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Microwave-assisted and conventional synthesis of novel antimicrobial 1,2,4-triazole derivatives containing nalidixic acid skeleton

DOI 10.1515/hc-2016-0019
Received March 25, 2016; accepted June 15, 2016; previously published online July 27, 2016

Abstract: Carbothioamides **4a**,**b**, obtained from nalidixic acid, were converted to the corresponding 1,3-thiazolidine derivatives **5a,b** by cyclocondensation with 2-bromo-1-(4-chlorophenyl)ethanone. Treatment of **4a,b** with base afforded 1,2,4-triazoles **6a,b**. The synthesis of 1,3-oxazolidine **7** was performed by the reaction of compound **4a** with ethyl bromoacetate. Treatment of **4a** with acid produced 1,3,4-thiadiazole **8**. The reaction of compounds **6a** and **6b** with several heterocyclic amines in the presence of formaldehyde gave the corresponding Mannich bases **9–15** containing various pharmacophore groups. Conventional and microwave-assisted methods were used for the synthesis. The effect of an acid catalyst on Mannich reactions was investigated. The structures of the newly synthesized compounds were elucidated on the basis of ^{1}H NMR, ^{13}C NMR, FTIR, EIMS techniques, and elemental analysis. All compounds were screened for their antimicrobial activity.

Keywords: 1,2,4-triazole; antimicrobial activity; Mannich base; nalidixic acid; norfloxacin.

Introduction

Nalidixic acid [1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid, **1**] was the first synthetic quinolone derivative introduced for the treatment of urinary tract infections in 1963 [1]. It inhibits DNA synthesis by promoting cleavage of bacterial DNA in the DNA-enzyme complexes of DNA gyrase, resulting in rapid bacterial death [2]. It is particularly effective against Gram-negative bacteria [3, 4]. Since the introduction of nalidixic acid for the treatment of bacterial infections, a number of derivatives have been synthesized. Among them, fluoroquinolones such as norfloxacin, pefloxacin, enoxacin, ofloxacin, and ciprofloxacin play a major role in the treatment of bacterial infections by displaying excellent pharmacokinetic properties, high antimicrobial activity, and low side effects [5–9]. Quinolones have been reported to have wide-ranging biological activities including antitubercular [10], fungicidal [11], antimalarial [12], anticancer and anti-HIV [12], antiviral [13], and antibacterial [14] properties.

The considerable biological importance of triazoles has stimulated a lot of interest in their derivatives [15]. A large number of 1,2,4-triazoles exhibit antibacterial, antifungal, antitubercular, analgesic, antiinflammatory, anticancer, anticonvulsant, antiviral, insecticidal, antidepressant, and central nervous system-affecting activities. 1,2,4-Triazoles, by virtue of their ambident nucleophilic centers, are good starting materials for the synthesis of several interesting *N*- and *S*-bridged heterocycles. The triazoles can be converted to thiazolotriazoles, triazolothiadiazoles, triazolooxazolidines, and various Mannich bases [16–20]. In addition, a 1,3,4-thiadiazole system constitutes the active part of several biologically active compounds [21–24]. A thiazolidinone ring is an attractive target for combinatorial synthesis as its structure-activity relationship belongs to an important class of *N*- and *S*-containing heterocycles, which are widely used in drug design and synthesis [25, 26]. Related oxazolidinones are also biologically active [27–30].

In this paper, we report the synthesis of new hybrid compounds which are derivatives of the systems mentioned above and possess an interesting profile of antimicrobial activity. The synthetic work was enhanced by

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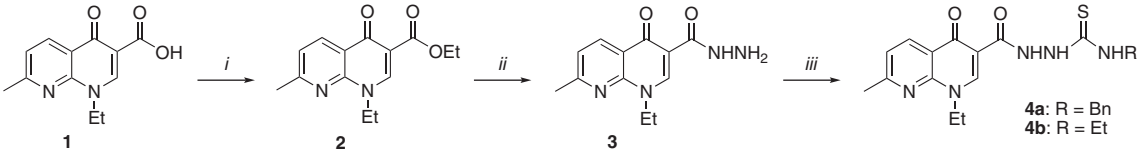
using Mannich reaction [31], homogeneous catalysis [32], and eco-friendly microwave irradiation [33].

Results and discussion

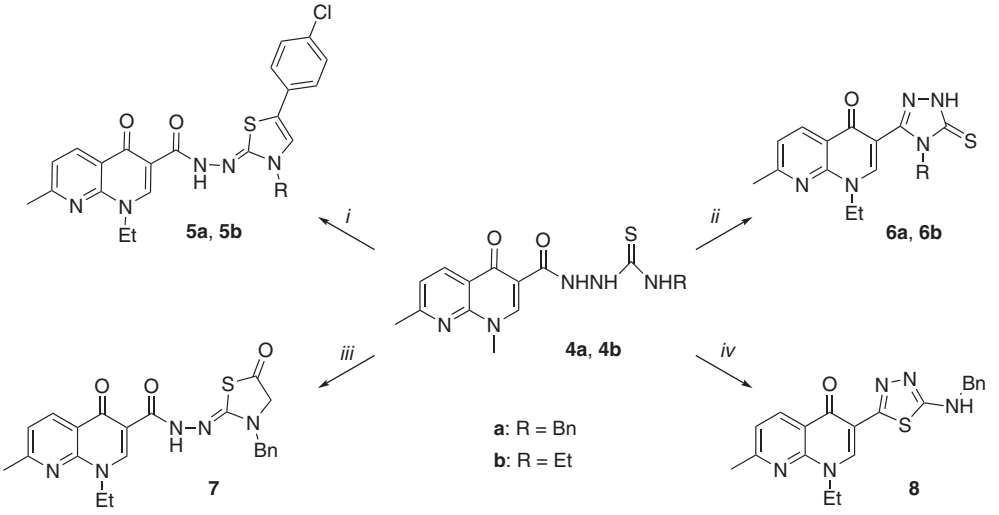
Chemistry

The main aim of this study was to synthesize antimicrobial hybrid molecules containing different heterocyclic moieties. The synthetic strategies adopted to obtain the target compounds are depicted in Schemes 1–3. The synthesis was achieved by using both microwave (eco-friendly) and conventional methods. The structures of all products were established on the basis of physicochemical, spectral, and elemental analysis data.

