Chem Biol Drug Des 2012; **79:** 384–397

Research Article

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doi: 10.1111/j.1747-0285.2011.01316.x

Synthesis of Novel Nalidixic Acid-Based 1,3,4-Thiadiazole and 1,3,4-Oxadiazole Derivatives as Potent Antibacterial Agents

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Novel nalidixic acid-based 1,3,4-thia(oxa)diazoles, their thio ethers, sulfones, *bis* mercapto, and Mannich bases were synthesized and characterized by Infrared spectra, ^{1}H NMR, ^{13}C NMR, and elemental analysis. These compounds were evaluated for their antibacterial activity against two Gram-positive and three Gram-negative bacteria. The preliminary bioassay showed that most of the compounds had better antibacterial activity than the parent compounds, 1,3,4-thia(oxa)diazoles, at the dosage 50 μg/mL toward five test bacteria. Four Mannich bases of nalidixic acid-based 1,3,4-thiadiazole exhibited maximum antibacterial activity against *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* with minimum inhibitory concentration in the range of 6.25–125 μg/mL.

Key words: 1,3,4-oxadiazole, 1,3,4-thiadiazole, antibacterial activity, Mannich bases, nalidixic acid

Received 16 March 2011, revised 5 December 2011 and accepted for publication 12 December 2011

The discovery of antibiotics in the 20th century marked a watershed in the treatment of infections. The rapid emergence of resistance to antibiotics among pathogens is still a major cause of deaths. Because of the resistant bacteria, particularly staphylococci, enterococci, *Klebsiella pneumonia*, and *Pseudomonas* species, many drugs failed to treat the infections. Quinolones are the first-line therapy that has made a major impact in the field of antimicrobial chemotherapy, particularly in the past few years. Nalidixic acid (1,8-naphthyridine derivative) was the first synthetic quinolone antibiotic introduced in 1960s by Leisher (1). It inhibits the DNA gyrase enzyme that is responsible for the initiation and propagation of DNA synthesis. It also shows promising activity against Gram-negative organisms including most of the enterobacteria mainly used in the treatment of urinary infection (2). A wide range of compounds with a wide spectrum of antibacterial activity have been developed from nalidixic acid (3,4).

Substituted 1,3,4-thiadiazoles and oxadiazoles have been widely used by the medicinal chemists in the past to explore their biological activities (5–11). 1,3,4-Thiadiazole derivatives exhibit various biological properties such as antimicrobial (12–14), antituberculosis (15), antidepressant (16), anti-inflammatory (17,18), anticonvulsants (19,20), antihypertensive (21,22), local anesthetic (23), anticancer (24,25), and hypoglycemic activities (26). 1,3,4-Oxadiazoles are widely used in pharmacy as antitubercular preparations, in the production of vat dyes and azo dyes, in color photography, in scintillator technology, and for the production of thermostable polymers. Also, several biologically relevant entities containing 1,3,4-oxadiazole ring are used as benzodiazepine receptor agonists (27), 5-HT receptor agonists (28), muscarinic agonists (29), 5-HT antagonists (30), human NK1 antagonists (31), antirhinoviral compounds (32), anti-inflammatory agents (10), angiogenic agents (33), and HIV integrase inhibitor (34). Some 1,3,4-oxadiazole derivatives and their Mannich bases are also reported to possess anti-inflammatory (8,35), antitubercular (36), antifungal (37), and anticancer activities (38). In order to combat the problem of drug resistance, there is an urgent need to develop and discover new antibiotic agents. Owing to the biopotential of quinolones and heterocyclic molecules, a series of title compounds were designed by combining two potential pharmacophores in a single matrix and their antibacterial activity evaluated.

Experimental

Materials and method

All the chemicals purchased from Sigma-Aldrich (St. Louis, MO, USA) were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on precoated Merck silica gel $60F_{254}$, and the spots were visualized either under UV light or by iodine vapor. Melting points were determined on an electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer (Waltham, MA, USA) model 2000 FT-IR spectrophotometer as KBr pellet and values are expressed as ν_{max} per centimeter. Mass spectra were recorded on a Joel (Tokyo, Japan) JMS-DX303 and micromass LCT, Mass Spectrometer/Data system. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrospin spectrometer (400 and 75.5 MHz), using tetramethylsilane as an internal standard. The chemical shift values were recorded on δ scale, and the coupling constants (J)

are in Hertz. Elemental analyses for all compounds were performed on Carlo Erba Model EA-1108 elemental analyzer, and data of C, H and N were within ±0.4% of calculated values.

Synthesis of nalidixic acid methyl ester

Nalidixic acid (2 g, 8.611 mmol) and tetrahydrofuran (50 mL) were placed in a 100-mL round-bottomed flask and mounted over a magnetic stirrer. Anhydrous potassium carbonate (5.96 g, 43.05 mmol) was added and the contents were stirred for 1 h. Dimethyl sulfate (1.63 mL, 12.92 mmol) was added to this stirred solution and the mixture was refluxed at 70 °C. The progress of the reaction was monitored by TLC using hexane/ethyl acetate (70:30) as the eluent. TLC showed complete disappearance of the starting material after 4 h and appearance of one new spot. After refluxing, the solvent was removed under reduced pressure on a rotary evaporator and the product was extracted with chloroform (3 × 10 mL). The chloroform layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give methyl nalidixate (1.95 g, 95%).

Synthesis of nalidixic acid hydrazide

Methyl nalidixate (2 g, 8.17 mmol) was dissolved in tetrahydrofuran (10 mL) in a 50-mL round-bottomed flask and hydrazine hydrate (0.5 mL, 12.25 mmol) was added. The contents were refluxed for 3 h. TLC analysis using hexane/ethyl acetate (70:30) as the eluent showed complete disappearance of methyl nalidixate after 3 h. To the resultant reaction mixture, cold water (50 mL) was added and stirred for 10 min. A solid separated out, which was filtered at pump and dried to give nalidixic acid hydrazide (1.7 g, 85%).

Synthesis of 1-ethyl-3-(5-mercapto-1,3,4-thiadiazol-2-yl)-7-methyl-1H-1,8-naphthyridin-4-one (2)

Nalidixic acid hydrazide, (5.0 mmol) was added to a solution of potassium hydroxide (10.0 mmol) in absolute ethanol (50 mL) placed in a 100-mL round-bottomed flask mounted over a magnetic stirrer. The reaction mixture was stirred for 1 h in an ice bath. Carbon disulfide (11.0 mmol) was added dropwise to the above precooled reaction mixture, which resulted in the formation of a pale yellow precipitate. The pale yellow precipitate was filtered and repeatedly washed with cold acetone (2 × 10 mL) and dried in an oven. The dried dithiocarbazinate salt (**1**) was added in very small portions to cooled sulfuric acid (15 mL, 0–5 °C) taken in a round-bottomed flask and stirred till the solution became homogenous. The homogenous mixture was poured into crushed ice (100 g). The separated precipitate was filtered at pump, washed with water, and dried to yield the desired product **2**. Its physiochemical properties are described below:

Yellow solid; M.p.: >300 °C; Yield: 80%; ^{1}H NMR (δ): 1.42 (t, 3H, N-$CH_2\underline{CH_3}$), 2.49 (s, 3H, 7-CH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 7.33 (d, J = 8.40 Hz, 1H, H-6-naphthyridine), 8.49 (d, J = 5.20 Hz, 1H, H-5-naphthyridine), 8.97 (s, 1H, H-2-naphthyridine), 14.32 (s, 1H, SH); IR (KBr, per cm) ν_{max}: 2923, 2367, 1604, 1570, 1539, 1429, 1251, 789; HRMS calculated for $C_{13}H_{12}N_4OS_2$: 304.0453, Observed: 305.1285 (M^+ + H).

General procedure for the synthesis of S-alkylated/arylated derivatives of 2-mercapto-1,3,4-thiadizole (3–10)

Product **2** (5 mmol) was added slowly to a cooled solution (10 °C) of potassium hydroxide (10 mmol) in absolute ethanol (50 mL) taken in a 50-mL round-bottomed flask mounted over a magnetic stirrer. The contents were stirred and cooled to 10 °C. A solution of alkyl iodide or allyl/propargyl/aryl bromides (5.5 mmol) in ethanol (20 mL) was added to the above precooled reaction mixture and stirred at ambient temperature. The completion of reaction was monitored by TLC using hexane/ethyl acetate (50:50) as solvent. The reaction was complete after 1.5 h. The solid thus obtained was filtered at pump, washed with water, and recrystallized from ethanol/water. The physiochemical properties and spectral data of compounds **3–10** are described below:

1-Ethyl-7-methyl-3-(5-methylsulfanyl-1,3,4-thiadiazol-2-yl)-1H-1,8-naphthyridin-4-one (3)

White solid; M.p.: 217 °C; Yield: 83%; ^{1}H NMR (δ): 1.59 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7-CH_3), 2.82 (s. 3H, S-CH_3), 4.65 (q, 2H, N-$\underline{CH_2}$), 7.34 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.70 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.26 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2956, 1621, 1578, 1425, 1366, 1250, 793; MS calculated for $C_{14}H_{14}N_4OS_2$: 318.0609; Observed MS-ES (m/z): 318.58 (M^+).

1-Ethyl-3-(5-ethylsulfanyl-1,3,4-thiadiazol-2-yl)-7-methyl-1H-1,8-naphthyridin-4-one (4)

White solid; M.p.: 218 °C; Yield: 78%; ^{1}H NMR (δ): 1.49 (t, 3H, N-$CH_2\underline{CH_3}$), 1.55 (t, 3H, S-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7-CH_3), 3.33 (q, 2H, S-$\underline{CH_2}$), 4.62 (q, 2H, N-$\underline{CH_2}$), 7.31 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.68 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.14 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 14.72, 15.37, 25.28, 28.77, 46.92, 112.73, 119.14, 121.37, 136.24, 141.54, 148.23, 160.33, 163.19, 165.64, 173.72; IR (KBr, per cm) ν_{max}: 2978, 1614, 1578, 1467, 1378, 1254, 791; MS calculated for $C_{15}H_{16}N_4OS_2$: 332.0766; Observed MS-ES (m/z): 332.48 (M^+).

