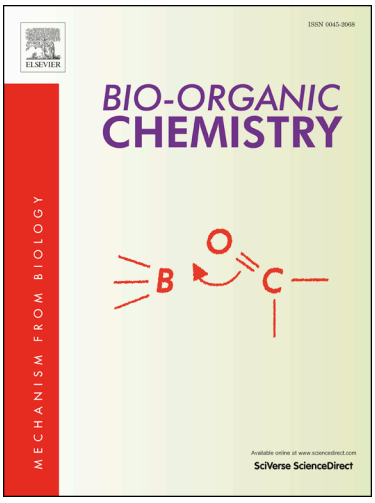

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Synthesis, Antimicrobial activity and Molecular Modeling Study of 3-(5-Amino-(*2H)*-1,2,4-triazol-3-yl]-naphthyridinones as Potential DNA-gyrase inhibitors

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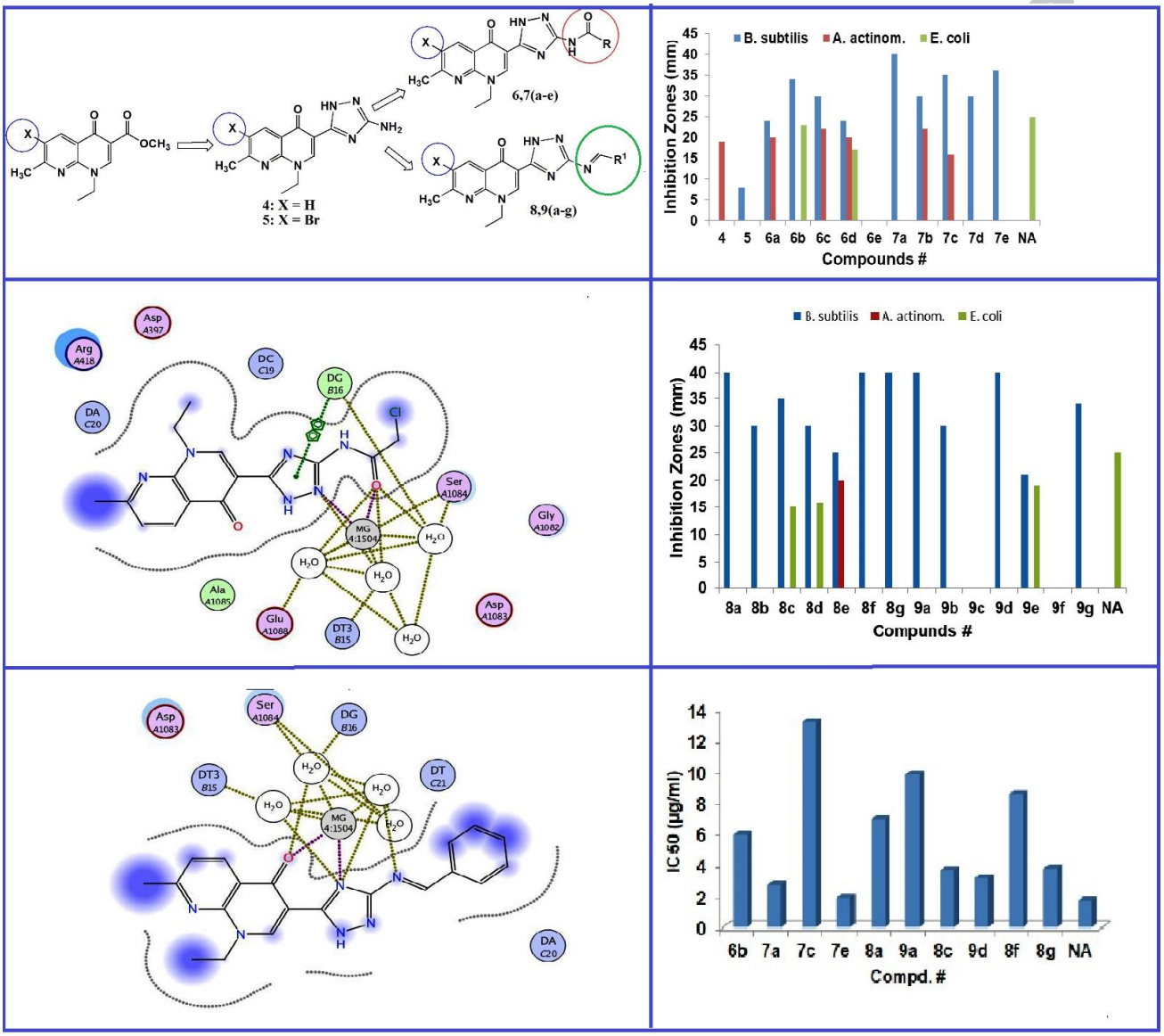
Abstract

Four series of triazolylnaphthyridinone derivatives were synthesized as structural surrogates of nalidixic acid. The targeted derivatives involve: 3-(5-acylamino-2H-1,2,4-triazol-3-yl)-naphtyridin-4-ones **6(a-e)**; 3-(5-benzylidineamino-2H-1,2,4-triazol-3-yl)-naphthyridin-4-ones **8(a-g)** and their 6-bromonaphthyridin-4-one analogs **7(a-e)**; **9(a-g).** The synthesized compounds were evaluated *In vitro* for their antimicrobial activity against selected resistant strains of *G+ve, G-ve*, and *Mycobacterium phlei*. The results revealed remarkable selectivity, of the tested compounds, against *Bacillus subtilis* and *Aggregati-bacter actinomycetemcomitans,* which are resistant to nalidixic acid. The growth inhibition zones were ranging from 20 – 40 *mm* at 10 *mg/ml* and the respective *MIC*-values ~ *3.68–6.3 µM*. The results illustrate that the 6-bromo derivatives **7(a-e)** and **9(a-g)** were more potent than the non-brominated counterparts **6(a-e)** and **8(a-e)** respectively. Inhibition of E. coli *DNA-gyrase* supercoiling activity is also evaluated. The 5-(4-methoxybanzamido)-triazolyl-6-bromonaphthyridinone **(7e)** exhibits IC_{50} = 1.94 *ug/ml*, which is comparable to that of nalidixic acid (IC_{50}:1.74 *ug/ml*). In addition, the most prominent IC_{50}-values are displayed by: (**7a;** IC_{50}:2.77 *µg/ml*); (**8g**; IC_{50}:3.78 *µg/ml*); and **(9d;** IC_{50}*:* 3.21 *µg/ml*). Molecular docking to the active site of *DNA–gyrase* cleavage complex of *Acinetobacter baumannii* (PDB code: 2xkk) co-crystallized with moxifloxacin revealed similar binding modes in addition to new interactions. Assessment of drug-likeness characteristics illustrate that the synthesized compounds showed agreement to Lipinski's and Veper's parameters. The study could offer an exceptional framework that may lead to the discovery of new potent antimicrobial agents.

Keywords: 1,8-naphthydridinone; 1,2,4-triazole; Antibacterial activity; DNA-gyrase inhibition; Molecular docking.

Graphical Abstract:



Highlights:

- Four series of triazolylnaphthyridinone derivative were synthesized as structural surrogates of nalidixic acid at the aim to attain New Chemical Entities (NCE) against resistant bacterial stains.
- The outcomes showed that some of the targeted compounds exhibit promising antibacterial activity against *A. actinomycetemcomitans which* is a resistant *G-ve* anaerobe often found in association with localized aggressive and chronic periodontitis.

- The DNA-gyrase inhibition assay results proved that compounds **7a & 7e** showed significant enzyme inhibition activity.
- A docking study illustrates binding modes, in the active site of *DNA-gyrase*, similar to that elicited by the known quinolone antibacterials.
- *In silico* computational assessment of molecular characteristics demonstrate acceptable bioavailability profile of the synthesized compounds. Undoubtedly, further optimization are planned to improve some physicochemical characteristics e.g. solubility.
- The results could contribute to future research for assisting design and synthesis of structurally manipulated quinolones with enhanced selectivity against resistant bacterial strains.

1. Introduction

Quinolones are the most widely prescribed antibacterial worldwide [1]. Unfortunately, their use is threatened by the increasing prevalence of target-mediated drug resistance [2,3]. Quinolones exert their antibacterial effect by binding to *DNA–Gyrase* or *Topoisomerase IV* (*Top IV*) cleavage complex converting the double stranded *DNA* of bacteria into permanent chromosomal breaks [4]. The most commonly seen resistance is target mediated and is initiated by specific point mutations in these two enzymes [5,6].

Katie J. Aldred et.al. reported a model for quinolone action and the most common cause of resistance (**Figure: 1**) [7]. In a study of the structure of a ternary *Acinetobacter baumannii* (*A. baumannii) topoisomerase IV*-moxifloxacin cleavage complex, it is recognized that the quinolone C3/C4 keto acid chelates a divalent metal ion, which interacts with the protein through water molecules that are coordinated by two highly conserved residues: serine (*Ser80*) and an acidic amino acid located four positions downstream (*Glu84*). Partial disruption of the water-metal ion bridge, resulting from mutation of the serine or acidic residue, significantly decreased the potency [8,9].

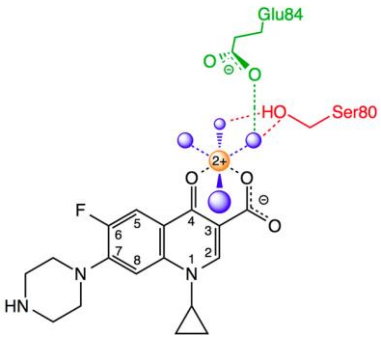


Fig. 1: Schematic of the water−metal ion bridge that mediates quinolone−topoisomerase IV Interaction [7].

Consequently, antibacterial quinolones have been enormously modified to enhance their antibacterial spectrum and attenuate the developed bacterial resistance. In this context, chemical manipulation of proven leads or drug candidate is a well-established strategy affording *NCE* with improved therapeutic characteristics.

We recently, reported the antibacterial and IC_{50} of *DNA-Gyrase* inhibition activity of a series of hybrid molecules comprising naphthyridinone skeleton and thiosemicarbazide or oxadiazole moiety, (**Figure: 2a**) [10]. The results demonstrate enhanced antibacterial spectrum against resistant strains e.g. *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus (B. cereus)* with respect to nalidixic acid, used in this study as a template for structural manipulation. Molecular docking of the synthesized hybrid molecules to the active site of *DNA-gyrase* cleavage complex of *S. aureus, Mycobacterium tuberculosis* (*Mtb*) and *Topoisomerase-IV of Klebsiella pneumoniae (K. pneumonia)* revealed comparable binding poses to the co-crystallized quinolone ligands and indicate good correlation of the binding energy (-dG) with the observed *MIC*-values of the active compounds [10]. Accordingly, we designed analogous series of naphthyridinone derivatives carrying aminotriazole moiety (**Figure: 2b**). The proposed design strategy is three folds: **i.** retaining the naphthyridinone skeleton as an essential anchoring unit for "water−metal ion Bridge", **ii.** Isosteric replacement of the COOH group by 5-membered heterocyclic nucleus as alternative anchoring site, finally, **iii.** Derivatization of the heterocyclic moiety into acyl-amides and/or benzylidineamino to attain additional binding functionalities to the targeted enzyme *DNA-Gyrase.*

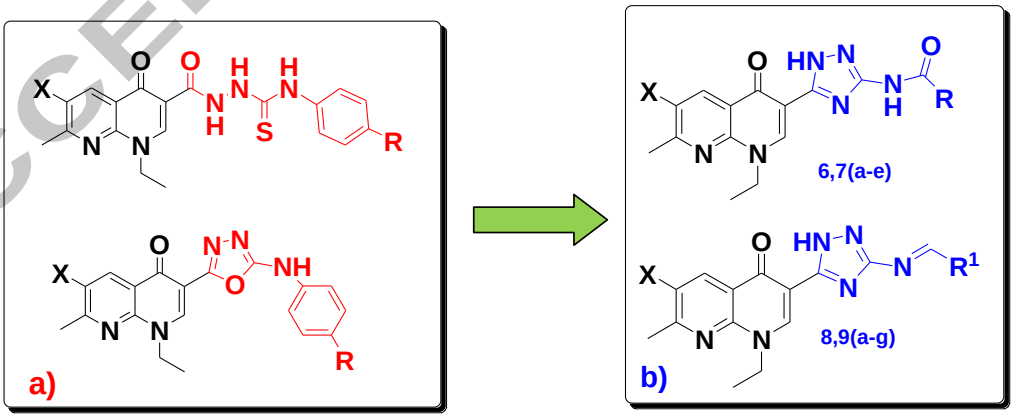


Fig. 2: a) Naphthyridone/6-bromonaphthyridone-3-thiosemicarbazide/oxadiazoles
b) Naphthyridone/6-bromonaphthyridone-3-Aminotriazoles

1,2,4-Triazole scaffold represents privileged fragment in modern heterocyclic chemistry principally due to its incorporation into a wide range of chemotherapeutic agents as

antibacterial [11-13], anti-TB [14], antifungal [15-17], antiviral [18] and anticancer [19]. Interestingly, synthesis of hybrid compounds by combining 1,2,4-triazole with other heterocycles such as thiazole, thiadiazole, imidazole, benzimidazole, naphthyridine and coumarine lead to enhanced biological activities [20,21]. Halogenation of lead compounds has been routinely adopted for improvement of the lipophilic and electronic characteristics resulting in enhanced drug-receptor interactions. Subsequently, a bromo substituent at C-6 of the naphtyridinone nucleus is implemented as to explore the effect of bromination on the antibacterial activity. Moreover, it is anticipated that, replacement of the 6-fluoro substituent in fluoroquinolones by the corresponding 6-bromo might attain a clue to overcome bacterial resistance. This assumption resides on the fact that bromo derivatives are rarely encountered within the molecular features of antibacterial agents.

