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Original article

Synthesis, antimicrobial evaluation and QSAR analysis of novel nalidixic acid based 1,2,4-triazole derivatives

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ABSTRACT

Novel nalidixic acid based 1,2,4-triazole derivatives were synthesized and characterized using spectral techniques like ^{1}H NMR, ^{13}C NMR, IR and mass spectrometry. All these compounds were screened for antimicrobial activity against five bacteria and two pathogenic fungi. Most of these compounds showed better antimicrobial activity than the parent compound, 4-amino-5-mercapto-1,2,4-triazole. Among all the screened compounds, 3-{6-(2-chlorophenyl)-1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (**23**) was emerged as promising antimicrobial agent (MIC = 16 μg/mL). Quantitative structure activity relationship (QSAR) analysis was carried out using various distance-based topological indices, steric and hydrophobic parameters. Based on the QSAR analysis it is indicative that lipophilic and steric parameters are the pre-requisites for these molecules to act as potent antimicrobial agents.

1. Introduction

Nalidixic acid (1,8-naphthyridine derivative) 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 particularly *Escherichia coli* and resistant to most of the *pseudomonas species* [3]. The development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles [3]. The considerable biological importance of triazoles has stimulated a lot of interest in its derivatives. 1,2,4-triazoles, being an important pharmacophores have a wide range of therapeutic properties like antibacterial [4–7], antifungal [8–13], antimycobacterial [14–19], antiviral [20], anti-inflammatory [21–24], anticonvulsant [25,26], antidepressant [27], antitubercular [19], antitumoral [28,29], antihypertensive [30], analgesic [31], enzyme inhibitor [32], hypoglycemic [33], sedative, hypnotic [34], antiparasitic, herbicidal [35], insecticidal [36,37] and plant growth activities [38–41]. Several compounds possessing 1,2,4-triazole nucleus are well known as drugs, for example, fluconazole, itraconazole, terconazole [42,43], ribavirin [44] and triazolam [45]. 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, triazolothiazines, triazolothiazepines and triazolothiadiazines. In the quest for biologically more potent antimicrobial agents and to increase the molecular diversity and analyze the structure–activity relationships, we envisioned to design and synthesize the nalidixic acid based 1,2,4-triazole derivatives.

2. Results and discussion

2.1. Chemistry

Nalidixic acid based 4-amino-5-mercapto-1,2,4-triazole (**1**), was prepared by the reaction of nalidixic acid hydrazide with carbon disulfide in ethanolic potassium hydroxide, a potassium dithiocarbazinate intermediate was found which on condensation with excess hydrazine hydrate produced the 3-(4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (**1**) as a white solid in good yield (Scheme 1).

Compound **1** was condensed with different aldehydes in dioxane in the presence of catalytic amounts of concentrated sulfuric acid to give the corresponding Schiff's bases (**2**–**21**, Scheme 2). Cyclization of

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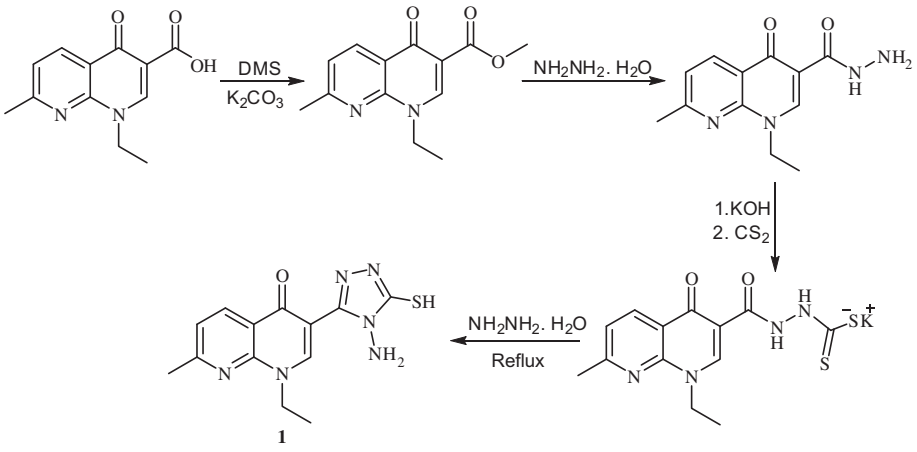


Scheme 1. Synthesis of nalidixic acid based 4-amino-1,2,4-triazole.

1 with aromatic acids in presence of polyphosphoric acid resulted in the formation of 4-(3-substituted-1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazol-6-yl) derivatives of nalidixic acid (**22**–**27**, Scheme 3). The effect of alteration at the C-6 position of triazolothiadiazoles was examined by preparing 3-substituted [1,2,4]-triazolo-[3,4-*b*] [1,3,4]thiadiazole-6-thiol (**28**) by the reaction of 4-amino-5-substituted-1,2,4-triazole-3-thiol (**1**) with carbon disulphide in refluxing pyridine (Scheme 4).

3-Substituted-4-amino-5-mercapto-1,2,4-triazole (**1**) on treatment with an equivalent amount of acetic anhydride underwent selective acylation of the amino group to form the monoacetyl derivative (**29**), whereas the treatment of compound **1** with benzoyl chloride in refluxing acetone using pyridine as a base afforded benzoyl amino derivative (**30**, Scheme 4). 1,2,4-Triazolo-[3,4-*b*] [1,3,4]-thiadiazine derivative (**31**) was readily prepared by reaction of compound **1** with phenacyl bromide in refluxing acetonitrile using potassium carbonate as a mild base. Furthermore, treatment of the 4-amino triazole (**1**) with oxalyl chloride in dry benzene resulted in the formation of corresponding triazolothiadiazine-6,7-dione (**32**, Scheme 4).

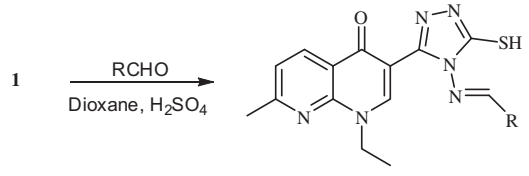
3-Substituted-4*H*-5-mercapto-1,2,4-triazoles (**33**) was synthesized by the cyclization of the nalidixic acid thiosemicarbazide with potassium hydroxide under reflux. The thiosemicarbazide, in turn, was obtained by the reaction of nalidixic acid hydrazide with potassium thiocyanate in the presence of dilute hydrochloride acid (Scheme 5).

All the synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The IR spectra (KBr) of compounds (**1**–**33**) showed the characteristic bands corresponding to the thiol, imine group, carbonyl and amide moieties etc. The C–H stretching vibration bands appeared at 2853–3059 cm^{-1} confirmed the presence of the CH_2 groups. The characteristic stretching vibrations of the C=N are at 1514–1652 cm^{-1}. The presence of thiol group was confirmed by S–H stretching vibration band in the range of 2329–2541 cm^{-1}. In the ^{1}H NMR spectra of the Schiff's bases (**2**–**21**), additional resonances assigned to the –HC=N-group at δ 8.89–9.12 were observed, which confirmed the condensation between the amino group and the carbonyl group. A downfield signal appearing at δ 12.93–13.61 was attributed to the –N=C–SH⇌–NH–C=S tautomer moiety. This signal was absent in the ^{1}H NMR spectra of triazolothiadiazoles (**22**–**27**) and triazolothiadiazines (**31**–**32**). The amide group of triazoles was confirmed by the signal appeared at δ 9.05 and 10.10.

2.2. In vitro antibacterial activity

All the synthesized compounds along with the 3-mercapto-1,2,4-triazole were subjected to the antibacterial screening using broth dilution technique. Streptomycin drug was used as the standard. The minimum inhibitory concentration (MIC) was noted by observing the lowest concentration of the drug at which there was more than 90% inhibition of bacteria. The compounds were screened against gram positive bacteria namely, *Staphylococcus aureus* ATCC 2937 and *Bacillus subtilis* ATCC 12711,and gram-negative bacteria namely, *E. coli* ATCC 8739, *Klebesilla pneumoniae* ATCC 31488 and *Pseudomonas aeruginos*a ATCC 9027 in nutrient broth medium. The results are listed in Table 1.