1-Ethyl-7-methyl-3-(5-propylsulfanyl-1,3,4-thiadiazol-2-yl)-1H-1,8-naphthyridin-4-one (5)

White solid; M.p.: 220 °C; Yield: 76%; ^{1}H NMR (δ): 1.05 (t, 3H, S-$(CH_2)_2\underline{CH3}$), 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 1.75–1.88 (m, 2H, S-$CH_2\underline{CH_2}$), 2.68 (s, 3H, 7-CH_3), 3.27 (t, 2H, S-$\underline{CH_2}$), 4.59 (q, 2H, N-$\underline{CH_2}$), 7.29 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.65 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.13 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 13.33, 15.35, 22.84, 25.26, 36.99, 46.90, 112.74, 119.17, 121.36, 136.6, 141.57, 148.25, 160.30, 163.18, 166.00, 173.73; IR (KBr, per cm) ν_{max}: 2970, 1628, 1576, 1465, 1372, 1252, 791; MS calculated for $C_{16}H_{18}N_4OS_2$: 346.0922; Observed MS-ES (m/z): 346.46 (M^+).

3-(5-Butylsulfanyl-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (6)

White solid; M.p.: 150 °C; Yield: 72%; ^{1}H NMR (δ): 0.96 (t, 3H, S-$(CH_2)_3\underline{CH_3}$), 1.57 (t, 3H, N-$CH_2\underline{CH_3}$), 1.61–1.81 (m, 4H, S-$CH_2\underline{CH_2CH_2}$), 2.68 (s, 3H, 7-CH_3), 3.32 (t, 2H, S-$\underline{CH_2}$), 4.63 (q, 2H, N-$\underline{CH_2}$), 7.30 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.69 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.14 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 13.55, 15.32, 21.89, 25.26, 31.43, 34.20, 46.87, 112.50, 119.27, 121.32, 136.27, 141.50, 147.36, 160.31, 163.15, 166.00, 173.52; IR (KBr, per

cm) ν_{max}: 2979, 1629, 1579, 1543, 1468, 1367, 1252, 793; MS calculated for $C_{17}H_{20}N_4OS_2$: 360.1079; Observed MS-ES (m/z): 360.5 (M^+).

1-Ethyl-7-methyl-3-(5-pentylsulfanyl-1,3,4-thiadiazol-2-yl)-1H-1,8-naphthyridin-4-one (7)

White solid; M.p.: 248 °C; Yield: 72%; ^{1}H NMR (δ): 0.89 (t, 3H, S-$(CH_2)_4\underline{CH_3}$), 1.42–1.45 (m, 2H, S-$(CH_2)_3\underline{CH_2}CH_3$), 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 1.58–1.82 (m, 4H, S-$CH_2\underline{CH_2}\underline{CH_2}$), 2.69 (s, 3H, 7-$CH_3$), 3.29 (t, 2H, S-$\underline{CH_2}$), 4.59 (q, 2H, N-$\underline{CH_2}$), 7.29 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.67 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.13 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2959, 1615, 1575, 1463, 1372, 1253, 798; MS calculated for $C_{17}H_{22}N_4OS_2$: 374.1235; Observed MS-ES (m/z): 374.16 (M^+).

3-(5-Allylsulfanyl-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (8)

White solid; M.p.: 238 °C; Yield: 82%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7-CH_3), 3.94 (d, J = 7.2 Hz, 2H, S-$\underline{CH_2}$), 4.62 (q, 2H, N-$\underline{CH_2}$), 5.13–5.21 (m, 3H, $\underline{CH{=}CH_2}$), 7.33 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.70 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.16 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2979, 1614, 1576, 1466, 1374, 1253, 791; MS calculated for $C_{16}H_{16}N_4OS_2$: 344.0776; Observed MS-ES (m/z): 344.26 (M^+).

1-Ethyl-7-methyl-3-(5-prop-2-ynylsulfanyl-1,3,4-thiadiazol-2-yl)-1H-1,8-naphthyridin-4-one (9)

White solid; M.p.: 166 °C; Yield: 85%; ^{1}H NMR (δ): 1.54 (t, 3H, N-$CH_2\underline{CH_3}$), 1.64 (s, 1H, terminal alkyne C), 2.70 (s, 3H, 7-CH_3), 4.04 (s, 2H, S-$\underline{CH_2}$), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.29 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.65 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.13 (s, 1H, H-2-naphthyridine); IR (KBr, ν_{max} per cm): 2970, 2315, 1613, 1573, 1545, 1468, 1372, 1254, 798; MS calculated for $C_{16}H_{14}N_4OS_2$: 342.0609; Observed MS-ES (m/z): 342.38 (M^+).

3-(5-Benzylsulfanyl-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (10)

White solid; M.p.: 268 °C; Yield: 68%; ^{1}H NMR (δ): 1.54 (t, 3H, N-$CH_2\underline{CH_3}$), 2.69 (s, 3H, 7-CH_3), 4.55 (s, 2H, S-$\underline{CH_2}$), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.23–7.26 (m, 3H, Ar-H), 7.28 (d, J = 7.2 Hz, 1H, H-6-naphthyridine), 7.30–7.45 (m, 2H, Ar-H), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.22 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2986, 1625, 1545, 1462, 1247, 79; MS calculated for $C_{20}H_{18}N_4OS_2$: 394.0922; Observed MS-ES (m/z): 394.60 (M^+).

General procedure for the synthesis of sulfone derivatives of 2-mercapto-1,3,4-thiadiazole (11–16)

Powdered potassium permanganate (5.6 mmol) was added in small portions over a period of 1 h to a stirred solution of 2-alkylthio compounds (1.89 mmol) in acetic acid (10 mL of 66%)) in a 50-mL round-bottomed flask mounted over a magnetic stirrer at 20 °C. The reaction mixture was stirred at room temperature. The progress of the reaction was monitored by TLC using hexane/ethyl acetate (30:70) as solvent system. The oxidation was complete within 2 h. The reaction mixture was decolorized with 12 mL of $NaHSO_3$ solution (20%). The resulting precipitate was filtered at pump and washed with water. It was recrystallized from ethanol to afford the desired compounds. Yield, melting point, and spectral data of these compounds are given below:

1-Ethyl-3-(5-methanesulfonyl-1,3,4-thiadiazol-2-yl)-7-methyl-1H-1,8-naphthyridin-4-one (11)

White solid; M.p.: 245 °C; Yield: 62%; ^{1}H NMR (δ): 1.57 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7-CH_3), 3.43 (t, 2H, -$SO_2\underline{CH_3}$), 4.65 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.68 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.25 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.41, 25.30, 43.51, 47.35, 111.27, 119.03, 121.96, 136.33, 142.83, 148.27, 163.78, 164.98, 167.52, 173.60; IR (KBr, per cm) ν_{max}: 2921, 1629, 1546, 1505, 1458, 1321, 1155, 794; MS calculated for $C_{14}H_{14}N_4O_3S_2$: 350.0507; Observed MS-ES (m/z): 350.28 (M^+).

3-(5-Ethanesulfonyl-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (12)

White solid; M.p.: 245 °C; Yield: 60%; ^{1}H NMR (δ): 1.31 (t, 3H, SO_2-$CH_2\underline{CH_3}$), 1.59 (t, 3H, N-$CH_2\underline{CH_3}$), 2.74 (s, 3H, 7-CH_3), 3.64 (q, 2H, -$SO_2\underline{CH_2}$), 4.66 (q, 2H, N-$\underline{CH_2}$), 7.38 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.71 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.29 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 14.13, 15.45, 25.34, 47.50, 50.01, 112.39, 119.60, 122.04, 137.64, 142.90, 148.50, 163.83, 165.18, 167.19, 173.65; IR (KBr, per cm) ν_{max}: 2942, 1615, 1525, 1501, 1458, 1394, 1125, 791; MS calculated for $C_{15}H_{16}N_4O_3S_2$: 364.0664; Observed MS-ES (m/z): 365.18 (M^+ + 1).

1-Ethyl-7-methyl-3-[5-(propane-1-sulfonyl)-1,3,4-thiadiazol-2-yl]-1H-1,8-naphthyridin-4-one (13)

White solid; M.p.: 225 °C; Yield: 58%; ^{1}H NMR (δ): 1.08 (t, 3H, SO_2-$(CH_2)_2\underline{CH_3}$), 1.59 (t, 3H, N-$CH_2\underline{CH_3}$), 1.66–1.92 (m, 2H, SO_2-$CH_2\underline{CH_2}CH_3$), 2.74 (s, 3H, 7-CH_3), 3.72 (t, 2H, -$SO_2\underline{CH_2}$), 4.66 (q, 2H, N-$\underline{CH_2}$), 7.38 (d, J = 7.6 Hz, 1H, H-6-naphthyridine), 8.73 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.30 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2932, 2459, 1613, 1576, 1455, 1329, 1148, 799; MS calculated for $C_{16}H_{18}N_4O_3S_2$: 378.0820; Observed MS-ES (m/z): 378.26 (M^+).

3-[5-(Butane-1-sulfonyl)-1,3,4-thiadiazol-2-yl]-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (14)

White solid; M.p.: 242 °C; Yield: 62%; ^{1}H NMR (δ): 0.89 (t, 3H, SO_2-$(CH_2)_3\underline{CH_3}$), 1.55 (t, 3H, N-$CH_2\underline{CH_3}$), 1.78–1.86 (m, 4H, SO_2-$CH_2\underline{CH_2}\underline{CH_2}$), 2.69 (s, 3H, 7-$CH_3$), 3.49 (t, 2H, -$SO_2\underline{CH_2}$), 4.63 (q, 2H, N-$\underline{CH_2}$), 7.34 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.68 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.25 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2872, 2325, 1613, 1575, 1546, 1454, 1336, 1149, 799; MS calculated for $C_{17}H_{20}N_4O_3S_2$: 392.0977; Observed MS-ES (m/z): 393.78 (M^+ + 1).

1-Ethyl-7-methyl-3-[5-(pentane-1-sulfonyl)-1,3,4-thiadiazol-2-yl]-1H-1,8-naphthyridin-4-one (15)

White solid; M.p.: 164 °C; Yield: 72%; ^{1}H NMR (δ): 0.88 (t, 3H, SO_2-$(CH_2)_4\underline{CH_3}$), 1.32–1.44 (m, 4H, SO_2-$CH_2\underline{CH_2CH_2}$), 1.58 (t, 3H, N-$CH_2\underline{CH_3}$), 1.86–1.90 (m, 2H, S-$CH_2\underline{CH_2}$), 2.73 (s, 3H, 7-CH_3), 3.52 (t, 2H, SO_2-$\underline{CH_2}$), 4.67 (q, 2H, N-$\underline{CH_2}$), 7.37 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.72 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.28 (s, 1H, H-2-naphthyridine); IR (KBr, ν_{max} per cm): 2858, 1614, 1575, 1499, 1427, 1333, 1149, 799; MS calculated for $C_{18}H_{22}N_4O_3S_2$: 406.1133; Observed MS-ES (m/z): 406.82 (M^+).

3-(5-(Benzylsulfonyl)-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (16)

White solid; M.p.: 246 °C; Yield: 68%; ^{1}H NMR (δ): 1.59 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7-CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 4.78 (s, 2H, SO_2-$\underline{CH_2}$), 7.27 (d, J = 7.2 Hz, 1H, H-6-naphthyridine), 7.30–7.37 (m, 5H, Ar-H), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.27 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.44, 25.3, 47.30, 62.05, 111.31, 118.98, 121.97, 126.39, 128.94, 129.28, 131.16, 136.29, 142.87, 148.21, 163.77, 165.37, 165.94, 173.55; IR (KBr, per cm) ν_{max}: 2914, 1612, 1575, 1547, 1451, 1332, 1159, 798; MS calculated for $C_{20}H_{18}N_4O_3S_2$: 426.0802; Observed MS-ES (m/z): 426.84 (M^+).

General procedure for the synthesis bis-(1,3,4-thia/oxadiazol-2-yl)-disulfides (17,18)

Bromine (6.4 mmol) in ethanol (25 mL) was added slowly to an ethanolic solution of 2-mercapto thiadiazole/oxadiazole (3.2 mmol) taken in a 50-mL round-bottomed flask mounted over a magnetic stirrer. After the completion of the reaction as monitored by TLC using hexane/ethyl acetate (50:50) as solvent system, water (25 mL) was added to the reaction mixture. The product was filtered at pump, washed with ethanol, and recrystallized from the ethanol. Yield, melting point, and spectral data of these compounds are given below:

3,3′-{5,5′-Disulfanediylbis(1,3,4-thiadiazole-5,2-diyl))bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (17)

Yellow solid; M.p.: 232 °C; Yield: 72%; ^{1}H NMR (δ): 1.56 (t, 6H, N-$CH_2\underline{CH_3}$), 2.70 (s, 6H, 7-CH_3), 4.35 (q, 4H, N-$\underline{CH_2}$), 7.34 (d, J = 8.4 Hz, 2H, H-6-naphthyridine), 8.69 (d, J = 8.0 Hz, 2H, H-5-naphthyridine), 9.20 (s, 2H, H-2-naphthyridine; ^{13}C NMR (δ): 15.53 (2C), 25.98 (2C), 47.20 (2C), 112.37 (2C), 119.16 (2C), 121.78 (2C), 136.28 (2C), 142.16 (2C), 143.01 (2C), 148.29 (2C), 163.34 (2C), 168.84 (2C), 173.52 (2C); IR (KBr, per cm) ν_{max}: 2958, 1612, 1578, 1546, 1456, 1360, 1255, 795; MS calculated for $C_{26}H_{22}N_8O_2S_4$: 606.0749; Observed MS-ES (m/z): 606.90 (M^+).

3,3′-{5,5′-Disulfanediylbis(1,3,4-oxadiazole-5,2-diyl)}bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (18)

Yellow solid; M.p.: 238 °C; Yield: 72%; ^{1}H NMR (δ): 1.40 (t, 6H, N-$CH_2\underline{CH_3}$), 2.57 (s, 6H, 7-CH_3), 4.41 (q, 4H, N-$\underline{CH_2}$), 7.18 (d, J = 8.0 Hz, 2H, H-6-naphthyridine), 8.39 (s, 2H, H-2-naphthyridine), 8.53 (d, J = 8.4 Hz, 2H, H-5-naphthyridine; IR (KBr, per cm) ν_{max}: 3046, 1647, 1575, 1523, 1445, 1389, 1259, 792; MS calculated for $C_{26}H_{22}N_8O_4S_2$: 574.1205; Observed MS-ES (m/z): 574.16 (M^+).

General procedure for the synthesis of bis-(1,3,4-thiadiazol-2-yl)-dialkylthiones (19–25)

A suspension of 2-mercapto-1,3,4-thiadiazole **2** (1 mmol) in methanol (10 mL) was placed in a 50-mL round-bottomed flask mounted over a magnetic stirrer. The reaction mixture was cooled in an ice bath. The aqueous solution of 1 M KOH (10 mL) was added to reaction mixture with vigorous stirring. When the solid was dissolved, the appropriate alkyl dihalide (0.5 mmol) was added dropwise and the temperature was allowed to rise up to 25 °C for 1 h. The reaction mixture was refluxed for 2 h. The completion of reaction was monitored by TLC using hexane/ethyl acetate (50:50) as eluent. The product was filtered at pump and repeatedly washed with water and oven-dried. The isolated products were confirmed by its IR, NMR, and mass spectra given below:

3,3′-{5,5′-(Ethane-1,2-diyl)bis(sulfanediyl)}bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (19)

Yellow solid; M.p.: 230 °C; Yield: 80%; ^{1}H NMR (δ): 1.57 (t, 6H, N-$CH_2\underline{CH_3}$), 2.70 (s, 6H, 7-CH_3), 3.64 (t, 2H, S-CH_2), 3.82 (t, 2H, S-CH_2), 4.61 (q, 4H, N-$\underline{CH_2}$), 7.25 (d, J = 8.0 Hz, 2H, H-6-naphthyridine), 8.75 (d, J = 8.4 Hz, 2H, H-5-naphthyridine), 9.03 (s, 2H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2985, 2364, 1616, 1578, 1543, 1468, 1373, 1254, 791; MS calculated for $C_{28}H_{26}N_8O_2S_4$: 634.1062; Observed MS-ES (m/z): 634.78 (M^+).

3,3′-{5,5′-(Propane-1,3-diyl)bis(sulfanediyl))bis(1,3,4-thiadiazole-5,2-diyl}bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (20)

White solid; M.p.: 244 °C; Yield: 85%; ^{1}H NMR (δ): 1.59 (t, 6H, N-$CH_2\underline{CH_3}$), 2.38–2.42 (m, 2H, S-$CH_2\underline{CH_2}CH_2$-S), 2.75 (s, 6H, 7-CH_3), 3.93 (t, 2H, S-CH_2), 3.95 (t, 2H, S-CH_2), 4.63 (q, 4H, N-$\underline{CH_2}$), 7.32 (d, J = 8.0 Hz, 2H, H-6-naphthyridine), 8.69 (d, J = 8.4 Hz, 2H, H-5-naphthyridine), 9.15 (s, 2H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2923, 1603, 1576, 1542, 1466, 1373, 1252, 790; MS calculated for $C_{29}H_{28}N_8O_2S_4$: 648.1218; Observed MS-ES (m/z): 648.42 (M^+).

3,3′-{5,5′-(Butane-1,4-diyl)bis(sulfanediyl)}bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (21)

White solid; M.p.: 252 °C; Yield: 83%; ^{1}H NMR (δ): 1.31 (t, 6H, N-$CH_2\underline{CH_3}$), 2.33–2.47 (m, 4H, S-$CH_2\underline{CH_2CH_2}CH_2$-S), 2.61 (s, 6H, 7-$CH_3$), 3.12 (t, 4H, S-$CH_2$), 4.39 (q, 4H, N-$\underline{CH_2}$), 7.08 (d, J = 8.4 Hz, 2H, H-6-naphthyridine), 8.38 (d, J = 8.0 Hz, 2H, H-5-naphthyridine), 8.91 (s, 2H, H-2-naphthyridine); ^{13}C NMR (δ): 15.16 (2C), 25.06 (2C), 28.11 (2C), 33.39 (2C), 46.69 (2C), 112.27 (2C), 118.82 (2C), 121.19 (2C), 135.52 (2C), 148.01 (2C), 160.15 (2C), 163.01 (2C), 164.366 (2C),

167.98 (2C), 173.50 (2C); IR (KBr, per cm) ν_{max}: 2919, 1624, 1581, 1545, 1434, 1364, 1254, 790; MS calculated for $C_{30}H_{30}N_8O_2S_4$: 662.1375; Observed MS-ES (m/z): 662.58 (M^+).