The newly synthesized compounds will be evaluated for their *in vitro* antimicrobial activity against *Gram positive (G+ve), Gram negative (G-ve)*; and *Mycobacterial* strains. Moreover, the most potent molecules will be tested for potential inhibition of *DNA-gyrase*. Finally, Molecular modeling study of the compounds, involving docking with bacterial *DNA–gyrase* cleavage complexes and estimation of their molecular characteristics and drug-likeness will be performed to explore their binding mode in a proof of design concept.

2. Experimental protocols:

2.1Chemistry:

Melting points were determined using electrothermal apparatus (Stuart scientific, England) and were uncorrected. Infrared spectra (IR) were recorded on thermo-scientific Nicolet 6700 FT-IR spectrometer as KBr pellets. ^{1}H-NMR spectra of compounds were recorded on Varian EM-360L NMR Spectrophotometer (60 MHz Varian, Palo Alto, CA, USA) and 400 MHz AVANCE-III High Performance FT-NMR spectrometer, (Brucker-Biospin International AG, Switzerland). ^{13}C-NMR spectra were carried on AVANCE-III High Performance FT-NMR spectrum (100MHz), (Brucker-Biospin International AG, Switzerland). Chemical shifts (δ) are reported in part per million (ppm), using TMS as reference in ^{1}H-NMR and are referenced relative to residual solvent (e.g., $CDCl_3$: δ C = 77.0 ppm, DMSO-d_6: δ C = 39.5 ppm) in ^{13}C-NMR. D_2O was used for the detection of exchangeable protons. Coupling constants are reported in Hertz (Hz) and spin multiplicities are represented by the following signals: Singlet (s), doublet (d), doublet of doublet (dd) and multiplet (m). Mass spectra were carried out, at the regional center for mycology and biotechnology (Al-Azhar University, Cairo/Egypt), using Direct Probe Controller Inlet part to single Quadropole mass

analyser in thermo Scientific GCMS model ISQLT with Thermo X-Caliber software. Elemental analyses were performed on Perkin Elmer 2400 CHN elemental analyzer and the values were within ±0.4 % of the theoretical values. The reactions and the purity of the products were monitored by TLC on silica gel coated aluminum sheets (Type 60 GF254, Fluka) and the spots were visualized using UV-lamp at *λ* 254 nm. All solvents and chemical are of reagent grade and used without further purification. Nalidixic acid methyl ester **(2)** and 6-bromonalidixic acid methyl ester **(3)** were prepared according to reported procedures [22,23].

3-(5-Amino-*2H*-1,2,4-triazole-3-yl)-naphtyridin-4-ones (4,5):

Aminoguanidine bicarbonate (5.04 g, 0.04 *mole*) was added portion-wise to a stirred ice cooled methanolic solution (30 *ml*) of freshly prepared sodium methoxide (0.84 g, 0.04 mole). A solution of the respective nalidixic acid methyl ester **(2)** or 6-bromo-nalidixic acid methyl ester **(3)** (0.01 *mole*) in methanol (10 *ml*) was then added dropwise. The ice bath was removed and the mixture was refluxed for 25 hours, poured into cooled water and neutralized (pH ~ 7) with 3N HCl. The precipitated product was filtered, washed with water, dried and crystallized from methanol.

3-(5-Amino-2H-1,2,4-triazol-3-yl)-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (4):

Yield 85%; *m.p:* 278-280°*C*. *IR* (KBr) υ (cm^{-1}): 3300 & 3244(broad bands, NH_2 & *NH*), 1624 (*C=O*). ^{1}*H-NMR* (DMSO-d_6) δ: 1.4(t, 3H, *J*=7.2 ,CH_3), 2.6(s, 3H, CH_3), 4.5(q, *J*= 6.8, 2H, CH_2), 5.2(s, 2H, D_2O-exchangeable, NH_2), 7.4(d,1H, *J*=8.4, H-6), 8.5(d, 1H,*J*=8, H-5), 8.8(s, 1H, H-2). ^{13}*C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.5(CH_2*CH_3*), 25.4(CH_3), 46.3(CH_2), 143.5(C-2), 121.4(C-3), 174.4(C_4=O), 119.4(C-4a), 136.5(C-5), 110.1(C-6), 163.1(C-7), 147.3(C-8a). Triazole: 148.3(C-3), 163.5(C-5). MS; M^+:269.99(100%), M+1: 271.01(17.58%). Anal. *Calcd* for $C_{13}H_{14}N_6O$ (270.29): C, 57.77; H, 5.22; N, 31.09: *Found* C, 58.4; H, 5.31; N, 31.34.

***3-(5-Amino-2H-1,2,4-triazol-3-yl)-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one* (5):** *Yield* 94%, *m.p* > 350 °*C*. *IR* (KBr) υ (*cm*$^{-1}$): 3300(*NH_2*), 3266(*NH*), 1624(C=O). ^{1}*H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=7.2, CH_3), 2.7(s, 3H, CH_3), 4.5(q, 2H, *J*=6.8, -CH_2), 5.2(s, 2H, D_2O-exchangeable, NH_2), 8.6(s,1H, H-5), 8.8(s, 1H, H-2). ^{13}*C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.4(CH_2*CH_3*), 26.1(CH_3), 46.3(CH_2), 144.5(C-2), 121.0(C-3), 173.9 (C_4=O), 117.1(C-4a), 138.5(C-5), 108.1(C-6), 165.2(C-7), 146.9(C-8a). Triazole: 148.1(C-3), 161.1(C-5). MS; M^+:348.96(6.98%), M+2:350.01(1.06%), M-2: 345.93(100%). Anal.

Calcd for $C_{13}H_{13}BrN_6O$ (349.19): C, 44.72; H, 3.75; N, 24.07. Found: C, 44.87; H, 3.78; N, 24.24.

Synthesis of the chloroactamides (6a,7a):

To a stirred solution of the respective aminotriazole **(4)** or **(5),** (0.7 mmole) in 10 ml $CHCl_3$ was added chloroacetylchloride (0.079 g, 0.7 mmole). The mixture was refluxed for 1.5 hour then cooled, filtered and crystallized from $CHCl_3$/DMF.

2-Chloro-N-[5-(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]acetamide **(6a)**: White crystalline powder, yield 70%, *m.p*: 244-246˚C. *IR* (KBr) υ (*cm*$^{-1}$); 32401 and 3330 (NH-amide & NH-triazole), 1697 and 1632 (C=O amide & C=O naphthyridinone). ^{1}*H-NMR* (DMSO–d_6) δ: 1.7(t, 3H, *J*=9, CH_3); 2.8(s, 3H, CH_3); 4.3(s, 2H, $COCH_2$); 4.9(q, 2H, *J*=9, -CH_2); 7.4(d, 1H, *J*=8, H-6); 8.7(d, 1H, *J*=8, H-5); 9.3(s, 1H, H-2); 11.5(s, 1H, NHCO). ^{13}*C-NMR* (DMSO–d_6) δ: Naphthyridinone : 13.1(CH_2*CH3*), 24.8(CH_3), 49.7(CH_2), 146.3(C-2), 118.4(C-3), 175.4(C_4=O), 119.4(C-4a), 138.2(C-5), 114.1(C-6), 162.6(C-7), 155.2(C-8a); triazole: 148.3(C-3), 163.5(C-5), 165.4(amide C=O), 42.3(CH_2Cl). MS; M^+: 346.95(20%), M+1: 347.95(36.84%), M+2: 348.96(7.36). Anal. *Calcd.* For $C_{15}H_{15}ClN_6O_2$ (346.77): C, 51.95; H, 4.36; N, 24.24. *Found:* C, 52.19; H, 4.29; N, 24.50.

N-[5-(6-Bromo-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-2-chloroacetamide **(7a)**: White crystalline powder, yield 75%, *m.p*: 242-244 ˚*C*. *IR* (KBr) υ (*cm*$^{-1}$): 3271 and 3248 (NH of amide & NH of triazole), 1647 and 1635 (amide C=O & naphthyridinone C=O). ^{1}*H-NMR* (DMSO-d_6) δ: 1.4(t, 3H, *J*=6.8, CH_3); 2.7(s, 3H, CH_3); 4.3(s, 2H, $COCH_2$); 4.5(q, 2H, *J*=6.4, -CH_2); 8.6(s, 1H, H-5); 8.9(s,1H, H-2); 10.7(s, 1H,NHCO); 13.6(s, 1H, triazole NH). ^{13}*C-NMR* (DMSO–d_6) δ: Naphthyridinone: 13.1 (CH_2 *CH_3*), 17.0(CH_3), 49.7(CH_2), 146.3(C-2), 118.8(C-3), 175.4(C_4=O), 119.8(C-4a), 141.1(C-5), 110.4(C-6), 165.2(C-7), 155.6(C-8a); triazole: 148.3(C-3), 163.5(C-5), 165.4(amide C=O), 42.3(CH_2Cl). MS; M^+: 425.95(100%), M+1:426.94(9.84%), M+2: 427.99(30.36). Anal. Calcd for $C_{15}H_{14}BrClN_6O_2$ (425.67): C, 42.32; H, 3.32; N, 19.74. *Found*: C, 42.06; H, 3.45; N, 19.58.

General method for the synthesis of arylamides 6,7(b–e):

To a stirred solution of the aminotriazoles **4** or **5,** (0.7 mmole) in 10 ml dioxane was added the respective acid chloride (1.4 mmole) and the reaction was refluxed for 3 hours and cooled to ambient temperature. The precipitated product was filtered, washed with 1% NaOH, water, dried and recrystallized from the appropriate solvent.

***N-[5-(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl] benzamide* (6b):** Yield 76%, *m.p*: 236-238°C (dioxane). IR (KBr) υ (cm^{-1}): 3200 and 3300 (NH- amide & NH-triazole); 1650 and 1690 (C=O amide & C=O naphthyridone). *^{1}H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*= 6.8 Hz, CH_3); 2.6(s, 3H, -CH_3); 4.5(q, 2H, *J*=7.2, -CH_2); 7.55(m, 5H, Ar-H); 8.0(d, 1H, *J*=7.6, H-6); 8.5(d, 1H, *J*=8, H-5); 9.1(s, 1H, H-2); 11.2(br s, 1H, NHCO, D_2O exchangeable). *^{13}C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.3(CH_2CH_3), 25.4 (CH_3), 46.2(CH_2), 144.5(C-2), 174.9(C_4=O), 119.0(C-3 & C-4a), 136.2(C-5), 114.1(C-6), 163.6(C-7), 155.6(C-8a). Triazole: 148.4(C-3), 154.2(C-5). Phenyl: 128.2-129.7 (C-1 - C-6), 166.1(amide C=O). MS: M^+:374.04(40.37%); M+1: 375.08(7.82%), M+2: 376.07 (1.09%). Anal. Calcd. For $C_{20}H_{18}N_6O_2$ (374.4): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.42; H, 4.91; N, 22.67.

***4-chloro-N-[5-(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]benzamide* (6c):** Yield 49%, m.p > 320°C (dioxane/water: 90/10). *IR* (KBr) υ (cm^{-1}): 3200 and 3300(NH of amide & NH of triazole), 1654 and 1600(C=O amide & C=O naphthyridinone). *^{1}H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=6.8, -CH_3); 2.6 (s, 3H, -CH_3); 4.5(q, 2H, *J*=7.2, -CH_2); 7.6(d, 2H, *J*=8.4, Ar–H); 8.0(d, 2H, *J*=8.4, Ar–H)); 7.4(d, 1H, *J*=8, H-6); 8.5(d, 1H, *J*=8, H-5); 9.0(s, 1H, H-2); 11.0(br s, 1H, NHCO, D_2O exchangeable). *^{13}C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.5 (CH_2CH_3), 25.4(CH_3), 46.4(CH_2), 144.7(C-2), 128.9 (C-3), 174.3(C_4=O), 121.9(C-4a), 137.5(C-5), 119.0(C-6), 163.5(C-7), 159.3*(C-8a). Triazole: 148.4(C-3), 159.3*(C-5). Phenyl: 132.5(C-1), 129.1-131.1(C-2,3,5,6), 136.2(C-4), 166.1(amide:C=O). MS: M^+: 408.02(32.70%), M+1: 409.03(25.51%), M+2:410.02(11.76%). Anal. Calcd. For $C_{20}H_{17}ClN_6O_2$ (408.84): C, 58.75; H, 4.19; N, 20.56. Found: C, 58.98; H, 4.25; N, 20.81.