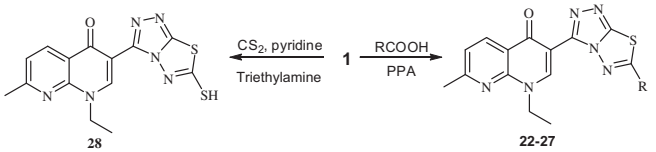
The investigation of antibacterial screening data revealed that all the tested compounds **1**–**33** showed moderate to good bacterial inhibition as compared to the standard drug streptomycin



2-21

2. R= -C_6H_5
3. R= 4-FC_6H_4
4. R= 4-ClC_6H_4
5. R= 3-BrC_6H_4
6. R= 4-BrC_6H_4
7. R= 3-$O_2NC_6H_4$
8. R= 4-$O_2NC_6H_4$
9. R= 4-NCC_6H_4
10. R= 4-$F_3CC_6H_4$
11. R= 4-HOC_6H_4
12. R= 4-$CH_3OC_6H_4$
13. R= 4-$CH_3CH_2OC_6H_4$
14.R= 4-$CH_3C_6H_4$
15. R= 4-$CH_3H_2CC_6H_4$
16. R= 4-$(CH_3)_2HCC_6H_4$
17. R= 4-$(CH_3)_3CC_6H_4$
18. R= 4-$(CH_3)_2NC_6H_4$
19. R= 4-$CH_3SC_6H_4$
20. R= 2,4-$Cl_2C_6H_3$
21. R= 2,6-$Cl_2C_6H_3$

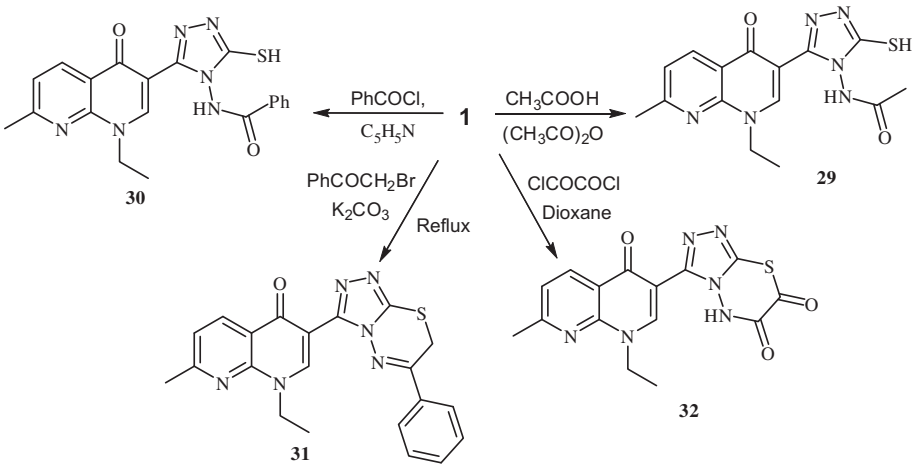
Scheme 2. Synthesis of nalidixic acid based 4-arylidene amino-4*H*-1,2,4-triazole-3-thiols (**2**–**21**).



28 **22-27**

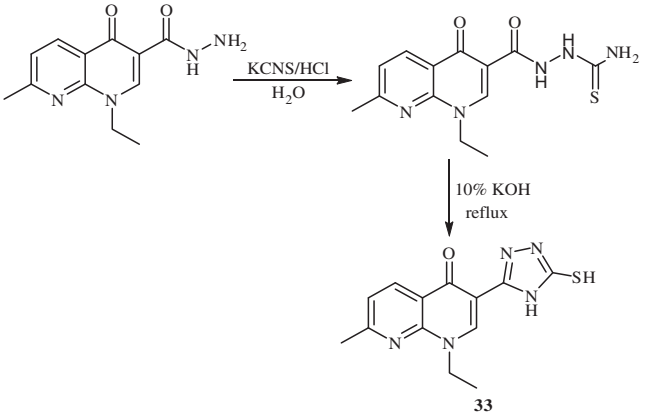
22. R= -C_6H_5
23. R= 2-ClC_6H_4
24. R=3-ClC_6H_4
25. R= 4-ClC_6H_4
26. R= 3-$O_2NC_6H_4$
27. R= 2,3-$Cl_2C_6H_3$

Scheme 3. Synthesis of nalidixic acid based 1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazole derivatives (**22**–**28**).



Scheme 4. Synthesis of nalidixic acid based acylamino-1,2,4-triazoles (**29**–**30**) and triazolothiadiazines (**31**–**32**).

(Tables 1–3). Most of the compounds exhibited significant antibacterial activity against *Pseudomonas aeruginosa* (MIC = 16–125 μg/mL). They showed moderate activity against *E. coli* and showed lower potency against *S. aureus*. The difference in the activity was observed due to molecular diversity. Conversion of free NH_2 group (compound **1**, MIC = 63–125 μg/mL) to phenylmethyleneamino moiety (compound **2,** MIC = 16 μg/mL) greatly increased the antibacterial activity against gram-negative bacteria but showed 3–4 fold decrease against gram-positive bacteria (Table 1). Among the azomethine derivatives of 1,2,4-triazole, compound **5** was the most potent against *S. aureus, B. subtilis, E. coli, K. pneumoniae* and *P. aeruginosa* with the MIC range of 125, 16, 31, 31 and 16 μg/mL Most of the compounds with electron-releasing substitutents showed better activity against all the tested microorganisms than the compound with unsubstituted phenyl ring. 3-Bromo substituted compound **5** showed highest activity. Replacing hydroxyl group with methoxy or ethoxy group (compounds **11**, **12** and **13**), resulted in marked enhancement in the antibacterial activity against *B. subtilis, K. pneumoniae* and *P. aeruginosa*. Some of the tested compounds, **3**, **6**, **10**, **19** and **20** showed very weak antibacterial activity. Triazolothiadiazoles depicted moderate to strong activity against all bacterial strains (Table 1). Compound **23** having chloro substitutent at 2-position on aromatic ring showed maximum antimicrobial potency against all tested microorganisms (MIC = 16 μg/mL) which was comparable to the standard streptomycin drug (MIC = 2–8 μg/mL). This could be due to the inductive and mesomeric effect. Compound **26** having electron withdrawing nitro group showed decreased antibacterial activity as compared to unsubstituted phenyl moiety. The benzamide derivative (compound **30**) showed effective bacterial activity against all bacteria (Table 1). This excellent inhibition by compound **30** could be attributed to the participation of the amide group. Conversion of triazole (**1**) to triazolothiadiazines *i.e.* the moieties linked through methylene spacers resulted in loss of activity against all tested bacterial species (Table 1).



Scheme 5. Synthesis of nalidixic acid based 3-mercapto-4*H*-1,2,4-traizole (**33**).

Table 1
In vitro antimicrobial activity of nalidixic acid based 1,2,4-Triazole derivatives.

Compd	Minimum Inhibitory Concentration (MIC, μg/mL)				
	Sa[a]	*Bs*[a]	*Ec*[a]	*Kp*[a]	*Pa*[a]
1	63	63	125	63	63
2	250	250	16	16	16
3	125	63	250	250	16
4	125	16	250	250	16
5	125	16	125	31	16
6	125	250	250	250	62
7	125	125	125	250	63
8	125	125	125	63	63
9	63	63	125	63	63
10	250	63	250	125	63
11	125	125	125	125	63
12	125	16	250	31	16
13	125	63	125	63	63
14	63	125	125	63	63
15	63	63	250	63	63
16	63	16	250	125	16
17	125	31	63	63	63
18	125	16	63	63	16
19	250	125	250	63	63
20	125	125	250	250	63
21	125	31	16	125	63
22	63	125	63	125	63
23	16	16	16	16	16
24	63	31	63	63	63
25	63	63	63	125	125
26	125	125	125	125	63
27	125	63	31	63	63
28	250	125	63	63	31
29	63	63	63	31	250
30	63	63	63	63	63
31	63	125	125	63	125
32	250	125	125	63	125
33	63	31	63	63	125
Streptomycin	15	5	13	2	4
Ciprofloxcin	10	12.5	12.5	2.5	1.25

[a] *Sa* = *Staphylococcus aureus*, *Bs* = *Bacillus subtillis*, *Ec* = *Escherichia coli*, *Kp* = *Klebsiella pneumoniae*, *Pa* = *Pseudomonas aeruginosa;* MIC = 90% inhibition.

Table 2
Antifungal activity of nalidixic acid based 1,2,4-triazole derivatives.

Compound	*Fusarium oxysporum*		*Aspergillus niger*	
	ED_{50}	Chi square	ED_{50}	Chi square
1	382.18	0.70	567.35	0.42
2	78.18	5.40	77.74	2.54
3	163.31	2.11	133.77	2.41
4	79.5	3.40	120.00	5.03
5	74.08	4.51	111.43	4.03
6	106.74	5.69	143.73	2.54
7	138.18	3.84	160.74	5.03
8	100.23	2.86	148.72	2.13
9	177.15	0.10	156.2	1.95
10	100.68	7.69	298.84	3.12
11	203.16	1.40	548.65	5.23
12	152.31	3.25	104.74	2.96
13	89.31	5.05	86.57	5.51
14	205.06	0.44	76.64	3.55
15	190.89	0.47	228.93	4.75
16	106.42	3.84	113.3	4.45
17	116.79	2.87	133.64	2.37
18	103.8	1.66	101.00	2.48
19	104.46	5.35	250.68	1.87
20	102.8	5.27	502.17	0.09
21	112.49	2.86	101.47	0.34
22	102.82	3.35	79.57	3.02
23	86.57	5.51	75.45	4.56
24	102.88	1.87	144.49	5.06
25	121.44	2.54	513.79	1.99
26	148.165	3.33	70.99	5.09
27	96.89	3.76	109.34	0.68
28	266.89	0.92	92.67	5.68
29	165.74	3.90	110.9	3.41
30	109.91	5.72	88.45	5.61
31	144.48	1.60	148.45	2.07
32	164.2	1.99	347.56	5.57
33	111.16	4.98	76.03	3.71

*ED_{50} values (μg/mL), calculated from mean percentage inhibition which is an average of four replicates and its standard deviation (±) ranged from ±0.09 to ± 5.87; Chi Square for Heterogeneity (tabular value at 0.05 level) = 5.99 (degrees of freedom = 3).

2.3. Antifungal activity

Antifungal activity was screened against two fungal species *Aspergillus niger* and *Fusarium oxysporum*. Hexaconazole was used as a standard antifungal agent. All the compounds showed moderate to weak activity against both fungi (Table 2). The results showed that aryldiaminotriazoles **2**–**21** possessed better activity against *F. oxysporum* than *A. niger*. In general, compounds with e-releasing substitutent showed better activity than e-withdrawing substitutent against both the tested fungi except compound **11** (R = 4–OHC_6H_4). Among the triazolothiadiazoles derivatives compound **23** performed better against both fungi as compared to their corresponding *para* and *meta* substitutent. On comparing the triazoles derivatives, five membered cylised products showed good fungicidal activity than the six membered thiadiazines compound. The amide derivatives (**29** and **30**) also showed promising inhibitory effect against both fungi as compared to the parent compound (**1**). The parent compound with free NH_2 group in the 4 position showed the lowest inhibitory effect against both fungi compared to its all the substituted derivatives.