3,3′-{5,5′-(Pentane-1,5-diyl)bis(sulfanediyl)}bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (22)

Yellow solid; M.p.: 238 °C; Yield: 72%; ^{1}H NMR (δ): 1.55 (t, 6H, N-$CH_2\underline{CH_3}$), 1.68 (pentet, 2H, CH_2), 1.81–1.94 (m, 4H, CH_2), 2.71 (s, 6H, 7-CH_3), 3.34 (t, 4H, S-CH_2) 4.62 (q, 4H, N-$\underline{CH_2}$), 7.31 (d, J = 8.0 Hz, 2H, H-6-naphthyridine), 8.67 (d, J = 8.4 Hz, 2H, H-5-naphthyridine), 9.16 (s, 2H, H-2-naphthyridine); ^{13}C NMR (δ): 15.36 (2C), 25.28 (2C), 27.77, 28.90 (2C), 34.05 (2C), 46.92 (2C), 112.66 (2C), 119.15 (2C), 121.36 (2C), 136.27 (2C), 141.64 (2C), 148.24 (2C), 160.38 (2C), 163.16 (2C), 165.67 (2C), 173.69 (2C); IR (KBr, per cm) ν_{max}: 2939, 1622, 1585, 1545, 1434, 1380, 1254, 789; MS calculated for $C_{31}H_{32}N_8O_2S_4$: 676.1531; Observed MS-ES (m/z): 677.48 (M^+ + 1).

3,3′-{5,5′-(Hexane-1,6-diyl)bis(sulfanediyl)}bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (23)

Yellow solid; M.p.: 233 °C; Yield: 78%; ^{1}H NMR (δ): 1.15–1.25 (m, 4H, CH_2CH_2), 1.53 (t, 6H, N-$CH_2\underline{CH_3}$), 1.57–1.85 (m, 4H, CH_2), 2.70 (s, 6H, 7-CH_3), 3.31 (t, 4H, S-CH_2), 4.61 (q, 4H, N-$\underline{CH_2}$), 7.30 (d, J = 8.0 Hz, 2H, H-6-naphthyridine), 8.67 (d, J = 8.0 Hz, 2H, H-5-naphthyridine), 9.13 (s, 2H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2952, 2358, 1613, 1572, 1538, 1430, 1374, 1255, 797; MS calculated for $C_{32}H_{34}N_8O_2S_4$: 690.1618; Observed MS-ES (m/z): 690.84 (M^+).

3,3′-{5,5′-(Octane-1,8-diyl)bis(sulfanediyl)}bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (24)

Yellow solid; M.p.: 235 °C; Yield: 72%; ^{1}H NMR (δ): 1.32–1.54 (m, 8H, CH_2), 1.57 (t, 6H, N-$CH_2\underline{CH_3}$), 1.80–1.86 (m, 4H, S-$CH_2\underline{CH_2}$), 2.70 (s, 6H, 7-CH_3), 3.32 (t, 4H, S-CH_2), 4.61 (q, 4H, N-$\underline{CH_2}$), 7.31 (d, J = 8.4 Hz, 2H, H-6-naphthyridine), 8.68 (d, J = 8.4 Hz, 2H, H-5-naphthyridine), 9.14 (s, 2H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2979, 2346, 1621, 1576, 1532, 1432, 1373, 1253, 792; MS calculated for $C_{34}H_{38}N_8O_2S_4$: 718.2001; Observed MS-ES (m/z): 718.88 (M^+).

3,3′-{5,5′-(1,4-Phenylenebis(methylene)}bis(sulfanediyl)bis(1,3,4-thiadiazole-5,2-diyl)bis(1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one) (25)

Yellow solid; M.p.: 240 °C; Yield: 68%; ^{1}H NMR (δ): 1.46 (t, 6H, N-$CH_2\underline{CH_3}$), 2.49 (s, 6H, 7-CH_3), 4.44 (s, 4H, S-CH_2), 4.53 (q, 4H, N-$\underline{CH_2}$), 7.21 (d, J = 8.4 Hz, 2H, H-6-naphthyridine), 7.26 (d, J = 8.0 Hz, 2H, Ar-H), 7.33 (d, J = 8.0 Hz, 2H, Ar-H), 8.57 (d, J = 8.0 Hz, 2H, H-5-naphthyridine), 9.07 (s, 2H, H-2-naphthyridine); ^{13}C NMR (δ): 15.29 (2C), 25.58 (2C), 46.87 (2C), 65.54 (2C), 112.38 (2C), 119.50 (2C), 121.18 (2C), 125.78 (2C), 129.41 (2C), 136.11 (2C), 138.26 (2C), 141.58 (2C), 148.20 (2C), 160.62 (2C), 163.87 (2C), 165.86 (2C), 173.41 (2C); IR (KBr, per cm) ν_{max}: 2925, 1620, 1581, 1515, 1431, 1366, 1253, 793; MS calculated for $C_{34}H_{30}N_8O_2S_4$: 710.1375; Observed MS-ES (m/z): 710.15 (M^+).

General procedure for the synthesis of Mannich bases of 1,3,4-thiadiazole (26–38)

Formalin 40% (200 μL, 4.2 mmol) was added to a stirred solution of 1,3,4-thiadiazole-2-thione (3 mmol) in DMSO (10 mL) and ethanol (20 mL) in a 100-mL round-bottomed flask mounted over a magnetic stirrer. An ethanolic solution (10 mL) of the appropriate amine (3 mmol) was added dropwise to the reaction mixture and the mixture was refluxed for 3 h. After the completion of reaction as monitored by TLC using hexane/ethyl acetate (30:70) as the solvent, the reaction mixture was allowed to cool and quenched with water. The precipitate formed was collected by filtration at pump, washed with water, and oven-dried. The structures of the isolated products were confirmed by IR, NMR, and mass spectra given below:

1-Ethyl-7-methyl-3-(4-phenylaminomethyl-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1H-1,8-naphthyridin-4-one (26)

Yellow solid; M.p.: 259 °C; Yield: 72%; ^{1}H NMR (δ): 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 4.57 (q, 2H, N-$\underline{CH_2}$), 5.25 (s, 2H, NH-$\underline{CH_2}$), 6.25 (s, 1H, NH), 6.89–7.28 (m, 5H, Ar-H), 7.29 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.66 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.14 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3241, 2980, 1620, 1546, 1463, 1425, 1266, 793; MS calculated for $C_{20}H_{19}N_5OS_2$: 409.1031; Observed MS-ES (m/z): 410.8 (M^+ + 1).

3-{4-[(2-Chloro-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (27)

Pink solid; M.p.: 215 °C; Yield: 64%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 5.54 (s, 2H, NH-$\underline{CH_2}$), 6.13 (s, 1H, NH), 7.29 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.31–7.45 (m, 4H, Ar-H), 8.67 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.16 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.32, 25.22, 46.90, 74.02, 112.07, 112.25, 118.99, 119.17, 121.54, 131.51, 136.07, 142.10, 142.33, 148.17, 151.85. 159.71, 163.24, 163.61, 173.51, 173.77; IR (KBr, per cm) ν_{max}: 3210, 2939, 1605, 1540, 1499, 1425, 1355, 1250, 790; MS calculated for $C_{20}H_{18}ClN_5OS_2$: 443.0641; Observed MS-ES (m/z): 443.85 (M^+), 445.25.

3-{4-[(4-Chloro-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1- ethyl-7-methyl-1H-1,8-naphthyridin-4-one (28)

Yellow solid; M.p.: 262 °C; Yield: 62%; ^{1}H NMR (δ): 1.51 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 4.70 (q, 2H, N-$\underline{CH_2}$), 5.58 (s, 2H, NH-$\underline{CH_2}$), 6.38 (s, 1H, NH), 7.17 (d, J = 7.8 Hz, 2H, Ar-H), 7.24 (d, J = 7.8 Hz, 2H, Ar-H), 7.29 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.73 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.26 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.19, 25.59, 46.21, 80.46, 110.05 (2C),

112.07, 119.17, 121.54, 125.87, 131.51 (2C), 133.59, 136.07, 139.92, 148.17, 152.60, 167.76, 173.71, 175.82; IR (KBr, per cm) ν_{max}: 3225, 2979, 1607, 1545, 1462, 1430, 1384, 1251, 792; MS calculated for $C_{20}H_{18}ClN_5OS_2$: 443.0641; Observed MS-ES (m/z): 443.23 (M^+), 445.19.

3-{4-[(4-Bromo-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1-ethyl-7-methyl-1H-1,8-naphthyridin-4-one (29)

Yellow solid; M.p.: 240 °C (charring); Yield: 65%; ^{1}H NMR (δ): 1.55 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 4.62 (q, 2H, N-$\underline{CH_2}$), 5.72 (s, 2H, NH-$\underline{CH_2}$), 6.20 (s, 1H, NH), 6.80 (d, J = 8.8 Hz, 2H, Ar-H), 7.32 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.42 (d, J = 7.6 Hz, 2H, Ar-H), 8.60 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.73 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3340, 2955, 1615, 1575, 1488, 1436, 1357, 1218, 787; MS calculated for $C_{20}H_{18}BrN_5OS_2$: 487.0136; Observed MS-ES (m/z): 487.51 (M^+), 489.42.

1-Ethyl-7-methyl-3-{4-[(4-nitro-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1H-1,8-naphthyridin-4-one (30)

Yellow solid; M.p.: 265 °C (charring); Yield: 65%; ^{1}H NMR (δ): 1.57 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7-CH_3), 4.65 (q, 2H, N-$\underline{CH_2}$), 5.81 (s, 2H, NH-$\underline{CH_2}$), 6.64 (s, 1H, NH), 6.99 (d, J = 9.0 Hz, 2H, Ar-H), 7.30 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.09 (d, J = 8.0 Hz, 2H, Ar-H), 8.69 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.96 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3250, 2942, 1617, 1601, 1445, 1436, 1362, 1245, 796; MS calculated for $C_{20}H_{18}N_6O_3S_2$: 454.0882; Observed MS-ES (m/z): 455.28 (M^+ + 1).

