Egypt. J. Chem. **Vol. 65,** No. 3 pp. 1 – 17 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

1,8-Diaminonaphthalene-derived pharmacophore as potent anti-MRSA with dual DNA gyrase and topoisomerase IV inhibition

Ebtehal M. Husseiny[a]*, Nagwan G. El Menofy[b], Samiha A. El-Sebaey[a]

[a] *Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, Cairo, Egypt*

[b] *Department of Microbiology and Immunology, Faculty of Pharmacy (Girls), Al-Azhar University, Nasr City, Cairo, Egypt*

Abstract

1,8-Diaminonaphthalene was used as an active binucleophile from which some perimidines and (naphthalene-1,8-diyl)bis(heterocycles) were designed, prepared, and assessed for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, different strains of methicillin-resistant *Staphylococcus aureus* (MRSA), and multidrug resistant (MDR) clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis. Compounds **1** and **6** exhibited significant antibacterial activity against Staphylococcus aureus* with MIC values of 62.5 and 15.63 µg/mL. Additionally, compound **2** showed a remarkable antimicrobial activity against MRSA 1 (MIC = 62.5 µg/mL). Additionally, compound **6** exerted extremely potent antimicrobial effect towards all resistant strains with MIC values range of 31.25-62.5 µg/mL that is much more potent than reference drugs amoxicillin and cephalexin (MIC > 500 µg/mL). Furthermore, compounds **2**, **3,** and **6** displayed powerful antibiofilm activity against both *Staphylococcus aureus* and MRSA where compound **2** presented the most potent antibiofilm action among the tested compounds. To investigate the mechanism of action for compounds **2** and **6**, inhibition of DNA gyrase together with topoisomerase IV were evaluated. Both compounds presented promising inhibitory action with IC_{50} at the micromolar range.

Keywords: Antibiofilm; antimicrobial; DNA gyrase; MRSA; naphthalene; perimidine; topoisomerase IV.

1. Introduction

The evolving antimicrobial resistance due to Gram-positive bacterial infections (GPBIs) is one of the major public health care concerns. Multidrug resistant Gram-positive bacteria can result in high morbidity and mortality rates in addition to high cost and long hospitalization [1,2]. Among numerous pathogenic Gram-positive bacteria, *Staphylococcus aureus* and enterococci are standing out as being responsible for the most challenging and resistant infections [3,4]. MRSA cause many infections such as osteomyelitis, meningitis, urinary tract infections, gastrointestinal tract infections, septicemia, pneumonia, endocarditis and infections of indwelling medical devices [5,6]. The World Health Organization (WHO) categorized MRSA as one of the most priority pathogens for which novel antibacterial agents should be designed [7].

Enterococci have elicited as significant nosocomial pathogens second to Staphylococci which are the leading source of nosocomial infections all over the world [8]. Approximately all Enterococci infections are provoked via *Enterococcus faecalis* and *Enterococcus faecium [9]*. *Enterococcus* is resistant to many antimicrobial agents and can transfer the drug resistance genes to *Staphylococcus aureus* [10]. In fact, many *Staphylococcus aureus* infections comprise formation of biofilms that are communities of bacteria attached to surfaces and enclosed in extracellular matrix usually formed of polysaccharides, proteins, lipids, and DNA [11]. The principal rational for antimicrobial resistance is the late distribution of antibiotics across the biofilm matrix [12]. Biofilm formation by staphylococcus resistant isolates is considered a significant virulence factor influencing its pathogenicity and persistence in host organisms [13]. Biofilm forming bacteria causes chronic infections that are resistant to many antimicrobial agents and accounts for more than 80 % of all types of infections [14]. The rising MRSA and enterococci resistance to the most antibiotics, biofilm formation along with the high infection rate, either hospital- or community-acquired, necessitate the discovery of

*Corresponding author e-mail: ebtehal.ouf@azhar.edu.eg

Receive Date: 04 November 2021, Revise Date: 22 November 2021, Accept Date: 06 December 2021

DOI: 10.21608/ejchem.2021.104410.4824

novel antimicrobial agents against these hazardous infections.

1,8-Diaminonaphthalene is considered as an active binucleophile from which many valuable nitrogens containing heterocyclic compounds were attained. Perimidine is a perinaphtho-fused pyrimidine structure that was firstly obtained in 1874 [15]. It was also synthesized from cyclocondensation of 1,8-diaminonaphthalene with different bicarbonyl compounds [16]. Perimidines are significant polynuclear heterocyclic compounds with versatile pharmacological and biological activities for example anticancer [17-21], antimicrobial [22,23], anti-inflammatory [24], antioxidant [25], and antiulcer [26]. Furthermore, (1*H*-perimidin-2-yl)methanethiol **I** was reported to exert potent antimicrobial activity, especially against *Bacillus subtilis* with inhibition percent equal 89-100 % [24]. Besides, pyridazinylperimidine derivative **II** showed promising growth inhibitory activity towards Gram-positive, Gram-negative and fungi strains with minimum inhibitory concentration (MIC) ranges from 0.06-1.95 µg/mL [27]. Moreover, compound **III** exhibited remarkable inhibitory activity towards *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Escherichia coli* as well as *Candida albicans* [28]. (Figure 1).

Moreover, quinoline motif was considered as a very important core in broad spectrum antibacterial drugs such as ciprofloxacin, gemifloxacin, sparfloxacin, levofolaxin, gatifloxacin, moxifloxacin and ofloxacin [29]. Also, 7-substituted quinolin-4-one derivatives showed potent antibacterial activity against MRSA [30]. Additionally, naphthoquinones have been known as substantial scaffold in drug design for treatment of many infectious diseases caused by pathogenic microorganisms [31,32]. So, isosteric replacement of quinoline by naphthalene is expected to provide compounds with similar antimicrobial activity.

On the other hand, naphthalene derivatives have caught the attention of many scientists owing to their broad pharmacological applications such as antimicrobial [33-39], anticancer [40], anti-inflammatory [41] and antiviral activities [42]. There are some marketed antimicrobial drugs containing naphthalene moiety for example Naftifine **IV**, antibacterial and antifungal drug that inhibited sterol biosynthesis [43]. Also, Tolnaftate **V** and Terbinafine **VI** are antifungal agents which are able to suppress squalene epoxidase, an essential enzyme for biosynthesis of ergosterol [44-46] (Figure 2). Previous studies revealed that Schiff base **VII** showed a potent antimicrobial action against *Staphylococcus aureus*with an inhibition zone of 10 ± 0.856 mm. The improved lipophilicity of **VII** encouraged its ability to penetrate the bacterial cell wall [47].

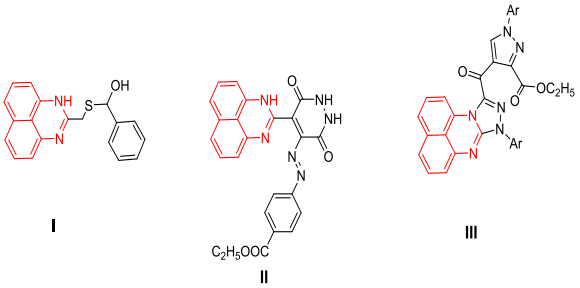


Figure 1: Perimidine containing antimicrobial agents

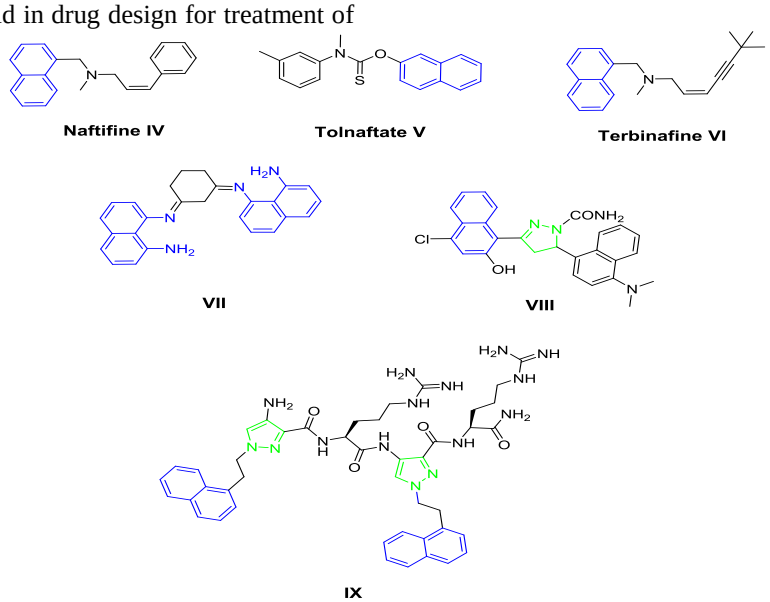


Figure 2: Naphthalene containing antimicrobial agents

Furthermore, dinaphthalene-1-ylsubstituted 2-pyrazolines **VIII** exhibited powerful antimicrobial potential against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabillis*, *Shigella dysentery* and

Salmonella typhii [48,49]. Also, pyrazole-arginine based peptidomimetics **IX** was investigated to exert good antimicrobial activity against antibiotic resistant resistant strains as MRSA, vancomycin-resistant *Enterococcus faecium* (VREF) and multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) two to four times better than melittin [50]. Moreover, 1,5-diphenylpyrrole containing analogues **X** were discussed to show high inhibitory action against drug-resistant Gram-positive and Gram-negative pathogens via DNA gyrase suppression [51]. Another study reported the promising antibacterial activity of pyridine-3-carboxamide derivatives **XI** as dual suppressors of DNA gyrase and DNA topoisomerase IV [52]. Additionally, *N*,*N*-bis(cyanoacetyl)hydrazine derivatives **XII** were reported to display a remarkable antimicrobial activity via suppression of DNA gyrase and topoisomerase IV [53] (Figure 3).

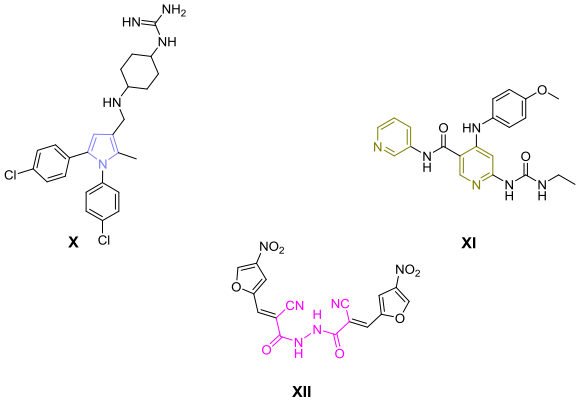


Figure 3 : Reported DNA gyrase and topoisomerase IV inhibitors

Guided by the aforementioned details, it was decided to use 1,8-diaminonaphthalene as an active precursor for the synthesis of novel perimidines and pharmacophore-linked perimidines derivatives to evaluate their antimicrobial activity and antibiofilm activity. Linked pharmacophores include bispyrroles, bispyridinones, naphthalenes, and bispyrazoles. The activity of the new molecules was assessed against different bacterial clinical isolates and standard strains including the realistic mode of action.

2. Experimental

2.1. Chemistry

Melting points were detected via Stuart device and were uncorrected. Infrared charts were carried out on Bruker FT-IR spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were applied in (DMSO-d_6) at 300 MHz on a Varian Gemini NMR spectrometer. Mass spectra were performed via Schimadzu GCMS-QP-1000EX mass spectrometer. Elemental analyses were determined on Elementar Vario analyzer. Progression of reactions were followed up through silica gel (60 GF 254, Merck), the eluent was ethyl acetate/hexane (2:1) and were viewed under UV-lamp.

9-Hydroxy-10-oxo-10*H*-pyrrolo[1,2-*a*]perimidine-8-carbonitrile (2).

A suspension of equimolar amounts of compound **1** (0.41 g, 2 mmol) and oxalyl chloride (0.25 g, 0.17 mL, 2 mmol) in absolute ethanol (20 mL) containing piperidine (3 drops) was refluxed for 50h. After cooling, the resulted powder was filtered and crystallized from DMF.

Brown powder; Rf = 0.38; yield 61%; m.p. 240-241°C. **IR** (KBr, cm^{-1}): 3425, 3387 (br. OH); 3010 (CH–Ar); 2200 (C≡N); 1656 (amidic C=O), 1593 (C=N); 1462 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 6.79-7.22 (m, 6H, Ar–H); 8.93 (s, 1H, OH, D_2O exchangeable). **^{13}C-NMR** (DMSO-d_6–δppm): 92.2 (C-8); 116.3 (C≡N);119.9 (C-1); 121.2 (C-3a$_1$); 122.0 (C-4); 124.0 (C-6); 126.5 (C-3); 129.4 (C-3a); 129.6 (C-5); 132.3 (C-2); 143.6 (C-11a); 148.1 (C-6a); 149.1 (C-7a); 152.4 (C=O); 161.2 (C-OH). **Molecular Formula**: $C_{15}H_7N_3O_2$. Analysis, **Calcd**. (%):C, 68.97; H, 2.70; N, 16.09. **Found** (%): C, 69.17; H, 2.67; N, 16.34.

10-Hydroxy-8,11-dihydropyrido[1,2-*a*]perimidine-8-carbonitrile (3).

A solution of compound **1** (0.41 g, 2 mmol) in DMF (15 mL) containing piperidine (1drop) was mixed with 1,3-dichloroacetone (0.25 g, 2 mmol). The mixture was refluxed for 26 h. After that, it was poured on crushed ice containing 3 drops of dil. HCl to give the targeted product. The resulted powder was filtered, rinsed out with plenty amount of water. The selected crystallization solvent was DMF / water mixture (1:1). Shiny black powder; Rf = 0.37; yield 70%; m.p. >300°C. **IR** (KBr, cm^{-1}): 3438 (br. OH); 3010 (CH–Ar); 2943 (CH–aliph.); 2165 (C≡N); 1636 (C=N); 1465 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 3.45-3.56 (m, 2H, pyridoperimidine–C_8–H); 4.02, 4.14 (2s, 2H, pyridoperimidine–C_{11}–H); 4.31-4.36 (m, 1H, pyridoperimidine–C_9–H); 7.36-7.60, 7.82-8.15 (2m, 6H, Ar–H); 9.29 (s, 1H, OH, D_2O exchangeable). **MS:** m/z (%): 262 (M+1, 17); 261($M^{\cdot+}$, 21); 223(32); 187(33); 166(46); 163(46); 149(34); 147(41); 134(75); 106(57); 97(55); 95(100). **Molecular Formula**: $C_{16}H_{11}N_3O$. Analysis, **Calcd**. (%):C, 73.55; H, 4.24; N, 16.08. **Found** (%):C, 73.81; H, 4.32; N, 16.21.

9-Hydroxy-11-oxo-8,11-dihydropyrido[1,2-*a*]perimidine-8-carbonitrile (4).

Compound **1** (0.41 g, 2 mmol) in benzene (20 mL) was added to diethyl malonate (0.64 g, 0.61 mL, 4 mmol) and the mixture was refluxed for 40 h., cooled, filtered and the attained powder was crystallized from DMF.

Brown powder; Rf = Rf = 0.36; yield 46%; m.p. 168-170°C. **IR** (KBr, cm^{-1}): 3367 (br. OH); 3049 (CH–Ar); 2924 (CH-aliph.); 2202 (C≡N); 1635 (C=O); 1616(C=N); 1585 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 2.72 (s, 1H, pyridoperimidine–C_8–H); 4.16 (s, 1H, pyridoperimidine–C_{10}–H); 6.97-7.31 (m, 6H, Ar–H); 11.69 (s, 1H, OH, D_2O exchangeable). **^{13}C-NMR** (DMSO-d_6–δppm): 28.6(C-8); 109.7 (C-10); 114.8 (C-6); 120.1 (C-1); 122.1 (C≡N);122.5 (C-3); 125.1 (C-4); 126.9 (C-$3a_1$); 129.9 (C-2); 133.5 (C-5); 141.8 (C-3a); 144.7 (C-6a); 146.3 (C-12a); 152.2 (C-7a); 159.0 (C=O); 196.1 (C-OH). **Molecular Formula**: $C_{16}H_9N_3O_2$. Analysis, **Calcd**. (%):C, 69.81; H, 3.30; N, 15.27. **Found** (%): C, 69.95; H, 3.34; N, 15.38.

11-Hydroxy-9-methylpyrido[1,2-*a*]perimidine-8-carbonitrile (5).

An equimolar mixture of compound **1** (0.41 g, 2 mmol) and ethyl acetoacetate (0.26 g, 0.25 mL, 2 mmol) in absolute ethanol (20 mL) containing ammonium acetate (0.15 g, 2 mmol) as a catalyst was refluxed for 12 h. The excess solvent was evaporated and the product was crystallized from DMF.

Pale brown powder; Rf = 0.43; yield 52%; m.p.200-202°C. **IR** (KBr, cm^{-1}): 3051 (CH–Ar); 2922 (CH-aliph.); 2204 (C≡N); 1633 (C=O); 1614 (C=N); 1469 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 1.23 (s, 3H, CH_3); 2.26 (s, 2H, CH_2); 6.52-7.31 (m, 6H, Ar–H). **MS:** m/z (%): 273($M^{\cdot+}$, 10); 207 (36); 182(92); 168(100); 167(30); 149(22); 140(44); 113(22); 95(22); 81(55); 69 (90). **Molecular Formula**: $C_{17}H_{11}N_3O$. Analysis, **Calcd**. (%):C, 74.71; H, 4.06; N, 15.38. **Found** (%):C, 74.89; H, 4.12; N, 15.49.

***N*,*N*'-(Naphthalene-1,8-diyl)bis(2-chloroacetamide) (6).**

A mixture of 1,8-diaminonaphthalene (1.58 g, 10 mmol) and chloroacetyl chloride (2.26 g, 1.59 mL, 20 mmol) was refluxed in dioxane for 1 h. Then, it was cooled and treated with crushed ice to attain the desired precipitate that was filtered, rinsed out with plenty amount of water. The selected crystallization solvent was DMF / H_2O (1:1) mixture.

Yellow powder; Rf = 0.30; yield 81%; m.p. 266-276°C. **IR** (KBr, cm^{-1}): 3123 (NH); 3003 (CH–Ar); 2966, 2823 (CH–aliph.); 1649 (C=O); 1587 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 4.45 (s, 4H, $2CH_2$); 6.71-7.38 (2m, 8H, Ar–H& 2NH). **Molecular Formula**: $C_{14}H_{12}Cl_2N_2O_2$. Analysis, **Calcd**. (%): C, 54.04; H, 3.89; N, 9.00. **Found** (%): C, 54.33; H, 3.91; N, 9.11.

***N*,*N*'-(Naphthalene-1,8-diyl)bis(2-cyanoacetamide) (7).**

Method A: A suspension of compound **6** (0.62 g, 2 mmol) in dry benzene (10 mL) and potassium cyanide (0.26 g, 4 mmol) in water (5 mL) was stirred at 50ºC for 6 h. The aqueous layer was separated and treated with ice-colled water containing 3 drops of 10% HCl. The resulted product was filtered, washed, and crystallized from DMF/H_2O (1:1) mixture.

Method B: A mixture of1,8-diaminonaphthalene (1.58 g, 10 mmol) and ethyl cyanoacetate (2.26 g, 2.13 mL, 20 mmol) in absolute ethanol (25 mL) was heated under reflux for 50 h. The resulted solution was cooled, filtered and the attained product was crystallized from DMF/H_2O (1:1) mixture.

Green powder; Rf = 0.31; yield 84%; m.p. >300°C. **IR** (KBr, cm^{-1}): 3378 (NH); 3039 (CH–Ar); 2920 (CH–aliph.); 2171 (C≡N); 1660 (C=O); 1579 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 3.99 (s, 4H, $2CH_2$); 6.55 (d, 2H, *J*= 6.9 Hz, naphthalene–$C_{2,7}$–H); 6.77-7.18 (m, 4H, naphthalene–$C_{3,4,5,6}$–H); 10.53 (s, 2H, 2NH, D_2O exchangeable). **^{13}C-NMR** (DMSO-d_6–δppm): 21.4 ($2CH_2$); 126.5 (C-2,7); 126.8 (C-8a); 128.1 (C-4,5); 129.3 (2C≡N);129.9 (C-3,6); 137.2 (C-1,8); 147.0 (C-4a); 155.1 (2C=O). **Molecular Formula**: $C_{16}H_{12}N_4O_2$. Analysis, **Calcd**. (%):C, 65.75; H, 4.14; N, 19.17. **Found** (%):C, 65.91; H, 4.24; N, 19.25.

***N*3,*N*3'-(Naphthalene-1,8-diyl)bis(1*H*-pyrazole-3,5-diamine) (8).**

To a suspension of compound **7** (0.29 g, 1 mmol) in absolute ethanol (20 mL), (0.5 g, 0.5 mL, 10 mmol) of hydrazine hydrate 99% was put in. The mixture was heated for 16 h. and the desired powder was filtered and crystallized from DMF/H_2O (1:1) mixture.

Brown powder; Rf = 0.36; yield 68%; m.p. >300°C. **IR** (KBr, cm^{-1}): 3367, 3205 (NH_2& NH); 3025 (CH–Ar); 1624 (C=N); 1543 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 4.70 (s, 2H, 2 pyrazole–C_4–H); 7.16 (d, 2H, *J*= 7.5 Hz, naphthalene–$C_{2,7}$–H); 7.41 (t, 2H, *J*= 7.5 Hz, naphthalene–$C_{3,6}$–H); 7.69 (d, 2H, *J*= 7.5 Hz, naphthalene–$C_{4,5}$–H); 10.41 (s, 4H, 2 NH_2, D_2O exchangeable); 10.88 (s, 2H, 2 NH, D_2O exchangeable); 12.54 (s, 2H, 2 Pyrazole-NH, D_2O exchangeable). **MS:** m/z (%): 320($M^{\cdot+}$, 7); 223(21); 207(27); 186(26); 173(26); 140(28); 114(51); 91(74); 76(21); 52(100). **Molecular Formula**: $C_{16}H_{16}N_8$. Analysis, **Calcd**. (%):C, 59.99; H, 5.03; N, 34.98. **Found** (%):C, 60.16; H, 5.10; N, 35.21.

1,1'-(Naphthalene-1,8-diyl)bis(5-(4-chlorophenyl)-2-oxo-2,3-dihydro-1*H*-pyrrole-3-carbonitrile) (9).

A suspension of compound **7** (0.29 g, 1 mmol) and 4-chlorophenacyl bromide (0.47 g, 2 mmol) in absolute ethanol (20 mL) including 5 drops of triethylamine as a catalyst was refluxed for 17 h. The resulted solid was filtered and crystallized from DMF/H_2O (1:1) mixture.

Brown powder; Rf = 0.41; yield 76%; m.p. >300°C. **IR** (KBr, cm^{-1}): 3055 (CH–Ar); 2206 (C≡N); 1670 (C=O); 1589 (C=C). **^{1}H-NMR** (DMSO-d_6-δppm): 2.73 (s, 2H, 2CHCN); 2.89 (s, 2H, 2pyrrole–C_4–H); 7.30-7.56 (m, 6H, naphthalene–H); 7.59 (d, 4H, *J*= 8.1 Hz, 4–Cl–C_6H_5–$C_{2,6}$–H); 7.96 (d, 4H, *J*= 8.1 Hz, 4–Cl–C_6H_5–$C_{3,5}$–H). **^{13}C-NMR** (DMSO-d_6–δppm): 29.0 (pyrrole-C-3,3'); 88.9 (pyrrole-C-4,4'); 103.2 (naphthalene-C-2,7); 106.0 (naphthalene-C-8a); 108.8 (2C≡N); 120.0 (naphthalene-C-4,5); 120.9(phenyl-C2,2',6,6'); 121.3 (naphthalene-C-3,6);128.6 (phenyl-C3,3',5,5'); 128.9 (pyrrole-C-5,5'); 130.0 (phenyl-C1,1'); 133.8 (phenyl-C4,4'); 137.9 (naphthalene-C-1,8); 140.8 (naphthalene-C-4a); 153.5 (2C=O).**MS:** m/z (%): 561($M^{\cdot+}$, 5); 208(21); 195(21); 174(27); 147(36); 128(46); 119(52); 104(30); 86(38); 80(50); 77(100). **Molecular Formula**: $C_{32}H_{18}Cl_2N_4O_2$. Analysis, **Calcd**. (%):C, 68.46; H, 3.23; N, 9.98. **Found** (%):C, 68.61; H, 3.30; N, 10.13.

1,1'-(Naphthalene-1,8-diyl)bis(6-amino-4-(2,4-dimethoxyphenyl)-2-oxo-1,2-dihydropyridine-3,5-dicarbonitrile); (11).

Compound **7** (0.29 g, 1 mmol) and 2-(2,4-dimethoxybenzylidene)malononitrile **(10)** (0.43 g, 2 mmol) were refluxed in absolute ethanol (20 mL) containing piperidine (3 drops) for 25 h. After cooling, the product was filtered, left to dry and crystallized from DMF/H_2O (1:1) mixture.

Dark brown powder; Rf = 0.45; yield 66%; m.p. >300°C. **IR** (KBr, cm^{-1}): 3394 (NH_2); 3059 (CH–Ar); 2931 (CH–aliph.); 2187 (C≡N); 1660 (C=O); 1581 (C=C); 1207, 1026 (C-O-C). **^{1}H-NMR** (DMSO-d_6-δppm): 3.84 (s, 12H, 4 OCH_3); 6.29-7.36 (2m, 12H, Ar-H); 8.31 (s, 4H, 2NH_2, D_2O exchangeable). **^{13}C-NMR** (DMSO-d_6–δppm): 49.1 (4OCH_3); 68.5 (pyridine-C-5,5'); 108.9 (phenyl-C-3,3'); 109.7 (phenyl-C-5,5'); 117.0 (naphthalene-C-2,7);120.1 (phenyl-C-1,1'); 121.9 (naphthalene-C-8a); 124.0 (pyridine-C-3,3'); 125.2 (4C≡N); 126.1 (naphthalene-C-4,5); 127.0 (naphthalene-C-3,6); 128.3 (phenyl-C-6,6'); 129.3 (naphthalene-C-1,8);133.1 (naphthalene-C-4a); 160.2 (pyridine-C-6,6'); 161.1 (2C=O); 162.2 (phenyl-C-2,2',4,4'); 163.1 (pyridine-C-4,4'). **Molecular Formula**: $C_{40}H_{28}N_8O_6$. Analysis, **Calcd**. (%):C, 67.03; H, 3.94; N, 15.63. **Found** (%):C, 67.34; H, 4.20; N, 15.82.

2.2. Antimicrobial assay:

Screening of minimum inhibitory concentration (MIC) for newly synthesized compounds was carried out via broth microdilution method according to the CLSI reference standards [54]. In brief, 100 µL of Muller-Hinton broth (MHB) (Oxoid® Limited, Basingstoke, UK) was dispersed in 96 multi-well microtiter plates, followed by the addition of 100 µL of tested compounds onto microtiter plate. After that, serial dilution was done from the 1st to the 12th well. Addition of 10 µL of freshly prepared bacterial suspension (1.5 x10^8 CFU / mL) was performed for all wells. Positive and negative controls were carried out for each bacterial strain. Incubation of plates were performed 18-24 h at 37˚C, Cephalexin, 1000 µg/ml and Amoxicillin 1000 µg/ml were used as reference standard antibiotics. The MICs were determined as the lowest concentration that showed no bacterial growth comparable with control. All the tests were run in duplicate.

2.2.1. Antibiofilm activity:

a-Biofilm formation

The antibiofilm activity of compounds **2**, **3** and **6** was detected against *Staphylococcus aureus* (ATCC 25923) a standard biofilm producer strain. Additionally, six MRSA, *Enterococcus faecium* and *E. faecalis* isolates were investigated for their biofilm formation by quantitative crystal violet microtiter plate assay [55]. The average optical densities for each isolate (OD) and control (ODc) were determined, and the biofilm-formation ability was detected through the reported method [56] where non-biofilm manufacturer OD ≤ ODc, weak biofilm manufacturer ODc < OD ≤ 2 × ODc, moderate biofilm manufacturer 2 × ODc < OD ≤ 4 × ODc and strong biofilm manufacturer OD > 4 × ODc.

b-Inhibition of bacterial attachment

Detection of antibiofilm activity was performed according to the reported procedure [57]. Briefly, 180 µL of tryptic soy broth (TSB) **(**Oxoid® Limited, Basingstoke, UK**)** with 1% glucose was added to polystyrene wells, flat-bottomed, 96-well microtiter plates followed by addition of 20 µL freshly prepared bacterial suspensions with turbidity equivalent to 0.5 McFarland standards (10^8 CFU/mL). Different concentrations of tested compounds that equal to 0.5× MIC, 1 × MIC and 2 × MIC were added and TSB broth with 1% glucose was considered as control. Incubation of plates at 37°C for 24 h. was performed. After that, wells decantation and washing three times with normal saline was performed to get

rid of non-adherent cells. Fixation of biofilms was applied via utilizing 150 µL of methanol for 15 min. All wells were stained with 150 µL of 0.1% crystal violet for 15 min followed by washing with deionized water and drying overnight at room temperature. Then, 150 µL of 95% ethanol solution was put in and the plates were left at 25°C for at least 30 min without shaking. Detection of absorbance at 630 nm was done utilizing Benchmark Microplate Reader (Bio-Rad, Hercules, CA, USA) to calculate percent reduction in biofilm formation aided by the following equation [58].

Percent reduction in biofilm formation =

$$\frac{\text{Ac - At}}{\text{Ac}}\ x\ 100$$

Where A_c is OD for positive control wells and A_t is OD of test wells

2.2.2. DNA gyrase supercoiling and ATPase assays:

The DNA gyrase supercoiling and ATPase assays were carried out at the confirmatory diagnostic unit, Vacsera-Egypt using Staphylococcus aureus DNA gyrase Inspiralis [59,60]. Circumstances of enzyme, plasmid, buffer and the estimation method were discussed in the reported literature [61]. IC_{50} values were calculated three times and the average was determined. Novobiocin was utilized as a reference.

2.2.3. Topoisomerase IV decatenation and ATPase assays:

The assays were carried out at confirmatory diagnostic unit, Vacsera-Egypt using Staphylococcus aureus topoisomerase IV decatenation kits (Inspiralis). The criteria of buffer, enzyme, plasmid, and the detailed procedure were described according to the reported publication [62,63]. IC_{50} values were detected three times for each and the average was taken. Novobiocin was utilized as a reference.

2.2.4. In-vitro cytotoxicity test:

Normal human lung fibroblast WI-38 (ATCC, Manassas, VA, USA) was implanted on Dulbecco's modified Eagle's medium (DMEM / Life Technologies) containing 10% fetal bovine serum (GIBCO, UK), 10 ug/ml of insulin (Sigma) and 1% penicillin–streptomycin (Life Technologies). The detailed procedure for MTT assay was carried out according to the reported publications [64,65].

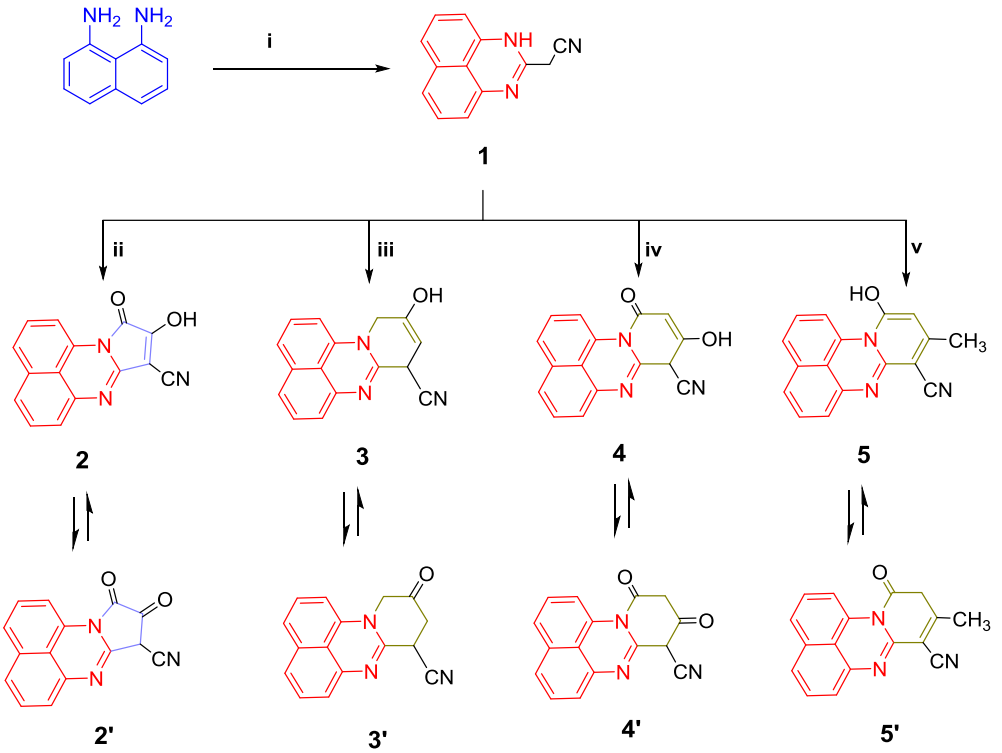
3. Results and discussion

3.1. Chemistry

1,8-Diaminonaphthalene is a precursor for biologically active compounds. For example, perimidine-2-cyanomethylperimidine **1** was synthesized from cyclocondensation of 1,8-diaminonaphthalene with cyanoacetamide [16, 66]. Literature survey revealed that, oxalyl chloride reacted with different bifunctional compounds to produce versatile heterocyclic derivatives via elimination of two molecules of HCl [67-71]. Nucleophilic substitution reaction of compound **1** with oxalyl chloride and dichloroacetone using basic catalyst afforded the corresponding pyrrolo[1,5-*a*]perimidine **2** and pyrido[1,2-*a*]perimidine **3** derivatives, respectively. The structures for these compounds were confirmed according to their spectral analyses. IR spectra for compounds **2** and **3** showed broad bands at υ 3387-3438 cm^{-1}assigned for tautomeric hydroxyl groups. Besides, there was amidic carbonyl functionality at υ 1656 cm^{-1}in compound **2**. ^{1}H-NMR spectrum for compound **2** presented D_2O exchangeable singlet at δ 8.93 ppm characteristic for OH proton.

Furthermore, compound **1** reacted with diethyl malonate in benzene to yield the targeted 9-hydroxy-11-oxopyrido[1,2-*a*]perimidine analog **4** . Its IR chart presented a broad band at υ 3367 cm^{-1} as well as another absorption at υ 1635 cm^{-1} assigned for hydroxyl and carbonyl groups, respectively. ^{1}H-NMR spectrum demonstrated two singlets at δ 2.72 and 11.69 ppm assigned for pyridoperimidine C-8 proton and OH proton that the latter one disappeared with D_2O. ^{13}C-NMR spectrum exhibited signal at δ 159.0 ppm characteristic for carbonyl carbon.

Moreover, the reaction of activated nitrile with ethyl acetoacetate was reported to be achieved in ethanol containing a catalytic amount of ammonium acetate to form the parallel pyridine analogs [72]. Therefore, cyclocondensation of compound **1** with ethyl acetoacetate was performed in ethanol containing ammonium acetate to afford compound **5**. The structure of compound **5** was proved via amidic carbonyl group in IR chart at υ 1633 cm^{-1}in addition to two singlets at δ 1.23 and 2.26 ppm corresponding to methyl and methylene protons, respectively. (Scheme 1)



Reagents and conditions: (i) $NCCH_2CONH_2$, fusion; **(ii)** $(COCl)_2$, C_2H_5OH / Pip.; **(iii)** $ClCH_2COCH_2Cl$, DMF / Pip.**; (iv)** $CH_2(CO_2C_2H_5)_2$, C_6H_6; **(v)** $CH_3COCH_2CO_2C_2H_5$, C_2H_5OH / NH_4OCOCH_3.

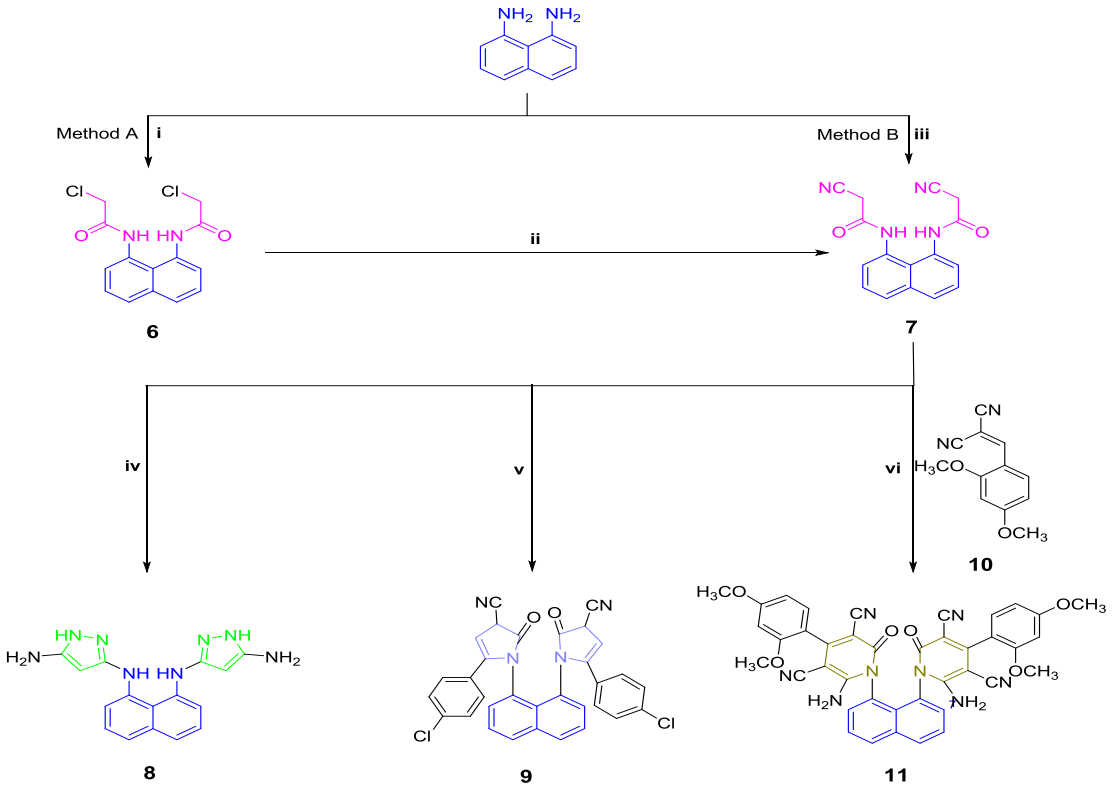
Scheme 1: synthetic protocol for perimidines synthesis

On the other hand, N-cyanoacetamide analogs were believed to be a significant precursor in heterocyclic chemistry [73]. Hence, *N,N′*-(naphthalene-1,8-diyl)bis(2-cyanoacetamide) **(7)** was preparaed via two methods. The first method was summarized in the reaction of 1,8-diaminonaphthalene with chloroacetyl chloride to yield bis(2-chloroacetamide) derivative **6** [74] that was subsequently converted into the corresponding bis(2-cyanoacetamide) analog **7** through stirring with potassium cyanide. The second method showed the nucleophilic substitution reaction of 1,8-diaminonaphthalene with ethyl cyanoacetate in ethanol to yield the parallel bis(2-cyanoacetamide) analog **7**. The structures of compounds **6** and **7** were proved through spectral and elemental data. IR charts of compounds **6** and **7** presented band at υ 2171 cm^{-1}in compound **7** due to cyano functions. Also, ^{1}H-NMR spectrum of compounds **7** presented singlet at δ 3.99 ppm characteristic for two methylene protons in addition to D_2O exchangeable singlet at δ 10.53 ppm attributed to two NH protons. Besides, the appearance of carbonyl carbon signal at δ 155.0 ppm in ^{13}C-NMR spectrum proved the structure.

Preparation of bis(1*H*-pyrazole-3,5-diamine) derivative **8** was accomplished via cyclocondensation of compound **7** with hydrazine hydrate. The reaction was presupposed to proceed via condensation reaction between carbonyl functionality of cyanoacetamide and amino function of hydrazine hydrate pursued by intramolecular cyclization via nucleophilic addition on cyano group. Compound **8** was identified by bands at υ 3367 and 3205 cm^{-1} in IR chart due to NH_2 and NH functionalities. As synthesis of pyrrole derivatives from cyanoacetamide precursor was previously reported [75], compound **7** reacted with 4-chlorophenacyl bromide to afford the target bis-pyrrole derivative **9** whose structure was confirmed via singlet at δ 2.89 ppm in ^{1}H-NMR spectrum attributed to pyrrole-C4 protons.

It was discussed that the reaction of cyanoacetamides with arylidene malononitriles yielded the parallel pyridine derivatives [76]. Herein, compound **7** reacted with 2-(2,4-dimethoxybenzylidene)malononitrile **(10)** in ethanol having 3 drops of piperidine to form the corresponding bis-pyridine analog **11**. The reaction was assumed to occur through Michael's addition of cyanoacetamide active methylene on the double bond

of arylidene malononitrile pursued by intramolecular cyclization. IR chart of compound **11** presented band at 3394 cm^{-1} characteristic for NH_2 group. Additionally, ^{1}H-NMR spectrum of compounds **11** presented singlet at δ 3.84 ppm characteristic for four methoxy protons and another singlet at δ 8.31 ppm assigned for two NH_2 protons that disappeared after D_2O addition (Scheme 2).



Reagents and conditions: (i) $ClCH_2COCl$, dioxane; **(ii)** KCN, C_2H_5OH; **(iii)** $CNCH_2CO_2C_2H_5$, C_2H_5OH; **(iv)** $NH_2NH_2.H_2O$ 99%, C_2H_5OH; **(v)** 4-chlorophenacyl bromide, C_2H_5OH / TEA; **(vi)** C_2H_5OH / Pip.

Scheme 2: Synthetic route for (naphthalene-1,8-diyl)bis(heterocycles)

3.2. Antimicrobial assay:

3.2.1. Primary antimicrobial investigation:

All compounds were tested for their *in-vitro* antibacterial effect towards one Gram-positive strain, *Staphylococcus aureus* (ATCC 25923) as well as one Gram-negative strain, *Escherichia coli* (ATCC 25922) using Cephalexin and Amoxicillin as reference standard antibiotics according to broth microdilution method. The results of antibacterial activity were assessed as the calculated minimum inhibitory concentration (MIC). (Table 1). The MIC was determined as the lowest concentration that prevented bacterial growth comparable with control cells.

Regarding the antibacterial activity against *Staphylococcus aureus*, it was apparent that pyrroloperimidine derivative **2** and 10-hydroxypyridoperimidine analog **3** exerted remarkable antibacterial activity with MIC equal 125 µg/mL. Noticeably, 2-(1*H*-perimidin-2-yl)acetonitrile **1** and bis(2-chloroacetamide) derivative **6** exhibited potent antibacterial activity with MIC 62.5 and 15.63 µg/mL, respectively. Also, compounds **4**, **5**, **7** and **11** showed mild antibacterial activity (MIC 250 µg/mL) however, compounds **8** and **9** have no antibacterial action.

On the other hand, compounds **4, 5**, **6** and **11** demonstrated reasonable antibacterial activity against *Escherichia coli* (MIC equal 125 µg/mL). Also, compounds **2**, **3**, **7** and **8** displayed mild antibacterial activity against the same strain with MIC value of 250

µg/mL. Additionally, compounds **1** and **9** presented no antibacterial action against *Escherichia coli.*

Table 1: Primary antimicrobial screening for all synthesized compounds (MIC- µg/ml)

Staphylococcus aureus **(ATCC 25923)**		***Escherichia coli*** **(ATCC 25922)**	
Compound No.	**MIC (µg/ml)**	**Compound No.**	**MIC (µg/ml)**
1	62.5	**1**	>500
2	125	**2**	250
3	125	**3**	250
4	250	**4**	125
5	250	**5**	125
6	15.63	**6**	125
7	250	**7**	250
8	500	**8**	250
9	500	**9**	>500
11	250	**11**	125
Amoxicillin	≤ 7.81	Amoxicillin	125
Cephalexin	7.81	Cephalexin	31.25

3.2.2. Antibacterial activity against some resistant strains:

Primary antimicrobial investigation showed the potential antimicrobial activity of compounds (**2**, **3** and **6**) against *Staphylococcus aureus*. Therefore, these compounds were further screened against different Gram positive bacterial strains that include one clinical methicillin sensitive *Staphylococcus aureus* isolates (MSSA), six MRSA and two MDR isolates of *Enterococcus faecium* as well as *E. faecalis. The results showed that all tested compounds were highly more potent than the references*, cephalexin and amoxicillin against all MRSA isolates, *Enterococcus faecium* and *E. faecalis MDR isolates.*

*In details, compounds **2** and **3** exerted moderate to potent antimicrobial activity against all tested strains except for MRSA 1 that its growth was by compound **2** with MIC equal 62.5* µg/mL. *It is clear that compound **6** exhibited extremely potent antimicrobial activity against all resistant strains with MIC ranges from 31.25 to 125* µg/mL. (Table 2)

3.2.3. Structure activity relationship:

According to the previously tabulated results, it was clear that naphthalene containing derivatives showed higher antibacterial activity than perimidineones. In other words, the cyclization of diamino groups of 1,8-diaminonaphthalene decreased the antibacterial activity. Hence, N,N'-(Naphthalene-1,8-diyl)bis(2-chloroacetamide) **(6)** demonstrated the highest antibacterial activity against all tested strains and its conversion into bis(2-cyanoacetamide) followed by transformation into bis(pyrazole) **8**, bis(pyrrole) **9** or bis(pyridine) **11** derivatives apparently decreased the antimicrobial activity.

Moreover, 2(1*H*-perimidin-2-yl)acetonitrile **1** exhibited remarkable antibacterial activity, especially towards *Staphylococcus aureus*. However, cyclization of active methylene group and NH moiety of compound **1** into pyrrole ring **2** or 10-hydroxypyridine ring **3** increased the antimicrobial effect against resistant strains. Additionally, compound **2** with pyrroloperimidine scaffold displayed higher antibacterial activity against MRSA 1 than pyridoperimidine containing derivative **3**. Noticeably, the addition of hydroxyl group or methyl function at position 9 of pyridoperimidine backbone in compounds **4** and **5**, respectively decreased the antibacterial activity against *Staphylococcus aureus* and increased the antimicrobial effect towards *Escherichia coli.* (Figure 4).

3.2.4. Antibiofilm estimation:

The inhibition of bacterial attachment of the three compounds **2**, **3** and **6** was tested against *Staphylococcus aureus* (ATCC 25923) and the aforementioned MRSA isolate through their capacity to disrupt biofilm formation. The tested compounds showed higher antibiofilm activity against *Staphylococcus aureus* (ATCC 25923) than clinical MRSA isolates. Among the compounds, pyrroloperimidine derivative **2** exhibited more potent antibiofilm activity than pyridoperimidine **3** and bis(2-chloroacetamide) **6** analogs.

Furthermore, compounds **2**, **3** and **6** displayed reduction in biofilm formation against *Staphylococcus aureus* by 34.2 %, 20.5 % and 27.5 % in 2 x MIC and by 22%, 15% and 26 % in 1 MIC, respectively (Figure 5). However, they presented reduced activity against MRSA by 14.8, 3.7% and 5.5% in 2 x MIC and 11.1 %, 1.5, 1.8% in 1x MIC, respectively (Figure 6).

Table 2: Antimicrobial assay for compounds 2,3 and 6 against resistant strains (MIC- µg/ml)

Bacterial strains/Compounds' No.	MIC (µg/ml)					
	2	**3**	**6**	**Amoxicillin**	**Cephalexin**	**DMSO**
MSSA	125	125	15.63	≤ 7.8125	15.625	250
MRSA 1	62.5	125	62.5	>500	>500	250
MRSA 2	125	125	62.5	>500	>500	250
MRSA 3	125	125	62.5	>500	>500	250
MRSA 4	125	125	62.5	>500	>500	250
MRSA 5	250	250	125	>500	>500	250
MRSA 6	250	250	62.5	>500	>500	250
Enterococcus faecium 33	125	125	62.5	>500	>500	250
E. faecalis 46	125	125	31.25	>500	>500	250

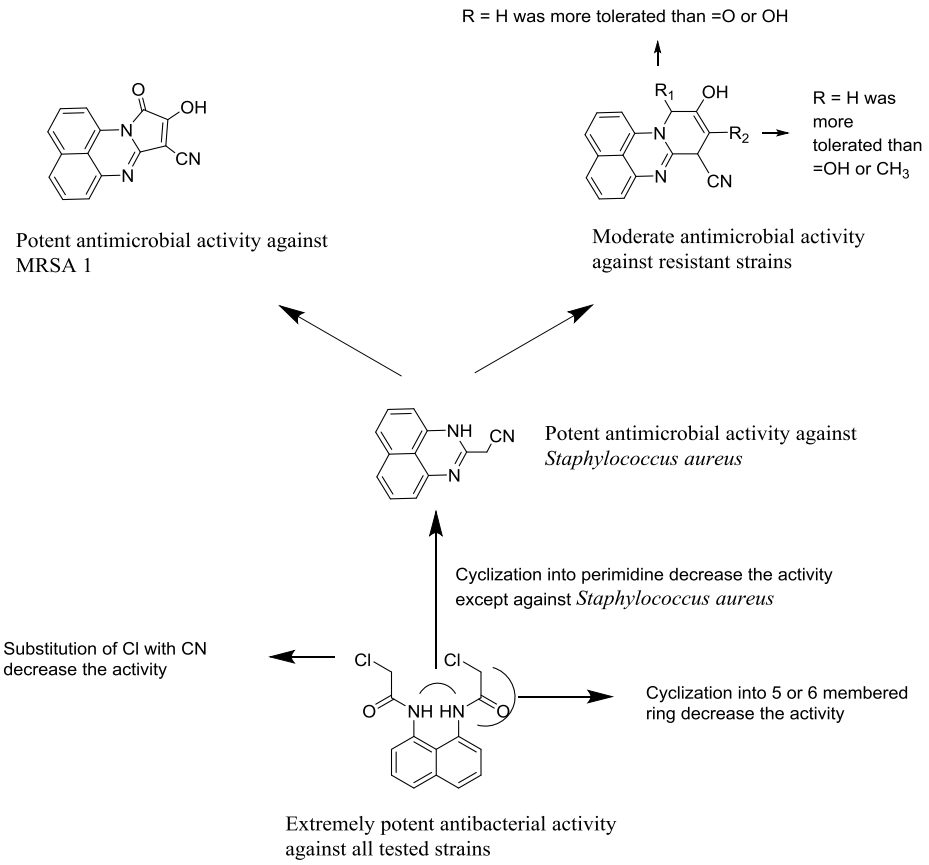


Figure 4: Structure activity relationship

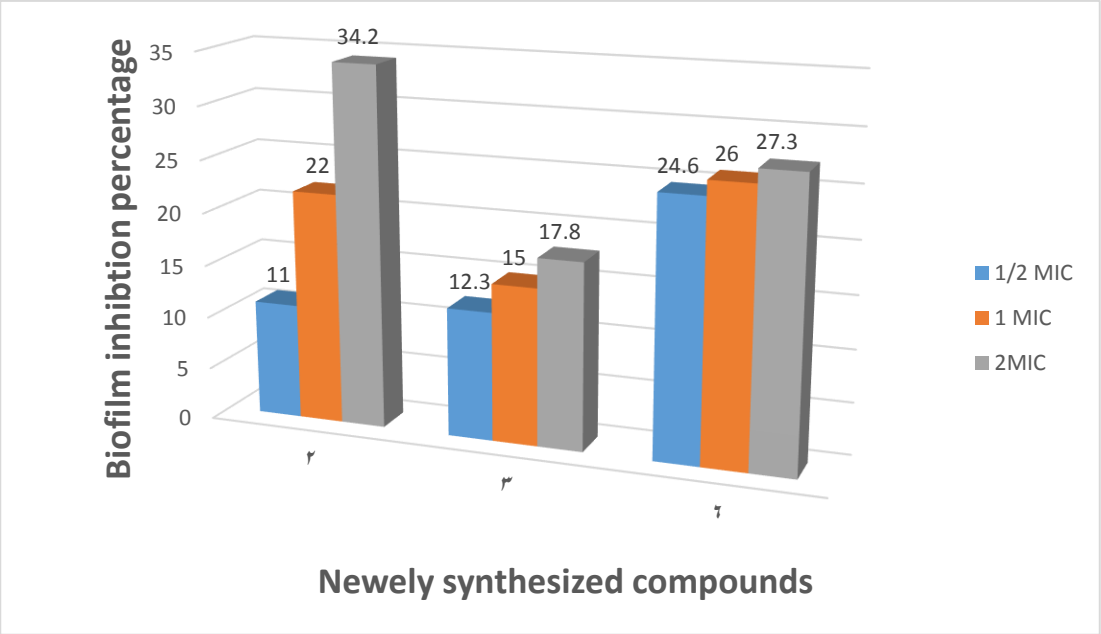


Figure 5: The percent reduction of biofilm formation against *Staphylococcus aureus* (ATCC 25923) by compounds 2, 3 and 6

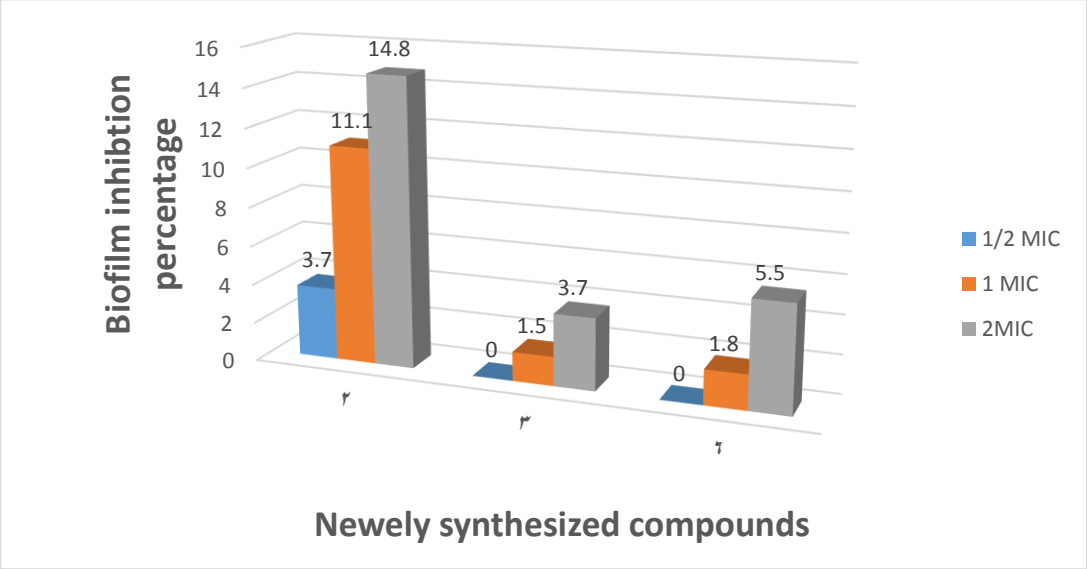


Figure 6: The percent reduction of biofilm formation against MRSA isolates by compounds 2, 3 and 6.

3.2.5. Enzyme inhibition assay:

The most active compounds **2** and **6** were subjected to further evaluation towards bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) to determine their mechanization.

3.2.5.1. DNA gyrase supercoiling assay:

DNA gyrase supercoiling assay was estimated for compounds **2** and **6** using *Staphylococcus aureus* DNA gyrase in addition to novobiocin as a standard antimicrobial agent. The results are deduced from table 3 showed that compound **2** exhibited potent inhibitory activity against DNA gyrase supercoiling with IC_{50} equal 0.167 µM while compound **6** exerted much higher inhibition activity with IC_{50} equal 0.079 µM.

3.2.5.2. DNA gyrase ATPase assay:

Similarly, compounds **2** and **6** were further examined for DNA gyrase ATPase assay utilizing *Staphylococcus aureus* DNA gyrase as well as

novobiocin as a standard antibacterial agent. As shown from table 3, compound **2** demonstrated DNA gyrase ATPase inhibitory action with IC_{50} equal 0.12 µM. In addition, compound **6** strongly inhibited DNA gyrase ATPase activity with IC_{50} equal 0.05 µM (nearly equipotent with novobiocin whose IC_{50} equal 0.06 µM). Noticeably, DNA gyrase was nearly two times more sensitive to naphthalene-1,8-diyl derivative **6** than pyrroloperimidine analog **2**.

Table 3: DNA gyrase coiling and DNA gyrase ATPase for compounds 2 and 6

Compound	IC_{50} (µM)	
	DNA gyrase supercoiling	DNA gyrase ATPase
2	0.167±0.01	0.12±0.007
6	0.079±0.004	0.05±0.003
novobiocin	0.042±0.002	0.06±0.002

3.2.5.3. Topoisomerase IV decatenation assay:

To detect whether the inhibitory activity of compounds **2** and **6** are gyrase specific or not, topoisomerase IV determination was estimated. This assay was applied to compounds **2** and **6** where novobiocin was used as a reference drug and topoisomerase IV was isolated from *Staphylococcus aureus*. The results disclosed that compound **2** exhibited good inhibitory activity with IC_{50} equal 6.63 µM while compound **6** exerted strong inhibitory activity (IC_{50} equal 1.13 µM) that was three folds more potent than novobiocin (IC_{50} equal 4.86 µM) as shown in table 4 This suggested that compound 6 exhibited its antibacterial activity through binding to topoisomerase IV active site and block its activity.

3.2.5.4. Topoisomerase IV ATPase assay:

Staphylococcus aureus topoisomerase IV was utilized to detect the ATPase inhibition for compounds **2** and **6** and reference drug (novobiocin). Both compounds **2** and **6** displayed potent inhibitory activity against topoisomerase IV ATPase with IC_{50} equal 0.66 µM and 0.31 µM, respectively. Apparently, topoisomerase IV was approximately twice more responsive to naphthalene-1,8-diyl derivative **6** than pyrroloperimidine analog **2** (table 4).

Table 4: Topoisomerase IV decatenation and topoisomerase IV ATPase for compounds 2 and 6

Compound	IC_{50} (µM)	
	Topoisomerase IV decatenation	Topoisomerase IV ATPase
2	6.63±0.3	0.66±0.07
6	1.31±0.06	0.31±0.06
novobiocin	4.86±0.24	0.26±0.04

3.2.6. In-vitro cytotoxicity test:

Regarding the cytotoxicity of compounds **2** and **6** on normal human lung fibroblast WI-38 using the MTT assay, they displayed safety profiles near (IC_{50} equal 17.33 µM) or higher than (40.75 µM) that of the reference drug, novobiocin (28.91 µM) (Table 5).

Table 5: Cytotoxicity of compounds 2 and 6 against WI-38 cell line using novobiocin as a reference drug:

Compound	WI-38 (IC_{50} µM).
2	17.33±1.84
6	40.75±3.06
Novobiocin	28.91±2.12

4. Conclusion:

1,8-Diaminonaphthalene was considered as an effective precursor for the synthesis of perimidines and (naphthalene-1,8-diyl)bis(heterocycles). All synthesized compounds were characterized via spectral and elemental data. Antimicrobial investigation was carried out for all prepared compounds against *Staphylococcus aureus* and *Escherichia coli*. Results of this study showed that (naphthalene-1,8-diyl)bis(2-chloroacetamide) **(6)** demonstrated the most promising effect towards *Staphylococcus aureus* with MIC value of 15.63 µg/mL. Further antimicrobial assay was performed for compounds **2**, **3,** and **6** against MRSA strains and MDR clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis. Pyrroloperimidine derivative **2** exerted a significant antimicrobial activity against MRSA 1 (MIC = 62.5* µg/mL), while bis(2-chloroacetamide) analog **6** demonstrated much more potent effect and broad antibacterial spectrum against all the resistant strains (*MIC ranges from 31.25 to 125* µg/mL). Furthermore, compounds **2**, **3,** and **6** displayed powerful antibiofilm activity against *Staphylococcus aureus* and MRSA in both 2 x MIC and 1 x MIC. On the other hand, compounds **2** and **6** showed potent dual DNA gyrase and topoisomerase IV inhibition with IC_{50} ranges of 0.05-0.167 µM and 0.31-6.63 µM, respectively. Also, they revealed safety profiles which are close to or even higher than that of the reference drug, novobiocin. These results supposed that compounds **2** and **6** should be considered as promising lead scaffolds for further antimicrobial research.

Conflict of interest:

The authors declare that there are no conflicts of interests.

Acknowledgment

The authors are very appreciative to VACSERA, Egypt for performing enzyme inhibition assay.

References:

1- Kulkarni, A.P.; Nagvekar, V.C.; Veeraraghavan, B.; Warrier, A.R.; Deepak, T.S.; Ahdal, J.; Jain, R. Current perspectives on treatment of Gram-positive infections in India: What is the way forward?. *Interdiscip. Perspect. Infect. Dis.*, **2019**, 1-8 (2019). https://doi.org/10.1155/2019/7601847

2- Nair, N.; Biswas, R.; Gotz, F.; Biswas, L. Impact of *Staphylococcus aureus* on pathogenesis in polymicrobial infections. *Infect. Immun.*, **82**(6), 2162–2169 (2014). DOI: 10.1128/IAI.00059-14

3- Jubeh, B.; Breijyeh, Z.; Karaman, R. Resistance of Gram-positive bacteria to current antibacterial agents and overcoming approaches. *Molecules*, **25**(12), 2888-2910 (2020). https://doi.org/10.3390/molecules25122888

4- Woodford, N.; Livermore, D.M. Infections caused by Gram-positive bacteria: a review of the global challenge. *Infection*, **59**, S4–S16 (2009). DOI: 10.1016/S0163-4453(09)60003-7

5- Tong, S.Y.C.; Davi, J.S.; Eichenberger, E.; Holland, T.L.; Fowler, V.G. *Staphylococcus aureus* Infections: Epidemiology, pathophysiology, clinical manifestations, and management. *Clin. Microbiol. Rev*., **28**, 603–661 (2015). DOI: 10.1128/CMR.00134-14

6- Sunagar, R.; Hegde, N.R.; Archana, G.J.; Sinha, A.Y.; Nagamani, K.; Isloor, S. Prevalence and genotype distribution of methicillin-resistant *Staphylococcus aureus* (MRSA) in India. *J. Glob. Antimicrob. Resist.*, **7**, 46–52 (2016). DOI: 10.1016/j.jgar.2016.07.008

7- Tacconelli, E.; Magrini, N.; Kahlmeter, G.; Singh, N. Global priority list of antibiotic resistant bacteria to guide research, discovery, and development of new antibiotics. *World Health Organization*, **27**, 1-7 (2017).

8- Sakka, V.; Tsiodras, S.; Galani, L. Risk factors and predictors of mortality in patients colonized with vancomycin resistant enterococci. *Clin. Microbiol. Infect.*, **14(1)**, 14-21 (2008). DOI: 10.1111/j.1469-0691.2007.01840.x

9- Tawfick, M.M.; El Menofy, N.G.; Omran, M.E.; Alsharony, O.A.; Abo-Shady, M.A. Phenotypic and molecular characterization of plasmid-mediated virulence and antimicrobial resistance traits among multidrug resistant Enterococcus Spp. in Egypt. *J. Pure Appl. Microbiol.*, **14**(3), 1649-1661 (2020). DOI: 10.22207/JPAM.14.3.03.

10- Guzman Prieto, A.M.; Schaik, W.V.; Rogers, M.R.; Coque, T.M.; Baquero, F.; Corander, J.; Willems, R.J.L. Global emergence and dissemination of Enterococci as nosocomial pathogens: Attack of the clones?. *Front. Microbiol.*, **7**(788), 1-15 (2016). https://doi.org/10.3389/fmicb.2016.00788

11- Cortes, M.E.; Bonilla, J.C.; Sinisterra, R.D. Biofilm formation, control and novel strategies for eradication. In: Mendez-Vilas A (ed) Science against microbial pathogens: communicating current research and technological advances. Formatex Research Center, Badajoz, **2**, 896–905 (2011).

12- Donlan, R.M.; Costerton, J.W. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.*, **15**, 167–193 (2002). DOI: 10.1128/CMR.15.2.167-193.2002

13- Cerca, N.; Jefferson, K.K.; Maira-Litran, T.; Pier, D.B.; Kelly-Quintos, C.; Goldmann, D.A.; Azeredo, J.; Pier, G.B. Molecular basis for preferential protective efficacy of antibodies directed to the poorly acetylated form of staphylococcal poly-N-acetyl-beta-(1-6)-glucosamine. *Infect. Immun.*, **75**(7), 3406–3413 (2007). DOI: 10.1128/IAI.00078-07

14- Davies, D. Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug. Discov.*, **2**(2), 114–122 (2003). DOI: 10.1038/nrd1008

15- de Aguiar, A. Ueber einige Abkommlinge des α-und β-Diamidonaphtalins. *Ber. Dtsch. Chem. Ges.*, **7**, 309–319 (1874). https://doi.org/10.1002/cber.187400701103

16- Sovic, I.; Pavlovic, G.; Papadopoulos, A.G.; Sisak, D.; Karminski-Zamola, G. 2-Substituted-1*H*-perimidines: Synthesis, crystal structure and DFT calculations. *J. Mol. Struct.*, **1041**, 156–163 (2013). DOI:10.1016/j.molstruc.2013.03.020

17- Wasulko, W.; Noble, A.C.; Popp, F.D. Synthesis of Potential Antineoplastic Agents. XIV. Some 2-Substituted 2,3-Dihydro-1*H*-perimidine. Potential Antineoplastic Agents. XIV., **9**, 599-601 (1966). DOI: 10.1021/jm00322a035

18- Farghaly, T.A.; Mahmoud, H.K. Synthesis, tautomeric structure, and antitumor activity of new perimidines. *Arch. Pharm. Chem. Life Sci.*, **346**, 392–402 (2013). DOI: 10.1002/ardp.201200486
19- Farghaly, T.A.; Abbas, E.M.H.; Dawood, K.M.; El-Naggar, T.B.A. Synthesis of 2-Phenylazonaphtho[1,8-*ef*][1,4]diazepines and 9-(3-arylhydrazono)pyrrolo[1,2-*a*]perimidines as antitumor Agents. *Molecules*, **19**, 740-٧55 (2014). DOI: 10.3390/molecules19010740
20- Kumar, A.; Banerjee, S.; Roy, P.; Sondhi, S.M.; Sharma, A. Solvent-free synthesis and anticancer activity evaluation of benzimidazole and perimidine derivatives. *Mol. Divers.*, **22**, 113–127 (2018). DOI: 10.1007/s11030-017-9790-3
21- Eldeab, H.A.; Eweas, A.F. A greener approach synthesis and docking studies of perimidine derivatives as potential anticancer agents. *J. Heterocycl. Chem.*, **55**, 431-439 (2018). https://doi.org/10.1002/jhet.3059
22- Azam, M.; Warad, I.; Al-Resayes, S.I.; Alzaqri, N.; Khan, M.R.; Pallepogu, R.; Dwivedi, S.; Musarrat, J.; Shakir, M. Synthesis and structural characterization of Pd(II) complexes derived from perimidine ligand and their in vitro antimicrobial studies. *J. Mol. Struct.*, **1047**, 48–54 (2013). https://doi.org/10.1016/j.molstruc.2013.04.064
23- Chan, A.H.; Wereszczynski, J.; Amer, B.P.; Yi, S.W.; Jung, M.E.; McCammon, J.A.; Clubb, R.T. Discovery of *Staphylococcus aureus* sortase A inhibitors using virtual screening and the relaxed complex scheme. *Chem. Biol. Drug Des.*, **82**(4), 418-428 (2013). DOI: 10.1111/cbdd.12167
24- Bassyouni, F.A.; Abu-Bakr, S.M.; Hegab, K.H.; El-Eraky, W.; El Beih, A.A.; Abdel Rehim, M.E. Synthesis of new transition metal complexes of 1*H*-perimidine derivatives having antimicrobial and anti-inflammatory activities. *Res. Chem. Intermed.*, **38**, 1527-1550 (2012). DOI: 10.1007/s11164-011-0482-9
25- Azam, M.; Warad, I.; Al-Resayes, S.; Zahin, M.; Ahmad, I.; Shakir, M. Syntheses, physico-chemical studies and antioxidant activities of transition metal complexes with a perimidine ligand. *Z. Anorg. Allg. Chem.*, **638**, 881-886 (2012). https://doi.org/10.1002/zaac.201100561
26- Ikeda, M.; Maruyama, K.; Nobuhara, Y.; Yamada, T.; Okabe, S. Synthesis and cytoprotective antiulcer activity of 2- or 4-(1*H*-pyrazol-1-yl)pyrimidine derivatives related to Mepirizole and Dulcerozine. *Chem. Pharm. Bull.*, **44**(9), 1700-1706 (1996). https://doi.org/10.1248/cpb.44.1700
27- Farghaly, T.A.; Abdallah, M.A.; Muhammad, Z.A. New 2-heterocyclic perimidines: synthesis and antimicrobial activity. *Res. Chem. Intermed.*, **41**, 3937–3947 (2015).
28- Sahiba, N.; Agarwal, S. Recent advances in the synthesis of perimidines and their applications. *Top Curr. Chem.*, **378** (44), 1-47 (2020). DOI: 10.1007/s41061-020-00307-5
29- Yadav, P.; Shah, K. Quinolines, a perpetual, multipurpose scaffold in medicinal chemistry. *Bioorg. Chem.*, **109**, 104639 (2021). https://doi.org/10.1016/j.bioorg.2021.104639
30- Gatadia, S.; Madhavia, Y.V.; Choprab, S.; Nanduri, S. Promising antibacterial agents against multidrug resistant *Staphylococcus aureus*. *Bioorg. Chem.*, **92**, 103252 (2019). https://doi.org/10.1016/j.bioorg.2019.103252
31- Chioma, F.; Ekennia, A.C.; Ibeji, C.U.; Okafor, S.N.; Onwudiwe, D.C.; Osowole, A.A.; Ujam, O.T. Synthesis, characterization, antimicrobial activity and DFT studies of 2-(pyrimidin-2-ylamino)naphthalene-1,4-dione and its Mn(II), Co(II), Ni(II) and Zn(II) complexes. *J. Mol. Struct.*, **1163**, 455-464 (2018). https://doi.org/10.1016/j.molstruc.2018.03.025
32- Erasmus, C.; Aucamp, J.; Smit, F.J.; Seldon, R.; Jordaan, A.; Warner, D.F.; N'Da, D.D. Synthesis and comparison of *in vitro* dual anti-infective activities of novel naphthoquinone hybrids and atovaquone. *Bioorg. Chem.*, **114**, 105118 (2021). https://doi.org/10.1016/j.bioorg.2021.105118
33- Karakurt, A.; Ozalp, M.; Isik, S.; Stables, J.P.; Dalkara, S. Synthesis, anticonvulsant and antimicrobial activities of some new 2-acetylnaphthalene derivatives. *Bioorg. Med. Chem.*, **18**, 2902–2911 (2010). https://doi.org/10.1016/j.bmc.2010.03.010
34- Rokade, Y.; Dongare, N. Synthesis and antimicrobial activity of some azetidinone derivatives with the β-naphthol. *Rasayan J. Chem.*, **3**(4), 641-645 (2010).

35- Budhiraja, A.; Kadian, K.; Kaur, M.; Aggarwal, V.; Garg, A.; Sapra, S.; Nepali, K.; Suri, O.P.; Dhar, K.L. Synthesis and biological evaluation of naphthalene, furan and pyrrole based chalcones as cytotoxic and antimicrobial agents. *Med. Chem. Res.*, **21**, 2133–2140 (2012). DOI:10.1007/s00044-011-9733-y

36- Fadda, A.A.; Afsah, E.M.; Awad, R.S. Synthesis and antimicrobial activity of some new benzo and naphthonitrile derivatives. *Eur. J. Med. Chem.*, **60**, 421-430 (2013). DOI: 10.1016/j.ejmech.2012.11.017

37- Kelley, C.; Lu, S.; Parhi, A.; Kaul, M.; Pilch, D.S.; Lavoie, E.J. Antimicrobial activity of various 4- and 5-substituted1-phenylnaphthalenes. *Eur. J. Med. Chem.*, **60**, 395-409 (2013). DOI: 10.1016/j.ejmech.2012.12.027

38- Ranjith, S.; Sugumar, P.; Rajagopal, G.; Udayakumar, M.; Ponnuswamy, M.N. Synthesis, growth, characterization, structure and molecular docking studies of 1-[(*E*)-{[4-(morpholin-4-yl)phenyl]imino}methyl]naphthalen-2-ol single crystal: A potential antimicrobial agent. *J. Mol. Struct.*, **1065-1066**, 21–28 (2014). DOI: 10.1016/j.molstruc.2014.02.025

39- Boopathy, M.; Selvam, R.; JohnSanthoshkumar, S.; Subramanian, K. Synthesis and evaluation of polyacrylamides derived from polycyclic pendant naphthalene, indole, and phenothiazine based chalcone moiety as potent antimicrobial agents. *Polym. Adv. Technol.*, **28**(6), 717-727 (2016). https://doi.org/10.1002/pat.3972

40- Abate, C.; Niso, M.; Lacivita, E.; Mosier, P.D.; Toscano, A.; Perrone, R. Analogues of σ receptor ligand 1-cyclohexyl-4-[3-(5-methoxy-1, 2, 3, 4- tetrahydronaphthalen-1-yl) propyl] piperazine (PB28) with added polar functionality and reduced lipophilicity for potential use as positron emission tomography radiotracers. *J. Med. Chem.*, **54**, 1022-1032 (2011). DOI: 10.1021/jm1013133

41- Goudie, A.C.; Gaster, L.M.; Lake, A.W.; Rose, C.J.; Freeman, P.C.; Hughes, B.O.; Miller, D. 4-(6-Methoxy-2-naphthyl)butan-2-one and related analogs, a novel structural class of antiinflammatory compounds. *J. Med. Chem.*, **21**, 1260-1264 (1978). DOI: 10.1021/jm00210a016

42- Debnath, A.K.; Radigan, L.; Jiang, S. Structure-based identification of small molecule antiviral compounds targeted to the gp41 core structure of the human immunodeficiency virus type 1. *J. Med. Chem.*, **42**, 3203-3209 (1999). DOI: 10.1021/jm990154t

43- Gupta, A.K.; Ryder, J.E.; Cooper, E.A. () Naftifine: a review. *J. Cutan. Med. Surg.*, **12**, 51-58 (2008). DOI: 10.2310/7750.2008.06009

44- Ryder, N.; Frank, I.; Dupont, M. Ergosterol biosynthesis inhibition by the thiocarbamate antifungal agents tolnaftate and tolciclate. *Antimicrob. Agents Chemother.*, **29**, 858-860 (1986). DOI: 10.1128/AAC.29.5.858

45- Petranyi, G.; Meingassner, J.G.; Mieth, H. Antifungal activity of the allylamine derivative terbinafine *in vitro*. *Antimicrob. Agents Chemother.*, **31**, 1365-1368 (1987). DOI: 10.1128/AAC.31.9.1365

46- Makar, S.; Saha, T.; Singh, S.K. Naphthalene, a versatile platform in medicinal chemistry: Sky-high perspective. *Eur. J. Med. Chem.*, **161**, 252-276 (2019). DOI: 10.1016/j.ejmech.2018.10.018

47- Zahoor, A.F.; Yousaf, M.; Mansha, A.; Naheed, S.; Ahmad, M.; Anjum, A.; Aftab, K.; Ghaffar, A.; Ahmad, S.; Irfan, A. Synthesis, characterization and antimicrobial potential of novel conjugated schiff bases. *Asian J. Chem.*, **26**, 6159-6162 (2014). http://dx.doi.org/10.14233/ajchem.2014.16992

48- Aazarifar, D.; Shebanzadeh, M. Synthesis and characterization of new 3,5-dinaphthyl substituted 2-pyrazolines and study of their antimicrobial activity. *Molecules*, **7**, 885-895 (2002). https://doi.org/10.3390/71200885

49- Rokade, Y.B.; Sayyed, R.Z. Naphthalene derivatives: A new range of antimicrobials with high therapeutic value. *Rasayan J. Chem.*, **2**(4), 972-980 (2009).

50- Ahn, M.; Gunasekaran, P.; Rajasekaran, G.; Kim, E.Y.; Lee, S-J.; Bang, G.; Cho, K.; Hyun, J-K.; Lee, H-J.; Jeon, Y.H.; Kim, N-H.; Ryu, E.K.; Shin, S.Y.; Bang, J.K. Pyrazole derived ultra-short antimicrobial peptidomimetics with potent anti-biofilm activity. *Eur. J. Med. Chem.*, **125**, 551-564 (2017). DOI: 10.1016/j.ejmech.2016.09.071

51- Masci, D.; Hind, C.; Islam, M.K.; Toscani, A.; Clifford, M.; Coluccia, A.; Conforti, I.; Touitou, M.; Memdouh, S.; Wei, X.; Regina, G.L.; Silvestri, R.; Sutton, J.M.; Castagnolo, D. Switching on the activity of 1,5-diaryl-pyrrole derivatives against drug-resistant ESKAPE bacteria: Structure-activity relationships and mode of action studies. *Eur. J. Med. Chem.*, **178**, 500-514 (2019). DOI: 10.1016/j.ejmech.2019.05.087

52- Narramore, S.; Stevenson, C.E.M.; Maxwell, A.; Lawson, D.M.; Fishwick, C.W.G. New insights into the binding mode of pyridine-3-carboxamide inhibitors of E. coli DNA gyrase. *Bioorg. Med. Chem.*, **27**, 3546–3550 (2019). DOI: 10.1016/j.bmc.2019.06.015

53- Metwally, N.H.; Abdallah, S.O.; Abdel Mohsen, M.M. Design, green one-pot synthesis and molecular docking study of novel *N*,*N*-bis(cyanoacetyl)hydrazines and bis-coumarins as effective inhibitors of DNA gyrase and topoisomerase IV. *Bioorg. Chem.*, **97**, 103672 (2020). DOI: 10.1016/j.bioorg.2020.103672

54- Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing, 28rd Informational Supplements, (CLSI Document M100-S28, Wayne PA) (2018).

55- Srdjan, S.; Vukovic, D.; Hola, V.; Di Bonaventura, G.; Djukic, S.; Cirkovic, I.; Ruzicka, F. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *Apmis* , **115**(8), 891-899 (2007). DOI: 10.1111/j.1600-0463.2007.apm_630.x

56- Shenkutie, A.M.; Yao, M.Z.; Siu, G.K.; Wong, B.K.C.; Leung, P.H. Biofilm-induced antibiotic resistance in clinical *Acinetobacter baumannii* isolates. *Antibiotics*, **9**(11), 817-1-15 (2020). https://doi.org/10.3390/antibiotics9110817

57- Costa, G.A.; Rossatto, F.C.; Medeiros, A.W.; Correa, A.P.F.; Brandelli, A.; Frazzon, A.P.G.; Motta, A.D.S. Evaluation antibacterial and antibiofilm activity of the antimicrobial peptide P34 against *Staphylococcus aureus* and *Enterococcus faecalis*. *Anais da Academia Brasileira de Ciencias*, **90**(1), 73-84 (2018). https://doi.org/10.1590/0001-3765201820160131

58- Kannan, B.; Thenmozhi, R.; Pandian, S.K. Effect of subinhibitory concentrations of fluoroquinolones on biofilm production by clinical isolates of *Streptococcus pyogenes*. *Indian J. Med. