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Research Article

Synthesis of 1,8-Naphthyridine Derivatives under Ultrasound Irradiation and Cytotoxic Activity against HepG2 Cell Lines

N. S. Ahmed,[1,2] K. O. AlFooty,[1] and S. S. Khalifah[1]

[1] *Chemistry Department, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia*
[2] *Medicinal Chemistry Department, National Research Center, Dokki, Cairo 12622, Egypt*

Correspondence should be addressed to N. S. Ahmed; nesreen69eg@yahoo.com

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Novel pyrazole derivatives **3a,b**, **5**, 1,3,4-oxadiazole **6**, 1,3,4-thiadiazole **8**, and 1,2,4-triazole **9a–c** incorporated into 1,8-naphthyridine have been synthesized using the versatile synthon 2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy) acetohydrazide **1**. An improvement in rates and yields was observed when the reactions were carried out under ultrasonic irradiation compared with the classical synthesis. The newly synthesized compounds were evaluated for HepG2 cell growth inhibition. The results obtained revealed that the tested compounds possess inhibitory effect on the growth of HepG2 liver cancer cells. The results were compared to doxorubicin (DOX) as a reference drug (IC_{50} : 0.04 μM). Compounds **9b** showed the highest inhibition activity against HepG2 cell line (IC_{50} : 0.048 μM) among all tested compounds.

1. Introduction

Naphthyridine derivatives have received significant attention due to their exceptionally broad spectrum of biological activity. For example 1,8-naphthyridine derivatives have promising medicinal properties, including anti-HIV [1], anticancer [2], anti-inflammatory [3], antimalarial [4], antibacterial [5], antiprotozoals [6], antimycobacterial [7], and antiplatelet [8]. In addition 1,8-naphthyridine derivatives were found to display cytotoxic activity against murine P388 leukemia cell line when changes were carried out at N-1 and C-7 positions [9, 10]. Moreover, it was recently found that 1,8-naphthyridine derivative vosaroxin (formerly SNS-595, AG-7352, AT-3639, or Voreloxin) (Figure 1) was found to have potential anticancer activity; it is currently subjected to clinical development. This drug is believed to exert its action via topoisomerase II inhibition [11]. Topoisomerase II is one of the well-known targets for antitumor agents like doxorubicin, etoposide, ellipticine, and amsacrine [12]. We have reported in a previous article that 1,8-naphthyridine substituted with Mannich bases, *N*-β-glycosides, and Schiff's bases showed potent cytotoxic activity against HepG2 cell line [13].

On the other hand, an important class of heterocyclic compounds such as pyrazoles [14, 15], 1,2,4-triazole derivative [16], 1,3,4-thiadiazoles [17], and 1,3,4-oxadiazoles [18] showed a remarkable anticancer effect [14–18]. Based on these observations it was of interest to incorporate the 1,8-naphthyridine ring system into the abovementioned nitrogen, sulphur, and oxygen heterocyclic systems in one molecule in a trial to obtain a new target and product of dual mode of biological function. The application of ultrasound in synthetic organic chemistry became more and more interesting. "Sonochemistry" is a new trend in organic chemistry, offering a versatile and pathway for a large variety of syntheses. Therefore, a large number of organic reactions can be carried out under ultrasonic irradiation in high yields, short time, and mild conditions [19–24].

As an extension of our efforts directed towards development of convenient synthetic approaches for the construction of biologically active heterocycles and as a part of growing interest in sonochemistry [25–27], our strategy is to develop a facile sonochemical synthesis and high yield procedure to prepare some novel 1,3,4-oxadiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and pyrazole incorporated into 1,8-naphthyridine

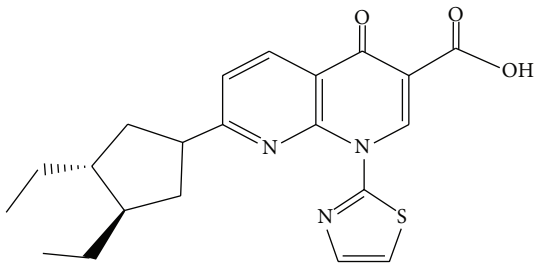


Figure 1: Vosaroxin.

and the findings of their biological activities in suppressing the growth of HepG2 liver cancer cells.

2. Results and Discussion

2.1. Chemistry. The starting material, namely, 2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy) acetohydrazide **1** [27], allowed to react with the diketone, namely, acetylacetone and benzoylacetone, under ultrasound irradiation at 65°C (Scheme 1). The IR spectra 2-((2,7-dimethyl-1,8- naphthyridin-4-yl)oxy)-1-(3,5-dimethyl-1*H*-pyrazol-1-yl) ethanone **3a** revealed the disappearance of $-NHNH_2$ bands of hydrazide and showed only one band at 1674 cm^{-1} characteristic for amidic CO group. ^{1}HNMR for compound **3a** showed the disappearance of the broad singlet signals from δ 3.99 and 9.28 corresponding to NH_2 and NH protons, respectively, in acid hydrazide **1** and the appearance of three singlet signals due to 2 methyl groups and CH-pyrazole at δ 1.69, 2.00, and 6.47, respectively, beside the original methyl groups, 3CH—of naphthyridine and the methylene protons. The mass spectrum of this compound showed molecular ion peak *m/z* 310 consistent with its molecular formula $C_{17}H_{18}N_4O_2$. Also, reaction of **1** with excess ethylacetoacetate under ultrasound irradiation at 60°C for 10 min gives only one isolable uncyclized product identified as ethyl-3-(2-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy)acetyl)hydrazono)butanoate **4** (Scheme 1). The structure of compound **4** was confirmed on the basis of its elemental and spectral data. The IR spectrum for **4** showed str. absorption band at 3200 cm^{-1} characteristic for NH and strong absorption band at 1722 cm^{-1} for CO of ester.^{1}HNMR of the compound **4** revealed one D_2O exchangeable signal at δ 9.05 due to NH, triplet and quartet signals at δ 1.32 and 4.24 for ethyl group, and two singlet signals at δ 2.15 and 3.40 for new methyl and methylene groups, respectively. Upon increasing the time of the foregoing reaction to 25 min under the same conditions the cyclized product, 1-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy)acetyl)-3-methyl-1*H*-pyrazol-5(4H)-one **5** ,was obtained (Scheme 1).

The structure of the new pyrazolone **5** was confirmed on the basis of its elemental analysis and IR, ^{1}HNMR,^{13}CNMR, and mass spectral data, and its ^{1}HNMR revealed the disappearance of the two singlet signals from δ 3.99 and 9.28 corresponding to NH_2 and NH protons and new two singlet signals at δ 2.17 and 3.39 for methyl group and CH_2-pyrazole, respectively.

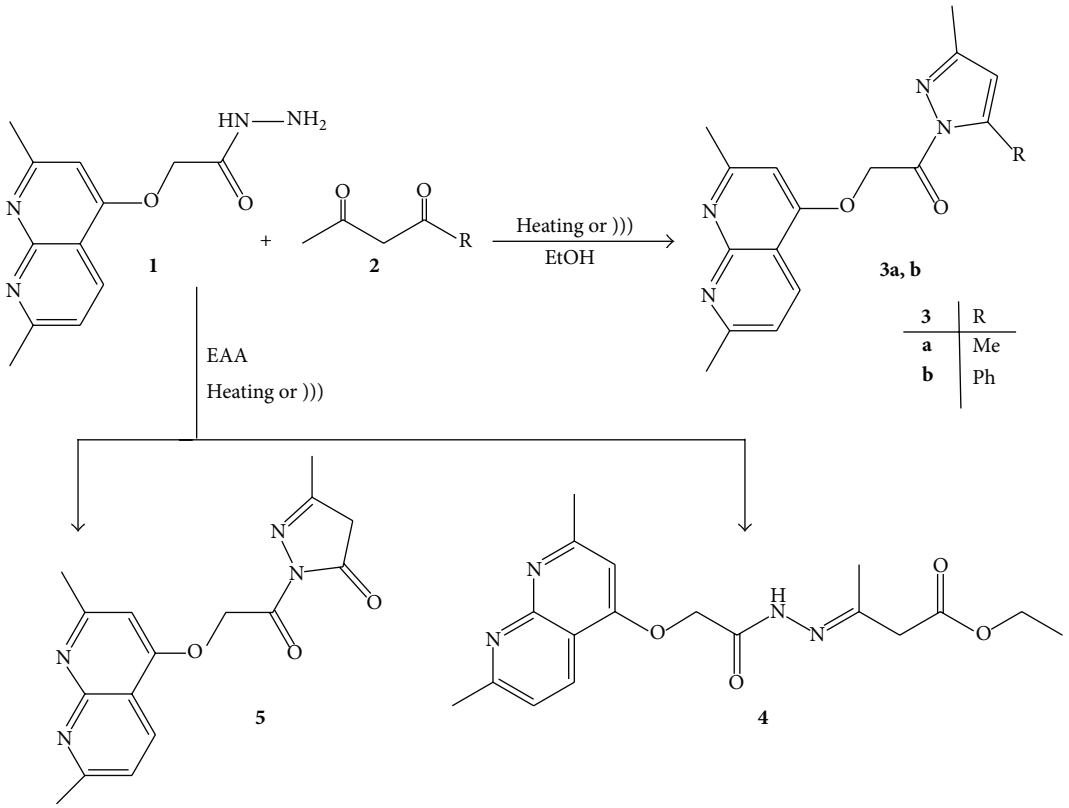
Table 1: Synthesis of pyrazole derivatives **3a-b**, **4,** and **5** under both ultrasonic irradiation and conventional method.

Compound	Ultrasonic irradiation		Conventional	
	Time (min.)	Yield %	Time (min.)	Yield %
3a	10	98	60	93
3b	10	97	60	91
4	10	93	60	87
5	25	96	180	83

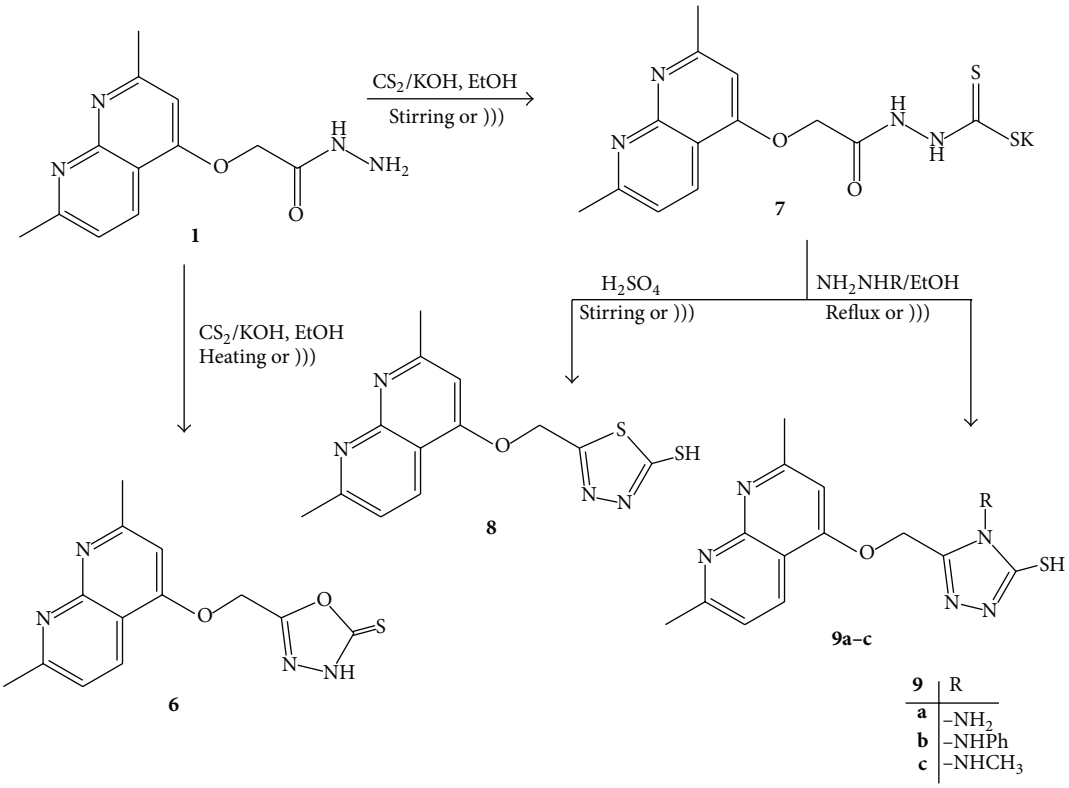
To find the specific effect of ultrasound on this reaction, all previously mentioned reactions were carried out under the same conditions in absence of ultrasound irradiations (Table 1). The data cited in Table 1 showed that the reaction time increased and the yields of the products decreased in absence of ultrasonic irradiation. Thus, the ultrasound irradiation was found to have beneficial effect on the synthesis of the pyrazole derivatives.

Furthermore a new series of 4-((oxymethyl)heterocyclo) 2,7-dimethyl-1,8-naphthyridines were synthesized under ultrasound irradiation. These compounds were obtained by reaction of **1** with CS_2 in the presence of ethanolic KOH (Scheme 2).

Upon subjecting the reaction mixture to ultrasonic irradiation at 65°C, the elemental analysis and spectral data of the reaction product were compatible only with the corresponding oxadiazole derivative **6**. IR spectra revealed that weak absorption band at 3200 cm^{-1} correspond to NH, and at 1293 cm^{-1}band due to C=S group. On the other hand the acid hydrazide **1** with excess CS_2 in ethanolic KOH was subjected to ultrasound irradiation at room temperature; it gave the dithiocarbazate potassium salt **7** (Scheme 2). The IR spectra for **7** showed band due to NH at 3132 cm^{-1}, C=O amidic at 1686 cm^{-1}, and C=S at 1291 cm^{-1}. The dithiocarbazate potassium salt **7** was added to concentrated H_2SO_4 at 0°C and then subjected to ultrasound irradiation (Scheme 2); it afforded 5-((2,7-dimethyl-1,8-naphthyridin-4-yl)oxy)-methyl)1,3,4-thiadiazole-2-thiol **8** methyl. The IR spectra showed the disappearance of NH band at 3200 cm^{-1} and new band at 2352 cm^{-1} for SH was observed. The ^{1}HNMR spectrum showed only one D_2O exchangeable signal due to SH at δ 10.5. Its mass spectrum revealed a peak corresponding to the molecular ion *m/z* 304. Furthermore, reaction of the potassium salt **7** with hydrazinehydrate in ethanol under ultrasound irradiation at 60–65°C afforded only one isolable product (as examined by TLC) identified as 4-amino-5-((2,7-dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4*H*-1,2,4-triazole-3-thiol **9a** (Scheme 2); under the same reaction conditions the pot salt **7** allowed to react with phenyl hydrazine and methyl hydrazine; it gave the corresponding aminophenyl and aminomethyltriazole derivative **9b** and **9c** (Scheme 2). All structures were established according elemental analysis and spectral data. The IR spectra of **9a** showed the disappearance of carbonyl group of potassium salt at 1660 cm^{-1} and two



Scheme 1



Scheme 2

Table 2: Synthesis of 1,3,4-oxadiazole **6**, 1,3,4-thiadiazole **8**, and 1,2,4-triazole **9a–c** derivatives under ultrasonic irradiation and conventional method.

Compound	Ultrasonic irradiation		Conventional	
	Time (min.)	Yield %	Time (h)	Yield %
6	45	91	24	76
7	25	96	8	93
8	10	89	2	73
9a	15	96	4	83
9b	15	91	3	77
9c	15	88	3	69

new stretching absorption bands at 3151 and 3244 cm^{-1} corresponding to NH_2 group. ^{1}HNMR for **9a** revealed two D_2O exchangeable singlet signals due to NH_2 and SH protons at δ 2.91 and 13.55, respectively. Time of reactions and yield are shown in Table 2. It is shown from this table that the ultrasound technique reduced the time of the reactions from several hours to minutes and improved the yield from 69–83% (under conventional conditions) to 88–96%.

Generally, ultrasound showed beneficial effect on the synthesis of some novel pyrazoles, 1,3,4-oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole incorporated into 1,8-naphthyridine which decreases the time of the above reactions from several hours in conventional procedure to few minutes with high yield under ultrasound irradiation. The improvement induced by ultrasound in the abovementioned reaction can be attributed to the well-established theory for the cavitation. These reactions according to sonochemical classification of Cabello et al. [28] and Ikawa et al. [29] are considered as false sonochemistry type in which cavitation effect provides the mechanical energy for all subsequent chemical reactions, including bond scission induced by viscous frictional forces.

2.2. Pharmacology. Preliminary screening showed that all selected compounds exhibited a moderate to strong growth inhibition activity on the tested cell line (IC_{50}: 0.048–0.091 μM) concentrations in comparison to the traditional anticancer drug doxorubicin (DOX). It can be deduced from the results cited in Table 3 that compound **9b** showed a growth inhibition activity quite similar to that observed to DOX. The pronounced activity might be due to the presence of N-aminophenyltriazole attached to (2,7-dimethyl-1,8-naphthyridine-4-yloxy) methyl moiety [30].

3. Conclusion

We have synthesized a class of novel substituted pyrazoles, 1,3,4-oxadiazole, 1,3,4-thiadiazole, and 1,2,4-triazole incorporated into 1,8-naphthyridine nucleus under both sonication and classical conditions. In general, improvements in rates and yield of the reactions are observed when reactions were carried out under sonication compared with classical condition. The cytotoxicity screening of the new compounds revealed that the selected compounds showed reasonable antitumor activity against HepG2 cancer cell line in comparison to the traditional anticancer drug DOX. Among all tested compounds, **9b** was found to have the highest inhibitory activity against HepG2 cell line with IC_{50} value 0.048 μM.

Table 3: IC_{50} (μM) of some selected new compounds against liver cancer cell line HepG2.

Compounds	IC_{50} (μM)
DOX	0.04
3a	0.071
3b	0.064
5	0.091
6	0.067
8	0.063
9a	0.058
9b	0.048
9c	0.067

4. Experimental

4.1. Chemistry

4.1.1. General. All melting points were measured on a Mel-Temp apparatus and were uncorrected. 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 on a FTIR, Perkin Elmer SP 100 spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Burker WM 350 and 600 MHz spectrometers using TMS (0.00 ppm). Chemical shift (δ) is given in ppm relative to the signal for TMS as standard and coupling constant in Hz. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 e.v. Sonication was performed by Daihan (Wiseclean, D-40 kHz).

*(1) Synthesis of 2-((2,7-Dimethyl-1,8-naphthyridin-4-yl)oxy)-1-(3,5-substituted-1H-pyrazol-1-yl)ethanone (**3a,b**)*

Method A: Silent Reactions. A mixture from acid hydrazide **1** (16 mmol) and appropriate 1,3-diketone, namely, acetylacetone and benzoylacetone (65 mmol) in absolute ethanol (10 mL), was heated at 100°C in a steam for a suitable time as examined by TLC. Then Cooled and treated with pet. ether. The solid precipitate formed was collected by filtration, washed with pet. ether, and dried. Recrystallization, from ethanol, afforded compounds **3a,b**.

Method B: Sonicated Reactions. In a 50 mL Erlenmeyer flask a mixture of acid hydrazide **1** (16 mmol) and appropriate 1,3-diketone, namely, acetylacetone and benzoylacetone (65 mmol), in absolute ethanol (10 mL), was subjected to ultrasound irradiation for suitable time (*cf.* Table 1) until the starting material was no longer detectable by TLC. All the reactions were kept at temperature 60–65°C (the temperature inside reaction vessel was 60°C). The precipitate formed was filtered off and washed with pet.

ether and finally recrystallized from ethanol to afford the corresponding 2-((2,7-dimethyl-1,8-naphthyridin-4-yl)oxy)-1-(3,5-substituted-1H-pyrazol-1-yl) ethanone **3a,b**.

The synthesized compounds (**3a,b**) with their physical data are listed below.

*2-((2,7-Dimethyl-1,8-naphthyridin-4-yl)oxy)-1-(3,5-dimethyl-1H-pyrazol-1-yl)ethanone (**3a**).* Off-white; crystals m.p 214°C. FTIR: 1674 (C=O), 1619 (C=N), 1606 (C=C); ^{1}H NMR (350 MHz, DMSO-d_6): δ_H = 1.69, 2.00 (6H, 2s, $2CH_3$ of pyrazole), 2.46, 2.51 (6H, 2s, $2CH_3$ of naphthyridine), 5.54 (2H, br.s, CH_2), 6.08 (1H, s, C_3-H, naphthyridine), 6.74 (1H, s, C_4-H, pyrazole); 7.24 (1H, d, C_6-H, J = 7.8 Hz); 8.31 (1H, d, C_7-H, J = 7.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 16.24, 21.46, 25.12, 26.98, 46.56, 91.89, 112.32, 118.42, 119.78, 135.83, 150.36, 151.69, 156.12, 161.72, 166.59, 178, 207.04; MS (*m/z*): 310 $M^{+\cdot}$. (Found: C, 65.84; H, 5.55; N, 17.97. $C_{17}H_{18}N_4O_2$ requires C, 65.79; H, 5.85; N, 18.05.)

*2-((2,7-Dimethyl-1,8-naphthyridin-4-yl)oxy)-1-(5-methyl-3-phenyl-1H-pyrazol-1-yl)ethanone (**3b**).* Pale yellow crystals; m.p. 220–222°C. FTIR: 1676 (C=O); 1607 (C=N); ^{1}H NMR (350 MHz, DMSO-d_6): δ_H = 2.07 (3H, s, C_5-CH_3), 2.42 (3H, s, C_7-CH_3), 2.55 (3H, s, C_2-$\underline{CH_3}$), 5.62 (2H, br.s, CH_2), 6.03 (1H, s, C_3-H) and 6.92 (1H, s, C_4-H of pyrazole), 7.16–7.31 (6H, m, C_6-H and ArH's), 8.27 (1H, d, C_5-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 16.14, 21.47, 25.10, 46.69, 112.28, 118.45, 119.75, 123.80, 127.00, 128.62, 132.29, 135.81, 142.88, 150.30, 151.59, 155.90, 161.56, 166.31, 178.03, 207.04; MS (*m/z*): 372 $M^{+\cdot}$. (Found: C, 71.25; H, 5.61; N, 15.34 $C_{22}H_{20}N_4O_2$requires C, 70.95; H, 5.41; N, 15.04.).

*(2) Ethyl-3-(2-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy) acetyl)hydrazono)butanoate (**4**)*

Method A: Silent Reaction. A mixture of acid hydrazide **1** (16 mmol) with ethylacetoacetate (65 mmol) was heated at 100°C in steam bath for 1 h. After cooling, the residue obtained was treated with pet. ether and the solid product obtained was filtered off and recrystallized from ethanol; it gives the title compound **6.**