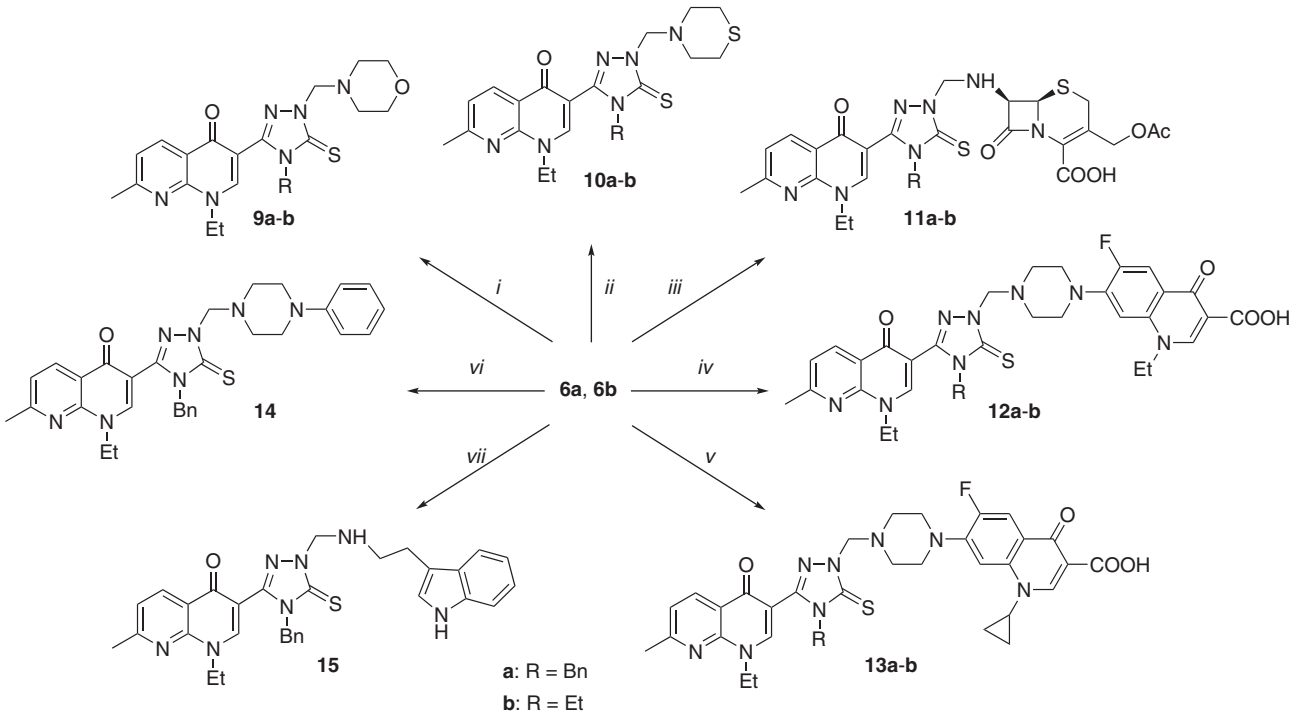
The esterification of nalidixic acid (**1**) with ethanol in the presence of a catalytic amount of sulfuric acid furnished ester **2**. Then, compound **2** was converted to the acetohydrazide **3** by treatment with hydrazine hydrate. Synthesis of **3** required 20–22 h of heating under reflux conditions and the yield was 75–78%. Under microwave irradiation at 100°C for 15 min, the yield was 88–98%. Compound **3** was converted to carbothioamides **4a,b** by treatment with benzylisothiocyanate and ethylisothiocyanate, respectively (Scheme 1). Again, the use of microwave irradiation was superior to the conventional synthesis. Condensation of compounds **4a,b** with 4-chlorophenacyl bromide in ethanolic solution furnished the corresponding thiazoles **5a,b** (Scheme 2). Again, the microwave method was preferred. The remaining preparations shown in Schemes 2 and 3 also benefited from the use of the microwave irradiation methodology. Triazoles **6a,b** were obtained by intramolecular cyclization of compounds **4a,b**. The aim was to introduce the 1,2,4-triazole nucleus to the nalidixic acid skeleton because it is known that more efficacious antibacterial compounds can be designed by joining two biologically active components together into a single molecular framework [34–38]. The synthesis of carbohydrazide **7** was performed by the reaction of compound **4a** with ethyl bromoacetate in ethanolic



Scheme 1 Reactions and conditions. *i*: H_2SO_4, EtOH, 22 h reflux or MW irradiation; *ii*: EtOH, H_2NNH_2, 6 h reflux or MW irradiation; *iii*: CH_2Cl_2, $PhCH_2NCS$ or EtNCS, 24 h room temprature or MW irradiation.



Scheme 2 Reactions and conditions. *i*: EtOH, $ClC_6H_4COCH_2Br$, CH_3COONa, reflux for 54 h (**5a**) or EtOH, C_2H_5Br, CH_3COONa, reflux for 45 h (**5b**) or MW irradiation; *ii*: 2 N NaOH in EtOH/H_2O, 16 h (**6a**) or 14 h (**6b**) reflux or MW irradiation; *iii*: $BrCH_2COOEt$ in EtOH, CH_3COONa, 24 h reflux or MW irradiation; *iv*: H_2SO_4, 3 h at room temperature or MW irradiation.



Scheme 3 Conditions: amine, CH_2O in DMSO (see Experimental). The amines: *i*, morpholine; *ii*, thiomorpholine; *iii*, 7-aminocephalosporanic acid; *iv*, norfloxacin; *v*, ciprofloxacin; *vi*, 1-phenylpiperazine; *vii*, tryptamine.

solution, while the cyclization of **4a** in an acidic medium yielded a 1,3,4-thiadiazole **8**.

Mannich bases are physiologically reactive agents [39–41]. Mannich bases **9–15** were obtained by treatment of compounds **6a,b** with formaldehyde and several biologically active amines (Scheme 3) [6, 8, 34, 42, 43]. Three different methods were used for the Mannich reactions. These were a conventional method in the absence of a catalyst, a conventional method in the presence of a catalyst, and microwave irradiation-assisted synthesis. The classical heating in the absence of any catalyst furnished the desired products in low-yield after 24 h. The same products were obtained in the yields of 53–97% at room temperature after 5 h in the presence of $InCl_3$ or *p*-toluenesulfonic acid as a Brønsted-Lowry acid catalyst. In the microwave irradiation-assisted method the temperature was held constant at 50°C, the reaction time was 5 min and the Mannich bases were obtained in yields of 43–99%. Thus, microwave irradiation provided more efficient and green way to Mannich products and a relatively higher yield.

Antimicrobial activity

All synthesized compounds were screened for their antimicrobial activity. Compounds **2, 3, 4a,b, 5a,b, 6a,b, 7,** and **8** are active against some of the test microorganisms with the minimal inhibition concentration (MIC) values in the range of 0.49–500 μg/mL (Table 1). Compounds **4a,b** and **6a,b** are exceptionally active against *Escherichia coli* (*E. coli*), Gram-negative bacteria. Mannich bases **9–15** show good-to-moderate activity against some of the test microorganisms. Among these, compounds **11a,b** with a β-lactam nucleus attached to nalidixic acid skeleton display remarkable antimicrobial activities against two Gram-positive bacteria. Compound **11a** is active against *Mycobacterium smegmatis* (*M. smegmatis*), a nonpigmented rapidly growing mycobacterium with the MIC value <1 μg/mL, which is better than that for standard drug streptomycin. Compound **11b** is active against *Enterococcus faecalis* (*E. faecalis*), for which the MIC value of 6 μg/mL is better than that for the reference drug ampicillin. Exceptional activity can be observed for some other Mannich bases (Table 1).

Conclusions

Several biologically active hybrid compounds containing a nalidixic acid moiety were obtained. The antimicrobial screening showed that compounds containing norfloxacine, ciprofloxacine or 7-aminocephalosporanic acid system display excellent antimicrobial activity. Several

Table 1 Antimicrobial activity of the compounds.