***N-[5-(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-4-fluorobenzamide (6d*):** Yield 75%, *m.p*: 228-230°C (dioxane). IR (KBr) υ (cm-^{1}): 3200 and 3300(NH amide & NH triazole), 1669 and 1604(C=O amide & C=O naphthyridone). *^{1}H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=6.8, -CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H, *J*=7.2, -CH_2); 7.4(d, 1H, *J*=8, H-6); 8.5(d, 1H, *J*=8, H-5); 7.8(m, 4H, Ar-H); 9.0(s, 1H, H-2); 11.2(s, 1H, NHCO, D_2O exchangeable). *^{13}C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.4(CH_2CH_3), 26.1(CH_3), 46.5(CH_2), 144.8 (C-2), 116.7 (C-3), 173.3 (C_4=O), 120.9 (C-4a), 137.5(C-5), 119.1(C-6), 163.5(C-7), 161.3(C-8a); phenyl: 129.1 – 129.8 (C-1,2,6), 115.5 (C-3,5), 166.2 (C-4); triazole: 148.0(C-3), 154.3(C-5), 164.1(amide C=O). MS: M^+:392.04(17.97%), M+1:393.06

(3.95%). Anal. Calcd. For $C_{20}H_{17}FN_6O_2$ (392.39): C, 61.22; H, 4.37; N, 21.42. Found: C, 61.49; H, 4.40; N, 21.7.

***N-[5-(1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-4-methoxybenzamide (6e)*:** Yield33%, *m.p:* 218-220˚*C* (DCM & dioxane: 80/20). *IR* (KBr) υ (*cm*$^{-1}$): 3200 and 3300(NH-amide & NH-triazole), 1683 and 1600 (C=O amide &C=O naphthyridinone). ^{1}*H-NMR* (DMSO–d_6) δ: 1.4(t, *J*=8, 3H, -CH_3); 2.6 (s, 3H, -CH_3); 3.8(s, 3H, OCH_3); 4.5(q, *J*=8, 2H, CH_2); 7.4(d, 1H, H-6); 8.5(d, 1H, H-5); 7.5(d, 2H, *J*=8, Ar-H); 8.0(d, 2H, *J*=8, Ar-H); 8.9(s, 1H, H-2); 10.3 (s, 1H, NHCO, D_2O exchangeable); 13.5(s, 1H, triazole-NH). ^{13}*C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.3 (CH_2*CH*$_3$), 25.9 (CH_3), 46.3 (CH_2), 144.2 (C-2), 117.0 (C-3), 173.4(C_4=O), 120.3(C-4a), 137.5(C-5), 118.6 (C-6), 162.5 (C-7), 161.1(C-8a); phenyl; 130.3 (C-1, C2,C6), 114.03(C-3,5), 166(C-4 and amide C=O), 55.9 (O-CH3); triazole: 147.1(C-3), 162.5(C-5). Anal. Calcd. For $C_{21}H_{20}N_6O_3$ (404.42): C, 62.37; H, 4.98; N, 20.78. Found: C, 62.58; H, 5.11; N 21.06.

N-[5-(6-bromo-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]benzamide (7b): Yield 65%, *m.p:* 295-297˚C (dioxane) *IR*,(KBr) υ (cm^{-1}): 3000 and 3300(NH-amide & NH-triazole), 1600 and 1660(C=O amide & C=O naphthyridinone). ^{1}*H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=7.2, -CH_3); 2.7(s, 3H, -CH_3); 4.5(q, 2H, *J*=9, -CH_2); 7.9(m, 5H, Ar-H); 8.7(s, 1H, H-5); 9(s, 1H, H-2); 10.5(br s, 1H, NHCO, D_2O exchangeable); 12.5(br s, 1H, triazole-NH, D_2O exchangeable). ^{13}*C-NMR* (DMSO–d_6) δ: Naphthyridinone: 15.3(CH_2*CH*$_3$), 25.4(CH_3), 57.0(CH_2), 144.5(C-2), 121.1(C-3,C-4a), 173.9(C_4=O) , 141.0(C-5), 115.5(C-6), 161.6(C-7), 154.6(C-8a); phenyl: 132.5(C-1), 128.3 – 132.8(C-2,3,4,5,6); triazole: 148.4(C-3), 154.2 (C-5), 167.2 (amide C=O). MS; M^+:453.96(88.10%), M+1:454.92(22.77%), M+2: 455.94(3.42%). Anal. Calcd for $C_{20}H_{17}BrN_6O_2$ (453.29): C, 52.99; H, 3.78; N, 18.54. Found: C, 53.17; H, 3.85; N, 18.73.

N-[5-(6-bromo-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-4-chlorobenzamide (7c): Yield 54%, *m.p:* 278-280˚*C* (ethanol/dioxane: 90/10). *IR*, (KBr) υ (*cm*$^{-1}$): 3100 and 3250 (NH-triazole& NH-amide), 1610 and 1690 (C=O amide & C=O naphthyridinone). ^{1}*H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=7, -CH_3); 2.8(s, 3H, CH_3); 4.5(q, 2H, *J*=7, -CH_2); 7.6(d, 2H, *J*=8.3 Hz, Ar–H); 8.0(d, 2H, *J*=8.3, Ar-H); 8.7(s, 1H, H-5); 9.0(s, 1H, H-2); 10.8(s, 1H, NHCO). ^{13}*C-NMR* (DMSO–d_6) δ; Naphtyridinone: 15.4 (CH_2*CH*$_3$), 26.0(CH_3), 46.5(CH_2), 144.5(C-2), 120.9(C-3), 173.2(C_4=O), 117.3(C-4a), 138.3(C-5), 109.9(C-6), 161.3(C-7), 154.2(C-8a). Triazole: 146.9(C-3), 150.4(C-5). Phenyl: 132.7 (phenyl:C-1), 129.1-131.6(Phenyl C-2,3,5,6), 137.4(phenyl:C-4), 165.1(amidic:C=O).

MS: M^+:487.90(26.52%), M+1:488.90(14.75%), M+2:489.90(6.22%). Anal. Calcd. For $C_{20}H_{16}BrClN_6O_2$ (487.74): C, 49.25; H, 3.31; N, 17.23. Found: C, 49.49; H, 3.37; N, 17.49.

N-[5-(6-bromo-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-4-fluorobenzamide (7d): Yield 77%, m.p: 244-246°C (dioxane). IR,(KBr) υ (cm^{-1}): 3100 and 3250(NH-triazole& NH-amide)1600 and 1690 (C=O amide & C=O naphthyridinone).*^{1}H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=7.2, -CH_3); 2.7(s, 3H, -CH_3); 4.5(q, 2H, *J*=7.2, -CH_2); 7.7(m, 4H, Ar-H); 8.7(s, 1H, H-5); 9.0 (s, 1H, H-2); 10.7(s, 1H, NHCO). *^{13}C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.4 (CH_2CH_3), 26.1(CH_3), 46.5(CH_2), 144.8(C-2), 116.7(C-3), 173.3(C_4=O), 117.4(C-4a), 138.5(C-5), 116.1(C-5), 115.7(C-6), 165.1(C-7), 161.4(C-8a,). Triazole: 147.1(C-3), 159.2(C-5). Phenyl: 128.0-131.5(C-1,2,6), 115.9(C-3), 166.1(C-4), 163.7(amide:C=O). MS; M^+:471.92(34.14%), M+1:472.92(26.80%), M+2: 473.92(9.28%). Anal. Calcd. For $C_{20}H_{16}BrFN_6O_2$ (471.28): C, 50.97; H, 3.42; N, 17.83; Found: C, 51.32; H, 3.46; N, 18.09.

N-[5-(6-bromo-1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridin-3-yl)-2H-1,2,4-triazol-3-yl]-4-methoxybenzamide (7e): Yield 33%, m.p: 252-254°C (DCM/dioxane: 80/20). *IR* (KBr) υ (*cm^{-1}*): 3212 and 3320(NH-triazole& NH-amide), 1603 and 1668(C=O amide & C=O naphthyridinone). *^{1}H-NMR* (DMSO–d_6) δ: 1.4(t, 3H, *J*=7.2, -CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H, *J*=7.2, -CH_2); 3.8(s, 3H, OCH_3); 7.0(d, 2H, *J*=8, Ar-H); 8.0(d, 2H, *J*=8, Ar-H); 8.7(s, 1H, H-5); 8.8(s, 1H, H-2); 11.9(s, 1H, NHCO). *^{13}C-NMR* (DMSO–d_6) δ: Naphthyridinone: 15.3 (CH_2CH_3), 25.9(CH_3), 46.3(CH_2), 144.8(C-2), 117.0(C-3), 173.4 (C_4=O), 121.3(C-4a), 138.6(C-5), 114.03*(C-6), 162.5*(C-7), 161.1(C-8a). Triazole: 147.1(C-3), 162.5*(C-5). Phenyl: 130.3(C-1,2,6),114.03*(C-3,C-5), 166.1(C-4, amide:C=O), 55.9(OCH_3). MS; M^+:482.90(13.79%), M+1:483.90(7.77%), M+2:484.90(1.68%). Anal. Calcd. For $C_{21}H_{19}BrN_6O_3$ (483.32): C, 52.19; H, 3.96; N, 17.39. Found: C, 52.41; H, 4.00; N, 17.54.

General Method for the synthesis of benzylideneaminotriazoles 8,9(a – g):

To a stirred solution of freshly prepared sodium methoxide (0.038 g, 0.7 mmole) in methanol (15 ml) was added the respective aldehyde (0.7 mmole) and aminotriazole **4 or 5** (0.7 mmole)**.**The mixture refluxed for 4-5 hours. After cooling the solvent was evaporated and the product was crystallized from the appropriate solvent.

3-((E)-[5-(benzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8a): Yield 45%, *m.p* 258-260°*C* (Ethanol). *IR*,(KBr), υ (*cm^{-1}*): 3227(NH) &

1622(C=O).^{1}H-NMR (DMSO–d_6) δ: 1.4(t, 3H, *J*= 8 Hz, CH_3); 2.6(s, 1H, CH_3); 4.6(q, 2H, *J*= 8 Hz, CH_2); 7.4(d, 1H, *J*= 8 Hz, H-6); 7.8(m, 5H, Ar-H); 8.6(d, 2H, *J*= 8 Hz, H-5); 9.0(s, 1H, H-2); 9.3(s, 1H, N=CH). *^{13}C-NMR* (DMSO–d_6) δ: Naphthyridinone: 15.3(CH_2*CH_3*), 24.8(CH_3), 49.7(CH_2), 146.2(C-2), 118.8(C-3), 175.4(C_4=O) , 118.6(C-4a), 138.2(C-5), 114.1(C-6), 162.6(C-7), 155.6(C-8a); phenyl: 133.8(C-1), 128.9(C-3,5), 129.2(C-2,6), 131.4 (C-4); triazole: 148.4 (C-3), 158.0(C-5), 160.1(CH=N). MS: M^+:358.06(29.78%), M+1:359.08 (5.40%), M+2: 360.06 (0.79%). Anal. Calcd. For $C_{20}H_{18}N_6O$ (358.4): C, 67.02; H, 5.06; N, 23.45. Found: C, 67.19; H, 5.13; N, 23.78.

3-((E)-[5-(4-chlorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8b): Yield 44%, m.p.272 – 274 ˚C (DCM/MeOH: 70/30). *IR*, (KBr), υ (*cm^{-1}*): 3190(NH) & 1610(C=O). ^{1}H-NMR (DMSO – d_6) δ: 1.4(t, 3H, *J*= 6.8 Hz, CH_3); 2.6(s, 3H, CH_3); 4.6(q, 2H,*J*= 7.2 Hz, CH_2); 7.4(d, 1H, *J*= 8.4, H-6); 7.5(d, 2H, *J*= 8.8 Hz, Ar - H); 7.9(d, 2H, *J*= 8.8 Hz, Ar - H); 8.5(d, 1H, *J*= 8.4, H-5); 8.8(s, 1H,H-2); 9.1(s, 1H, N=CH). *^{13}C-NMR* (DMSO–d_6) δ: Naphthyridinone: 15.3(CH_2*CH_3*), 24.8(CH_3), 49.7(CH_2), 146.2(C-2), 118.8(C-3), 175.4(C_4=O), 118.6(C-4a), 138.2(C-5), 114.1(C-6), 162.6(C-7), 155.6(C-8a); phenyl: 131.9(C-1), 129.2(C-3,5), 130.6(C-2,6), 136.6(C-4); triazole: 148.4 (C-3), 158.0(C-5), 160.1(CH=N). Anal. Calcd. For $C_{20}H_{17}ClN_6O$ (392.84): C, 61.15; H, 4.36; N, 21.39. Found C, 61.43; H, 4.41; N, 21.67.