Consequent to the observation of high unit activity against *Pseudomonas aeurogenosa* and *F. oxysporum* QSAR analysis was carried out utilizing the activity data with respect to above two pathogens.

2.3.1. QSAR analysis

The 2D QSAR study requires the calculation of a variety of molecular descriptors (e.g., topological indices, hydrophobic and steric parameters) which are used as independent variables in QSAR modeling [46]. The DRAGON 5.4 software [47] and Marvin Sketch [48] were used to generate the descriptors for model development. In Tables 3 and 4, the calculated values for topological indices and steric parameters along with the LogP have been reported. The following QSAR model, based on antibacterial activity and various physicochemical parameters for hydrophobic and steric properties, were developed using stepwise linear regression analysis technique [49–51]. The predictive ability of the model is discussed on the basis of predictive correlation coefficient (Table 5).

$$MIC_{E.Coli} = -21.641\ \log P(\pm 5.911) + 144.245(\pm 23.022) \quad (1)$$

$$n = 33, r = 0.549, r^2 = 0.302, F = 13.405, SE = 39.43$$

In equation (1), n stands for number of compounds, r is the correlation coefficient, SE the standard error of estimate, F the ratio of correlation respectively. In QSAR equation, the log P value is negatively correlated, indicating that the compounds having high log P values will have less MIC value. Substitutions favoring the lipophilic phase will be leading to increase in activity. The negative contribution of log P values may be due to fact that compounds require to penetrate the bacterial cell wall to interact with the actual target inside the cell.

Initial regression analysis for the antifungal activity indicated that out of 16 descriptors used molecular weight in combination of other physiochemical descriptor plays a dominating role in imparting the activity to the molecule. The intercorelatedness among molecular descriptors with the activity showed that except hydrogen bond acceptor all other physicochemical parameters are mutually well correlated (Table 6). The successive regression analysis indicates that a tri-parametric model including MW, log P and MV yielded still better results (Table 7).

$$EC_{50}\ \text{for FO} = -1.344*MW + 705.423 \quad (2)$$

$$EC_{50}\text{for FO} = -2.047*MW + 27.289*\log P + 897.246 \quad (3)$$

$$EC_{50}\text{for FO} = -2.154*MW + 45.051*\log P + (-0.592)*MV + 1042.814 \quad (4)$$

The mol wt and molar volume have negative correlation with the EC_{50} value *i.e.* increase in molecular weight resulted in decrease in EC_{50} value. The log P has positive correlation with the EC_{50} value *i.e.* increase in log P value resulted in increase in EC_{50} value. Thus steric parameters of the molecules strongly influenced the antifungal activity than hydrophobic and other topological parameters.

3. Conclusions

A series of nalidixic acid based triazole derivatives like triazolothiadiazoles, triazolothiadiazines, arylideneamino triazoles, triazolothiadiazine-6,7-dione were synthesized in good yield and evaluated for *in vitro* antimicrobial activity. The study revealed that all tested compounds showed moderate to good antibacterial activity against pathogenic strains. Among the synthesized compounds, **5** and **23** possessed the most prominent and consistent activity. Most of the compounds showed lower fungicidal activity than the bactericidal activity. Therefore, such compounds would represent a fruitful matrix for the development of a new class of antibacterial agents and with the suitable molecular modification potency of these compounds may be further increased.

Table 3
Physiochemical parameters used in the study.

Compd no.	MW	MR	η	MV	ST	*D*	Pr	Polar 2D SA	Polar 3D SA	Hb D
1	302	87.23	1.79	193.1	63.9	1.56	546	89.93	380.34	2
2	390.46	115.84	1.71	285	54.4	1.36	774	76.27	502.29	0
3	408.45	116.06	1.71	287.8	52.3	1.41	774	76.27	509.24	0
4	424.91	120.65	1.73	294.2	55.4	1.44	802.9	76.27	518.34	0
5	469.36	123.47	1.74	297.5	57	1.57	817.9	76.27	522.53	0
6	469.36	123.47	1.74	207.5	57	1.57	817.6	76.27	522.7	0
7	435.46	123.17	1.75	290.2	63.5	1.5	819.5	122.09	542.88	0
8	435.46	123.17	1.75	290.2	63.5	1.5	819.5	122.09	542.87	0
9	415.47	121.57	1.73	297.8	57.4	1.39	819.8	100.06	519.44	0
10	458.46	121.82	1.66	315	47.7	1.45	828.1	76.27	552.06	0
11	406.46	117.82	1.74	282.2	58.2	1.44	779.7	96.5	513.55	0
12	420.49	122.31	1.7	306.6	52.2	1.37	824.3	85.5	550.44	1
13	434.514	127.06	1.69	322.7	51.1	1.34	862.9	85.5	581.5	0
14	404.49	120.88	1.71	300.1	51.7	1.34	805.1	115.07	538.21	0
15	418.52	125.49	1.68	316.7	50.6	1.32	843.8	76.27	564.97	0
16	432.54	130.03	1.69	331.4	48.4	1.3	874.8	76.27	594.48	0
17	446.18	134.31	1.68	351.9	46.8	1.26	920.8	76.27	629.67	0
18	433.55	130.27	1.67	326.1	50.7	1.32	870.4	79.51	558.19	0
19	436.55	128.6	1.72	314.6	54.4	1.38	854.6	101.57	533.82	0
20	459.35	125.45	1.73	303.5	56.3	1.51	831.7	76.27	532.51	0
21	459.35	125.45	1.73	303.5	56.3	1.51	831.7	76.27	589.07	0
22	388.11	141.55	1.78	262.5	63.3	1.48	740	104.52	490.93	0
23	422.89	146.35	1.79	271.5	64.2	1.55	768.8	104.52	506.52	0
24	422.89	146.35	1.79	271.5	64.2	1.55	768.8	104.52	507.21	0
25	422.89	146.35	1.79	271.5	64.2	1.55	768.8	104.52	507.28	0
26	433.44	148.87	1.82	267.5	74.2	1.61	785.4	150.34	531.77	0
27	457.33	151.16	1.8	280.8	65	1.62	797.7	104.52	522.14	0
28	344.42	113.93	1.87	203.1	72.1	1.69	591.9	104.52	400.08	0
29	344.39	95.61	1.74	229.6	58.1	1.49	633.9	93.01	442.71	1
30	406.46	116.28	1.74	282.2	58.2	1.44	779.7	90.21	513.83	0
31	402.47	118	1.85	205	79.9	1.73	613	101.57	513.83	0
32	356.36	94.33	1.76	278.3	61.2	1.44	778.6	135.38	417.88	1
33	287.34	81.33	1.68	203.7	74.8	1.41	599.6	71.87	357.53	0

MW = Molecular weight, MR = Molar Refractivity, η = Index of Refraction, MV = Molar Volume, ST = Surface Tension, *D* = Density, Pc = Parachor, Polar 2D SA = polar 2D surface area, Polar 3D SA = polar 3D surface area, Hb D = Hydrogen bond donor.

Table 4
Calculated values of distance-based topological indices used in the present study.

Compd no.	*J*	*W*	Sz	1χ	log *P*	POL
1	1.54	848	1461	10.02	1.49	30.52
2	1.38	2024	3177	13.58	3.93	41.74
3	1.32	2266	3539	13.97	4.07	41.41
4	1.32	2266	3539	13.97	4.53	43.68
5	1.33	2266	3495	13.97	4.73	44.49
6	1.32	2244	3539	14.88	4.07	44.49
7	1.24	2744	4191	13.97	3.87	43.64
8	1.21	2810	4323	14.88	3.87	43.64
9	1.26	2537	3930	14.51	3.79	43.57
10	1.37	3085	4718	15.18	4.81	42.8
11	1.32	2266	3539	13.97	3.63	42.36
12	1.26	2537	3930	14.51	3.77	44.27
13	1.37	2838	4351	15.01	4.13	46.11
14	1.32	2266	3539	13.97	4.44	43.2
15	1.26	2537	3930	14.51	4.89	45.34
16	1.21	2810	4323	14.88	5.18	47.18
17	1.37	3085	4718	15.18	5.48	49.03
18	1.21	2810	4323	14.88	4.04	46.56
19	1.26	2537	3930	14.51	4.56	46.62
20	1.29	2468	3819	14.38	5.14	45.65
21	1.31	2424	3731	14.4	5.14	45.66
22	1.21	1988	3414	13.65	3.21	40.48
23	1.18	2181	3719	14.06	3.72	42.45
24	1.17	2203	3763	14.05	3.72	42.44
25	1.16	2225	3807	14.05	3.72	42.43
26	1.08	2693	4521	14.96	3.16	42.41
27	1.14	2399	4072	14.47	4.24	44.44
28	1.47	1093	1889	11.08	1.71	33.6
29	1.64	1245	1993	11.42	1.2	34.52
30	1.37	2184	3391	13.99	3.06	41.95
31	1.18	2161	3838	14.15	2.64	42.56
32	1.36	1388	2504	11.99	0.96	33.68
33	1.58	752	1320	9.6	1.79	29.32

J = Balaban index, *W* = Wiener index, Sz = Szeged index, 1χ = Connectivity index, log *P* = octanol/water partition coefficient, POL = polarzibility.

