicle for dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HePG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 μg/mL streptomycin sulphate and 25 μg/mL amphotericin B), and 1% L-glutamine in 96-

well flat bottom microplate at 37^{o}C under 5% CO_2.After 48h of incubation, the medium was aspirated, 40 μL of MTT salt (2.5 μg/mL) were added to each well and incubated for further 4h at 37^{o}C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37^{o}C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm [12,13]. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. The results are presented in Table 1 as LC_{50} μg which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

4. Conclusion

The aim of the present investigation was to synthesize some naphthyridine and pyrimidonaphthyridin derivatives to be evaluated for their *in vitro* cytotoxic and antiviral activities. Such target was verified by obtaining fourteen compounds with variable degree of cytotoxic potential against a panel of three cancer cell lines namely; human colon carcinoma (HT29), hepatocellular carcinoma (Hep-G2) and Caucasian breast adenocarcinoma (MCF7), among which the analogs compounds **9, 11** and **12** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. Moreover concerning the antiviral activity, only two derivatives **10** and **12** were able to inhibit the hepatitis-C virus RNA (+) and (–) strands at 10-100 μg/mL concentration range.

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