1-Ethyl-7-methyl-3-{4-[(4-cyano-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1H-1,8-naphthyridin-4-one (31)

Yellow solid; M.p.: 278 °C; Yield: 66%; ^{1}H NMR (δ): 1.56 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7-CH_3), 4.63 (q, 2H, N-$\underline{CH_2}$), 5.77 (s, 2H, NH-$\underline{CH_2}$), 6.22 (s, 1H, NH), 7.01 (d, J = 8.4 Hz, 2H, Ar-H), 7.48 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.60 (d, J = 7.6 Hz, 2H, Ar-H), 8.76 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.84 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.23, 25.12, 47.25, 58.44, 111.07, 113.29 (2C), 114.48, 118.93, 121.94, 129.24, 133.59 (2C), 135.96, 141.44, 148.09, 148.85, 152.54, 163.73, 163.91, 174.33; IR (KBr, per cm) ν_{max}: 3340, 2955, 1615, 1575, 1488, 1436, 1357, 1218, 787; MS calculated for $C_{21}H_{18}N_6OS_2$: 434.0984; Observed MS-ES (m/z): 434.26 (M^+).

1-Ethyl-7-methyl-3-(5-thioxo-4-((4-(trifluoromethyl)phenylamino)methyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (32)

Yellow solid; M.p.: 215 °C; Yield: 66%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.69 (s, 3H, 7-CH_3), 4.61 (q, 2H, N-$\underline{CH_2}$), 5.77 (s, 2H, NH-$\underline{CH_2}$), 6.28 (s, 1H, NH), 7.02 (d, J = 8.4 Hz, 2H, Ar-H), 7.43 (d, J = 8.8 Hz, 2H, Ar-H), 7.32 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.58 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.69 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3322, 2928, 1608, 1574, 1441, 1316, 1246, 788; MS calculated for $C_{21}H_{18}F_3N_5OS_2$: 477.0905; Observed MS-ES (m/z): 477.18 (M^+).

3-(4-((4-Acetylphenylamino)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (33)

Yellow solid; M.p.: 207 °C; Yield: 65%; ^{1}H NMR (δ): 1.57 (t, 3H, N-$CH_2\underline{CH_3}$), 2.65 (s, 3H, $CO\underline{CH_3}$), 2.73 (s, 3H, 7-CH_3), 4.75 (q, 2H, N-$\underline{CH_2}$), 5.82 (s, 2H, NH-$\underline{CH_2}$), 6.43 (s, 1H, NH), 7.00 (d, J = 8.0 Hz, 2H, Ar-H), 7.32 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.37 (d, J = 7.6 Hz, 2H, Ar-H), 8.61 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.12 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.51, 25.85, 29.71, 47.24, 59.69, 111.66, 113.20 (2C), 119.16, 120.30, 120.57, 121.54, 129.39 (2C), 136.12, 142.46, 145.32, 151.84, 154.70, 163.38, 174.28, 186.67; IR (KBr, per cm) ν_{max}: 3345, 2985, 1667, 1620, 1544, 1441, 1340, 1252, 789; MS calculated for $C_{22}H_{21}N_5O_2S_2$: 451.1137; Observed MS-ES (m/z): 451.28 (M^+).

3-(4-((4-Aminophenylamino)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (34)

Yellow solid; M.p.: 268 °C; Yield: 66%; ^{1}H NMR (δ): 1.55 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7-CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 5.66 (s, 2H, NH_2), 5.68 (s, 2H, NH-$\underline{CH_2}$), 6.17 (s, 1H, NH), 6.95 (d, J = 8.4 Hz, 2H, Ar-H), 7.28 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.14 (d, J = 8.0 Hz, 2H, Ar-H), 8.58 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.74 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.45, 25.29, 47.19, 60.18, 112.54, 114.44, 115.52, 116.94 (2C), 117.17 (2C), 121.73, 129.02, 136.02, 136.19, 141.15, 141.86, 148.21, 163.61, 186.65; IR (KBr, per cm) ν_{max}: 3363, 3238, 2920, 1620, 1583, 1548, 1440, 1347, 1232, 788; MS calculated for $C_{20}H_{20}N_6OS_2$: 424.1140; Observed MS-ES (m/z): 424.84 (M^+).

3-(4-((3-Chloro-4-fluorophenylamino)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (35)

Yellow solid; M.p.: 272 °C; Yield: 69%; ^{1}H NMR (δ): 1.47 (t, 3H, N-$CH_2\underline{CH_3}$), 2.53 (s, 3H, 7-CH_3), 4.66 (q, 2H, N-$\underline{CH_2}$), 5.64 (s, 2H, NH-$\underline{CH_2}$), 5.88 (s, 1H, NH), 6.98 (s, 1H, Ar-H), 7.39 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.42 (d, J = 8.4 Hz, 1H, Ar-H), 8.08 (d, J = 6.8 Hz, 1H, Ar-H), 8.38 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.52 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3287, 3097, 1610, 1575, 1441, 1364, 1253, 789; MS calculated for $C_{20}H_{17}ClFN_5OS_2$: 461.0547; Observed MS-ES (m/z): 461.10 (M^+), 463.25.

3-(4-[(2,4-Dinitrophenylamino)methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (36)

Yellow solid; M.p.: 270 °C; Yield: 66%; ^{1}H NMR (δ): 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7-CH_3), 4.65 (q, 2H, N-$\underline{CH_2}$), 5.89 (s, 2H, NH-$\underline{CH_2}$), 6.21 (s, 1H, NH), 7.38 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.74 (d, J = 6.8 Hz, 1H, Ar-H), 8.28 (d, J = 7.2 Hz, 1H, Ar-H), 8.76 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.87 (s, 1H, Ar-H), 9.26 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3241, 2976, 1604,

1575, 1469, 1433, 1327, 1254, 790; MS calculated for $C_{20}H_{17}N_7O_5S_2$: 499.0733; Observed MS-ES (m/z): 499.52 (M^+).

1-Ethyl-7-methyl-3-(4-(morpholinomethyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (37)

Yellow solid; M.p.: 240 °C (charring); Yield: 66%; ^{1}H NMR (δ): 1.54 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 2.81 (t, 4H, CH_2), 3.69 (t, 4H, CH_2), 4.61 (q, 2H, N-$\underline{CH_2}$), 5.27 (s, 2H, N-$\underline{CH_2}$), 7.29 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.15 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.44, 25.27, 47.21, 50.54 (2C), 66.92 (2C), 70.54, 111.47, 119.16, 121.66, 136.22, 141.12, 148.23, 151.50, 163.55, 174.30, 188.53; IR (KBr, per cm) ν_{max}: 2945, 1604, 1545, 1444, 1362, 1252, 790; MS calculated for $C_{18}H_{21}N_5O_2S_2$: 403.1137; Observed MS-ES (m/z): 403.9 (M^+).

1-Ethyl-7-methyl-3-(4-((methyl(phenyl)amino)methyl)-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)-1,8-naphthyridin-4(1H)-one (38)

Yellow solid; M.p.: 213 °C; Yield: 61%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 3.65 (s, 3H, N-CH_3), 4.66 (q, 2H, N-$\underline{CH_2}$), 5.75 (s, 2H, N-$\underline{CH_2}$), 6.89–7.25 (m, 5H, Ar-H), 7.29 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.56 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.85 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2980, 1607, 1545, 1462, 1384, 1251, 792; MS calculated for $C_{21}H_{21}N_5OS_2$: 423.1188; Observed MS-ES (m/z): 423.21 (M^+).

Synthesis of 1-ethyl-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-methyl-1H-1,8-naphthyridin-4-one (39)

To a solution of potassium hydroxide (10.0 mmol) in absolute ethanol (20 mL) in a round-bottomed flask, nalidixic acid hydrazide, (5.0 mmol) was added and the mixture was stirred for 1 h in an ice bath. To the above reaction mixture, carbon disulfide (11.0 mmol) was added dropwise, which resulted in the formation of a pale yellow precipitate. The reaction mixture was heated under reflux for 4 h, till the mixture became clear. The solution was concentrated by distillation and acidified with dilute hydrochloric acid to give the desired product. Its yield, melting point, and spectral data are given below:

White solid; M.p.: 278 °C; Yield: 60%; ^{1}H NMR (δ): 1.37 (t, 3H, N-$CH_2\underline{CH_3}$), 2.49 (s, 3H, 7-CH_3), 4.52 (q, 2H, N-$\underline{CH_2}$), 7.44 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.47 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.85 (s, 1H, H-2-naphthyridine), 11.24 (s, 1H, SH), ^{13}C NMR (δ): 15.40, 25.30, 46.34, 105.19, 120.02, 121.95, 136.35, 146.54, 146.84, 158.72, 163.26, 172.66, 177.06; MS calculated for $C_{13}H_{12}N_4O_2S$: 288.0681; Observed MS-ES (m/z): 288.32 (M^+).

General procedure for the synthesis of S-alkylated/arylated derivative of 2-mercapto-1,3,4-oxadiazole (40–42)

The S-alkylation and benzylation of 1-ethyl-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-methyl-1*H*-1,8-naphthyridin-4-one (**39**) were carried out as reported for alkylation of compound **2** for the preparation of compounds **3–10**. The yield, melting point, and spectral data of these compounds are given below:

1-Ethyl-7-methyl-3-{5-(methylsulfanyl)-1,3,4-oxadiazol-2-yl}-1,8-naphthyridin-4(1H)-one (40)

White solid; M.p.: 225 °C; Yield: 68%; ^{1}H NMR (δ): 1.44 (t, 3H, N-$CH_2\underline{CH_3}$), 2.61 (s, 3H, 7-CH_3), 2.69 (s. 3H, S-CH_3), 4.53 (q, 2H, N-$\underline{CH_2}$), 7.26 (d, J = 8.8 Hz, 1H, H-6-naphthyridine), 8.68 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.95 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3045, 2360, 1604, 1524, 1444, 1319, 1254, 797; MS calculated for $C_{14}H_{14}N_4O_2S$: 302.0837; Observed MS-ES (m/z): 301.00 (M^+-1).

3-{5-(Allylsulfanyl)-1,3,4-oxadiazol-2-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (41)

White solid; M.p.: 228 °C; Yield: 83%; ^{1}H NMR (δ): 1.50 (t, 3H, N-$CH_2\underline{CH_3}$), 2.67 (s, 3H, 7-CH_3), 3.76 (d, J = 7.2 Hz, 2H, S-$\underline{CH_2}$), 4.49 (q, 2H, N-$\underline{CH_2}$), 5.03–5.16 (m, 3H, $\underline{CH{=}CH_2}$),7.34 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.65 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.85 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3027, 2361, 1622, 1577, 1478, 1437, 1339, 1229, 791; MS calculated for $C_{16}H_{16}N_4O_2S$: 328.0994; Observed MS-ES (m/z): 328.39 (M^+).