3-(E)-[5-(4-fluorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8c): Yield 77%, *m.p* 238–240˚*C* (MeOH). *IR* (KBr) υ (*cm^{-1}*): 3216(NH) & 1615(C=O). ^{1}H-NMR (DMSO–d_6) δ: 1.4(t, 3H, *J*= 6.9 Hz, CH_3); 2.6(s, 3H, CH_3); 4.6(q, 2H, *J*= 6.9 Hz, CH_2); 7.4(d, 1H, *J*= 8.1 Hz, H-6); 7.9 (m, 4H, Ar-H); 8.5(d, 1H, J= 8.1 Hz, H-5); 8.9(s, 1H, H-2); 9.2(s, 1H, N=CH). *^{13}C-NMR* (DMSO–d_6) δ: Naphthyridinone: 15.3(CH_2*CH_3*), 24.8(CH_3), 49.7(CH_2), 146.2(C-2), 118.8(C-3), 175.4 (C_4=O), 118.6(C-4a), 138.2(C-5), 114.1(C-6), 162.6(C-7), 155.6(C-8a); phenyl: 129.4(C-1), 115.6 (C-3,5), 129.8(C-2,6), 165.2(C-4); triazole: 148.4(C-3), 158.0(C-5), 160.1(CH=N). MS: M+: 376.04(3.57%), M-1: 375.02(24.78%), M-2: 374.00(100.00%). Anal. Calcd. For $C_{20}H_{17}FN_6O$ (376.14): C, 63.82; H, 4.55; N, 22.33. Found: C, 64.09; H, 4.62; N, 22.60.

3-(E)-[5-(4-bromobenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8d): yield 25%, *m.p* > 350˚C (MeOH). *IR* (KBr) υ (*cm^{-1}*): 3350(NH) & 1646(C=O). ^{1}H-NMR (DMSO – d_6) δ: 1.4(t, 3H, *J*= 6.5 Hz, CH_3); 2.6(s, 3H, CH_3); 4.5 (q, 2H, *J* = 6.6 Hz, CH_2); 7.4(d, 1H, *J*= 7.8 Hz, H-6); 7.5(d, 2H, *J*= 7.84 Hz, Ar-H);

7.9(d, 2H, *J*= 7.84 Hz, Ar-H); 8.5(d, 1H, *J*= 7.8 Hz, H-5); 8.8(s, 1H, H-2); 9.1(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ: Naphthyridinone: 15.5(CH_2*CH_3*), 25.9(CH_3), 45.9(CH_2), 143.3(C-2), 121.3(C-3), 173.7(C_4=O), 117.7(C-4a), 138.8(C-5), 115.9(C-6), 167.3(C7), 154.6(C8a); phenyl: 136.9(C-1), 130.2(C-2,6), 132.1(C-3,5), 123.9(C-4); triazole: 146.5(C-3), 157.1(C-5), 160(N=CH). MS: M+: 436.94(100.00%), M+1: 437.95(27.80%), M+2: 438.97(5.24%). Anal. Calcd. For $C_{20}H_{17}BrN_6O$ (437.29): C, 54.93; H, 3.92; N, 19.22. Found: C, 55.20; H, 3.99; N, 19.51.

3-(E)-[5-(2,4-dichlorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8e): Yield 57%, *m.p* 242–243°*C* (MeOH). *IR* (KBr) υ (*cm^{-1}*): 3250(NH) & 1622(C=O). ^{1}H-NMR (DMSO–*d_6*) δ: 1.4(t, 3H, *J*= 6.7 Hz, CH_3); 2.6(s, 3H, CH_3); 4.6(q, 2H, *J*= 7 Hz, CH_2); 7.3(d, 1H, *J*=7.8 Hz, H-6); 7.5(d, 2H, *J*= 8.4 Hz, Ar-H); 8.2(d, 1H, *J*= 8.1 Hz, Ar-H); 7.6(s, 1H, Ar-H); 8.5(d, 2H, *J*= 7.5, H-5); 8.7(s, 1H, H-2); 9.4(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.6(CH_2*CH_3*), 25.3(CH_3), 45.7 (CH_2), 143.8(C-2), 120.6(C-3), 174.7(C_4=O), 119.7(C-4a), 137.5(C-5), 115.0(C-6), 167.1(C-7), 156.8(C-8a). Triazole: 148.1(:C-3), 162.5(C-5, CH=N). Phenyl: 131.5(C-1), 135.4(C-2), 130.5(C-3, C-5), 136.4(C-4), 132.0(C-6). MS: M^+: 427.04(25.42%), M+1:428.03(2.87%), M+2:429.06(2.39%). Anal. Calcd. For $C_{20}H_{16}Cl_2N_6O$ (427.29): C, 56.22; H, 3.77; N, 19.67. Found: C, 56.45; H, 3.80; N, 19.88.

3-(E)-[5-(4-methoxybenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8f): Yield 39%, *m.p* 236-238°*C* (MeOH). *IR* (KBr) υ (*cm^{-1}*): 3211(NH), 1623(C=O). ^{1}H-NMR (DMSO–*d_6*) δ: 1.4(t, 3H,*J*= 6.5 Hz, CH_3); 2.6(s, 3H, CH_3); 3.8(s, 1H, OCH_3); 4.6(q, 2H, *J*= 6.6 Hz, CH_2); 7.4(d, 2H, *J*= 8.1 Hz, Ar-H); 7.9(d, 2H, *J*= 8.1 Hz, Ar-H); 7.1(d, 1H,*J*= 8 Hz, H-6); 8.5(d, 1H, *J*= 8 Hz, H-5); 9.0(s, 1H, H-2); 9.1(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.3(CH_2*CH_3*), 25.9(CH_3), 46.3(CH_2), 146.2(C-2), 118.6(C-3), 173.4(C_4=O), 118.6(C-4a), 138.2(C-5), 114.6 (C-6), 162.5(C-7), 155.6(C-8a); phenyl: 126.1(C-1), 130.2(C-2,6), 114.4(C-3,5), 163.2(C-4), 55.9 (O-CH3); triazole: 147.1(C-3), 158.0(C-5), 160.1(CH=N). MS: M^+: 388.06(30.76%), M+1: 389.07 (5.48%). Anal. Calcd. For $C_{21}H_{20}N_6O_2$ (388.42): C, 64.94; H, 5.19; N, 21.64. Found: C, 65.21; H, 5.26; N, 21.91.

3-(E)-[5-(2-hydroxybenzylideneamino)-2H-1,2,4-triazol-3-yl]-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (8g): Yield 43%, m.p. 310-312°C (MeOH). IR (KBr), υ (cm^{-1}): 3219 & 3445(NH & OH), 1621(C=O). ^{1}H-NMR (DMSO–*d_6*) δ: 1.4(t, 3H, *J*=7 Hz, CH_3);

2.6(s, 3H, CH_3); 4.6(q, 2H, *J*= 6.9 Hz, CH_2); 7.0(s, 1H, OH); 7.6(m, 4H, Ar-H); 7.4(d, 1H, *J*= 8 Hz, H-6); 8.5(d, 1H, *J*= 8.1 Hz, H-5); 9.0(s, 1H, H-2); 9.4 (s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.3(CH_2*CH_3*), 25.9(CH_3), 46.3 (CH_2), 146.3(C-2), 118.8 (C-3), 175.4(C_4=O), 118.6(C4a), 138.2(C-5), 114.4(C-6), 162.6(C-7), 155.6(C-8a); phenyl; 118.5(C-1), 161.1(C-2), 116.0(C-3), 132.5(C-4), 121. 5(C-5), 130.6(C-6); triazole: 147.1 (C-3), 158.5(C-5), 160.1(CH=N). MS: M+: 374.00(100.00%), M+1:375.02(24.78%), M+2: 376.04(3.57%). Anal. Calcd. For $C_{20}H_{18}N_6O_2$ (374.4): C, 64.16; H, 4.85; N, 22.45. Found: C, 64.38; H, 4.90; N, 22.68.

3-(E)-[5-(benzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9a): Yield 48%, m.p. 238-240°C (DCM & MeOH: 80/20). IR (KBr) υ (cm^{-1}): 3190(NH) & 1610(C=O of naphthyridinone). ^{1}H-NMR (DMSO – *d_6*) δ: 1.4(t, 3H, *J*= 9Hz, CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H, *J*= 9 Hz, CH_2); 7.8(m, 5H, Ar-H); 8.7(s, 1H, H-5); 8.9(s, 1H, H-2); 9.2(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.3 (CH_2*CH_3*), 17.0(CH_3), 49.7(CH_2), 146.3(C-2), 118.8(C-3), 175.4(C_4=O) , 119.8(C-4a), 141.1 (C-5), 110.4(C-6), 165.2(C-7), 154.7(C-8a); phenyl: 133.8(C-1), 128.9(C-3,5), 129.2(C-2,6), 131.4(C-4); triazole: 148.4(C-3), 158.0(C-5), 160.1(CH=N). MS; M+: 437.93(31.26%), M+1: 438.94(6.33%). Anal calcd. For $C_{20}H_{17}BrN_6O$ (437.29): C, 54.93; H, 3.92; N, 19.22. Found: C, 55.09; H, 3.96; N, 19.41.

3-(E)-[5-(4-chlorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9b): Yield 59%, m.p.298-300°C (MeOH). IR (KBr) υ (cm^{-1}): 3250(NH) & 1650(C=O of naphthyridinone). ^{1}H-NMR (TFA) δ: 1.6(t, 3H, *J*= 9 Hz, CH_3); 3.0(s, 3H, CH_3); 5(q, 2H,*J*= 9 Hz, CH_2); 7.6(d, 2H, *J*= 7.2 Hz, Ar-H); 7.9(d, 2H, *J*= 7.2 Hz, Ar-H); 9.0(s, 1H, H-5); 9.5(s, 1H, H-2); 9.9(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.3(CH_2*CH_3*), 17.0(CH_3), 49.7(CH_2), 146.2(C-2), 118.8(C-3), 175.4 (C_4=O), 119.6(C-4a), 141.2(C-5), 110.1(C-6), 166.2(C-7), 154.7(C-8a); phenyl: 131.9(C-1), 129.2(C-3,5), 130.6(C-2,6), 136.6(C-4); triazole: 148.4 (C-3), 158.0(C-5), 160.1(CH=N). MS; M^+: 471.94(43.00%), M+1: 472.92(8.25%), M+2:473.97(1.36). Anal. Calcd. For $C_{20}H_{16}BrClN_6O$ (471.74): C, 50.92; H, 3.42; N, 17.82. Found: C, 51.23; H, 3.49; N, 18.10.

3-(E)-[5-(4-fluorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9c): Yield 67%, m.p.>300°C (MeOH). IR (KBr) υ (cm^{-1}): 3190(NH) & 1610(C=O of naphthyridinone). ^{1}H-NMR (TFA) δ: 1.6(t, 3H, *J*= 9 Hz, CH_3); 3.0(s, 3H, CH_3); 5.0(q, 2H, *J*= 9Hz, CH_2); 8.0(m, 4H, Ar-H); 9.0(s, 1H, H-5); 9.5(s,1H, H- 2);

9.9(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.3(CH_2*CH_3*), 24.8(CH_3), 49.7(CH_2), 146.2(C-2), 118.8(C-3), 175.4(C_4=O), 119.2(C-4a), 141.2(C-5), 110.4(C-6), 166.6(C-7), 154.6(C-8a); phenyl: 129.4(C-1), 115.6(C-3,5), 130.8(C-2,6), 166.2(C-4); triazole: 148.4(C-3), 158.0(C-5), 160.1(CH=N). MS; M^+:454.92(82.65%), M+1:455.93 (33.64%), M+2:456.95(6.21%). Anal. Calcd. For $C_{20}H_{16}BrFN_6O$ (455.28): C, 52.76; H, 3.54; N, 18.46. Found: C, 52.89; H, 3.58; N, 18.71.

3-(E)-[5-(4-bromobenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9d): Yield 30%, m.p. 274-276°C (MeOH). IR (KBr) υ (cm^{-1}): 3300(NH) & 1615(C=O). ^{1}H-NMR (DMSO – *d_6*) δ: 1.4(t, 3H, *J*= 8Hz, CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H,*J*= 8Hz, CH_2); 7.6(d, 2H, *J*= 7.8 Hz, Ar-H); 7.8(d, 2H, *J*= 7.8 Hz, Ar-H); 8.6(s, 1H, H-5); 8.7(s, 1H, H-2); 9.0(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.5 (CH_2*CH_3*), 25.9(CH_3), 45.9(CH_2), 143.3(C-2), 121.3(C-3), 173.7(C_4=O), 117.7(C-4a), 138.8(C-5), 115.9(C-6), 167.3(C-7), 154.6(C-8a); Phenyl: 136.9(C-1), 130.2(C-2,6), 123.9 (C-4), 132.1(C-3,5); Triazole: 146.5(C-3), 157.1(C-5). 160(CH=N). MS: M^+: 516.90 (51.22%), M+1:517.90(15.24%), M+2:518.88(2.68%). Anal. Calcd. For $C_{20}H_{16}Br_2N_6O$ (516.19): C, 46.54; H, 3.12; N, 16.28. Found: C, 46.79; H, 3.15; N, 16.45.