Comp.	Minimal inhibition concentration (μg/mL)								
	Ec	Yp	Pa	Sa	Ef	Bc	Ms	Ca	Sc
2	3.9	15.6	–	–	–	125	–	250	500
3	1.9	3.9	125	62.5	125	31.3	–	62.5	62.5
4a	0.49	7.81	500	31.25	–	7.81	62.5	15.65	15.65
4b	0.49	7.81	–	125	125	15.65	62.5	31.25	15.65
5a	7.8	3.9	–	–	–	78	–	–	–
5b	7.8	31.25	–	–	–	39	–	–	–
6a	0.98	7.81	–	125	–	31.25	–	250	–
6b	0.98	3.91	125	62.5	–	31.25	62.5	–	–
7	7.8	31.25	–	–	156	39	–	–	–
8	7.8	3.9	–	–	312.5	>1000	125	78	39
9a	7.8	31.25	–	–	–	39	–	–	–
9b	3.9	62.5	156	–	–	>1000	62.5	–	–
10a	15.6	3.9	–	–	–	625	–	–	156
10b	3.9	62.5	156	–	312.5	>1000	–	–	–
11a	125	7.8	–	–	156	>1000	<1	–	312.5
11b	15.6	31.25	156	–	6	39	–	–	–
12a	<1	3.9	9.7	78	9.7	19	1.9	–	156
12b	<1	3.9	9.7	–	9.7	19	1.9	–	–
13a	<1	<1	9.7	9.7	–	9.7	<1	–	156
13b	<1	<1	9.7	–	9.7	9.7	<1	–	–
14	7.8	3.9	–	–	–	156	–	–	–
15	7.8	62.5	–	–	–	39	–	–	312.5
Amp	10	32	>128	35	10	15			
Strep							4		
Flu								<8	<8

Ec, *E. coli* ATCC 35218; Yp, *Y. pseudotuberculosis* ATCC 911; Pa, *P. aeruginosa* ATCC 10145; Sa, *S. aureus* ATCC 25923; Ef, *E. faecalis* ATCC 29212; Bc, *B. cereus* 709 Roma; Ms, *M. smegmatis* ATCC607; Ca, *C. albicans* ATCC 60193; Sc, *S. cerevisiae* RSKK 251; Amp, ampicillin; Strep, streptomycin; Flu, fluconazole; (–), no activity at test concentrations.

compounds are more active against *E. coli* and *M. smegmatis* than the reference drug ampicillin and streptomycin.

Experimental

All chemicals were purchased from Fluka Chemie AG (Buchs, Switzerland) and used without further purification. Melting points were determined in open capillaries on a Büchi B-540 melting point apparatus and are uncorrected. Reactions were monitored by thin layer chromatography (TLC) on silica gel using 60 F_{254} aluminum sheets. The mobile phase was ethyl acetate/diethyl ether (1:1) and detection was under UV light. FT-IR spectra were recorded using a Perkin Elmer 1600 series FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were registered in DMSO-d_6 on a Bruker Avene II 400 MHz NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer.

Ethyl 1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylate (2)

Method 1 A solution of nalidixic acid (**1**, 2.31 g, 0.01 mol) and concentrated sulfuric acid (4 drops) in ethanol was heated under reflux for 22 h, then cooled to room temperature and concentrated under reduced pressure. The resultant white residue of **2** was crystallized from ethanol.

Method 2 A solution of compound **1** (1 mmol) and sulfuric acid (4 drops) in ethanol was microwave irradiated in a closed vessel at 100°C for 15 min and then poured into ice water. The resultant solid product **2** was collected by filtration and crystallized from ethanol.

Yield 76% (method 1); yield 88% (method 2); mp 207–208°C; IR (υ_{max}, cm^{-1}): 3072, 2980, 1720, 1683, 1616; ^{1}H NMR: δ 1.3 (t, 3H J = 7.2 Hz), 1.4 (t, 3H, J = 6.9 Hz), 2.6 (s, 3H), 4.2 (q, 2H, J = 6.9 Hz,), 4.5 (q, 2H, J = 7.2 Hz), 7.4 (d, 1H, J = 8.1 Hz,), 8.4 (d, 1H, J = 8.1 Hz), 8.8 (s, 1H); ^{13}C NMR: δ 14.3, 23.6, 27.9, 41.1, 44.5, 110.8, 118.2, 125.6, 136.2, 148.9, 154.6, 148.1, 162.2, 176.3. Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.25; H, 5.93; N, 10.41.

1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (3)

Method 1 A solution of compound **2** (2.60 g, 0.01 mol) and hydrazine hydrate (1.46 mL, 0.03 mol) in ethanol was heated under reflux for 20 h, then cooled and kept in the refrigerator for 12 h. The resultant solid was collected by filtration and crystallized from ethanol.

Method 2 A solution of compound **2** (1 mmol) and hydrazine hydrate (3 mmol) in ethanol was microwave-irradiated in a closed vessel at 100°C for 15 min. After the completion of the reaction, as monitored by TLC, the mixture was concentrated and the residue was crystallized from ethanol.

Yield 75% (method 1); yield 98% (method 2); mp 163–165°C; IR (υ_{max}, cm^{-1}): 3316, 3222, 3033, 2958, 1685, 1604; ^{1}H NMR: δ 1.32 (s, 3H), 2.60 (s, 3H), 4.51 (s, 2H), 7.40 (s, 1H), 8.42 (s, 1H), 8.94 (s, 1H), 10.50 (s, 2H), 12.50 (s, 1H); ^{13}C NMR: δ 14.3, 24.8, 40.1, 111.6, 119.4, 122.5, 135.8, 147.9, 153.6, 147.2, 163.1, 175.8. Anal. Calcd for $C_{12}H_{14}N_4O_2$: C, 58.53; H, 5.73; N, 22.75. Found: C, 58.40; H, 5.93; N, 22.48.

General synthesis of compounds 4a,b

Method 1 To a solution of compound **3** (2.46 g, 0.01 mol) in dichloromethane, benzyl isothiocyanate (for **4a**, 2.46 g, 0.01 mol) or ethyl isothiocyanate (for **4b**, 1.75 g, 0.02 mol), was added and the mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure. The residue was crystallized from ethanol (**4a**) or from dimethyl sulfoxide/water (1:3) (**4b**).

Method 2 A mixture of compound **3** (1 mmol) and the corresponding isothiocyanate (1 mmol) in ethanol was irradiated in a closed vessel with the pressure control at 100°C for 10 min. After cooling the mixture was poured into ice water and the resultant solid was crystallized from an appropriate solvent.

***N*-Benzyl-2-[(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)carbonyl]hydrazinecarbothioamide (4a)** Yield 80% (method 1); yield 98% (method 2); mp 197–200°C; IR (υ_{max}, cm^{-1}): 3137, 3062, 2974, 1649, 1541, 1187; ^{1}H NMR: δ 1.40 (t, 3H, *J* = 8.0 Hz), 2.71 (brs, 3H), 4.73 (d, 2H, *J* = 4.0 Hz), 4.94 (s, 2H), 7.29 (d, 1H, *J* = 8.0 Hz), 7.31–7.38 (m, 5H), 7.42 (d, 1H, *J* = 8.0 Hz), 8.58 (q, 1H, *J* = 8.0 Hz), 9.04 (s, 1H), 9.18 (s, 1H), 09.47 (s, 1H); ^{13}C NMR: δ 15.5, 25.5, 46.6, 48.5, 119.1, 122.6, 123.4, 127.6, 127.7, 128.7, 129.3, 136.5, 148.6, 163.9, 165.1, 166.1, 150.0, 175.9, 178.5, 183.1. Anal. Calcd for $C_{20}H_{21}N_5O_2S$: C, 60.74; H, 5.35; N, 17.71. Found: C, 60.40; H, 5.83; N, 17.48.

***N*-Ethyl-2-[(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)carbonyl]hydrazinecarbothioamide (4b)** Yield 75% (method 1); yield 85% (method 2); mp 180–182°C; IR (υ_{max}, cm^{-1}): 3144, 3058, 2986, 1679, 1541, 1189; ^{1}H NMR: δ 1.07 (t, 3H, *J* = 8.0 Hz), 1.39 (t, 3H, *J* = 8.0 Hz), 2.69 (brs, 3H), 3.41 (brs, 2H), 4.57 (q, 2H, *J* = 8.0 Hz), 7.44–7.47 (m, 4H), 7.56 (d, 1H, *J* = 8.0 Hz), 7.92 (brs, 2H), 8.53 (q, 1H, *J* = 8.0 Hz), 9.00 (s, 1H), 9.15 (s, 1H), 10.54 (s, 1H); ^{13}C NMR: δ 14.8, 15.5, 25.5, 46.4, 47.2, 121.8, 123.0, 136.3, 148.5, 163.9, 165.1, 149.9, 175.8, 178.5, 182.1. Anal. Calcd for $C_{15}H_{19}N_5O_2S$: C, 54.04; H, 5.74; N, 21.01. Found: C, 54.40; H, 5.93; N, 21.10.

General synthesis of compounds 5a,b

Method 1 4-Chlorophenacyl bromide (2.33 g, 0.01 mol) and sodium acetate (4.1 g, 0.05 mol) were added to the solution of compound **4a** (3.95 g, 0.01 mol, for **5a**) or compound **4b** (3.33 g, 0.01 mol, for **5b**) in ethanol and the mixture was heated under reflux for 54 h (for **5a**) or 45 h (for **5b**), as monitored by TLC. Then, the mixture was cooled to room temperature and concentrated under reduced pressure. The resultant solid was filtered, washed with water and crystallized from dimethyl sulfoxide/water (1:3).

Method 2 A solution of 4-chlorophenacyl bromide (1 mmol), sodium acetate (5 mmol), compound **4a** (1 mmol) or compound **4b** (1 mmol) in ethanol was microwave-irradiated in a closed vessel at 150°C for 15 min. After completion of the reaction, as monitored by TLC, the mixture was poured into ice water. The crude product was filtered and crystallized from dimethyl sulfoxide/water (1:3).

***N*′-[3-Benzyl-5-(4-chlorophenyl)-1,3-thiazol-2(3*H*)-ylidene]-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (5a)** Yield 65% (method 1); yield 80% (method 2); mp 175–177°C; IR (υ_{max}, cm^{-1}): 3043, 3079, 2980, 1716, 1521, 1129; ^{1}H NMR: δ 1.41 (brs, 3H), 2.67 (t, 3H, *J* = 8.0 Hz), 4.24 (brs, 2H, 4.41 (q, 2H, *J* = 8.0 Hz), 5.03 (s, 1H), 7.05 (d, 1H, *J* = 8.0 Hz), 7.17–7.63 (m, 9H), 7.62 (d, 1H, *J* = 8.0 Hz), 7.98 (brs, 1H), 9.17 (s, 1H); ^{13}C NMR: δ 14.7, 25.5, 43.6, 47.3, 110.0, 117.2, 123.1, 127.8, 128.3, 129.5, 130.2, 130.3, 135.4, 136.1, 136.3, 140.3, 147.6, 148.1, 160.0, 166.2, 149.8, 166.8, 168.8, 176.1, 178.6. Anal. Calcd for $C_{28}H_{24}ClN_5O_2S$: C, 65.45; H, 4.56; N, 13.21. Found: C, 65.31; H, 4.78; N, 13.01.

***N*′-[5-(4-Chlorophenyl)-3-ethyl-1,3-thiazol-2(3*H*)-ylidene]-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (5b)** Yield 70% (method 1); yield 87% (method 2); mp 192–193°C; IR (υ_{max}, cm^{-1}): 3068, 3068, 2971, 1702, 1519; ^{1}H NMR: δ 1.05 (t, 3H, *J* = 8.0 Hz), 1.41 (t, 3H *J* = 4.0 Hz), 2.70 (brs, 3H), 3.67 (brs), 4.60 (q, 2H, *J* = 8.0 Hz), 5.03 (s, 1H), 7.48–7.58 (m, 6H), 7.58 (brs, 1H), 9.18 (s, 1H); ^{13}C NMR: δ 13.3, 15.5, 25.5, 46.6, 47.3, 98.9, 109.1, 119.5, 120.0, 121.0, 123.1, 129.4, 129.5, 130.1, 131.0, 136.1, 148.8, 159.9, 150.1, 163.8, 166.0, 171.6, 178.6. Anal. Calcd for $C_{23}H_{22}ClN_5O_2S$: C, 59.03; H, 4.74; N, 14.97. Found: C, 59.33; H, 4.38; N, 14.69.

General synthesis of compounds 6a,b

Method 1 A solution of carbothioamide **4a** (3.95 g, 0.01 mol, for **6a**) or **4b** (3.33 g, 0.01 mol, for **6b**) in ethanol/water (1:1) was heated under reflux in the presence of 2 N NaOH for 16 h (for **6a**) or 14 h (for **6b**) with the progress of the reaction monitored by TLC. Then, the solution was cooled to room temperature and acidified to pH 7 with 37% HCl. The precipitate formed was filtered off, washed with water, and crystallized from methanol (for **6a**) or ethanol /water (1:3) (for **6b**).

Method 2 A mixture of compound **4a** or **4b** (1 mmol) and 2N NaOH (2 mmol) in ethanol (10 mL) was microwave-irradiated in a closed vessel at 150°C for 4 min with the progress of the reaction monitored by TLC. Then the resulting solution was cooled to room temperature and acidified to pH 5 with 37% HCl. The precipitate formed was filtered off, washed with water, and crystallized from an appropriate solvent indicated above.

3-(4-Benzyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (6a) Yield 63% (method 1); yield 86% (method 2); mp >270°C; IR (υ_{max}, cm^{-1}): 3061, 2985, 2959, 1717, 1517, 1128; ^{1}H NMR: δ 1.17 (s, 3H), 2.50 (s, 3H), 4.33 (brs, 2H), 5.25 (s, 2H), 7.13–7.33 (m, 7H), 8.34 (s, 1H), 9.16 (s, 1H); ^{13}C NMR: δ 15.4, 25.5, 45.6, 47.3, 109.2, 118.8, 121.6, 123.1, 127.2, 127.9, 128.8, 136.1, 136.9, 146.5, 148.8, 163.7, 150.0, 166.1, 174.7, 178.5. Anal. Calcd for $C_{20}H_{19}N_5OS$: C, 63.64; H, 5.07; N, 18.55. Found: C, 63.28; H, 5.38; N, 18.89.

1-Ethyl-3-(4-ethyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl)-7-methyl-1,8-naphthyridin-4(1*H*)-one (6b) Yield 67% (method 1); yield 88% (method 2); mp 216-218°C; IR (υ_{max}, cm^{-1}): 3090, 2988, 2957, 1717 2, 1537, 1130; ^1H NMR: δ 1.14 (t, 3H, *J* = 8.0 Hz), 1.41 (t, 3H, *J* = 8.0 Hz), 2.66 (s, 3H), 3.93 (q, 2H, *J* = 8.0 Hz), 4.49 (q, 2H, *J* = 8.0 Hz), 7.44–7.61 (m, 2H), 8.61 (d, 1H, *J* = 8.0 Hz), 9.19 (s, 1H); ^{13}C NMR: δ 13.8, 15.5, 25.5, 45.9, 47.3, 108.8, 119.5, 121.9, 136.2, 149.1, 163.6, 148.5, 167.1, 174.9, 178.6. Anal. Calcd for $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; N, 22.21. Found: C, 57.28; H, 5.87; N, 22.60.

N'-[3-Benzyl-5-thioxo-1,3-oxazolidin-2-ylidene]-1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carbohydrazide (7)

Method 1 Ethyl bromoacetate (1.28 mL, 0.01 mol) and sodium acetate (4.1 g, 0.05 mol) were added to the solution of compound **4a** (3.95 g, 0.01 mol) in ethanol and the mixture was heated under reflux for 24 h. Then, the mixture was cooled to room temperature and concentrated under reduced pressure. The residue was filtered, washed with water, and crystallized from dimethyl sulfoxide/water (1:3).

Method 2 A mixture of compound **4a** (1 mmol), ethyl bromoacetate (1 mmol) and sodium acetate (5 mmol) in ethanol was irradiated in a closed vessel at 150°C for 13 min with the progress of the reaction monitored by TLC. After the completion of the reaction, the mixture was poured into ice water and the resultant precipitate was filtered and crystallized from dimethyl sulfoxide/water (1:3).

Yield 55% (method 1); yield 76% (method 2); mp 230–232°C; IR (υ_{max}, cm^{-1}): 3031, 1698 2, 1495, 1127; ^1H NMR: δ 1.42 (s, 3H), 4.23 (s, 2H), 4.63 (t, 3H, *J* = 8.0 Hz), 4.79 (s, 2H), 7.26-742 (m, 7H), 8.59 (t, 1H, *J* = 8.0 Hz), 9.03 (s, 1H); ^{13}C NMR: δ 15.5, 25.4, 40.4, 44.1, 45.9, 109.1, 118.8, 123.1, 127.0, 127.3, 127.6, 127.7, 128.0, 136.1, 136.3, 148.8, 160.1, 150.1, 165.1, 166.1, 172.2, 178.6. Anal. Calcd for $C_{22}H_{21}N_5O_3S$: C, 60.67; H, 4.86; N, 16.08. Found: C, 60.32; H, 4.49 H; N, 16.28.

3-[5-(Benzylamino)-4,5-dihydro-1,3,4-thiadiazol-2-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (8)

Method 1 A solution of carbothioamide **4a** (3.95 g, 0.01 mol) in concentrated sulfuric acid (11 mL, 0.20 mol) was stirred for 15 min at 0°C, then allowed to reach room temperature and stirred for an additional 3 h. The solution was poured into ice-cold water and made alkaline to pH 7 with ammonia. The precipitated product was filtered, washed with water, and crystallized from ethanol/water (1:3).

Method 2 A solution of compound **4a** (1 mmol) in concentrated sulfuric acid (1.5 mL) was irradiated in a closed vessel at 50°C for 4 min with the progress of the reaction monitored by TLC. After completion of the reaction, the mixture was poured into ice water and the resultant crude product was collected by filtration and crystallized from ethanol/water (1:3).

Yield 74% (method 1); yield 83% (method 2); mp 193–195°C; IR (υ_{max}, cm^{-1}): 3197, 1715, 1518, 1080; ^1H NMR: δ 1.41 (s, 3H), 2.67 (t, 3H, *J* = 8.0 Hz), 3.45 (brs, 2H), 4.63 (brs, 2H), 7.16–7.56 (m, 7H), 8.55 (brs, 1H), 9.16 (s, 1H); ^{13}C NMR: δ 15.5, 25.5, 46.6, 47.9, 109.1, 119.9, 123.1, 127.3, 127.7, 128.6, 129.3, 136.0, 142.6, 148.9, 163.0, 165.1, 149.9, 166.0, 172.8, 178.5. Anal. Calcd for $C_{20}H_{19}N_5O_2$: C, 66.47; H, 5.30; N, 19.38. Found: C, 66.32; H, 4.89; N, 16.08.

General synthesis of compounds 9–13 (a,b)

Method 1 To a solution of compound **6a** or **6b** (0,01 mol) in dimethyl sulfoxide (10 mL), morpholine (for **9a** and **9b**, 0.87 mL, 0.01 mol) or thiomorpholine (for **10a** and **10b**, 0.94 mL, 0.01 mol) or 7-aminocephalosporanic acid (for **11a** and **11b**, 2.72 g, 0.01 mol) or norfloxacin (for **12a** and **12b**, 3.19 g, 0.01 mol) or ciprofloxacin (for **13a** and **13b**, 3.31 g, 0.01 mol) was added in the presence of formaldehyde (37%, 3.72 mL, 0.05 mol) and the mixture was stirred at room temperature for 22 h. The solution was poured into ice-cold water and the resultant solid was crystallized from ethanol/water (1:3) (for **9a,b, 10a**,**b**), from ethyl acetate (for **11a,b**) and from dimethyl sulfoxide/water (1:3) (for **12a,b, 13a**,**b**).

Method 2 To a solution of compound **6a**,**b** (1 mmol) in dimethyl sulfoxide (10 mL), morpholine (for **9a** and **9b**, 1 mmol) or thiomorpholine (for **10a** and **10b**, 1 mmol) or 7-aminocephalosporanic acid (for **11a** and **11b**, 1 mmol) or norfloxacin (for **12a** and **12b**, 1 mmol) or ciprofloxacin (for **13a** and **13b**, 1 mmol) was added in the presence of formaldehyde (5 mmol) and the mixture was stirred at room temperature for 15 min. Then, a catalytic amount of a suitable catalyst ($InCl_3$ or *p*-TsOH) was added and the stirring was continued for an additional 5 h. The mixture was poured into ice water and a resultant solid was crystallized from an appropriate solvent indicated above.

Method 3 The mixture, obtained as described above for method 2, was irradiated at 50°C in a microwave oven for 5 min with the progress of the reaction monitored by TLC. After cooling the solution was poured into ice water and the resultant precipitate was filtered off and crystallized from an appropriate solvent indicated above.