3-{5-(Benzylsulfanyl)-1,3,4-oxadiazol-2-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (42)

White solid; M.p.: 215 °C; Yield: 62%; ^{1}H NMR (δ): 1.59 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7-CH_3), 4.30 (q, 2H, N-$\underline{CH_2}$), 4.55 (s, 2H, S-CH_2), 7.19–7.28 (m, 5H, Ar-H), 7.30 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.68 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.95 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 2934, 1646, 1582, 1440, 1324, 1255, 803; MS calculated for $C_{20}H_{18}N_4O_2S$: 378.1150; Observed MS-ES (m/z): 378.45 (M^+).

General procedure for the synthesis of Mannich bases of 1,3,4-oxadiazole (43–45)

These compounds were synthesized following the same procedure reported for the synthesis of compounds (**26–38**) except that the starting material was 1-ethyl-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-methyl-1*H*-1,8-naphthyridin-4-one (**39**). Yield, melting point, and spectral data are given below:

3-{4-[(4-Chlorophenylamino)methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (43)

Yellow solid; M.p.: 270 °C; Yield: 66%; ^{1}H NMR (δ): 1.56 (t, 3H, N-$CH_2\underline{CH_3}$), 2.58 (s, 3H, 7-CH_3), 4.52 (q, 2H, N-$\underline{CH_2}$), 5.51 (s, 2H, NH-$\underline{CH_2}$), 6.51 (s, 1H, NH), 6.87 (d, J = 8.8 Hz, 2H, Ar-H), 7.26 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.46 (d, J = 7.6 Hz, 2H, Ar-H), 8.60 (d, J = 7.6 Hz, 1H, H-5-naphthyridine), 8.99 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3148, 2928, 1612, 1565, 1442, 1428, 1323, 1252, 793; MS calculated for $C_{20}H_{18}ClN_5O_2S$: 427.0870; Observed MS-ES (m/z): 427.64 (M^+), 429.85.

4-[(5-(1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) methyl amino]benzonitrile (44)

Yellow solid; M.p.: 263 °C; Yield: 66%; ^{1}H NMR (δ): 1.42 (t, 3H, N-$CH_2\underline{CH_3}$), 2.60 (s, 3H, 7-CH_3), 4.52 (q, 2H, N-$\underline{CH_2}$), 5.65 (s, 2H, NH-$\underline{CH_2}$), 6.55 (s, 1H, NH), 6.75 (d, J = 7.6 Hz, 2H, Ar-H), 7.22 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.32 (d, J = 8.0 Hz, 2H, Ar-H), 8.53 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.91 (s, 1H, H-2-naphthyridine); IR (KBr, per cm) ν_{max}: 3189, 2948, 1617, 1545, 1458, 1428, 1355, 1265, 793; MS calculated for $C_{21}H_{18}N_6O_2S$: 418.1212; Observed MS-ES (m/z): 418.28 (M^+).

3-{4-[(2,4-Dinitrophenylamino)methyl]-5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (45)

Yellow solid; M.p.: 268 °C; Yield: 70%; ^{1}H NMR (δ): 1.32 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7-CH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 5.82 (s, 2H, NH-$\underline{CH_2}$), 6.86 (s, 1H, NH), 7.07 (d, J = 6.0 Hz, 1H, H-6-naphthyridine), 7.56 (d, J = 7.2 Hz, 1H, Ar-H), 8.40 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.43 (d, J = 7.2 Hz, 1H, Ar-H), 8.67 (s, 1H, Ar-H), 9.25 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.24, 25.23, 46.08, 47.08, 111.03, 118.71, 120.73, 121.56, 129.35, 133.56, 136.16, 136.43, 141.55, 147.68, 151.48, 155.30, 163.95, 165.82, 176.38, 178.42; IR (KBr, per cm) ν_{max}: 3285, 2960, 1606, 1584, 1495, 1442, 1323, 1252, 793; MS calculated for $C_{20}H_{17}N_7O_6S_2$: 483.0961; Observed MS-ES (m/z): 483.79 (M^+).

Antibacterial activity

Agar disk diffusion method

Disk diffusion is one of the most common assays used for the evaluation of the antibacterial activity (39). The bacterial strains used included Gram-positive bacteria, namely *Staphylococcus aureus* ATCC 2937 and *Bacillus subtilis* ATCC 12711, and Gram-negative bacteria, namely *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, and *Klebsiella pneumoniae* ATCC 31488. Stock cultures were maintained at 4 °C on slopes of nutrient agar (NA). Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth (NB) that were incubated without agitation for 24 h at 37 °C. The cultures were diluted with fresh NB to achieve optical densities corresponding to 2×10^6 colony-forming units (CFUs/mL) for bacteria. *In vitro* antimicrobial activity was screened by using NA obtained from Himedia (Delhi, India). The NA plates were prepared by pouring 25 mL of molten media into sterile petri plates. The plates were allowed to solidify for 3–4 h. On the surface of the media, microbial suspension was spread with the help of sterilized L-shaped loop. All the synthesized compounds (50 μg/mL) were loaded on 6-mm sterile disk. The loaded disk was placed on the surface of the medium, and the compound was allowed to diffuse for 5 min and the plates were then kept for incubation at 37 °C for 24 h. DMSO was used as a solvent for all the compounds and as a control. After 24 h, inhibition zones formed around the disk were measured with a transparent ruler in millimeter. The studies were performed in triplicate.

Broth dilution method

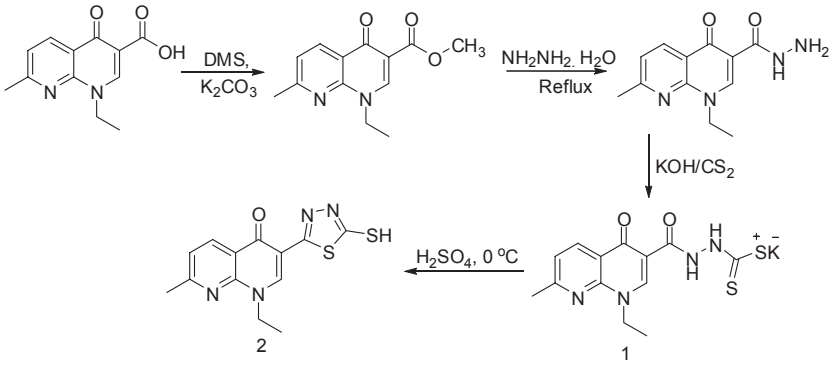
Antibacterial susceptibility testing was carried out using microdilution broth assay against five pathogenic strains: *S. aureus, B. Subtilis, E. coli, K. pneumoniae and P. aeruginosa.* Initially, bacteria were grown at 37 °C in NB (HIMEDIA) until exponential growth. This culture was used to inoculate 100 mL of NB so that an initial number of 2×10^6 CFUs/mL could be achieved. Test compound, dissolved in DMSO/water, was added to 20 mL of inoculated media to obtain a final concentration of 250 μg/mL. Twofold dilution of this compound was achieved by transferring 10 mL of this 250 μg/mL test compound containing media to another 10 mL inoculated media to acquire a final concentration of 125 μg/mL of the test compound. All other dilutions of the test compound were prepared in a similar fashion to obtain the minimal dilution of 15.68 μg/mL. And 10 mL each of inoculated media along with the test compound was dispensed equally into three screw-capped 10-mL glass culture tubes. Controls without the test compound, only media and in the presence of streptomycin 5 μg/mL were set up simultaneously. All culture tubes were incubated at 37 °C for 24 h. After incubation, the growth of bacteria was determined by measuring optical density at 600 nm using UV-Specord 200 (Analytik JENA Instruments®, Jena, Germany). The lowest concentration, at which there was 99% inhibition of bacteria as compared to the culture without test compound, was taken up as the minimal inhibitory concentration (MIC).

Results and Discussion

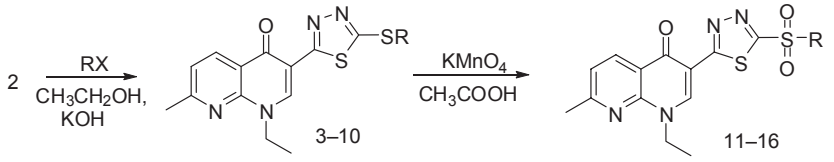
Synthesis of title compounds

1-Ethyl-3-(5-mercapto-1,3,4-thiadiazol-2-yl)-7-methyl-1*H*-1,8-naphthyridin-4-one (**2**) was synthesized by the reaction of nalidixic acid hydrazide with carbon disulfide and potassium hydroxide to give potassium dithiocarbazinate (**1**) followed by its cyclization in acidic medium (Scheme 1). The 2-mercapto derivative (**2**) on alkylation with alkyl, allyl, propargyl, and benzylic halides in the presence of a base using ethanol as a solvent gave sulfides (**3–10**, Scheme 2), which on oxidation with acidic potassium permanganate gave corresponding sulfones (**11–16**, Scheme 2). The 2-mercapto derivatives (**2 and 39**) on oxidation with bromine in ethanol gave disulfides (**17,18**). The thiols were also coupled *via* spacers by reaction with terminal dihalides in the presence of a base (**19–25**, Scheme 3). The corresponding Mannich bases (**26–38**, Scheme 4) were synthesized by the reaction of **2** with formaldehyde in the presence of primary and secondary amines.