3-(E)-[5-(2,4-dichlorobenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9e): Yield 50%, m.p. 280-282°C (MeOH). IR (KBr) υ (cm^{-1}): 3150(NH) & 1621(C=O). ^{1}H-NMR (DMSO – *d_6*) δ: 1.4(t, 3H, *J*= 8 Hz, CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H, *J*= 8Hz, CH_2); 7.5(d, 2H, *J*= 8Hz, Ar-H); 8.2(d, 2H, *J*= 8Hz, Ar-H); 7.6(s, 1H, Ar-H); 8.6(s, 1H, H-5); 8.7(s, 1H, H-2); 9.4(s,1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; Naphthyridinone: 15.6(CH_2*CH_3*), 25.3(CH_3), 45.7(CH_2), 143.8(C-2), 120.6(C-3), 174.7 (C=O), 119.7(C-4a), 137.5(C5), 115(C-6), 167(C7), 156.8(C8a); phenyl: 128.4-136.4 (dichlorophenyl); triazole:148.1(C-3), 162.5(C-5,CH=N). Anal. Calcd. For $C_{20}H_{15}BrCl_2N_6O$ (506.18): C, 47.46; H, 2.99; N, 16.60. Found: C, 47.72; H, 2.97; N, 16.86.

3-(E)-[5-(4-methoxybenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9f): Yield 95%, m.p. 288-290°C (MeOH). IR (KBr) υ (cm^{-1}): 3190(NH), 1610(C=O). ^{1}H-NMR (TFA) δ: 1.6(t, 3H, *J*= 9 Hz, CH_3); 3.0(s, 3H, CH_3); 4.1(s, 3H, OCH_3); 5.0(q, 2H, *J*= 6 Hz, CH_2); 7.2(d, 2H, *J*= 9 Hz, Ar-H); 8.0(d, 2H, *J*= 9 Hz, Ar-H); 9.0(s, 1H, H-5); 9.5(s, 1H, H-2); 9.8(s, 1H, N=CH). *^{13}C-NMR* (DMSO–*d_6*) δ; MS; Naphthyridinone: 15.3(CH_2*CH_3*), 25.9(CH_3), 46.3(CH_2), 146.3(C-2), 118.8(C-3), 175.4 (C_4=O), 119.8(C4a), 141.1(C-5), 110.4(C-6), 165.2(C-7), 154.7(C-8a); phenyl; 126.1(C-1),

130,2(C-2,6), 114.4(C-3,5), 163.0(C-4), 55.9(O-CH3); triazole: 147.1(C-3), 158.5(C-5), 160.1(CH=N). M^+:466.95(100.00%), M+1: 467.90(17.35%). Anal. Calcd. For $C_{21}H_{19}BrN_6O_2$ (467.32): C, 53.97; H, 4.10; N, 17.98. Found: C, 54.32; H, 4.16; N, 18.21.

3-(E)-[5-(2-hydroxybenzylideneamino)-2H-1,2,4-triazol-3-yl]-6-bromo-1-ethyl-7-methyl-1,8-naphthyridin-4(1H)-one (9g): Yield 57%, m.p. 262-264°C (MeOH). IR (KBr) υ (cm^{-1}): 3180(NH & OH), 1610(C=O). ^{1}H-NMR (DMSO – d_6) δ: 1.6 (t, 3H,*J*= 6Hz, CH_3); 2.7(s, 3H, CH_3); 4.5(q, 2H,*J*= 9 Hz, CH_2); 7.9(m, 4H, Ar-H); 8.1 (s, 1H, OH); 8.6(s, 1H, H-5); 9.0(s, 1H, H-2); 10.3(s, 1H, N=CH). *^{13}C-NMR* (DMSO–d_6) δ; Naphthyridinone: 15.3(CH_2*CH_3*), 25.9(CH_3), 46.3(CH_2), 146.3(C-2), 118.8(C-3), 175.4(C_4=O), 119.8(C4a), 141.1(C-5), 110.4 (C-6), 165.2(C-7), 154.7(C-8a); phenyl; 118.5(C-1), 161.1(C-2), 116.0(C-3), 132.5(C-4), 121.5(C-5), 130.6(C-6); triazole: 147.1(C-3), 158.5(C-5), 160.1(CH=N). MS; MS; M^+: 453.93(43.78%), M+1:454.95(11.84%), M+2: 455.95(4.75%). Anal. Calcd. For $C_{20}H_{17}BrN_6O_2$ (453.29): C, 52.99; H, 3.78; N, 18.54. Found: C, 53.23; H, 3.74; N, 18.79.

2.2 Antibacterial evaluation:

In vitro assessment of the antimicrobial activity of the synthesized compounds has been carried out at the department of microbiology, faculty of pharmacy, October 6 University. The tested bacterial strains involve *S. aureus, B. subtilis, Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Mycobacterium Phlei (M. Phlei)* (Clinical isolates from Cairo university hospital) and *Aggregatibacter actinomycetemcomitans* (ATCC 29524) (*A. actinomycetemcomitans)*. Organisms were cultured on tryptose phosphate or trypticase soy broth. Maintenance of the culture was done by either; weekly subculture of the strain on brain heart blood agar plates; or maintained at -20 °C in a broth medium with glycerol (1:1 v/v) for up to 6 months.

2.2.1 Microbial growth inhibition activity

The synthesized compounds and nalidixic acid (as reference drug) were dissolved in dimethylsulfoxide (*DMSO*) to afford stock solutions (10 *mg/ml).* A fresh culture of each organism at a cell density of 10^6 to 10^7 cfu/ml was surface-inoculated into brain heart blood agar plates. The Plates left to dry and then cups were made aseptically using sterile cork borer. 100 µl of each compound was aseptically dropped into the cups. The diameters of inhibition zones were measured by a ruler on the undersurface of petri dishes. A reading of 6 mm indicates no zone and the end point is taken as complete inhibition of growth.

2.2.2 Determination of minimum inhibitory concentration

Compounds exhibiting growth inhibition zones > 20 mm were selected for assessment of *MIC-values* using modified cup diffusion method [24]. A fresh culture of each organism at a cell density of 10^6 to 10^7 cfu/ml was surface-inoculated into brain heart blood agar plates. Plates were dried and then cups were made aseptically using sterile cork borer. Serial dilutions of the stock solutions (40 *mg/ml*) in DMSO were prepared, so that the final concentrations of the tested compounds/100 µl are: ***20; 10; 5; 2.5; 1.25; and 0.625 mg*** respectively and were aseptically dropped into the cups. Inoculated Plates were incubated for 3 to 7 days at 37 °C under microaerophilic conditions and were examined daily (1st day and 2nd day) for the absence of bacterial growth. The *MICs* were defined as the lowest concentrations of tested compounds preventing visible bacterial growth after 24 hrs. of incubation.

2.3 DNA-gyrase supercoiling assay

The supercoiling assay of DNA gyrase has been carried out at the confirmatory diagnostic R&D sector (*Vacsera-Egypt*) using *E. coli* Gyrase Microplate Assay Kit (Inspiralis®) [25]. Details of the materials; enzyme, DNA substrate & the assessment procedure are described in previous publication [10] (Supporting information). Seven dilutions of each compound were prepared (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 µg/ml) using DMSO as solvent. IC_{50} values were determined at a final concentration of 50, 10, 2, and 0.4 µg/ml. Nalidixic acid was used as a reference drug.

2.4 Molecular Docking:

Docking studies were carried out at the department of Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut/Egypt. All molecular modeling studies were carried out on an Intel(R) Xeon(R) CPU E5-1650 3.20 GHz processor, 16 GB memory with Windows 7 Professional operating system. Molecular Operating Environment (MOE 2014.0901, Chemical Computing Group, Montorial-Canada) was used as the computational software. The crystal structures of DNA-gyrase cleavage complex of *Acinetobacter A. baumannii (A. baumannii)* co-crystallized with moxifloxacin (PDB code: 2xkk)

The Studied compounds were built using the builder interface of the MOE software and subjected to conformational search. Conformers were subjected to energy minimization until a RMSD gradient of 0.01 Kcal/mol and RMS distance of 0.1 Å with MMFF94X force-field and the partial charges were automatically calculated. The obtained database was

then saved as MDB file to be used in the docking calculations. Docking of the energy minimized conformations was done using MOE-dock wizard. The vicinity of the co-crystallized ligand, moxifloxacin, was specified to be the docking site. London ΔG was used as the scoring function and it estimates the free energy of binding of the ligand from a given pose. The saved pose for the ligand-target complex of each compound was subjected to detailed 2D and 3D analysis for its interactions with the target.

3. Results and discussion

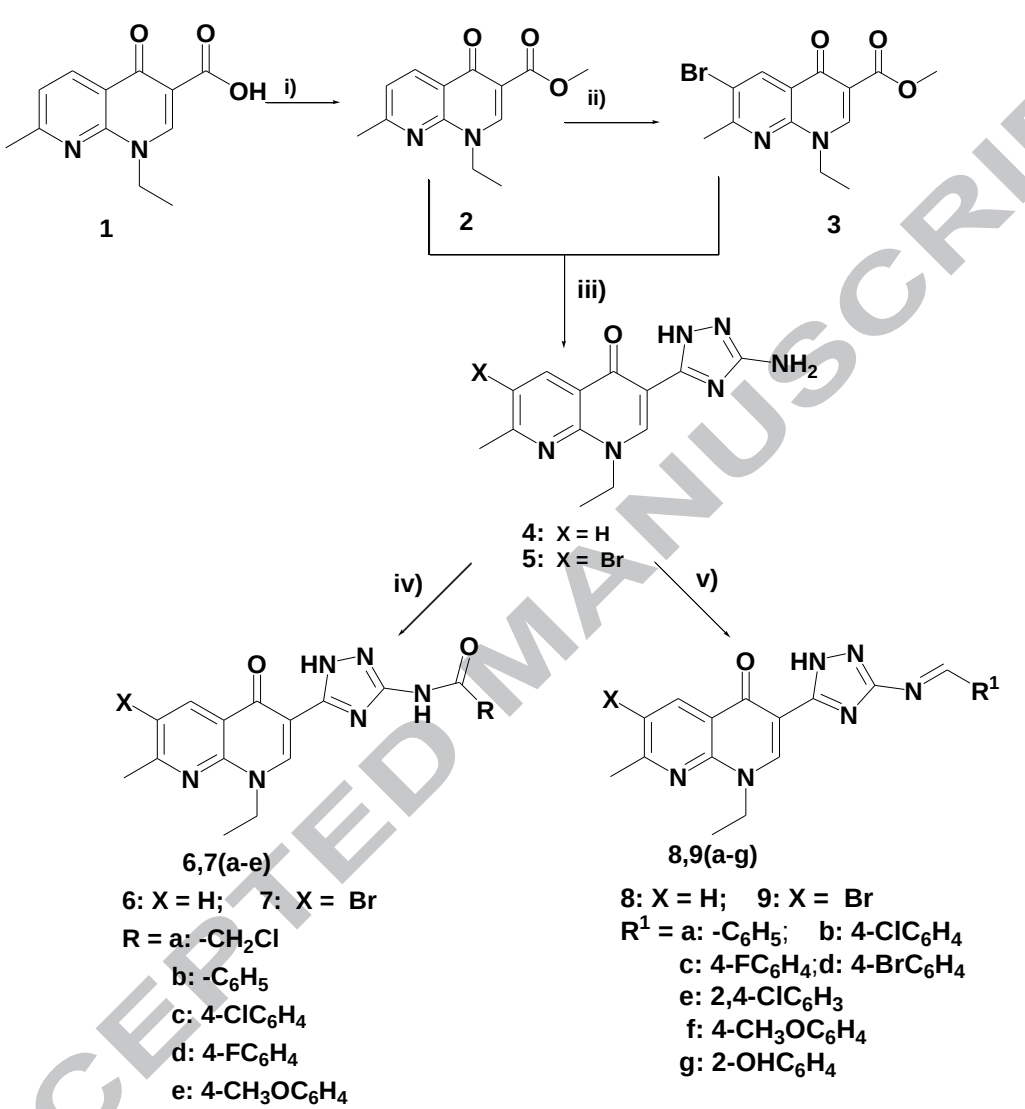
3.1 Chemistry

The target compounds were prepared as outlined in **scheme 1.** The starting ester intermediate **(2)** has been prepared from nalidixic acid as reported [22], then treated with 3 equivalents of bromine to afford the corresponding 6-bromo analog **(3)** [23]. The chemical structures of **(2)** & **(3)** were established by IR &^{1}H-NMR (Supporting information) and in comparison to the reported data.