3-[4-Benzyl-1-(morpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (9a) Yield 73% (method 1); yield 75% (method 2, $InCl_3$); yield 77% (method 2, *p*-TsOH); yield 90% (method 3); mp 106–108°C; IR (υ_{max}, cm^{-1}): 1629, 1498, 1152, 1078; ^1H NMR: δ 1.19 (t, 3H, *J* = 4.0 Hz), 2.56 (s, 4H), 2.73 (s, 3H), 3.36 (s, 4H), 4.37 (brs, 2H), 5.14 (s, 2H), 5.31 (s, 2H), 6.97 (brs, 2H), 7.15–7.30 (m, 5H), 7.46 (d, 2H *J* = 8.0 Hz), 8.52 (s, 1H); ^{13}C NMR: δ 15.4, 25.3, 45.7, 48.6, 50.8, 66.6, 69.6, 108.6, 119.3, 121.8, 127.5, 127.8, 128.0, 128.8, 136.2, 136.3, 147.6, 148.8, 146.5, 163.7, 169.1, 174.7. Anal. Calcd for $C_{25}H_{28}N_6O_2S$: C, 63.00; H, 5.92; N, 17.63. Found: C, 63.28; H, 6.01; N, 17.32.

1-Ethyl-3-[4-ethyl-1-(morpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-7-methyl-1,8-naphthyridin-4(1*H*)-one (9b) Yield 65% (methods 1 and 2); yield 66% (method 2, *p*-TsOH); yield 85% (method 3); mp 209–210°C; IR (υ_{max}, cm^{-1}): 1710, 1519, 1162, 1081; ^1H NMR: δ 1.17 (s, 3H), 1.40 (s, 3H), 2.68 (s, 3H), 3.35 (s, 4H), 3.57 (s, 4H), 3.95 (brs, 2H), 4.49 (s, 2H), 5.06 (s, 2H), 7.46 (d, 2H, *J* = 8.0 Hz), 8.46 (brs, 1H), 8.65 (s, 1H); ^{13}C NMR: δ 13.6, 15.4, 25.4, 46.0, 50.8, 52.0, 66.5, 69.1, 108.2, 119.4, 121.6, 136.2, 147.2, 149.1, 146.8, 163.6, 168.1, 178.5. Anal. Calcd for $C_{20}H_{26}N_6O_2S$: C, 57.95; H, 6.32; N, 20.27. Found: C, 57.71; H, 6.01; N, 20.01.

3-[4-Benzyl-1-(thiomorpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (10a) Yield 40% (method 1); yield 53% (method 2, $InCl_3$ or *p*-TsOH); yield 47% (method 3); mp 100–102°C; IR (υ_{max}, cm^{-1}): 3069, 2957, 1627, 1496, 1131; ^1H NMR: δ 1.41 (t, 3H *J* = 8.0 Hz), 2.61 (s, 4H), 2.71 (s, 3H), 3.36 (s, 4H), 4.29 (q, 2H, *J* = 8.0 Hz), 4.64 (brs, 2H),

5.27 (s, 2H), 6.45 (brs, 1H), 7.00–7.95 (m, 7H), 8.51 (s, 1H); ^{13}C NMR: δ 15.3, 25.3, 40.7, 45.7, 48.3, 52.3, 70.0, 108.7, 111.3, 117.8, 121.8, 127.2, 127.5, 127.7, 128.8, 132.9, 136.2, 136.2, 147.6, 146.5, 148.8, 163.7, 174.7. Anal. Calcd for $C_{25}H_{28}N_6OS_2$: C, 60.95; H, 5.73; N, 17.06. Found: C, 60.62; H, 5.91; N, 17.38.

1-Ethyl-3-[4-ethyl-1-(thiomorpholin-4-ylmethyl)-5-thioxo-4,5-dihydro-1*H*-1,2,4 -triazol-3-yl]-7-methyl-1,8-naphthyridin-4(1*H*)-one (10b) Yield 35% (method 1); yield 55% (method 2, $InCl_3$); yield 56% (method 2, *p*-TsOH); yield 43% (method 3); mp 192–193°C; IR (υ_{max}, cm^{-1}): 3087, 2980, 1710, 1518, 1128; ^{1}H NMR: δ 1.17 (s, 3H), 1.40 (s, 3H), 2.67 (s, 3H), 2.99 (s, 4H), 3.34 (s, 4H), 3.95 (s, 2H), 4.49 (s, 2H), 5.08 (s, 2H), 7.45 (d, 2H, *J* = 8.0 Hz), 8.48 (brs, 1H), 8.63 (s, 1H); ^{13}C NMR: δ 13.6, 15.5, 25.3, 46.0, 50.6, 52.8, 66.6, 70.4, 108.2, 119.4, 121.6, 136.2, 147.2, 149.1, 146.8, 163.6, 167.9, 178.5. Anal. Calcd for $C_{20}H_{26}N_6OS_2$: C, 55.79; H, 6.09; N, 19.52. Found: C, 55.61; H, 6.33; N, 19.28.

3-[(Acetoxy)methyl]-7-({[4-benzyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (11a) Yield 55% (method 1); yield 64% (method 2, $InCl_3$); yield 60% (method 2, *p*-TsOH); yield 74% (method 3); mp 148–150°C; IR (υ_{max}, cm^{-1}): 3305, 3057, 1771, 1715 4, 1556, 1130; ^{1}H NMR: δ 1.41 (t, 3H, *J* = 8.0 Hz), 1.91 (s, 3H), 2.73 (brs, 3H), 3.43 (q, 2H, *J* = 4.0 Hz), 3.96 (s, 1H), 4.34 (brs, 2H), 5.02 (d, 2H, *J* = 8.0 Hz), 5.29 (brs, 2H), 5.45 (s, 2H), 5.54 (s, 1H), 7.04-7.95 (m, 7H), 8.58 (s, 1H), 9.17 (s, 1H), 14.04 (s, 1H); ^{13}C NMR: δ 14.7, 22.01, 26.3, 27.0, 45.7, 47.3, 59.2, 63.2, 68.1, 71.3, 108.9, 109.1, 119.4, 123.0, 125.0, 127.3, 127.9, 128.8, 136.0, 136.2, 146.4, 148.8, 148.8, 120.5, 150.0, 163.5, 163.7, 167.3, 168.1, 174.7, 178.5. Anal. Calcd for $C_{31}H_{31}N_7O_6S_2$: C, 62.26; H, 4.72; N, 14.82. Found: C, 62.01; H, 4.45; N, 14.51.