The nalidixic acid-based 1,3,4-oxadiazole, namely 1-ethyl-3-(5-mercapto-1,3,4-oxadiazol-2-yl)-7-methyl-1*H*-1,8-naphthyridin-4-one (**39**), was prepared by refluxing potassium dithiocarbazinate (**1**) in alcoholic potassium hydroxide. The 2-mercapto-1,3,4-oxadiazole derivative (**39**) on alkylation/benzylation with alkyl/benzyl halide in the presence of alcoholic KOH gave compounds **40–42**. The 2-mercapto-1,3,4-oxadiazole derivative (**39**) on treatment with formaldehyde and primary amines gave Mannich bases (**43–45**, Scheme 5). All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis. The IR spectra (KBr) of compounds **2–45** generally showed the bands at 1524–1601 per cm for C=N stretching. The characteristic stretching



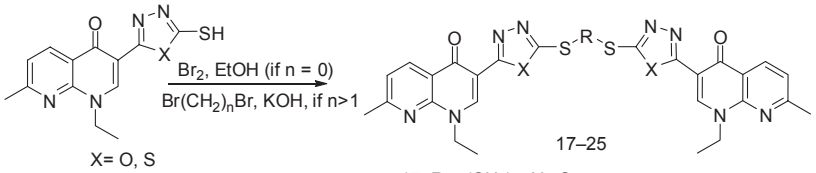
Scheme 1: Synthesis of nalidixic acid based 2-mercapto thiadiazole.



3. R= CH_3-
4. R= CH_3CH_2-
5. R= $CH_3(CH_2)_2$-
6. R= $CH_3(CH_2)_3$-
7. R= $CH_3(CH_2)_4$-
8. R= CH_2=CH-CH_2-
9. R= HC≡C–CH_2-
10. R= $C_6H_5CH_2$-

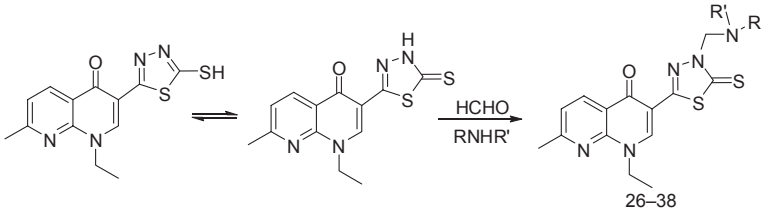
11. R= CH_3-
12. R= CH_3CH_2-
13. R= $CH_3(CH_2)_2$-
14. R= $CH_3(CH_2)_3$-
15. R= $CH_3(CH_2)_4$-
16. R= $C_6H_5CH_2$-

Scheme 2: Synthesis of S-alkylated/arylated and 2-sulphone derivatives of 2-mercapto-1,3,4-thiadizole.



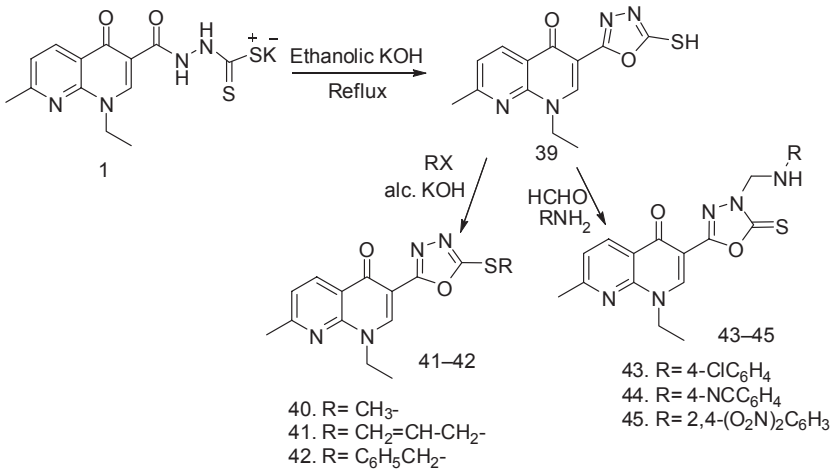
17. R= -$(CH_2)_0$- X= S
18. R= -$(CH_2)_0$- X= O
19. R= -$(CH_2)_2$- X= S
20. R= -$(CH_2)_3$- X= S
21. R= -$(CH_2)_4$- X= S
22. R= -$(CH_2)_5$- X= S
23. R= -$(CH_2)_6$- X= S
24. R= -$(CH_2)_8$- X= S
25. R= -$CH_2C_6H_4CH_2$-, X= S

Scheme 3: Synthesis of the bis-(1,3,4-thia/oxadiazol-2-yl)-disulfide.



26. R= -C_6H_5, R'= H
27. R= 2-ClC_6H_4, R'= H
28. R= 4-ClC_6H_4, R'= H
29. R= 4-BrC_6H_4, R'= H
30. R= 4-$O_2NC_6H_4$, R'= H
31. R= 4-NCC_6H_4, R'= H
32. R= 4-$F_3CC_6H_4$, R'= H
33. R= 4-$CH_3COC_6H_4$, R'= H
34. R= 4-$H_2NC_6H_4$, R'= H
35. R= 3-Cl-4-FC_6H_3, R'= H
36. R= 2,4-$(O_2N)_2C_6H_3$, R'= H
37. R, R'= -$(CH_2)_2O(CH_2)_2$-
38. R= -C_6H_5, R'= CH_3

Scheme 4: Synthesis of Mannich bases of 1,3,4-thiadiazole.



Scheme 5: Synthesis of nalidixic acid based 2-mercapto oxadiazole and its derivatives.

vibration for C=S and NH appears at 1218–1266 per cm and 3210–3363 per cm. In the Mannich bases, the ^{1}H NMR spectra of compounds **26–38** displayed broad exchangeable singlet owing to NH proton; the signal of CH_2 protons neighboring thiadiazole ring was observed at 5.54–5.89 as a singlet in addition to the aromatic protons of phenyl moiety. All other aromatic and aliphatic protons were observed at expected regions. Furthermore, the ^{13}C NMR and mass spectral data are in accordance with the expected structures of the obtained compounds.

Biological activity

All the synthesized compounds along with the starting nalidixic acid-based 1,3,4-thiadiazole (**2**) and 1,3,4-oxadiazole (**39**) were evaluated for their antibacterial activity using disk diffusion method by measuring the zone of inhibition in millimeters, and MIC was computed by observing the lowest concentration at which there was 99% inhibition of bacteria using broth dilution method. Streptomycin was used as the reference standard drug. The compounds were screened against Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli, P. aeruginosa*, and *K. pneumoniae*) in NA medium. The results are reported in Tables 1 and 2.

All the synthesized compounds **2–45** showed significant inhibitory activity against tested bacterial strains. The antibacterial data (Table 1) revealed that newly synthesized compounds have a significant influence on the antibacterial profile of Gram-negative bacteria. The compounds showed a significant inhibitory activity against *P. aeruginosa* and a moderate to good activity against *E. coli* and *K. pneumoniae*. The data indicated that a change in the substituent affects the antibacterial activity of title compounds (**2** and **39**). Among substituted sulfides, compound **7** (R=heptyl) exhibited moderately comparable antibacterial activity (MIC- 125–750 μg/mL) against both Gram-positive and Gram-negative organisms, while other substituents showed mild activity against Gram-negative bacteria and no activity against Gram-positive bacteria. The correlation between different partition coefficient values and antibacterial activity of compounds was examined by plotting zone of inhibition versus ClogP values (Figure 1). The higher the ClogP values, the higher the zone of inhibition of compounds at 50 μg/mL concentration against all test bacteria, indicating a positive correlation between the activity and partition coefficient (steric parameter). The sulfide derivatives of 1,3,4-oxadiazoles with the same substituents showed poor or no activity against all bacterial species (compounds **40–42**).

The sulfone analogs (**11–16**) were found to be inactive against Gram-positive bacteria or showed minimum activity even at a concentration of 200 μg/mL. The conversion from sulfides to sulfones produced variable, but not significant, changes in the antibacterial activity toward Gram-negative bacteria (Figure 2).

Compounds **17–25** showed better antibacterial activity (Table 1) than the sulfide and sulfone analogs against both tested bacteria. Table 1 shows that *bis* mercapto thiadiazole (compound **17**) performed better than *bis* mercapto oxadiazole (compound **18**). On introducing the alkyl spacers, that is, $(CH_2)_n$ group, into the disulfide linkages between the two thiadiazole moieties, activity profiles decreased. It has been reported that the antibacterial activity of hydrophobic compounds depends on their water/octanol partition coefficients (measure of lipophilicity) (40,41). The water/octanol partition coefficients of the *bis*-(1,3,4-thiadiazol-2-yl)-dialkylthiones derivatives used in this study are listed in Table 3. However, no such correlation was observed in our study (Figure 3). Increase in chain length up to the hexyl was accompanied by a steady enhancement of antibacterial activity. The compound with octyl group exhibited no activity against all tested organisms. Compound **25** with 1,4-*bis*-(methylene)benzene group as a spacer between two 1,3,4-thiadiazoles showed remarkable antibacterial activity (MIC, 31.25–125 μg/mL) against all the tested organisms (Figure 4).