Method B: Sonicated Reaction. A mixture of acid hydrazide **1** (16 mmol) and ethylacetoacetate (65 mmol) in 50 mL Erlenmeyer flask was subjected to ultrasound irradiation for suitable time (*cf.* Table 1) until the starting material was no longer detectable by TLC. All the reactions were kept at temperature 60–65°C (the temperature inside reaction vessel was 60°C). The precipitate formed was filtered off and washed with pet. ether and finally recrystallized from ethanol to afford the corresponding ethyl-3-(2-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy)acetyl)hydrazano)butanoate **4**,), m.p. 179–180.5°C. FTIR: 3200 (N–H); 1722 (C=O of ester); 1625 cm^{-1} (C=O amidic); ^{1}H NMR (350 MHz, $CDCl_3$): δ_H = 1.32 (3H, q, $-CH_2\underline{CH_3}$, J = 7.2 Hz), 2.15 (3H, s, –N=C–$\underline{CH_3}$), 2.40, 2.58 (6H, 2s for C_2-CH_3, C_7-CH_3), 3.40 (2H, s, –N=C–$\underline{CH_2}$–CO), 4.24 (2H, q, $-\underline{CH_2}CH_3$, J = 7.2 Hz), 5.63 (2H, s, $-O\underline{CH_2}CO$), 6.26 (1H, s, C_3-H), 7.15 (1H, d, C_6-H, J = 7.8 Hz), 8.55 (1H, d, C_5-H, J = 7.8 Hz), 9.05 (1H, s, –NH, D_2O exchangeable); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 14.22, 21.40, 25.05, 30.94, 44.47, 46.04, 59.49, 61.80, 112.48, 118.40, 119.99, 135.86, 150.07, 151.19, 161.94, 168.48, 178.11, 207.09; MS (*m/z*): 358 $M^{+\cdot}$. (Found: C, 60.02; H, 6.43; N, 15.37 $C_{18}H_{22}N_4O_4$ requires C, 60.32; H, 6.19; N, 15.63.)

*(3) 1-(2-(2,7-Dimethyl-1,8-naphthyridin-4-yloxy)acetyl)-3-methyl-1H-pyrazol-5(4H)-one (**5**)*

Method A: Silent Reaction. A mixture of acid hydrazide **1** (16 mmol) with ethylacetoacetate (65 mmol) was heated in steam bath for 3 h; the mixture was left to cool to room temperature. The yellow precipitate so formed was collected by filtration, washed with pet. ether, and dried. Recrystallization, from ethanol, afforded compound **5**.

Method B: Sonicated Reaction. A mixture of acid hydrazide **1** (16 mmol) and ethylacetoacetate (65 mmol) in 50 mL Erlenmeyer flask was subjected to ultrasound irradiation for suitable time (*cf.* Table 1) until the starting material was no longer detectable by TLC. All the reactions were kept at temperature 60–65°C. The yellow precipitate so formed was filtered off and washed with pet. ether and finally recrystallized from ethanol to afford the corresponding 1-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy)acetyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one **5**, m.p. 194–196.2°C. FTIR: 1604 (C=C); 1620 (C=N); 1625 (C=O amidic); ^{1}H NMR (600 MHz, $CDCl_3$): δ_H = 2.17, 2.43, 2.60 (9H, 3s, $3CH_3$), 3.39 (2H, s, CH_2 of pyrazole), 5.64 (2H, br.s, $-O\underline{CH_2}CO$), 6.26 (1H, s, C_3-H), 7.14 (1H, d, C_6-H, J = 7.8 Hz), 8.53 (1H, d, C_5-H, J = 7.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C = 12.91, 21.33, 25.12, 29.70, 45.91, 112.29, 118.40, 119.86, 135.80, 150.07, 151.19, 161.86, 161.94, 168.48, 178.11, 207.09; MS (*m/z*): 312 $M^{+\cdot}$. (Found: C, 61.67; H, 5.03; N, 17.64 $C_{16}H_{16}N_4O_3$ requires C, 61.53; H, 5.16; N, 17.94.)

*(4) 5-((2,7-Dimethyl-1,8-naphthyridin-4-yl)oxy)methyl)-1,3,4-oxadiazole-2(3H)-thione (**6**)*

Method A: Silent Reaction. The hydrazide **1** (2 mmol) was dissolved in hot ethanolic potassium hydroxide solution (0.11 gm KOH in 5.5 mL absolute ethanol). Carbon disulfide (23 mmol) was added to the reaction mixture and heated under refluxed on water bath until the evolution of H_2S ceased. The reaction mixture was cooled and acidified with acetic acid. The formed solid precipitate was filtered off, washed with water, and recrystallized from methanol to give the title compound as off-white crystals.

Method B: Sonicated Reaction. A mixture of acid hydrazide **1** (2 mmol) was dissolved in hot ethanolic potassium hydroxide solution (0.11 gm KOH in 5.5 mL absolute ethanol) and carbon disulfide (23 mmol) in 50 mL Erlenmeyer flask. The mixture was subjected to ultrasound irradiation for suitable time (*cf.* Table 2) until the evolution of H_2S ceased and the starting material was no longer detectable by TLC. The reaction was kept at temperature 60–65°C. The reaction mixture was cooled and acidified

with acetic acid. The formed solid precipitate was filtered off, washed with water, and recrystallized from methanol to afford the corresponding 5-((2,7-dimethyl-1,8-naphthyridin-4-yl)oxy)methyl)-1,3,4-oxadiazole-2(3H)-thione **6**, m.p. 261–263°C. FTIR: 1293 (C=S), 1607 (C=N), 3200 (–NH); MS (*m/z*): 288$M^{+\cdot}$. (Found: C, 54.53; H, 3.90; N, 19.20 $C_{13}H_{12}N_4O_2S$ requires C, 54.15; H, 4.20; N, 19.43.)

(5) Potassium2-(2-(2,7-dimethyl-1,8-naphthyridin-4-yloxy) acetyl)hydrazinecarbodithioate (7)

Method A: Silent Reaction. Carbon disulphide (0.6 mL, 10 mmol) was added dropwise to an ice cold solution of potassium hydroxide (0.56 gm, 10 mmol) in absolute ethanol (20 mL) containing the respective hydrazide **1** (2.46 gm, 10 mmol). The mixture was stirred at room temperature for 8 h. The separated solid was filtered off and washed several times with ether. The product obtained in quantitative yield was employed in the next reactions without further purification.

Method B: Sonicated Reaction. Carbon disulphide (0.6 mL, 10 mmol) was added dropwise to an ice cold solution of potassium hydroxide (0.56 gm, 10 mmol) in absolute ethanol (20 mL) containing the respective hydrazide **1** (2.46 gm, 10 mmol). The mixture subjected to ultrasound irradiation at room temperature for a suitable time (*cf.* Table 2). The separated solid was filtered off, washed several time with ether, and dried. FTIR: 1686 (C=O amidic); 1291 (C=S); 3132 (N–H); (Found: C, 43.61; H, 3.33; N, 15.22 $C_{13}H_{13}KN_4O_2S_2$ requires C, 43.31; H, 3.63; N, 15.54.)

(6) 5-((2,7-Dimethyl-1,8-naphthyridin-4-yl)oxy)methyl)-1,3,4-thiadiazole-2-thiol (8)

Method A: Silent Reaction. The potassium salt **7** (0.36 gm, 1 mmol) was added in small portions to H_2SO_4 (d = 1.836, 1.75 mL) under gentle stirring at 0°C. After complete addition of the salt the stirring was continued for further 2 h.; then the solution was poured into ice. The precipitate formed was filtered off, washed with water, dried, and recrystallized from ethanol to give the title compound as chestnut crystals.

Method B: Sonicated Reaction. The potassium salt **7** (0.36 gm, 1 mmol) was added in small portions to H_2SO_4 (d = 1.836, 1.75 mL) in 50 mL Erlenmeyer flask under gentle stirring at 0°C. After complete addition of the salt, the mixture was subjected to ultrasound irradiation at room temperature for suitable time (*cf.* Table 2) until the starting material was no longer detectable by TLC. The solution was poured into ice. The precipitate formed was filtered off, washed with water, dried, and recrystallized from ethanol to afford the corresponding 5-((2,7-dimethyl-1,8-naphthyridin-4-yl)oxy)methyl)-1,3,4-thiadiazole-2-thiol **8**, m.p. 223–225°C. FTIR: 1604 (C=C); 1630 (C=N) and 2352 (S–H);^{1}H NMR (600 MHz, $CDCl_3$: DMSO-d_6): δ_H = 2.40, 2.61 (6H, 2s, $2CH_3$), 5.79 (2H, s, $-CH_2$), 6.22 (1H, s, C_3-H), 7.5 (1H, d, C_6-H, J = 7.8 Hz), 8.50 (1H, d, C_5-H, J = 7.8 Hz), 10.5 (1H, s, S–H, D_2O exchangeable), ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 24.52, 25.33, 43.28, 111.89, 118.00, 120.33, 135.36, 144.51, 152.27, 158.52, 161.86, 167, 188; MS (*m/z*): 304 $M^{+\cdot}$. (Found: C, 51.63; H, 3.71; N, 18.06 $C_{13}H_{12}N_4OS_2$ requires 51.30; H, 3.97; N, 18.41.)

(7) Synthesis of 4-N-Substituted-5-((2,7-dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4H-1,2,4-triazole-3-thiol (9a–c)

Method A: Silent Reaction. A mixture of the potassium salt **7** (0.51 gm, 1.3 mmol), appropriate hydrazine derivative (2.6 mmol), and water (0.2 mL) in 10 mL absolute ethanol was refluxed with stirring for a suitable time (*cf.* Table 2) until the starting material was no longer detectable by TLC. The reaction mixture was cooled and the solid precipitate was filtered off, dried, and finally recrystallized from glacial acetic acid to give the title compounds **9a–c.**

Method B: Sonicated Reaction. A mixture of the potassium salt **7** (0.51 gm, 1.3 mmol), appropriate hydrazine derivative (2.6 mmol), and water (0.2 mL) in 10 mL absolute ethanol in 50 mL Erlenmeyer flask was subjected to ultrasound irradiation for suitable time (*cf.* Table 2) until the starting material was no longer detectable by TLC. All the reactions were kept at temperature 60–65°C (the temperature inside reaction vessel was 60°C). The reaction mixture was cooled and the solid precipitate was filtered off, dried, and finally recrystallized from glacial acetic acid to afford the corresponding 4-N-substitueted-5-((2,7-dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4H-1,2,4-triazole-3-thiol **9a–c**.

The synthesized compounds (**9a–c**) with their physical data are listed below.

4-Amino-5-((2,7-dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4H-1,2,4-triazole-3-thiol (9a). Brown crystals; m.p 286–288°C. FTIR: 1601 (C=N); 2363 (S–H); 3151, 3244 ($-NH_2$); ^{1}H NMR (600 MHz, DMSO-d_6): δ_H = 2.35, 2.44 (6H, 2s, $2CH_3$), 2.91 (2H, s, NH_2, D_2O exchangeable), 5.75 (2H, s, $-CH_2$), 6.15 (1H, s, C_3-H), 7.28 (1H, d, $-CH_2$, J = 7.8 Hz), 8.33 (1H, d, C_5-H, J = 7.8 Hz) and 13.55 (1H, s, S–H, D_2O exchangeable); ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 20.58, 24.62, 51.25, 111.55, 117.92, 120.01, 135.24, 148.82, 149.70, 152.86, 161.81, 166.74, 176.30; MS (*m/z*): 302 $M^{+\cdot}$. (Found: C, 51.84; H, 4.86; N, 27.86 $C_{13}H_{14}N_6OS$ requires C, 51.64; H, 4.67; N, 27.80.)

5-((2,7-Dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4-(phenylamino)-4H-1,2,4-triazole-3-thiol (9b). Yellow crystals; m.p 221.6–222.9°C. FTIR: 1293 (C=S), 1605 (C=N), 2363 (S–H), 3649 (N–H); ^{1}H NMR (600 MHz, $CDCl_3$: DMSO-d_6): δ_H = 2.57, 3.12 (6H, 2s, $2CH_2$); 2.69 (1H, s, –NHPh, D_2O exchangeable), 5.81 (2H, s, $-CH_2$), 6.22 (1H, s, C_3-H); 7.23, 7.41 (6H, m, PhH's and S–H), 7.6 (1H, d, C_6-H, J = 7.8 Hz); 8.48 (1H, d, C_5-H, J = 7.8 Hz); MS (*m/z*): 302 $M^{+\cdot}$. (Found: C, 60.65; H, 4.32; N, 22.63 $C_{19}H_{18}N_6OS$ requires C, 60.30; H, 4.79; N, 22.21.)

5-((2,7-Dimethyl-1,8-naphthyridin-4-yloxy)methyl)-4-(methylamino)-4H-1,2,4-triazole-3-thiol (9c). Yellow crystals; m.p 233–235°C. FTIR: 1602 (C=N), 2364 (S–H), 3280 (N–H); ^{1}H NMR (600 MHz, $CDCl_3$: DMSO-d_6): δ_H = 2.07

(3H, s, –NHCH$_3$), 2.45, 2.56 (6H, 2s, 2CH$_3$), 3.5 (1H, s, –NH, D$_2$O exchangeable), 5.79 (2H, s, –CH$_2$), 6.16 (1H, s, C$_3$-H), 7.27 (1H, d, C$_6$-H, J = 7.8 Hz), 8.35 (1H, d, C$_5$-H, J = 7.8 Hz), 10.27 (1H, s, –SH); ^{13}C NMR (150 MHz, DMSO-d$_6$): δ_C = 21.25, 24.71, 30.74, 36.93, 111.15, 117.84, 120.05, 135.28, 147.87, 150.08, 152.80, 161.40, 166.29, 176; MS (m/z): 316 M$^{+\cdot}$. (Found: C, 53.45; H, 4.86; N, 26.39 $C_{14}H_{16}N_6OS$ requires 53.15; H, 5.10; N, 26.56.)

4.2. Cytotoxicity

Measurement of Potential Cytotoxicity by SRB Assay. The selected 1,8-naphthyridine derivatives, compounds (**3a**, **3b**, **5**, **6**, **8**, **9a**, **9b**, **and 9c**), were subjected to a screening system for evaluation of their antitumor activity against liver HepG2 cancer cell line in comparison to the known anticancer drugs, doxorubicin (DOX). Potential cytotoxicity of the selected 1,8-naphthyridine derivatives was tested using the method of Skehan et al. [31] as follows: cells were plated in 96-multiwell plate (10^4 cells/well) for 24 h before treatment with compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (5, 12.5, 25, and 50 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. Cultures were fixed with trichloroacetic acid and stained for 30 min with 0.4% (wt/vol) sulforhodamine B (SRB) dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and protein-bound dye was extracted with 10 μM unbuffered Tris base [tris(hydroxymethyl) aminomethane] for determination of optical density in a computer-interfaced, 96-well microtiter plate reader. The SRB assay results were linear with the number of cells and with values for cellular protein measured by both the Lowry and Bradford assays at densities ranging from sparse subconfluence to multilayered supraconfluence. The signal-to-noise ratio at 564 nm was approximately 1.5 with 1,000 cells per well. The relation between surviving fraction and drug concentration is plotted to get the survival curve of both cancer cell lines after the specified compound.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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