The key intermediates 3-(5-amino-*1H*-1,2,4-triazol-3-yl)-naphthyridin-4-ones **(4,5)** were prepared from the esters (**2,3),** analogous to reported procedures [26,27], through reflux with aminoguanidine bicarbonate in presence of sodium methoxide in methanol for 24 hours. Synthesis of the anticipated triazoles **(4,5)** directly from nalidixic acid or its 6-bromo analog and aminoguanidine is postponed based on previous experience that nalidixic acid is susceptible to decarboxylation upon drastic reaction conditions (Supporting Literature). Moreover, acylation of aminoguanidine with aliphatic acids is straightforward, whereas aromatic acids should be converted firstly to the corresponding acid chlorides and subsequent cyclization of the resulting acylaminoguanidine by heating at 250^{o}C [28].

The structures of the key intermediates (**4,5)** were confirmed by the IR, NMR spectroscopy (Supporting information), MS and elemental analysis. The IR spectrum revealed broad bands at υ ~ 3300–3200 cm^{-1} assigned for the NH_2 and NH groups, and a sharp band at 1624 cm^{-1} of C=O of naphthyridinone, in addition to disappearance of ester band at 1692 cm^{-1}. ^{1}H-NMR spectra characterized by a singlet at δ 5.2 ppm corresponding to (NH_2) group disappeared by addition of D_2O. The triazolyl (NH) signal displayed in some compounds **(7a, 7b & 6e)** downfield shifted (δ ~ 12.5 – 13.6 ppm) which assign its acidic character. Otherwise, in most of the synthesized compounds this signal is not detectable, that might be attributed to possible rapid exchange with DMSO-d_6 used as solvent. The structures of these compounds were further confirmed through mass spectral analyses (Supporting

information); whereby the molecular ion peak corresponding to the proposed formula. Moreover, the results of elemental analysis ascertain their constitution.



Reagents & Conditions: i) Ethylchloroformate, Triethylamine (TEA), MeOH, RT; ii) Br_2, MeOH, RT; iii) Aminoguanidine bicarbonate, NaOMe, MeOH, reflux 24 hrs.; iv) RCOCl , dioxane, reflux ; v) R^1-CHO, NaOMe, MeOH, reflux.

Scheme 1: Synthesis of the target compounds

It is mentions worthy, that the observed chemical shifts of the exocyclic (NH_2) and triazolyl (NH) supported the assignment of the synthesized naphthyridinyl-aminotriazoles (**4,5**) to the tautomeric form (**A**), Figure **(3, left)**. In a study involving ^{1}H-NMR and X-ray crystallography of a series (het)aryl-aminotriazoles, it has been concluded that form (**A**), is the predominant tautomer specifically in cases of possible intramolecular H-bonding between the (NH) and a neighboring acceptor atom or group [29]. In the synthesized

aminotriazoles (**4,5**), the oxygen of the naphtyridin-4-one moiety acts as the respective H-bond acceptor resulting in building of a 6-membered ring structure (**Fig. 3, right**).

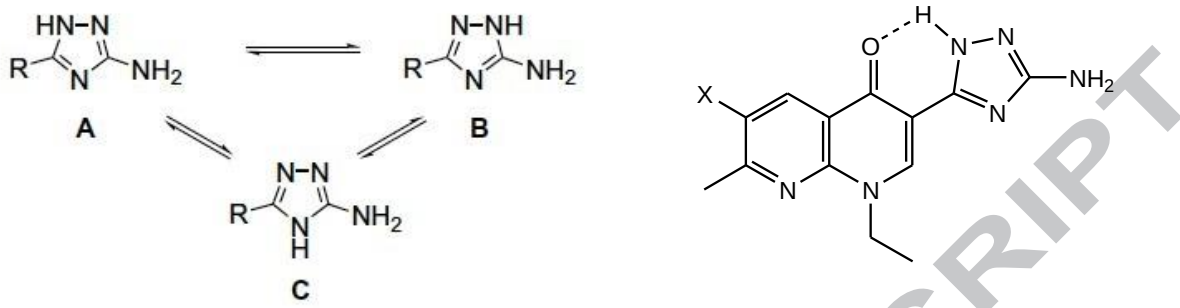


Fig. 3: The possible tautomers of amino-1,24-triazoles

The resulting aminotriazoles **(4,5)** were then subjected to further derivatization to afford the anticipated acylamino derivatives **6,7(a–e)** and benzylidineaminotriazoles **8,9(a–g)**. Amidation of aminotriazoles may take in consideration the presence of two nucleophilic nitrogens represented by the primary exocyclic (NH_2) and the endocyclic (NH). Consequently, the reaction with acid chlorides can afford two positional isomers as shown in **figure (4).** It is reported that the N-acyl-5-amino-1,2,4-triazole (**A**) experiences trans-acylation to the thermodynamically stable, 5-acylamino-1,2,4-triazole (**B)** at elevated reaction temperature [30]**.** Since we are interested in the 5-acylamino derivatives **6,7(a–e)**, the reactions have been carried out in dry dioxane under reflux for 3 hours.

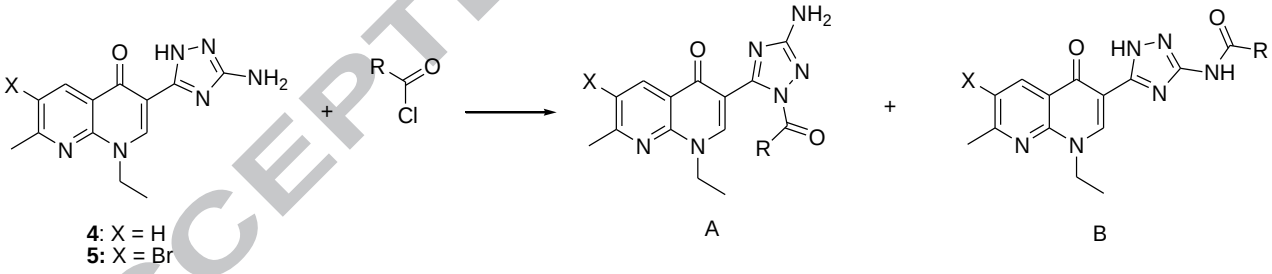


Figure 4: The expected acylation products (**A**) & (**B**) of aminotriazoles.

The structures of the resulting acylamino derivatives **6,7(a–e)** were confirmed by IR, NMR spectroscopy (Supporting information), MS and elemental analysis. IR spectra show the characteristic bands in the range of ~ 3300 – 3200 cm^{-1} resulting from the stretching vibration of amidic and triazolyl (NH) groups. In addition, absorption bands at ~ 1600 -1690 cm^{-1} indicating the presence of two C=O groups (naphthyridinone ring and amide) are also observed. The ^{1}H-NMR illustrates generally the disappearance of the singlet signal of (NH_2)

at δ 5.2 ppm and appearance of new broad band between δ = 10.5 -12.0 ppm indicating the amidic-H.

The synthesis of the targeted benzylidineamino-triazoles **8,9(a–g)** was affected through condensation of the aminotriazoles (**4,5**) and a series of aromatic aldehydes in a methanolic solution of sodium methoxide affording the desired products in good yields. Trials for preparation according to reported conventional methods, using acidic catalysts e.g. glacial acetic acid [31], H_2SO_4 [32] and HCl [33,34] as well as basic catalysts e.g. KOH and NaOH [35,36], failed to afford the anticipated targeted compounds **8,9(a–g)**. The IR of the prepared compounds showed sharp bands at ~ 3150 and 1650 cm^{-1} characteristic for the triazolyl (NH) and naphthyridinone C=O groups respectively. Their ^{1}H-NMR spectra illustrate disappearance of the signal at δ ~ 5.2 ppm of the exocyclic (NH_2). Alternatively, sharp singlets of the benzylidine-H at δ ~ 9.0 – 10.3 ppm have been observed as new feature.

Additional structural assessment of the synthesized amides **6,7(a–e)** and Benzylidine-aminotriazoles **8,9(a–g)** was achieved through ^{13}C-NMR and MS spectral analyses (Supporting information). The spectra show the characteristic peaks of the amidic C=O at δ ~ 163 -165 ppm and CH=N at δ ~ 160 -162 ppm respectively. In addition the signal characteristic for C-atoms of the naphthyridinone skeleton, triazole nucleus, and the aryl substituent were assigned to the respective δ-values, on basis of reported chemical shifts of related compounds [37], as described in details in the experimental section.

3.2 Antibacterial Activity

The synthesized 3-(5-Amino-2H-1,2,4-triazol-3-yl)-naphthyridinones (**4,5)** and their 5-substituted-amino derivatives **6,7(a-e); 8,9(a – g)** were tested *In vitro* for their antibacterial activity in comparison with nalidixic acid (**NA**) using modified cup diffusion method [24]. The investigated bacterial strains involve *Gram positive (G+ve) (S. aureus and B. subtilis)*, *Gram negative (G-ve)* (*E. coli*, *P. aeruginosa* and *Ag. actinomycetemcomitans),* in addition to *M. Phlei.* The antibacterial activity has been primarily evaluated as the observed growth inhibition zones (*mm*) resulting from 100 µl of stock solution (~ *10 mg/ml*) of the tested compounds. Minimum inhibitory concentrations (***MIC***) were then determined for compounds exhibiting significant growth inhibition zones ~ 20 - 40 mm. Figure **5a,b** illustrates the antibacterial activity of the studied 3-(5-acylaminotriazolyl)-naphtyridinones **6,7(a-e)** and the 3-(benzylidineaminotriazolyl)-naphthyridones **8,9(a-g)** respectively.

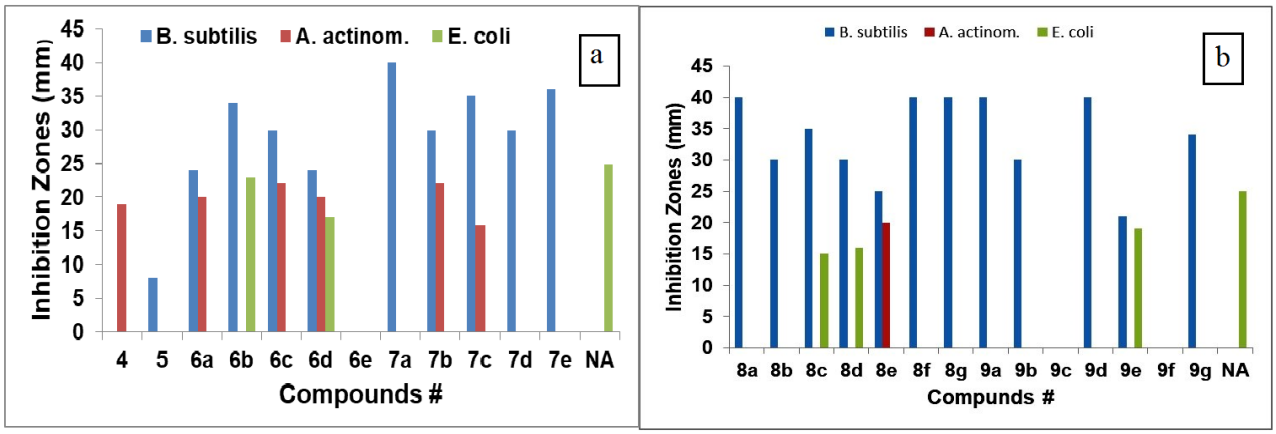


Figure 5: a) Growth inhibition zone of 3-(aminotriazolo)-naphthyridinones (**4,5**) & their amides; **b)** Growth inhibition zone of 3-(Benzylidinaminotriazolo)-naphthyridinones.

As demonstrated in ***Fig. 5a & 5b*** the tested compounds exhibit remarkable selectivity against *B. subtilis,* which is resistant to nalidixic acid**.** *B. subtilis* is a "*strict aerobe*" often considered as the *G+ve* equivalent of *E. coli* [38]. Meanwhile, *A. actinomycetemcomitans* is a *G-ve*, facultative anaerobe, non-motile bacterium that is often found in association with localized aggressive and chronic periodontitis [39,40]. It possesses certain virulence factors that enable it to invade tissues. leukotoxin A (LtxA) is one of its pore-forming toxin, which has been isolated from women with bacterial vaginosis and also regarded as an etiologic agent in endocarditis [41]. Moreover, LtxA is thought to be a trigger of rheumatoid arthritis due to its ability to stimulate protein citrullination, resulting in post-translational modification targeted by auto-antibodies in the disease [42,43].