2-Thione derivatives of 1,3,4-thiadiazole and 1,3,4-oxadiazole (compounds **26–38**, **43–45**, Tables 1 and 2) showed moderate to good activity. These compounds inhibited the Gram-positive bacteria more effectively than the Gram-negative bacteria. The compound with unsubstituted phenyl ring (compound **26**; MIC, 6.25–62.5 μg/mL)

Table 1: Inhibition zone in millimeters as a criterion for antibacterial activity of newly synthesized nalidixic acid-based 1,3,4-thiadiazole and 1,3,4-oxadiazole derivatives

	Zone of inhibition in millimeters				
	Gram-positive		Gram-negative		
Compound	*Sa*	*Bs*	*Ec*	*Kp*	*Pa*
2	0	0	0	0	0
3	0	0	0	0	5
4	0	0	5	5	7
5	0	0	6	6	8
6	0	0	7	8	9
7	6	9	9	11	12
8	0	0	0	8	7
9	0	0	6	6	5
10	5	8	8	10	11
11	0	0	6	6	0.2
12	0	0	8	8	5
13	0	0	5	8	6
14	0	0	8	8	8
15	0	0	8	7	10
16	0	0	11	9	9
17	12	13	12	9	12
18	0	0	5	6	8
19	8	7	6	8	8
20	0	0	5	11	9
21	10	8	7	9	10
22	6	0	0	0	8
23	11	11	8	10	12
24	0	0	0	0	0
25	18	36	15	18	20
26	19	41	17	43	25
27	2	8	0	0	0
28	21	45	14	31	28
29	15	17	0	0	0
30	8	10	0	0	0
31	16	23	13	21	21
32	0	0	0	0	0
33	19	22	12	27	27
34	2	24	11	17	18
35	17	25	13	25	22
36	6	9	0	0	0
37	0	0	0	0	0
38	17	45	15	25	25
39	0	0	0	0	0
40	7	9	0	0	0
41	0	4	0	0	0
42	0	8	8	0	9
43	0	5	0	0	0
44	0	8	0	0	0
45	0	7	0	0	0
Streptomycin	25	37	22	27	23

Sa, Staphylococcus aureus; Bs, Bacillus subtilis; *Ec, Escherichia coli*; *Kp, Klebsiella pneumoniae*; *Pa, Pseudomonas aeruginosa.*

Table 2: Minimal inhibitory concentration (MIC) of nalidixic acid-based 1,3,4-thiadiazole and 1,3,4 oxadiazole derivatives against five bacterial strains

	MIC (μg/mL)				
Compound	*Sa*	*Bs*	*Ec*	*Kp*	*Pa*
2	>1000	>1000	>1000	>1000	>1000
3	>1000	>1000	>1000	>1000	750
4	>1000	>1000	750	750	250
5	>1000	>1000	750	750	250
6	>1000	>1000	750	500	250
7	750	750	250	250	125
8	1000	>1000	>1000	500	250
9	>1000	>1000	750	500	750
10	1000	750	250	250	125
11	>1000	1000	750	750	>1000
12	>1000	>1000	500	500	500
13	>1000	>1000	1000	500	250
14	>1000	>1000	500	500	250
15	>1000	>1000	500	500	125
16	>1000	>1000	125	250	250
17	250	250	125	250	125
18	1000	>1000	750	750	250
19	500	1000	750	750	500
20	1000	1000	750	250	250
21	250	750	500	250	125
22	750	>1000	>1000	>1000	250
23	250	500	500	250	125
24	>1000	>1000	>1000	>1000	>1000
25	62.5	31.25	62.5	125	62.5
26	62.5	25	62.5	6.25	12.5
27	>1000	750	>1000	>1000	>1000
28	62.5	12.5	125	12.5	6.25
29	125	250	>1000	>1000	>1000
30	750	750	>1000	>1000	750
31	125	125	125	125	62.5
32	>1000	>1000	>1000	>1000	>1000
33	62.5	125	125	12.5	12.5
34	1000	125	125	125	62.5
35	62.5	125	125	31.25	31.25
36	750	750	>1000	>1000	>1000
37	>1000	>1000	>1000	>1000	>1000
38	62.5	125	62.5	100	12.5
39	>1000	>1000	>1000	>1000	>1000
40	750	750	>1000	>1000	750
41	>1000	>1000	>1000	>1000	>1000
42	>1000	750	500	>1000	250
43	>1000	1000	>1000	>1000	>1000
44	>1000	750	>1000	>1000	>1000
45	>1000	750	>1000	750	>1000
Streptomycin	15	5	13	2	4

Sa, Staphylococcus aureus; Bs, Bacillus subtilis; *Ec, Escherichia coli*; *Kp, Klebsiella pneumoniae*; *Pa, Pseudomonas aeruginosa.*

showed remarkable enhancement in the activity profile compared with compounds with substituted phenyl ring (compounds **27–36**), except compound with *p*-chloro substituent in phenyl ring (compound **28**; MIC, 6.25–125 μg/mL). The antibacterial activity of compounds **26**, **28**, **33**, and **38** was comparable with the reference standard drug streptomycin against all test organisms. The introduction of nitro groups into the phenyl ring reduced their activity considerably (compounds **30** and **36**). Compound **37** with a morpholine group was completely inactive against all tested bacteria. The compounds having substituents *p*-bromo, *p*-nitro, *p*-trifluoromethyl, and *p*-amino showed poor activity against all bacterial species. Compound **35** with both chloro and fluoro substituents in one phenyl ring showed considerable activity (MIC, 31.25–125 μg/mL). It is clear that the antibacterial activity also depends

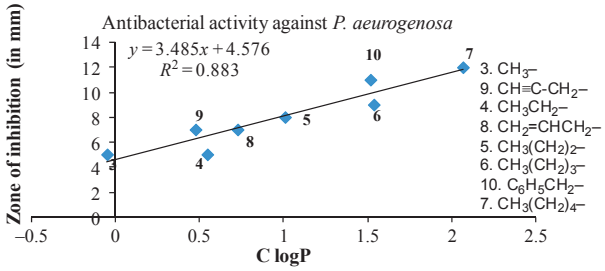


Figure 1: ClogP versus zone of inhibition at concentration 50 μg/mL against *Pseudomonas aeruginosa*.

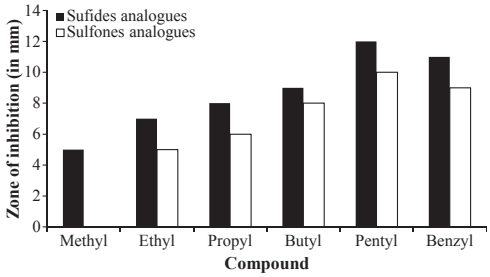


Figure 2: Comparison of sulfide and sulfone analogs of 1,3,4-thiadiazole against the *Pseudomonas aeruginosa*.

Table 3: Partition coefficients of the *bis*-(1,3,4-thiadiazol-2-yl)-dialkylthiones derivatives

Compound	R	X	ClogP
17	–	S	–1.658
18	–	O	–3.034
19	$(CH_2)_2$	S	–0.3857
20	$(CH_2)_3$	S	0.268
21	$(CH_2)_4$	S	0.2722
22	$(CH_2)_5$	S	0.8012
23	$(CH_2)_6$	S	1.330
24	$(CH_2)_8$	S	2.388
25	1,4-*bis*(methyl)benzene	S	0.9022

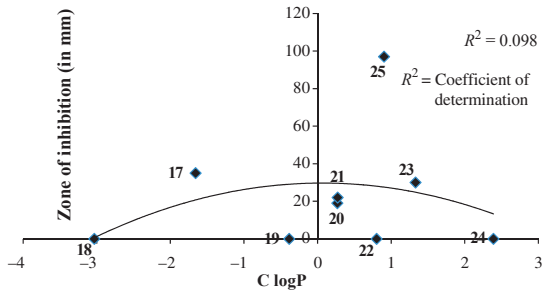


Figure 3: Graph plot ClogP of compounds **17–25** versus zone of inhibition at concentration 50 μg/mL against *Bacillus subtilis*.

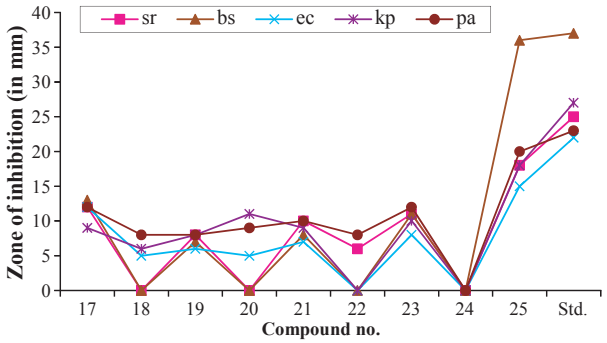


Figure 4: Effect of chain length on antibacterial activity against all micro-organisms.

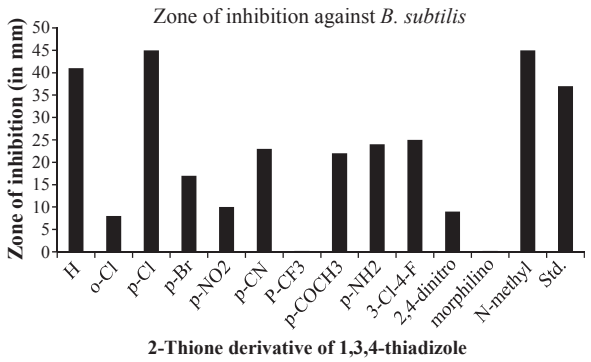


Figure 5: Effect of subsutituents on 2-thione derivatives of 1,3,4-thiadiazole aginst *Bacillus subtilis*.

on the nature of substituents besides the basic skeleton of the molecules (Figure 5).

Replacement of sulfur atom with oxygen atom in the five-membered heterocyclic ring, that is, 1,3,4-oxadiazole, resulted in decreased activity (compounds **2** and **39**). A comparison of 2-thione derivatives of 1,3,4-thiadiazole and oxadiazole revealed that thiadiazoles show marked enhancement in the potency of their analogs.

Conclusions

A variety of S-alkylated derivatives, their corresponding sulfides, sulfones, Mannich bases of 1,3,4-thiadiazole and oxadiazoles, and their coupled products with or without spacers were synthesized in good yields. All the compounds showed moderate to good antibacterial activity against tested pathogenic bacteria. 1,3,4-Thiadiazole derivatives were found to be better antimicrobial agents as compared to 1,3,4-oxadiazoles derivatives. 3-{4-[(4-Chloro-phenylamino)-methyl]-5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl}-1-ethyl-7-methyl-1*H*-1,8-naphthyridin-4-one has emerged as a potent antibacterial compound with MIC 6.25–125 μg/mL against all test micro-organisms.

Acknowledgment

NA is thankful to Council of Scientific and Industrial Research, New Delhi, India, for granting the senior research fellowship.

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