3-[(Acetoxy)methyl]-7-({[4-ethyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}amino)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid (11b) Yield 58% (method 1); yield 69% (method 2, $InCl_3$); yield 70% (method 2, *p*-TsOH); yield 81% (method 3); mp 140–142°C; IR (υ_{max}, cm^{-1}): 3280, 3047, 1768, 1710 4, 1562, 1130; ^{1}H NMR: δ 1.15 (t, 3H, *J* = 8.0 Hz), 1.41 (t, 3H, *J* = 8.0 Hz), 1.91 (s, 3H), 2.71 (brs, 3H), 3.32 (brs, 2H), 3.92 (s, 1H), 4.47 (brs, 2H), 4.63 (brs, 2H), 5.01 (d, 2H, *J* = 8.0 Hz), 5.53 (s, 1H), 7.44 (d, 1H, *J* = 8.0 Hz), 7.59 (d, 1H, *J* = 8.0 Hz), 8.60 (s, 1H), 9.16 (s, 1H), 13.82 (s, 1H); ^{13}C NMR: δ 13.8, 15.4, 21.5, 25.5, 27.1, 45.9, 47.3, 58.2, 64.3, 69.1), 72.0, 108.8, 109.8, 118.8, 123.1, 136.2, 148.5, 149.1, 119.4, 150.0, 163.5, 165.1, 166.0, 167.1, 174.9, 178.6. Anal. Calcd for $C_{26}H_{29}N_7O_6S_2$: C, 52.07; H, 4.87; N, 16.35. Found: C, 52.31; H, 4.45; N, 16.51.

6-(4-{[4-Benzyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-1-ethyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (12a) Yield 82% (method 1); yield 92% (method 2, $InCl_3$); yield 91% (method 2, *p*-TsOH); yield 98% (method 3); mp 200–202°C; IR (υ_{max}, cm^{-1}): 3451, 1720, 1562, 1130; ^{1}H NMR: δ 1.39 (t, 3H, *J* = 8.0 Hz), 1.96 (s, 3H), 2.64 (brs, 3H), 2.98 (s, 4H), 3.39 (brs, 4H), 4.34 (brs, 2H), 4.60 (brs, 2H), 5.25 (d, 4H, *J* = 8.0 Hz), 6.53 (s, 1H), 7.15–7.57 (m, 7H), 7.83 (brs, 1H), 8.47 (s, 1H), 8.92 (s, 1H), 14.89 (s, 1H); ^{13}C NMR: δ 14.8 (CH_3), 15.3 (CH_3), 25.5 (CH_3), 44.8 (N-2CH_2), 46.4 (CH_2), 47.2 (N-2CH_2), 49.5, 49.9, 50.2, 106.0, 110.4, 112.3, 118.7, 119.6 and 119.7 (d, CH, *J* = 7.0 Hz), 121.8, 123.0, 126.1, 127.2 and 127.6 (d, CH, *J* = 47.0 Hz), 128.6 and 128.8 (d, CH, *J* = 17.0 Hz), 135.0 (CH), 135.9 and 136.2 (d, C, *J* = 26.0 Hz), 142.3 (C), 145.9 (CH), 146.5 and 147.7 (d, C, *J* = 122.0 Hz), 151.9 and 153.6 (d, C, *J* = 161.0 Hz), 165.1, 107.5, 108.4, 149.1, 149.9, 166.6, 169.1, 174.7, 176.5, 178.5. Anal. Calcd for $C_{37}H_{37}FN_8O_4S$: C, 62.70; H, 5.26; N, 15.81. Found: C, 62.31; H, 5.06; N, 16.03.

1-Ethyl-6-(4-{[4-ethyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (12b) Yield 85% (method 1); yield 98% (method 2, $InCl_3$); yield 92% (method 2, *p*-TsOH); yield 96% (method 3); mp 220–222°C; IR (υ_{max}, cm^{-1}): 3372, 1710, 1517, 1130; ^{1}H NMR: δ 1.17 (s, 3H), 1.39 (t, 3H *J* = 8.0 Hz), 1.97 (s, 3H), 2.66 (brs, 3H), 2.95 (s, 4H), 3.36 (brs, 4H), 3.96 (s, 2H), 4.48 (brs, 2H), 4.56 (brs, 2H), 5.18 (s, 2H), 7.13 (s, 1H), 7.42 (brs, 1H), 7.54 (brs, 1H), 7.80 (brs, 1H), 8.88 (s, 1H), 9.03 (s, 1H), 15.27 (s, 1H); ^{13}C NMR: δ 13.6, 14.8, 15.4, 25.5, 43.3, 45.8, 48.0, 49.5, 49.9, 50.2, 106.2, 109.8, 110.8, 119.4 and 119.5 (d, C, *J* = 15.0 Hz), 121.6 and 122.9 (d, CH, *J* = 136.0 Hz), 135.9 and 136.1 (d, CH, *J* = 17.0 Hz), 137.5, 145.8, 149.1 and 149.9 (d, C, *J* = 84.0 Hz), 154.1 and 156.1 (d, C, *J* = 200.0 Hz), 163.6 and 165.1 (d, C, *J* = 153.0 Hz), 107.5, 108.2, 149.2, 149.9, 166.5, 168.1, 174.9, 176.5, 178.4. Anal. Calcd for $C_{32}H_{35}FN_8O_4S$: C, 59.43; H, 5.45; N, 17.33. Found: C, 59.31; H, 5.15; N, 17.03.

6-(4-{[4-Benzyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-1-cyclopropyl-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (13a) Yield 82% (method 1); yield 90% (method 2, $InCl_3$); yield 97% (method 2, *p*-TsOH); yield 97% (method 3); mp >270°C; IR (υ_{max}, cm^{-1}): 3300, 1716 3, 1481, 1130; ^{1}H NMR: δ 1.18 (s, 2H), 1.32 (s, 2H), 1.41 (t, 3H, *J* = 8.0 Hz), 2.69 (brs, 3H), 2.99 (s, 4H), 3.38 (brs, 4H), 3.83 (brs, 2H), 4.33 (d, 4H, *J* = 8.0 Hz), 4.66 (brs, 1H), 5.25 (s, 2H), 6.98 (s, 1H), 7.13–7.84 (m, 7H), 7.95 (s, 1H), 8.61 (s, 1H), 9.01 (s, 1H), 15.15 (s, 1H); ^{13}C NMR: δ 8.0 (2CH_2), 15.3, 25.5, 36.3, 44.3 (N-2CH_2), 47.3 (N-2CH_2), 48.4, 49.9, 50.2, 106.9, 118.8, 119.0 and 120.3 (d, C, *J* = 129.0 Hz), 122.7, 123.9, 127.2, 127.6, 127.9 and 127.9 (d, CH, *J* = 7.0 Hz), 128.7 and 128.8 (d, CH, *J* = 6.0 Hz), 136.0, 136.1 (2C), 138.5 and 139.5 (d, C, *J* = 100.0 Hz), 144.5, 146.1 and 147.7 (d, C, *J* = 162.0 Hz), 148.6, 162.8 and 163.7 (d, C, *J* = 88.0 Hz)], 107.2, 118.6, 148.3, 148.8, 166.4, 169.1, 174.7, 176.7, 178.5. Anal. Calcd for $C_{37}H_{37}FN_8O_4S$: C, 63.32; H, 5.17; N, 15.55. Found: C, 63.28; H, 5.06; N, 15.21.

1-Cyclopropyl-6-(4-{[4-ethyl-3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-1-yl]methyl}piperazin-1-yl)-5-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (13b) Yield 79% (method 1); yield 85% (method 2, $InCl_3$); yield 88% (method 2, *p*-TsOH);. yield 95% (method 3); mp 228–230°C; IR (υ_{max}, cm^{-1}): 3456, 1710 3, 1558, 1159; ^{1}H NMR: δ 1.18 (s, 2H), 1.32 (s, 2H), 1.40 (t, 3H, *J* = 8.0 Hz), 1.96 (s, 3H), 2.69 (brs, 3H), 2.97 (s, 4H), 3.33 (brs, 4H), 3.82 (brs, 2H), 4.49 (d, 4H, *J* = 8.0 Hz), 4.64 (brs, 1H), 5.25 (s, 2H), 7.44–7.79 (m, 4H), 8.61 (s, 1H), 9.05 (s, 1H), 15.08 (s, 1H); ^{13}C NMR: δ 8.0, 15.3, 25.4, 27.7, 36.4, 43.8, 45.9, 47.2, 48.8, 49.7, 51.2, 105.9, 109.8, 119.0 and 119.6 (d, C, *J* = 50.0 Hz), 121.7, 123.9, 125.9 and 125.9 (d, CH, *J* = 7.0 Hz), 128.1 and 128.8 (d, CH, *J* = 76.0 Hz), 135.0, 139.5 and 141.5 (d, C, *J* = 200.0 Hz), 146.1 and 148.6 (d, C, *J* = 253.0 Hz), 161.9 and 162.6 (d, C, *J* = 72.0 Hz), 107.7, 108.6, 143.6, 149.1, 166.2, 169.1, 173.9, 175.8, 178.3. Anal. Calcd for $C_{33}H_{35}FN_8O_4S$: C, 60.17; H, 5.36; N, 17.01. Found: C, 60.31; H, 5.15; N, 17.37.

General synthesis of compounds 14 and 15

Method 1 To a solution of compound **6a** (0.01 mol) in dimethyl sulfoxide, 1-phenylpiperazine (for **14**, 1.52 mL, 0.01 mol) or tryptamine (for **15**, 1.60 g, 0.01 mol) was added in the presence of formaldehyde

(37%, 3.72 mL, 0.05 mol) and the mixture was stirred at room temperature for 24 h, then poured into ice-cold water. The resultant precipitate was crystallized from ethanol/water (1:3).

Method 2 To a solution of compound **6a** (1 mmol) in dimethyl sulfoxide, 1-phenylpiperazine (for **14**, 1 mmol) or tryptamine (for **15**, 1 mmol) was added in the presence of formaldehyde (5 mmol) and the mixture was stirred at room temperature for 15 min. Then, a catalytic amount of a catalyst ($InCl_3$ or *p*-TsOH) was added and the stirring was continued for an additional 5 h. The mixture was poured into ice water and the resultant precipitate was crystallized from ethanol/water (1:3).

Method 3 A solution, prepared as described above, was irradiated at 50°C in the microwave oven for 5 min, then cooled to room temperature and poured into ice water. The resultant precipitate was filtered off and crystallized from ethanol/water (1:3).

3-{4-Benzyl-1-[(4-phenylpiperazin-1-yl)methyl]-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (14) Yield 74% (method 1); yield 78% (method 2, $InCl_3$); yield 80% (method 2, *p*-TsOH); yield 93% (method 3); mp 106–108°C; IR (υ_{max}, cm^{-1}): 1726, 1495, 1155; ^1H NMR: δ 1.37 (t, 3H, *J* = 8.0 Hz), 2.59 (s, 3H), 2.92 (s, 4H), 3.13 (brs, 4H), 4.35 (brs, 2H), 5.23 (s, 2H), 5.32 (s, 2H), 6.77 (brs, 1H), 6.95 (d, 3H, *J* = 8.0 Hz), 7.15–7.29 (m, 7H), 7.45 (brs, 1H), 7.95 (s, 1H); ^{13}C NMR: δ 15.4, 25.3, 47.3, 48.3, 48.8, 50.4, 69.4, 108.6, 116.1, 119.3, 119.4, 121.8, 127.4, 127.5, 127.8, 127.9, 128.6, 128.7, 128.8, 129.3, 136.2, 136.3, 147.6, 148.8, 151.5, 146.5, 163.7, 169.1, 174.7. Anal. Calcd for $C_{31}H_{33}N_7OS$: C, 67.49; H, 6.03; N, 17.77. Found: C, 67.28; H, 6.06; N, 17.98.

3-[4-Benzyl-1-({[2-(1*H*-indol-3-yl)ethyl]amino}methyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (15) Yield 71% (method 1); yield 80% (method 2, $InCl_3$), yield 82% (method 2, *p*-TsOH); yield 99% (method 3); mp 163–165°C; IR (υ_{max}, cm^{-1}): 3029 2, 1710, 1503, 1155; ^1H NMR: δ 1.20 (t, 3H, *J* = 8.0 Hz), 2.66 (s, 3H), 4.36 (t, 2H, *J* = 8.0 Hz), 4.64 (t, 2H, *J* = 8.0 Hz), 5.12 (s, 2H), 5.30 (s, 2H), 5.54 (s, 2H), 6.99 (brs, 3H), 7.14–7.31 (m, 7H), 7.48 (d, 2H, *J* = 8.0 Hz), 8.54 (s, 1H), 9.01 (s, 1H), 9.21 (s, 1H), 14.93 (s, 1H); ^{13}C NMR: δ 15.3, 25.3, 25.5, 45.6, 47.3, 48.2, 71.3, 108.7, 119.3, 121.8, 123.1, 127.6 (2CH), 128.0 (2CH), 128.8 (2CH), 136.1, 136.2 (2CH), 146.4 (2CH), 147.9, 148.8, 152.2, 163.7, 150.1, 164.9, 167.9, 174.7. Anal. Calcd for $C_{31}H_{31}N_7OS$: C, 67.73; H, 5.68; N, 17.84. Found: C, 67.49; H, 6.05; N, 17.97.

Antimicrobial activity

The following test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey): *E. coli* ATCC35218, *Yersinia pseudotuberculosis (Y. pseudotuberculosis*) ATCC911, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC43288, *E. faecalis* ATCC29212, *Staphylococcus aureus* (*S. aureus*) ATCC25923, *Bacillus cereus* (*B. cereus)* 709 Roma, *M. smegmatis* ATCC607, *Candida albicans* (*C. albicans*) ATCC60193, and *Saccharomyces cerevisiae* (*S. cerevisia*) RSKK 251. All synthesized compounds were weighed and dissolved in hexane to prepare a stock solution of 20.0 μg/mL. The antimicrobial effects of the substances were tested quantitatively by using double microdilution; the MIC values (μg/mL) were determined. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI, USA) at pH 7.3 and buffered yeast nitrogen base (Difco, Detroit, MI, USA) at pH 7.0, respectively. The microdilution test plates were incubated for 18–24 h at 35°C. Brain heart infusion broth (BHI) (Difco, Detriot, MI, USA) was used for *M. smegmatis*; incubation was for 48–72 h at 35°C [44]. Ampicillin, streptomycin, and fluconazole were used as standard antibacterial and antifungal drugs. Dimethyl sulfoxide with a dilution of 1:10 was used as a solvent control.

Acknowledgments: The financial support was provided by Scientific and Technological Research Council of Turkey (TUBITAK, Project no: 113Z181).

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