The studied compounds showed limited antibacterial activity against *A. actinomycete-mcomitans* and *E. coli* as compared to that observed in case of *B. subtilis*. Only some compoundsof the amide series (**6a, 6c, 6d, 7b, 7c)**, (**fig. 5a*)***, in addition to 3-(5-amino-2H-1,2,4-triazol-3-yl)-naphtyridin-4-one **(4)** showed growth inhibition of *A. actinomycete-mcomitans*. The observed inhibition zones ranging from ***16–22*** *mm* and the respective *MIC-values* are ***4.25 – 6.3*** *µM/ml*. Concerning, the antibacterial activity against *E. coli,* only the non-brominated benzamide (**6b**) and its 4-fluoroanalog (**6d**), (***Figure 5a***), are active with inhibition zones ~ 23 and 17 *mm* respectively. Likewise, in the Benzylidineamino series **8,9(a-g)**, (***Figure 5b***), only three derivatives **8c, 8d** and **9e** showed moderate inhibition of the growth of *E. coli* (inhibition zones: 15; 16; and 19 *mm* respectively).

3.3 Enzyme inhibition activity

Quinolone antibacterials are highly specific inhibitors of *DNA-gyrase*. Mutations leading to high-level drug resistance are exclusively in *gyrase-A*, the structural gene for subunit A [44]. The observed selectivity of the tested compounds towards nalidixic acid-resistant *B. subtilis* and *A. actinomycetemcomitans*, inspires evaluation of their potential inhibitory activity (IC_{50}) against the target enzyme. Consequently, *DNA-gyrase* assay has been proposed and executed using a reported high throughput plate assay [25]. *E. Coli* DNA-gyrase microplate assay kit (Inspiralis®) has been used according to the optimized protocol by the manufacturer (Supporting information).The method based upon the fact that negatively-supercoiled plasmids form intermolecular triplex DNA more readily than do relaxed plasmids under some conditions. The assay overcomes some of the problems of gel-based methods, which are time consuming and are therefore inherently low-throughput. In this assay the substrate is the relaxed pNO1, a modified form of pBR322 which contains a 'triplex-forming sequence'.

The assay can be used to determine the activity of compounds as inhibitors of DNA-gyrase either as an initial screen or in the determination of IC_{50} values. Representative compounds of the synthesized series, specifically those having the lowest *MIC*-values were used for assessment of their IC_{50}. The results are represented in **Figure (6),** All compounds showed ~ 50% - 75% inhibition at concentration of ~ 10.0 *µg/ml*.

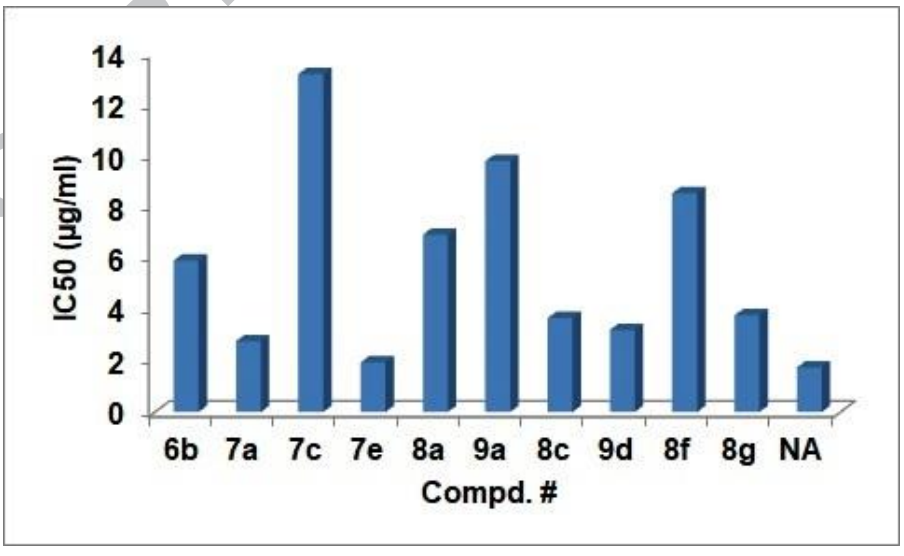


Figure 6: IC_{50} of the most active compounds against *E. coli* DNA–Gyrase

3.4 Structure-Activity Relationship

The *in vitro* antibacterial activities, expressed as growth inhibition zones (*mm*), against the tested organisms are summarized in ***Table 1*****.** The results demonstrate that *B. subtilis* is more susceptible to the 3-(benzylidineaminotriazolyl)-naphthyridinones **8,9(a–g)** than the amides **6,7(a-e**). Additionally, It is also remarkable that bromination of the naphthyridine skeleton at C-6 boosted the antibacterial activity against *B. subtilis* within the amide series, Figures **(5a)**. The brominated compounds **7(a-e)** showed higher inhibition zones than the non-brominated counterparts **6(a-e)**.

Table 1: In vitro antimicrobial activity and docking results of the studied compounds

Cpd. #	Inhibition Zones[a]; (*MIC-values*[b])			Score	E-refine
	B. subt.[c]	*A. actin.*[c]	*E. coli*[c]		
4	0	19		-7.0713	-30.6380
5	8			-6.7719	-25.6302
6a	24	20 (*6.50*)		-8.0302	-36.6972
6b	34		23	-7.4003	-29.6146
6c	30	22		-7.9338	-34.9221
6d	24	20	17	-7.2843	-29.0654
6e	0			-7.0894	-31.0654
7a	40 (*4.46*)			-8.4347	-38.1783
7b	30	22 (*4.25*)		-7.5624	-30.5788
7c	35	16		-8.0774	-36.3195
7d	30			-7.3729	-28.3771
7e	36			-7.7185	-31.6063
8a	40 (*5.30*)			-7.0056	-28.4873
8b	30			-7.4218	-29.8822
8c	35		15	-5.5698	-6.4450
8d	30		16	-7.0093	-25.6541
8e	25	20 (*4.50*)		-7.4115	-30.7913
8f	40 (*4.78*)			-7.3569	-29.3394
8g	40 (*4.90*)			-8.0438	-37.6573
9a	40 (4.25)			-7.5867	-30.6020
9b	30			-7.6046	-20.9404
9c	0			-5.9760	-15.4860
9d	40 (*3.68*)			-7.0456	-19.6408
9e	21		19 (*4.5*)	-7.9246	-33.0737
9f	0			-5.8120	-6.8853
9g	34			-7.9334	-32.3282
NA	0	0	25	-6.6211	-29.7519
MXF.				-6.3736	-59.1971

[a]: (mm); [b]: (µM); [c]: Bacillus subtilis, Aggregatibacter actinomycetemcomitans, Escherichia coli; NA: Nalidixic acid; MXF: Moxifloxacin

The most active compound is the 6-bromo-3-(5-(2-chloroactamido)triazolyl) derivative **7a**: inhibition zone: ***40 mm;*** *MIC:* ***6.5 µM/ml***), whereas, its non-brominated analog (**6a**): inhibition zone: ***24*** *mm****;*** *MIC: 6.3 µM/ml*). In case of the benzylidineamino derivatives **8,9(a–g),** Figure **(5b)**, there is no significant difference between the non-brominated **8(a-g)** and the corresponding brominated derivatives **9(a-g)**. Maximum growth inhibition zones (***40 mm;*** *MIC:* ***3.68 – 5.3 µM/ml***) were elicited by **8a, 8f, 8g**, **9a** and **9d** respectively**.** Regarding, the effect of the substituent on the phenyl moiety of the acyl or the arylidene residues the results revealed that maximum growth inhibition (~40 mm) has been attained by the unsubstituted benzylidenes **(8a,9a)** as well as those having electron donating substituents (**7a,8f, 8g)**.

The results of *DNA-gyrase* inhibitory activity are listed in ***table 2.*** The results revealed that the potent compounds within amide series **6,7(a-e)** are the chloroacetamide derivative (**7a:** IC_{50} = 2.77 µg/ml) and 4-methoxybenzamide **(7e:** IC_{50} = 1.94 *µg/ml*), matching the value of the reference drug (nalidixic acid **NA**: IC_{50} = 1.74 µg/ml). Meanwhile, the unsubstituted benzamide (**6b)** showed 2 fold lower inhibitory activity (IC_{50} = 5.9 *µg/ml*) than the most potent compound (**7e).** However, the presence of electron withdrawing substituent as in compound **(7c)** greatly decreased the inhibitory effect (IC_{50} = 13.2 *µg/ml*). Conversely, The IC_{50}-values of the benzylidineaminotriazole series **8,9(a-g)** didn't show the same pattern of enzyme inhibition activity. Compounds with aryl moiety bearing electron withdrawing substituent e.g. (**8c)** and **(9d)** elicit the lowest IC_{50}*-values*: 3.67 µg/ml and 3.21µg/ml respectively. The unsubstituted counterparts (**8a)** and (**9a)** as well as 4-methoxybenzylidineamino derivative (**8f)** are the least inhibitors.

Table 2: Inhibitory activity (IC_{50}; µg/ml) against *E. Coli DNA-gyrase.*

Cpd. #	6b	7a	7c	7e	8a	9a	8c	9d	8f	8g	NA
IC_{50}	5.92	2.77	13.2	1.94	6.93	9.8	3.67	3.21	8.55	3.78	1.74

3.5 Molecular docking study

Bacterial *DNA-gyrase* represents an important target of antibacterial agents, including fluoroquinolones. This *DNA-enzyme* complex is an *ATP*-dependent enzyme that acts by creating a transient double-stranded DNA break through catalyzing the negative supercoiling and is essential for efficient DNA replication, transcription, and recombination. It is a tetrameric A_2B_2 protein, in which the *A* subunit carries the breakage-reunion active

site, whereas the *B* subunit promotes *ATP* hydrolysis [9]. In most bacterial species, fluoroquinolones inhibit *DNA-gyrase* and *topoisomerase IV* and cause bacterial cell death. Unfortunately, their use is threatened by the increasing prevalence of target-mediated drug resistance. The most widespread resistance-conferring mutations occur at a highly conserved serine residue in the *A* subunit of the enzyme. In general, alterations at these amino acids are observed in >90% of quinolone resistant clinical bacterial isolates [45,46].

Recent study of the structure of a ternary *A. baumannii topoisomerase IV*-moxifloxacin cleavage complex identified a chelated Mg^{2+} ion that appeared to be coordinated to four water molecules (**Fig. 1**). Furthermore, two of these water molecules were situated close enough to *Ser1084* and *Glu1088* to form hydrogen bonds [45]. It was concluded that this water−metal ion interaction, which "bridges" the drug to the enzyme plays a pivotal role in mediating quinolone activity [9].

Accordingly, it was interesting to delineate the mechanistic aspects of the observed growth inhibition of resistant bacterial strains (*B. subtilis & A. actinomycetemcomitans*) by the synthesized compounds. The crystal structures of *DNA-gyrase* cleavage complex of *A. baumannii* co-crystallized with Moxifloxacin (PDB code: 2xkk) has been downloaded from protein data bank and used for the present docking study.

Analysis of the co-crystallized *DNA-gyrase* cleavage complex of *A. baumannii* with moxifloxacin revealed the following binding modes [10] (***Supporting information***):

- C-3 (COOH)/C-4 (C=O): Mg^{2+}/($4H_2O$) – bridge/*Ser*(*A1084*) and *Glu*(*A1088*).
- C-3(COOH)/C-4 (C=O): Mg^{2+}/($4H_2O$) – bridge/*Thymidine DT*(*C15*).
- Ionic interaction COO^-/*Arg*(*B1123*).
- Arene-arene interaction: Pyridone ring/*Guanine DG*(*C15*).
- H-arene interaction: C-8(OCH_3)/*Guanine DG*(*C15*).
- H-bonding: Pyrrolidino-piperidine/*Adenine DA*(*D20*).

In the present investigation the proposed docking algorithm was initially validated by self-docking of the co-crystallized ligand (*moxifloxacin*) to the active site of *DNA-gyrase* cleavage complex of *A. baumannii*. Thereby, the ligand was removed from the complex and then docked back into the active site. Heavy-atom root mean square deviation (RMSD) values between top-ranked poses and the experimental crystal structure ranged from ~ 1.02 – 1.60 Å. Subsequently, docking procedures have been accomplished for the studied compounds using Molecular Operating Environment software (*MOE-dock tool, ver. 2014.0901,* Chemical Computing Group, Montreal, Canada) to detect their binding modes

to bacterial *DNA-gyrase* cleavage complex active site. The study have successfully identified comparable interaction modes for the studied 3-(5-un/substituted aminotriazolyl)-naphthyridinones illustrating that the designed compounds could potentially bind to the active site with analogous or even higher strengths.

The Docking of the synthesized compounds to the active site of *DNA-gyrase* cleavage complex of *A. baumannii* (PDB code: 2xkk) showed that non-derivatized 3-aminotriazolyl moiety (**4** & **5**) bind to *DNA-gyrase* cleavage complex through dipole ion interaction (Mg^{+2}-water bridge) between the naphthyridinone C=O group and Mg^{+2}. This is further co-ordinated to water molecules forming hydrogen bond with *Ser*(*A1084*); *DNA-guanine* (DG:B16) and *Thymidine* (DT3: B15) (supporting information). This binding mode resembles that observed with the co-crystallized *moxifloxacin*. Acylation of the amino group into amides **6,7(a-e**) changed the mode of interaction **(Figure 7a)**. In this respect, the C=O group of the amide moiety and N_1 of triazole ring chelate Mg^{+2} ion, Which is then linked to *Ser*(*A1084*), *Glu(A1088*) and *DT3*(*B15*) through water bridges. Additionally, there are hydrophobic interaction of the naphthyridinone ring as well as arene-arene interaction between triazole ring and *DNA* residues: *DG*(*B16*). The shift of binding group to the amide moiety might imply an explanation for growth inhibition of the resistant bacterial strains by the tested compounds. On the other hand, docking of benzylideneaminotriazolyl naphthyridinones **8–9(a-g)** showed that Mg^{+2}-water bridge is initiated between C=O group of the naphthyridinone, N_1 of triazole and coordinated to the water molecules forming hydrogen bonds with *Ser*(*A1084), DT3*(*B15*) and *DG*(*B16*) **(Figure 7b)**.

Table (1), summarizes the docking scores and the respective growth inhibition zones of the tested compounds**.** The interaction free energy of the ligand-target complex represents a measurable value for the binding strength that preferably might be matched with observed biological activity. Consequently, docking simulations based on London -dG as a scoring function (S) were performed to estimate the free energy of interactions of the studied 3-(5-acylamino-triazolyl)-naphtyridinones **6,7(a-e)** and the corresponding benzylidineaminotriazolyl derivatives **8,9(a-g)** from given poses with *DNA-gyrase* cleavage complex of *A. baumannii* (PDB code 2xkk). Molecular data base files were generated for each of the studied compounds and the best conformer representing ligand-target interaction were allocated. The selection criteria are based primarily on RMSD-value < 2.0 and comparable interaction to the co-crystallized ligand. An additional scoring function expressed as *E-refine* values is also taken in consideration. This parameter considered as more representative for the binding energy, since it involves energy minimization of the ligand-target complex. Consequently, it illustrates the least energy interaction value of the selected pose.

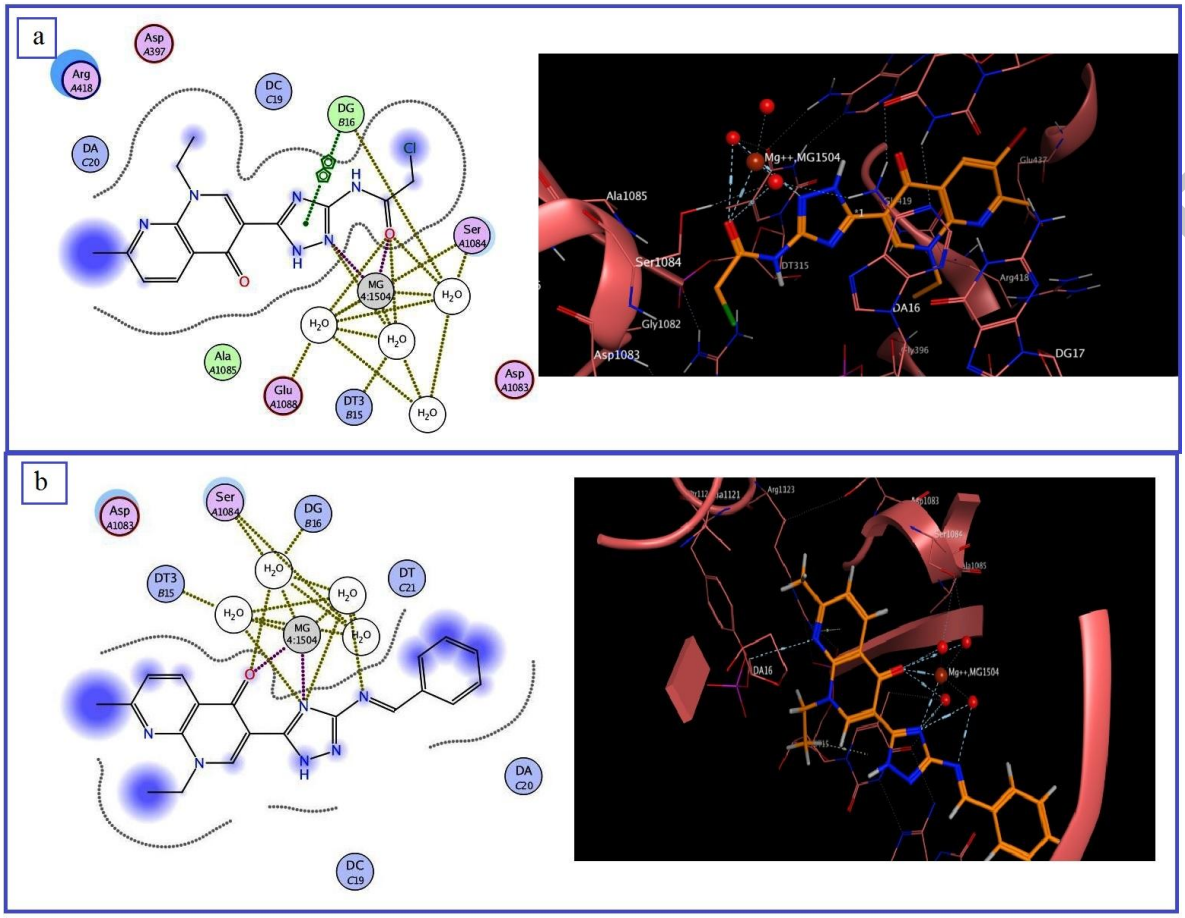


Figure (7): **a)** 2D & 3D representation of **(6a)** docked into DNA-gyrase cleavage complex of *A. baumannii* (PDB code 2xkk); **b)** 2D & 3D representation of **(8a)** docked into DNA-gyrase cleavage Complex of *A. baumannii* (PDB code 2xkk).

The results in ***table (1)*** indicate that, there is a good correlation between the docking scores and the observed antibacterial activity expressed as growth inhibition zones. The most active compounds against *B. subtilis* (**7a, 8f, 8g, 9a** and **9d)** with growth inhibition zones (40 *mm*) showed the lowest dG values = -7.0 – (-8.4) *kcal/mol*, which is superior than that of the co-crystalized ligand, moxifloxacin (dG: -6.37 *kcal/mol)*. It is obvious that the reference compound, nalidixic acid, which is practically inactive against *B. subtilis,* exhibits lower binding with the DNA-gyrase cleavage complex (dG: -6.62 *kcal/mol)*.

3.6 Molecular properties and drug-likeness

Molecular properties are a complex balance of various structural features which determines whether a particular molecule is similar to the known drugs or not. Hydrophobicity, molecular size, flexibility, and presence of various pharmacophoric features are the main physical properties that influence the behavior of molecules in a

living organism. Good bioavailability can be achieved with an appropriate balance between solubility and partitioning properties. In addition, topological polar surface area (*TPSA*) and number of rotatable bonds (*nrotb*) have been linked to drug bioavailability [47]. Thus, the compliance of the newly synthesized compounds to Lipinski's rule of five was evaluated [48].

Molecular properties (*TPSA, nrotb, LogP, OH–NH interaction, molecular weight*) of the newly synthesized compounds were calculated using *MOE* software program and compared to the values of the standard drug, nalidixic acid (**Table 3**). *TPSA* is calculated based on the methodology published by Ertl et al [49] as the surface areas that are occupied by oxygen and nitrogen atoms and by hydrogen atoms attached to them. It is considered as a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, and blood–brain barrier penetration. Molecules with TPSA values around 140 $Å^2$ or more are expected to exhibit poor intestinal absorption [47]. The results shown in **table 3** indicate that all the synthesized compounds have TPSA values <140 $Å^2$; thus, they are expected to have good intestinal absorption. Molecules with more than 10 rotatable bonds may have problems with bioavailability [47]. All compounds under investigation (**Table 3**) have two to six rotatable bonds and they might not have problems with bioavailability. *LogP* values are based on summation of fragment-based contributions and correction factors. It has been shown that for the compound to have a reasonable probability of being well absorbed, *LogP* value must be in the range of -0.4 to 5 [47]. On this basis, all the tested compounds were found to have *LogP* values within the acceptable criteria and are expected to have reasonable oral absorption. It is worth mentioning that all analyzed compounds have one or zero violation of Lipinski's rule; therefore, they are expected not to have problems with bioavailability.

Finally, drug score is a computational value combining: drug-likeness, *LogP*, solubility, molecular weight, and toxicity risks, in one handy value that may be used to judge the compound's overall potential to qualify for a drug [50]. A value of 0.5 or more makes the compound a promising lead for future development of safe and efficient drugs. The overall drug score values for the synthesized compounds were calculated using *molsoft* software (*http://molsoft.com/ mprop/*) and compared to that of the parent drug, nalidixic acid (**Table 3**). Almost all the synthesized compounds possess good drug score values above 0.5

Table 3: Molecular characteristics* of the studied triazolylnaphthyridinones

Cpd. #	*M. wt*	*HBA*	*HBD*	*#RT*	*Log P*	*TPSA (Å²)*	*Sol. (mg/L)*	*Drug Score*
4	270.29	4	2	2	1.96	102.48	278.02	0.69
5	349.19	4	2	2	1.16	102.48	23.43	0.72
6a	346.77	5	2	5	1.88	105.56	17.13	1.08
6b	374.4	5	2	5	1.61	105.56	2.66	1.10
6c	408.84	5	2	5	1.73	105.56	0.32	1.44
6d	392.39	6	2	5	1.55	105.56	0.85	1.37
6e	404.42	6	2	6	2.01	114.79	1.58	1.01
7a	425.67	5	2	5	1.31	105.56	1.37	1.05
7b	453.3	5	2	5	1.79	105.56	0.21	1.07
7c	487.74	5	2	5	2.06	105.56	0.02	1.53
7d	471.28	6	2	5	1.9	105.56	0.07	1.45
7e	483.32	6	2	6	3.33	114.79	0.12	1.08
8a	358.40	5	1	4	3.03	88.82	1.13	0.77
8b	392.84	5	1	4	2.32	88.82	0.14	1.02
8c	376.39	6	1	4	2.28	88.82	0.36	0.97
8d	437.29	5	1	4	2.51	88.82	0.10	0.75
8e	427.29	5	1	4	2.64	88.82	0.03	0.65
8f	388.42	6	1	5	2.51	98.05	0.68	0.58
8g	374.40	6	2	4	1.94	109.05	3.38	0.49
9a	437.29	5	1	4	2.25	88.82	0.09	0.89
9b	471.74	5	1	4	2.58	88.82	0.01	1.17
9c	455.28	6	1	4	2.56	88.82	0.03	1.10
9d	516.19	5	1	4	2.69	88.82	0.01	1.07
9e	506.18	5	1	4	2.95	88.82	0.00	0.70
9f	467.32	6	1	5	2.81	98.05	0.05	0.70
9g	453.29	6	2	4	1.93	109.05	0.27	0.77
NA	232.08	4	1	2	1.23	53.67	758.43	0.82

* ***Mwt:*** molecular weight, ***Log P:*** lipophilicity parameter, ***HBD:*** # of hydrogen bond donor, ***HBA:*** # of hydrogen bond acceptor, ***TPSA:*** topological polar surface area, # RT: # of rotatable bonds.

4. Conclusion

The *in vitro* biological evaluation of the studied series of hybrid compounds combining naphthyridinone skeleton and 3-amino-1,2,4-triazole revealed selective antibacterial activity against two resistant bacterial strains. The *G +ve*, *B. subtilis,* was the most susceptible bacterial strain, whereby 21 compounds exhibited growth inhibition zones ~20-40 *mm* (*MIC:* 33.5-5.5 *µM)* against this. Meanwhile, the growth of *A. actinomycete-mcomitans* has been arrested by 7 compounds (Inhibition zones 15-20 *mm*; *MIC* values: 4.3-6.3 *µM).* Furthermore, the introduction of bromine at C-6 of the naphthyridinone skeleton enhanced the antibacterial activity. DNA-gyrase inhibition assay of the most active compounds showed that they possess a moderate to high inhibitory effect having IC_{50} range from 1.7 *µg/ml* to 13.2 *µg/ml*. Compound **7a** and **7e** were the most potent

(IC_{50} = 2.77 and 1.94 µg/ml respectively). Molecular docking results revealed that 1,2,4-triazole is a bioisostere to COOH group that maintains the essential binding interaction with *DNA–gyrase* cleavage complex similar to that observed with the co-crystallized ligand, *moxifloxacin*. Derivatization into amides and imines introduced additional binding interactions with the enzyme with lower binding energy which might represent mechanistic basis for the observed antibacterial activity. Computational results of molecular characteristics indicate that the synthesized compounds are primarily of acceptable bioavailability and possess drug-likeness potential.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

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