Table 5
Correlation matrix for antibacterial activity against *P. aeurogenosa* independent variables.

	PA	MW	log *P*	2D SA	3D SA	MR	POL	Hb D	*J*	*W*	Sz	1χ	MV	Pr	*η*	ST	*D*
PA	1																
MW	−0.409	1															
log *P*	−0.549	0.838	1														
2D SA	0.227	−0.097	−0.399	1													
3D SA	−0.375	0.856	0.869	−0.177	1												
MR	−0.371	0.727	0.595	0.214	0.637	1											
POL	−0.449	0.912	0.896	−0.182	0.959	0.713	1										
Hb D	0.302	−0.553	−0.581	0.084	−0.487	−0.557	−0.566	1									
W	0.389	−0.649	−0.473	−0.388	−0.553	−0.844	−0.644	0.469	1								
J	−0.412	0.877	0.827	−0.044	0.945	0.672	0.928	−0.528	−0.646	1							
Sz	−0.382	0.876	0.781	0.054	0.918	0.755	0.913	−0.543	−0.746	0.984	1						
1χ	−0.41	0.922	0.807	−0.024	0.925	0.75	0.948	−0.565	−0.726	0.959	0.967	1					
MV	−0.407	0.639	0.762	−0.167	0.807	0.441	0.767	−0.35	−0.373	0.8	0.744	0.712	1				
Pr	−0.431	0.819	0.82	−0.126	0.866	0.555	0.87	−0.461	−0.501	0.872	0.828	0.848	0.904	1			
η	0.154	−0.198	−0.486	0.593	−0.418	0.187	−0.336	0.103	−0.235	−0.386	−0.262	−0.263	−0.63	−0.568	1		
ST	0.324	−0.427	−0.623	0.521	−0.598	−0.075	−0.541	0.036	−0.086	−0.521	−0.408	−0.465	−0.759	−0.709	0.802	1	
D	0.195	−0.057	−0.39	0.375	−0.405	0.106	−0.322	0.054	−0.146	−0.346	−0.243	−0.235	−0.692	−0.571	0.875	0.784	1

4. Experimental

4.1. Materials and methods

All the chemicals used were purchased from Sigma–Aldrich and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated Merck silica gel $60F_{254}$ and the spots were visualized either under UV or by iodine vapour. Melting points were determined on the Digital Image Processing Tech model EZ-120 automated melting point apparatus and are uncorrected. Infra-red (IR) spectra were recorded on Perkin–Elmer model 2000 FT-IR spectrophotometer as KBr pellet and values are expressed as ν_{max} cm^{-1}. Mass spectra were recorded on a Joel (Japan) JMS-DX303 and micro mass LCT, Mass Spectrometer/Data system. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrospin spectrometer (400 MHz), using tetramethylsilane as an internal standard. The chemical shift values were recorded on δ scale and the coupling constants (*J*) are in Hz. Elemental analysis was performed on a Carlo Erba Model EA-1108 elemental analyzer and data of C, H and N were within ±0.4% of calculated values.

4.2. Synthesis

4.2.1. Synthesis of nalidixic acid methyl ester

To the solution of nalidixic acid (2 g, 8.611 mmol) in tetrahydrofuran (50 mL) anhydrous potassium carbonate (5.96 g, 43.05 mmol) was added and stirred for 1 h. After 1 h dimethyl sulphate (1.63 mL, 12.92 mmol) was added to it and refluxed at 70 °C. After refluxing, the solvent was removed under reduced pressure on a rotary evaporator and the product was extracted with chloroform. The chloroform layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure to give methyl nalidixate (1.95 g, 95%).

4.2.2. Synthesis of nalidixic acid hydrazide

The methyl nalidixate (2 g, 8.17 mmol) was dissolved in tetrahydrofuran (10 mL) and hydrazine hydrate (0.5 mL, 12.25 mmol) was added to it. The contents were refluxed for 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 (1.7g, 85%).

4.2.3. Synthesis of nalidixic acid based 4-amino-3-mercapto-1,2,4-triazole (**1**)

To a solution of potassium hydroxide (10.0 mmol) in absolute ethanol (50 mL), nalidixic acid hydrazide (5.0 mmol) and carbon disulphide (11.0 mmol) was added to it. The mixture was stirred for 4–6 h at room temperature followed by addition of dry acetone. The precipitated potassium dithiocarbazinate was filtered, washed with acetone and dried under vacuum. The potassium salt was obtained in quantitative yield and was used in the next step without further purification. A suspension of the potassium salt (10 mmol) and hydrazine hydrate (15 mmol) was heated under

Table 6
Correlation matrix for antifungal activity against *F. oxysporum*.

	FO	MW	log *P*	2D SA	3D SA	MR	POL	Hb AcP	*J*	*W*	Sz	1χ	MV	Pr	*η*	ST	*D*
FO	1																
MW	−0.823	1															
log *P*	−0.562	0.852	1														
2D SA	0.126	−0.263	−0.481	1													
3D SA	−0.643	0.809	0.878	−0.341	1												
MR	−0.629	0.695	0.591	0.099	0.57	1											
POL	−0.716	0.885	0.907	−0.337	0.947	0.67	1										
Hb AcP	−0.004	−0.125	−0.338	0.618	−0.289	0.352	−0.256	1									
W	0.51	−0.588	−0.439	−0.316	−0.466	−0.806	−0.583	−0.354	1								
J	−0.675	0.839	0.828	−0.177	0.929	0.621	0.91	−0.116	−0.585	1							
Sz	−0.706	0.839	0.78	−0.075	0.894	0.713	0.891	−0.004	−0.7	0.981	1						
1χ	−0.757	0.897	0.815	−0.178	0.903	0.697	0.933	−0.066	−0.674	0.953	0.962	1					
MV	−0.52	0.576	0.734	−0.248	0.782	0.377	0.73	−0.232	−0.298	0.775	0.712	0.671	1				
Pr	−0.676	0.79	0.803	−0.221	0.844	0.504	0.849	−0.224	−0.436	0.852	0.802	0.83	0.89	1			
η	0.279	−0.379	−0.584	0.563	−0.618	0.063	−0.523	0.576	−0.137	−0.571	−0.442	−0.473	−0.766	−0.719	1		
ST	0.211	−0.313	−0.574	0.638	−0.53	0.067	−0.451	0.577	−0.24	−0.442	−0.307	−0.358	−0.725	−0.668	0.957	1	
D	0.111	−0.145	−0.438	0.343	−0.536	0.052	−0.436	0.433	−0.096	−0.466	−0.356	−0.355	−0.783	−0.669	0.884	0.877	1

Table 7
Regression parameters and quality of correlation of the proposed models.

Model	Parameters	*n*	SE	*R*	R^2	*F*
1	MW	29	37.22	0.823	0.667	56.716
2	MW, log *P*	29	33.563	0.865	0.768	38.486
3	MW, log *P*, MV,	29	29.611	0.90	0.811	35.527

reflux for 5 h. Hydrogen sulphide gas was evolved and a homogenous solution was obtained which was diluted with 50 mL of water and acidified with dilute hydrochloric acid to give a white precipitate which was filtered at pump, washed repeatedly with water (3 × 10 mL) and dried to yield 3-(4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1*H*)-one (1).

*4.2.3.1. 3-(4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**1**).* Pale Yellow solid; M.p.: >300 °C; Yield: 88%; ^{1}H NMR (δ): 1.49 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7−CH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 5.70 (s, 2H, NH_2), 7.45 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 8.50 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.67 (s, 1H, H-2-naphthyridine), 13.87 (s, 1H, SH); ^{13}C NMR (δ): 15.42, 25.38, 46.03, 108.57, 119.36, 121.77, 136.00, 146.28, 147.28, 148.30, 163.63, 164.48, 174.78; IR (KBr, cm^{-1}) ν_{max}: 3287, 3053, 2358, 1635, 1609, 1537, 1439, 1292, 802; HRMS calculated for $C_{13}H_{14}N_6OS$: 302.0950, Observed 302.1870 [M^+].

*4.2.4. General synthesis of nalidixic acid based Schiff bases of 4-amino-3-mercapto-1,2,4-triazole (**2**–**21**)*

A suspension of substituted benzaldehyde (5 mmol) in dioxane (10 mL) along with triazole (1, 5 mmol) was heated until a clear solution was obtained. A few drops of conc. sulfuric acid were added as a catalyst and the solution was refluxed for 3 h on a water bath. After the completion, the reaction was quenched with water and cooled. The precipitated solid was filtered, thoroughly washed with water and dried.

*4.2.4.1. 3-{4-(Benzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H}-one (**2**) [52].* Yellow solid; M.p.: 298 °C; Yield: 80%; ^{1}H NMR (δ): 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7−CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.45−7.82 (m, 5H, Ar-H), 8.25 (d, *J* = 7.6 Hz, 1H, H-5-naphthyridine), 8.92 (s, 1H, H-2-naphthyridine), 9.07 (s, 1H, N]$\underline{CH}$), 13.25 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2932, 2348, 1670, 1603, 1525, 1479, 1250, 790; MS calculated for $C_{20}H_{18}N_6OS$: 390.1263, Observed ESI-MS (*m*/*z*): 390.52 [M^+].

*4.2.4.2. 1-Ethyl-3-{4-(4-fluorobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**3**).* White solid; M.p.: 192 °C; Yield: 78%; ^{1}H NMR (δ): 1.54 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7−CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.14 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.81 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.22 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.68 (s, 1H, H-2-naphthyridine), 9.04 (s, 1H, N]$\underline{CH}$), 13.26 (s, 1H, SH); ^{13}C NMR (δ): 15.34, 25.25, 47.14, 115.88, 115.92 (2C), 116.14, 120.16, 121.60, 129.64, 129.72, 130.28 (2C), 136.30, 147.17, 147.97, 160.86, 161.62, 163.70, 176.75; IR (KBr, cm^{-1}) ν_{max}: 2954, 2541, 1680, 1603, 1533, 1506, 1443, 1229, 829; MS calculated for $C_{20}H_{17}FN_6OS$: 408.1169, Observed ESI-MS (*m*/*z*): 408.42 [M^+].

*4.2.4.3. 3-{4-(4-Chlorobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**4**) [52].* Yellow solid; M.p.: 180.5 °C; Yield: 75%; ^{1}H NMR (δ): 1.55 (t, 3H, N-$CH_2\underline{CH_3}$), 2.69 (s, 3H, 7−CH_3), 4.59 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.38 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.76 (d, *J* = 8.4 Hz, 2H, Ar-H), 8.21 (s, 1H, H-2-naphthyridine), 8.68 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 9.05 (s, 1H, N]$\underline{CH}$), 13.19 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2914, 2378, 1607, 1558, 1432, 1252, 791; MS calculated for $C_{20}H_{17}ClN_6OS$: 424.0873, Observed ESI-MS (*m*/*z*): 424.22 [M^+], 426.36.

*4.2.4.4. 3-{4-(3-bromobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**5**).* Yellow solid; M.p.: 188.3 °C; Yield: 72%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7−CH_3), 4.54 (q, 2H, N-$\underline{CH_2}$), 7.25 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.30−7.33 (m, 1H, Ar-H), 7.45 (dd, *J* = 7.2 Hz, 1H, Ar-H), 7.99−8.19 (m, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 8.50 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.64 (s, 1H, H-2-naphthyridine), 8.94 (s, 1H, N]$\underline{CH}$), 13.28 (s, 1H, SH); ^{13}C NMR (δ): 15.09, 25.11, 47.35, 119.43, 121.19, 123.06, 125.24, 127.45, 128.40, 130.22, 132.79, 133.99, 135.77, 137.78, 146.25, 148.32, 148.73, 160.67, 163.33, 178.52; IR (KBr, cm^{-1}) ν_{max}: 2888, 2385, 1624, 1607, 1566, 1441, 1254, 791; MS calculated for $C_{20}H_{17}BrN_6OS$: 468.0368, Observed ESI-MS (*m*/*z*): 469.85 [M^+ + 1], 471.58.

*4.2.4.5. 3-{4-(4-Bromobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**6**) [52].* Yellow solid; M.p.: 217.5 °C; Yield: 75%; ^{1}H NMR (δ): 1.49 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7−CH_3), 4.52 (q, 2H, N-$\underline{CH_2}$), 7.31 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.55 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.72 (d, *J* = 7.6 Hz, 2H, Ar-H), 8.35 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.82 (s, 1H, H-2-naphthyridine), 9.06 (s, 1H, N]$\underline{CH}$), 13.28 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2954, 2382, 1626, 1585, 1497, 1256, 794; MS calculated for $C_{20}H_{17}BrN_6OS$: 468.0368, Observed ESI-MS (*m*/*z*): 468.22 [M^+], 470.44.

*4.2.4.6. 1-Ethyl-3-{5-mercapto-4-(3-nitrobenzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**7**).* Yellow solid; M.p.: 258.6 °C; Yield: 73%; ^{1}H NMR (δ): 1.57 (t, 3H, N−$CH_2\underline{CH_3}$), 2.73 (s, 3H, 7−CH_3), 4.62 (q, 2H, N-$\underline{CH_2}$), 7.33 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.78−7.80 (m, 1H, Ar-H), 8.18−8.24 (m, 1H, Ar-H), 8.35 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.52−8.55 (m, 1H, Ar-H), 8.73 (s, 1H, Ar-H), 8.74 (s, 1H, H-2-naphthyridine), 9.12 (s, 1H, N]$\underline{CH}$), 13.14 (s, 1H, SH); ^{13}C NMR (δ): 15.34, 25.27, 47.24, 111.73, 120.14, 121.76, 123.26, 125.80, 128.25, 129.62, 130.28, 132.95, 134.17, 136.33, 145.54, 148.70, 148.16, 160.55, 163.87, 176.76; IR (KBr, cm^{-1}) ν_{max}: 2926, 2402, 1628, 1614, 1530, 1440, 1255, 805; MS calculated for $C_{20}H_{17}N_7O_3S$: 435.1114, Observed ESI-MS (*m*/*z*): 436.42 [M^+ + 1].

*4.2.4.7. 1-Ethyl-3-{5-mercapto-4-(4-nitrobenzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**8**).* Yellow solid; M.p.: 189.7 °C; Yield: 77%; ^{1}H NMR (δ): 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7−CH_3), 4.61 (q, 2H, N-$\underline{CH_2}$), 7.37 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.98 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.34 (d, *J* = 8.8 Hz, 2H, Ar-H), 8.68 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.72 (s, 1H, H-2-naphthyridine), 9.05 (s, 1H, N]$\underline{CH}$), 13.40 (s, 1H, SH); IR (KBr, cm−1) ν_{max}: 2939, 2343, 1613, 1520, 1443, 1256, 795; MS calculated for $C_{20}H_{17}N_7O_3S$: 435.1114, Observed ESI-MS (*m*/*z*): 436.1 [M^+ + 1].

*4.2.4.8. 4-{(3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-mercapto-4H-1,2,4-triazol-4-ylimino)methyl}benzonitrile (**9**).* Yellow solid; M.p.: 236.7 °C; Yield: 77%; ^{1}H NMR (δ): 1.47 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7−CH_3), 4.55 (q, 2H, N-$\underline{CH_2}$), 7.34 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.52 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.81 (d, *J* = 8.0 Hz, 2H, Ar-H), 8.44 (s, 1H, H-2-naphthyridine), 8.55 (d, *J* = 8.4 Hz, 1H, H-5-naphthyridine), 9.06 (s, 1H, N]$\underline{CH}$), 13.30 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 3242, 2343, 1623, 1568, 1440, 1256, 790; MS calculated for $C_{21}H_{17}N_7OS$: 415.1215, Observed ESI-MS (*m*/*z*): 416.2 [M^+ + 1].

4.2.4.9. 1-Ethyl-3-{5-mercapto-4-(4-(trifluoromethyl)benzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one

(**10**). Pale yellow solid; M.p.: 226.4 °C; Yield: 70%; ^{1}H NMR (δ): 1.49 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7–CH_3), 4.62 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.65 (d, J = 8.4 Hz, 2H, Ar-H), 7.92 (d, J = 8.0 Hz, 2H, Ar-H), 8.27 (s, 1H, H-2-naphthyridine), 8.68 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.05 (s, 1H, N]$\underline{CH}$), 13.30 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2949, 2342, 1682, 1612, 1532, 1444, 1256, 797; MS calculated for $C_{21}H_{17}F_3N_6OS$: 458.1137, Observed ESI-MS (m/z): 458.4 [M$^+$].

4.2.4.10. 1-Ethyl-3-{4-(4-hydroxybenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-7-methyl –1,8-naphthyridin-4(1H)-one (**11**). Yellow solid; M.p.: >300 °C; Yield: 68%; ^{1}H NMR (δ): 1.51 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7–CH_3), 4.57 (q, 2H, N-$\underline{CH_2}$), 6.96 (d, J = 8.4 Hz, 2H, Ar-H), 7.30 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.65 (d, J = 8.4 Hz, 2H, Ar-H), 8.13 (s, 1H, H-2-naphthyridine), 8.68 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.99 (s, 1H, OH), 9.01 (s, 1H, N]$\underline{CH}$), 13.07 (s, 1H, SH); ^{13}C NMR (δ): 15.34, 25.3, 46.8, 113.3, 116.02 (2C), 119.01, 120.6, 122.0, 125.47, 129.70 (2C), 136.7, 145.9, 147.65, 148.95, 156.5, 160.33, 163.0, 176.22; IR (KBr, cm^{-1}) ν_{max}: 3324, 2935, 2363, 1660, 1607, 1515, 1444, 1253, 796; MS calculated for $C_{20}H_{18}N_6O_2S$: 406.1212, Observed ESI-MS (m/z): 407.3 [M$^+$ + 1].

4.2.4.11. 1-Ethyl-3-{5-mercapto-4-(4-methoxybenzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl –1,8-naphthyridin-4(1H)-one (**12**) [52]. Yellow solid; M.p.: 163.1 °C; Yield: 85%; ^{1}H NMR (δ): 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.72 (s, 3H, 7–CH_3), 3.81 (s, 3H, OCH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 6.86 (d, J = 8.0 Hz, 2H, Ar-H), 7.39 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.82 (d, J = 8.0 Hz, 2H, Ar-H), 8.24 (s, 1H, H-2-naphthyridine), 8.64 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.95 (s, 1H, N]$\underline{CH}$), 13.07 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 3043, 2366, 1672, 1612, 1528, 1424, 1256, 795; MS calculated for $C_{21}H_{20}N_6O_2S$: 420.1368, Observed ESI-MS (m/z): 420.5 [M$^+$].

4.2.4.12. 3-{4-(4-ethoxybenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**13**). Yellow solid; M.p.: 207.7 °C; Yield: 73%; ^{1}H NMR (δ): 1.35 (t, 3H, O-$CH_2\underline{CH_3}$), 1.40 (t, 3H, N-$CH_2\underline{CH_3}$), 2.67 (s, 3H, 7–CH_3), 4.07 (q, 2H, O-$\underline{CH_2}$), 4.52 (q, 2H, N-$\underline{CH_2}$), 6.99 (d, J = 8.8 Hz, 2H, Ar-H), 7.52 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.68 (d, J = 8.8 Hz, 2H, Ar-H), 8.33 (s, 1H, H-2-naphthyridine), 8.59 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.08 (s, 1H, N]$\underline{CH}$), 12.97 (s, 1H, SH); ^{13}C NMR (δ): 15.04, 15.50, 25.37, 47.10, 63.72, 112.0, 115.17 (2C), 118.40, 122.32, 127.24, 129.34 (2C), 130.57, 135.41, 136.43, 148.54, 148.96, 151.35, 160.82, 164.00, 173.46; IR (KBr, cm^{-1}) ν_{max}: 2980, 2359, 1607, 1514, 1439, 1250, 834; MS calculated for $C_{22}H_{22}N_6O_2S$: 434.1525, Observed ESI-MS (m/z): 434.1 [M$^+$].

4.2.4.13. 1-Ethyl-3-{5-mercapto-4-(4-methylbenzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**14**) [52]. Yellow solid; M.p.: 174.0 °C; Yield: 71%; ^{1}H NMR (δ): 1.54 (t, 3H, N-$CH_2\underline{CH_3}$), 2.41 (s, 3H, CH_3), 2.72 (s, 3H, 7–CH_3), 4.61 (q, 2H, N-$\underline{CH_2}$), 6.99 (d, J = 7.6 Hz, 2H, Ar-H), 7.33 (d, J = 7.6 Hz, 1H, H-6-naphthyridine), 7.53 (d, J = 7.6 Hz, 2H, Ar-H), 8.14 (s, 1H, H-2-naphthyridine), 8.69 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 8.89 (s, 1H, N]$\underline{CH}$), 12.93 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2957, 2345, 1623, 1583, 1440, 1254, 795; MS calculated for $C_{21}H_{20}N_6OS$: 4045.1419, Observed ESI-MS (m/z): 404.8 [M$^+$].

4.2.4.14. 1-Ethyl-3-{4-(4-ethylbenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**15**). Yellow solid; M.p.: 192.5 °C; Yield: 80%; ^{1}H NMR (δ): 1.27 (t, 3H, CH_3), 1.52 (t, 3H, N-$CH_2\underline{CH_3}$), 2.65 (q, 2H, CH_2), 2.70 (s, 3H, 7–CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.24 (d, J = 8.0 Hz, 2H, Ar-H), 7.33 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.73 (d, J = 8.4 Hz, 2H, Ar-H), 8.20 (s, 1H, H-2-naphthyridine), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.03 (s, 1H, N]$\underline{CH}$), 13.09 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2985, 2357, 1633, 1608, 1585, 1441, 1253, 794; MS calculated for $C_{22}H_{22}N_6OS$: 418.1576, Observed ESI-MS (m/z): 419.3 [M$^+$ + 1].

4.2.4.15. 1-Ethyl-3-{4-(4-isopropylbenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**16**). Yellow solid; M.p.: 174.4 °C; Yield: 82%; ^{1}H NMR (δ): 1.24 (d, 6H, $2CH_3$), 1.51 (t, 3H, N-$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7–CH_3), 2.90 (heptet, 1H, CH), 4.58 (q, 2H, N-$\underline{CH_2}$), 7.24 (d, J = 8.0 Hz, 2H, Ar-H), 7.31 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.72 (d, J = 8.4 Hz, 2H, Ar-H), 8.18 (s, 1H, H-2-naphthyridine), 8.64 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.01 (s, 1H, N]$\underline{CH}$), 13.06 (s, 1H, SH), ^{13}C NMR (δ): 15.31, 23.79 (2C), 25.25, 34.13, 47.08, 112.20, 120.14, 121.53, 126.91 (2C), 127.94 (2C), 128.62, 131.61, 136.29, 147.91, 148.49, 151.49, 152.45, 161.45, 163.60, 176.73; IR (KBr, cm^{-1}) ν_{max}: 2956, 2353, 1675, 1606, 1531, 1442, 1255, 796; MS calculated for $C_{23}H_{24}N_6OS$: 432.1732, Observed ESI-MS (m/z): 432.1 [M$^+$].

4.2.4.16. 3-{4-(4-tert-Butylbenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**17**). Yellow solid; M.p.: 158.2 °C; Yield: 80%; ^{1}H NMR (δ): 1.35 (s, 9H, $3CH_3$), 1.39 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7–CH_3), 4.59 (q, 2H, N-$\underline{CH_2}$), 7.44 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.49 (d, J = 8.0 Hz, 2H, Ar-H), 7.67 (d, J = 8.4 Hz, 2H, Ar-H), 8.34 (s, 1H, H-2-naphthyridine), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 9.06 (s, 1H, N]$\underline{CH}$), 13.01 (s, 1H, SH); ^{13}C NMR (δ): 15.87, 25.62, 31.14 (3C), 34.33, 47.55, 111.74, 118.78, 121.75, 126.03 (2C), 127.55 (2C), 129.94 132.01, 136.17, 146.19, 148.51, 148.93, 156.17, 160.94, 163.95, 176.14; IR (KBr, cm^{-1}) ν_{max}: 2948, 2329, 1690, 1613, 1533, 1440, 1250, 793; MS calculated for $C_{24}H_{26}N_6OS$: 446.1889; Observed ESI-MS (m/z): 446.5 [M$^+$].

4.2.4.17. 3-{4-(4-(Dimethylamino)benzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**18**). Red-orange solid; M.p.: 252.8 °C; Yield: 88%; ^{1}H NMR (δ): 1.51 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7–CH_3), 3.68 (s, 6H, $2CH_3$), 4.53 (q, 2H, N-$\underline{CH_2}$), 6.68 (d, J = 8.0 Hz, 2H, Ar-H), 7.32 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.42 (d, J = 8.4 Hz, 2H, Ar-H), 8.29 (s, 1H, H-2-naphthyridine), 8.60 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.94 (s, 1H, N]$\underline{CH}$), 13.61 (s, 1H, SH); ^{13}C NMR (δ): 15.12, 25.28, 46.51, 66.90 (2C), 108.74, 109.43 (2C), 119.54, 121.21 (2C), 122.30, 125.63, 129.51, 135.88, 136.01, 144.33, 146.18, 148.29, 148.59, 163.38, 175.24; IR (KBr, cm^{-1}) ν_{max}: 2930, 2385, 1621, 1567, 1479, 1255, 791; MS calculated for $C_{22}H_{23}N_7OS$: 433.1685; Observed ESI-MS (m/z): 433.2 [M$^+$], 434.4 [M$^+$ + 1].

4.2.4.18. 1-Ethyl-3-{5-mercapto-4-(4-(methylthio)benzylideneamino)-4H-1,2,4-triazol-3-yl}-7-methyl-1,8-naphthyridin-4(1H)-one (**19**). Yellow solid; M.p.: 214.9 °C; Yield: 82%; ^{1}H NMR (δ): 1.51 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7–CH_3), 2.53 (s, 3H, S-CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.24 (d, J = 8.4 Hz, 2H, Ar-H), 7.34 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 7.72 (d, J = 8.4 Hz, 2H, Ar-H), 8.18 (s, 1H, H-2-naphthyridine), 8.66 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.03 (s, 1H, N]$\underline{CH}$), 13.12 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2902, 2376, 1688, 1610, 1530, 1441, 1254, 796; MS calculated for $C_{21}H_{20}N_6OS_2$: 436.1140; Observed ESI-MS (m/z): 436.2 [M$^+$].

4.2.4.19. 3-{4-(2,4-dichlorobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**20**). Yellow solid; M.p.: 275.0 °C; Yield: 82%; ^{1}H NMR (δ): 1.49 (t, 3H, N-$CH_2\underline{CH_3}$), 2.69 (s, 3H, 7–CH_3), 4.60 (q, 2H, N-$\underline{CH_2}$), 7.27 (d, J = 7.2 Hz, 1H, Ar-H), 7.35 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.82 (s, 1H, Ar-H), 8.16 (d, J = 7.2 Hz, 1H, Ar-H), 8.21 (s, 1H, H-2-naphthyridine), 8.57 (d, J = 8.4 Hz, 1H, H-5-naphthyridine), 9.03

(s, 1H, N]CH), 13.15 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2892, 2386, 1632, 1609, 1585, 1494, 1253, 794; MS calculated for $C_{20}H_{16}Cl_2N_6OS$: 458.0482; Observed ESI-MS (*m*/*z*): 458.4 [M^+], 460.2, 462.7.

*4.2.4.20. 3-{4-(2,6-Dichlorobenzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**21**).* Yellow solid; M.p.: 135.6 °C; Yield: 68%; ^{1}H NMR (δ): 1.48 (t, 3H, N-CH_2CH_3), 2.69 (s, 3H, 7–CH_3), 4.58 (q, 2H, N-CH_2), 7.35 (d, *J* = 8.0 Hz, 1H, H-6-naphthyridine), 7.53–7.75 (m, 3H, Ar-H), 8.16 (s, 1H, H-2-naphthyridine), 8.62 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 9.01 (s, 1H, N]CH), 13.10 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 2986, 2369, 1611, 1525, 1435, 1254, 793; MS calculated for $C_{20}H_{16}Cl_2N_6OS$: 458.0482; Observed ESI-MS (*m*/*z*): 458.2 [M^+], 460.4, 462.1.

*4.2.5. General synthesis of nalidixic acid based 1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-6-yl (**22**–**27**)*

A mixture of 4-amino-5-substituted-1,2,4-triazole-3-thiol (1, 5 mmol) and polyphosphoric acid (20 mL) was heated to 50–60 °C with stirring. Substituted benzoic acid (6 mmol) was added portion wise. The mixture was then heated at 180–200 °C for 3 h with stirring. After completion of the reaction, the reaction mixture was poured into ice and neutralized with concentrated aqueous ammonia solution. The crude product filtered, washed with water and recrystallized from hexane/ethyl acetate to give 4-(3-substituted-1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazole) derivatives.

*4.2.5.1. 1-Ethyl-7-methyl-3-(6-phenyl-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazol-3-yl)-1,8-naphthyridin-4(1H)-one (**22**).* Brown solid; M.p.: 122.3 °C; Yield: 78%; ^{1}H NMR (δ): 1.51 (t, 3H, N-CH_2CH_3), 2.70 (s, 3H, 7–CH_3), 4.60 (q, 2H, N-CH_2), 7.25 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.28 (dd, *J* = 5.6 Hz, 1H, Ar-H) 7.30–7.35 (m, 2H, H-3, Ar-H), 8.24–8.27 (m, 2H, Ar-H), 8.65 (d, *J* = 8.4 Hz, 1H, H-5-naphthyridine), 9.01 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.02, 25.23, 47.34, 111.31, 119.52, 121.66, 122.00, 128.50, 129.32, 130.20, 131.67, 133.81, 136.28, 148.59, 151.36, 163.78, 164.82, 168.25, 172.14, 176.26; IR (KBr, cm^{-1}) ν_{max}: 2962, 1693, 1652, 1574, 1452, 1272, 801; MS calculated for $C_{20}H_{16}N_6OS$: 388.1106; Observed ESI-MS (*m*/*z*): 388.4 [M^+].

*4.2.5.2. 3-{6-(2-Chlorophenyl)-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**23**).* Brown solid; M.p.: 195.6 °C; Yield: 70%; ^{1}H NMR (δ): 1.58 (t, 3H, N-CH_2CH_3), 2.65 (s, 3H, 7–CH_3), 4.60 (q, 2H, N-CH_2), 7.40 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.63–7.67 (m, 2H, Ar-H) 7.68–7.70 (m, 1H, Ar-H), 8.10 (dd, *J* = 8.8 Hz, 1H, Ar-H), 8.45 (d, *J* = 8.4 Hz, 1H, H-5-naphthyridine), 8.71 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.34, 25.55, 47.76, 111.38, 119.59, 121.17, 122.96, 128.71, 129.02, 129.51, 130.07, 131.42, 136.65, 145.35, 148.05, 160.70, 163.12, 168.49, 174.45, 176.20; IR (KBr, cm^{-1}) ν_{max}: 2874, 1612, 1549, 1481, 1445, 1340, 792; MS calculated for $C_{20}H_{15}ClN_6OS$: 422.0717; Observed ESI-MS (*m*/*z*): 423.2 [$M^+ + 1$], 425.7.

*4.2.5.3. 3-{6-(3-Chlorophenyl)-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**24**).* Yellow-brown solid; M.p.: 110.3 °C; Yield: 71%; ^{1}H NMR (δ): 1.55 (t, 3H, N-CH_2CH_3), 2.70 (s, 3H, 7–CH_3), 4.58 (q, 2H, N-CH_2), 7.28 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.40–7.44 (m, 1H, Ar-H), 7.53–7.55 (m, 1H, Ar-H), 8.13 (dd, *J* = 1.6 Hz, 1H, Ar-H), 8.40 (s, 1H, Ar-H), 8.71 (d, *J* = 8.4 Hz, 1H, H-5-naphthyridine), 8.80 (s, 1H, H-2-naphthyridine); IR (KBr, cm^{-1}) ν_{max}: 2853, 1614, 1547, 1435, 1253, 789; MS calculated for $C_{20}H_{15}ClN_6OS$: 422.0717; Observed ESI-MS (*m*/*z*): 422.1 [M^+], 424.3.

*4.2.5.4. 3-{6-(4-Chlorophenyl)-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**25**).* Brown solid; M.p.: 200.5 °C; Yield: 70%; ^{1}H NMR (δ): 1.54 (t, 3H, N-CH_2CH_3), 2.69 (s, 3H, 7–CH_3), 4.59 (q, 2H, N-CH_2), 7.30 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.55 (dd, *J* = 8.0 Hz, 2H, Ar-H) 8.10 (d, 2H, Ar-H), 8.58 (d, *J* = 8.0 Hz, 1H, H-5-naphthyridine), 8.70 (s, 1H, H-2-naphthyridine); IR (KBr, cm^{-1}) ν_{max}: 2977, 1608, 1541, 1433, 1250, 789; MS calculated for $C_{20}H_{15}ClN_6OS$: 422.0717; Observed ESI-MS (*m*/*z*): 422.1 [M^+], 424.2.

*4.2.5.5. 1-Ethyl-7-methyl-3-{6-(3-nitrophenyl)-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl}-1, 8-naphthyridin-4(1H)-one (**26**).* Yellow-brown solid; M.p.: 150.0 °C; Yield: 81%; ^{1}H NMR (δ): 1.54 (t, 3H, N-CH_2CH_3), 2.71 (s, 3H, 7–CH_3), 4.60 (q, 2H, N-CH_2), 7.28 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 7.60–7.64 (m, 1H, Ar-H), 8.13 (dd, *J* = 1.2 Hz, 1H, Ar-H), 8.16 (dd, *J* = 1.2 Hz, 1H, Ar-H), 8.48 (s, 1H, Ar-H), 8.65 (d, *J* = 8.4 Hz, 1H, H-5-naphthyridine), 8.85 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.21, 25.23, 47.04, 111.02, 119.43, 121.57, 125.14, 127.53, 128.88, 129.47, 130.20, 132.55, 133.75, 134.54, 136.84, 143.10, 147.33, 162.39, 166.90, 172.15; IR (KBr, cm^{-1}) ν_{max}: 2977, 1682, 1598, 1452, 1281, 777; MS calculated for $C_{20}H_{15}N_7O_3S$: 433.0957; Observed ESI-MS (*m*/*z*): 433.1 [M^+].

*4.2.5.6. 3-{6-(2,3-Dichlorophenyl)-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl}-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (**27**).* Yellow-brown solid; M.p.: 119.2 °C; Yield: 71%; ^{1}H NMR (δ): 1.53 (t, 3H, N-CH_2CH_3), 2.70 (s, 3H, 7–CH_3), 4.58 (q, 2H, N-CH_2), 7.32 (d, *J* = 7.2 Hz, 1H, H-6-naphthyridine), 7.39–7.44 (m, 1H, Ar-H), 7.55–7.67 (m, 1H, Ar-H), 7.91–7.99 (m, 1H, Ar-H), 8.64 (d, *J* = 7.6 Hz, 1H, H-5-naphthyridine), 8.81 (s, 1H, H-2-naphthyridine); ^{13}C NMR (δ): 15.14, 25.06, 46.29, 108.54, 120.82, 121.38, 124.77, 125.34, 127.80, 129.54, 130.05, 132.86, 134.44, 136.26, 144.94, 148.71, 162.01, 163.18, 173.47, 174.34; IR (KBr, cm^{-1}) ν_{max}: 2875, 1632, 1556, 1439, 1255, 790; MS calculated for $C_{20}H_{14}Cl_2N_6OS$: 456.0327; Observed ESI-MS (*m*/*z*): 456.2 [M^+], 458.1, 460.1.

*4.2.6. Synthesis of 3-substituted-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazole-6-thioles (**28**)*

A solution of 4-amino-5-substituted-1,2,4-triazole-3-thiol (1, 5 mmol) in pyridine (20 mL) was taken and carbon disulfide (8 mmol) with few drops of triethylamine were added to the reaction mixture. The reaction mixture was refluxed at 100 °C with stirring for 6 h. Then the reaction mixture was cooled, poured into ice-cold water and acidified with dil. HCl till the solution was slightly acidic. The solid product was obtained which was filtered, washed with water and dried to yield the 1-ethyl-3-(6-mercapto-1,2,4-triazolo [3,4-*b*] [1,3,4]thiadiazol-3-yl)-7-methyl-1,8-naphthyridin-4(1*H*)-one.

*4.2.6.1. 1-Ethyl-3-(6-mercapto-1,2,4-triazolo [3,4-b] [1,3,4]thiadiazol-3-yl)-7-methyl-1,8-naphthyridin-4 (1H)-one (**28**).* Yellow solid; M.p.: 273.0 °C; Yield: 72%; ^{1}H NMR (δ): 1.25 (t, 3H, N-CH_2CH_3), 2.69 (s, 3H, 7–CH_3), 4.57 (q, 2H, N-CH_2), 7.56 (d, *J* = 8.4 Hz, 1H, H-6-naphthyridine), 8.60 (d, *J* = 7.6 Hz, 1H, H-5-naphthyridine), 8.69 (s, 1H, H-2-naphthyridine), 13.06 (s, 1H, SH); ^{13}C NMR (δ): 15.40, 25.38, 46.64, 113.4, 120.9, 129.59, 130.7, 136.7, 145.6, 160.2, 163.34, 166.76, 174.17, 184.6; IR (KBr, cm^{-1}) ν_{max}: 2863, 2531, 1644, 1611, 1442, 1255, 796; MS calculated for $C_{14}H_{12}N_6OS_2$: 344.0514; Observed ESI-MS (*m*/*z*): 345.4 [$M^+ + 1$].

*4.2.7. Synthesis of N-{3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-mercapto-4H-1,2,4-triazol-4-yl}acetamide (**29**)*

To a solution of 4-amino-4*H*-1,2,4-triazole-3-thiol (5 mmol) in glacial acetic acid (3 mL), acetic anhydride (10 mmol) was added to it and refluxed for 2 h. After refluxing the reaction mixture was cooled and poured into ice-cold water. The solid product formed

was filtered, washed with water and oven dried to give the title compound.

White solid; M.p.: 198.4 °C; Yield: 66%; ^{1}H NMR (δ): 1.53 (t, 3H, N-$CH_2\underline{CH_3}$), 2.18 (s, 3H, CH_3), 2.70 (s, 3H, 7–CH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 7.33 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.67 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.83 (s, 1H, H-2-naphthyridine), 9.05 (s, 1H, NH), 12.78 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 3441, 2362, 1687, 1603, 1531, 1445, 1254, 796; MS calculated for $C_{14}H_{16}N_6O_2S$: 344.1055; Observed ESI-MS (m/z): 345.2 [$M^+ + 1$].

4.2.8. Synthesis of N-{3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5-mercapto-4H-1,2,4-triazol-4-yl}benzamide (**30**)

To a solution of 1,2,4-triazole (1, 5 mmol) in acetone and pyridine, benzoyl chloride (6 mmol) was slowly added to it and refluxed for 6–8 h. The reaction was quenched by adding water to it. A solid separated out which was treated with aqueous sodium bicarbonate solution for the removal of excess of benzoic acid. The crude product was filtered, washed with water and oven dried to get the desired product.

White solid; M.p.: 192.5 °C; Yield: 66%; ^{1}H NMR (δ): 1.33 (t, 3H, N-$CH_2\underline{CH_3}$), 2.61 (s, 3H, 7–CH_3), 4.40 (q, 2H, N-$\underline{CH_2}$), 7.13 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.16–7.20 (m, 2H, Ar-H) 7.33–7.38 (m, 1H, Ar-H), 8.07–8.12 (m, 2H, Ar-H), 8.42 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.68 (s, 1H, H-2-naphthyridine), 10.10 (s, 1H, NH), 13.53 (s, 1H, SH); IR (KBr, cm^{-1}) ν_{max}: 3417, 3059, 2324, 1625, 1606, 1573, 1253, 795; MS calculated for $C_{20}H_{18}N_6O_2S$: 406.1212; Observed ESI-MS (m/z): 406.1 [M^+].

4.2.9. Synthesis of 1-ethyl-7-methyl-3-(6-phenyl-7H-1,2,4-triazolo [3,4-b] [1,3,4] thiadiazin-3-yl)-1,8-naphthyridin-4(1H)-one (**31**)

A mixture of triazole (5 mmol), anhydrous potassium carbonate (11.5 mmol) and phenacyl bromide (5 mmol) in dry acetonitrile (25 mL) was refluxed for 6–7 h. The reaction mixture was poured into crushed ice. Solid product obtained was filtered, washed with water and oven dried.

White solid; M.p.: >300.0 °C; Yield: 75%; ^{1}H NMR (δ): 1.35 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7–CH_3), 4.55 (q, 2H, N-$\underline{CH_2}$), 4.65 (s, 2H, CH_2), 7.35 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 7.41–7.71 (m, 5H, Ar-H), 8.66 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.78 (s, 1H, H-2-naphthyridine); IR (KBr, cm^{-1}) ν_{max}: 2928, 1673, 1609, 1442, 1255, 797; MS calculated for $C_{21}H_{18}N_6OS$: 402.1263; Observed ESI-MS (m/z): 402.1 [M^+].

4.2.10. Synthesis of 3-(1-ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridin-3-yl)-5H-[1,2,4]-triazolo [3,4-b] [1,3,4]-thiadiazine-6,7-dione (**32**)

A mixture of **1** (1 mmol) and oxalyl chloride (1.2 mmol) in dry benzene (10 mL) was heated under reflux for 6 h. The reaction mixture was cooled to room temperature. The solvent was removed under reduced pressure, the solid product obtained was filtered and recrystallized from ethanol to afford the final product.

White solid; M.p.: 180.3 °C; Yield: 70%; ^{1}H NMR (δ): 1.50 (t, 3H, N-$CH_2\underline{CH_3}$), 2.70 (s, 3H, 7–CH_3), 4.58 (q, 2H, N-$\underline{CH_2}$), 7.35 (d, J = 8.0 Hz, 1H, H-6-naphthyridine), 8.57 (d, J = 7.6 Hz, 1H, H-5-naphthyridine), 8.29 (s, 1H, H-2-naphthyridine), 8.98 (s, 1H, NH); ^{13}C NMR (δ): 15.17, 25.21, 46.32, 109.44, 119.99, 120.76, 128.93, 131.56, 133.91, 136.42, 148.41, 149.02, 162.81, 174.57, 184.24; IR (KBr, cm^{-1}) ν_{max}: 3248, 3056, 1678, 1609, 1580, 1442, 1255, 790; MS calculated for $C_{15}H_{12}N_6O_3S$: 356.0692; Observed ESI-MS (m/z): 356.1 [M^+].

4.2.11. Synthesis of 1-ethyl-3-(5-mercapto-4H-1,2,4-triazol-3-yl)-7-methyl-1,8-naphthyridin-4(1H)-one (**33**)

A mixture of nalidixic acid hydrazide (5 mmol), potassium thiocyanate (10 mmol), conc. HCl (10 mL) and water (20 mL) was refluxed for 3 h. After cooling, the resulting nalidixic acid thiosemicarbazide was filtered, washed with water and dried. It was recrystallized from ethanol. A mixture of nalidixic acid thiosemicarbazide (5 mmol) and 10% KOH (50 mL) was then refluxed for 3 h. The mixture was then cooled to room temperature and filtered to remove the unwanted impurities. The filtrate was neutralized by the gradual addition of glacial acetic acid. The resulting solid 5-mercapto-4*H*-1,2,4-triazole (**33**) was filtered, washed with water, dried and recrystallized from ethanol.

Pale Yellow solid; M.p.: >300 °C; Yield: 82%; ^{1}H NMR (δ): 1.37 (t, 3H, N-$CH_2\underline{CH_3}$), 2.68 (s, 3H, 7–CH_3), 4.48 (q, 2H, N-$\underline{CH_2}$), 7.42 (d, J = 8.4 Hz, 1H, H-6-naphthyridine), 8.49 (d, J = 8.0 Hz, 1H, H-5-naphthyridine), 8.77 (s, 1H, H-2-naphthyridine), 12.81 (s, 1H, NH), 13.59 (s, 1H, SH); ^{13}C NMR (δ): 15.40, 25.49, 46.13, 107.75, 119.48, 121.69, 136.15, 144.49, 147.59, 148.59, 163.43, 166.00, 174.19; IR (KBr, cm^{-1}) ν_{max}: 3360, 3039, 1629, 1605, 1561, 1441, 1255, 792; MS calculated for $C_{13}H_{13}N_5OS$: 287.0841, Observed 288.4 [$M^+ + 1$].

4.3. In vitro antibacterial activity

Agar dilution method: Antibacterial susceptibility testing was carried out using National Committee for Clinical Laboratory Standards (NCCLS) micro dilution broth assay against five pathogenic strains *S. aureus* ATCC 2937, *B. subtilis* ATCC 12711, *E. coli* ATCC 8739, *Pseudomonas aeruginos*a ATCC 9027 and *K. pneumoniae* ATCC 31488. Initially bacteria were grown at 37 °C in Nutrient Broth (HIMEDIA) until exponential growth. This culture was used to inoculate 100 mL of Nutrient Broth so that an initial number of 2×10^6 CFU/mL could be achieved. Test compound was dissolved in DMSO/Water and was added to 20 mL inoculated media to get a final concentration of 250 μg/mL. Two-fold 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 get a final concentration 125 μg/mL of the test compound. All other dilutions of the test compound were prepared in a similar fashion to get the minimal dilution to 15.68 μg/mL. Each 10 mL inoculated media along with the test compound was dispensed equally into 3 screw capped 10 mL glass culture tubes. Controls without the test compound, only media and in the presence of streptomycin 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 200/1 (Analytik JENA Instruments®). The lowest concentration at which there was more than 90% inhibition of bacteria as compared to the culture without test compound was taken up as minimal inhibitory concentration (MIC).

4.4. Invitro antifungal activity

The antifungal activity of the compounds was evaluated *in vitro* using food poison technique [53].

4.5. QSAR

All the topological indices used were calculated using all hydrogen suppressed graph. These molecular graphs were obtained by deleting all the carbon hydrogen as well as heteroatomic hydrogen bonds from the structure of the triazole derivatives. The calculations of these indices are well documented in the literature and therefore are not given here.

4.5.1. Physicochemical parameters

Various physicochemical parameters (descriptors) *viz*, MW (molecular weight), MR (molar refraction), MV (molar volume), Pc (parachor), η(refractive index), Surface tension (ST), density (d), Pol

(polarizability), LogP (logarithm of octanol/water partition coefficient), H Bnd Acp (hydrogen bond acceptor), 2D SA (Polar 2D surface area), and 3D SA (Polar 3D surface area), were calculated using DRAGON evaluation version software [47] and Marvin sketch [48]. The expressions for the calculation of these parameters are available in the literature.

4.5.2. Statistical analysis

The regression analysis is made using maximum R^2 method and stepwise regression analysis [49–51] for obtaining statistically significant models.

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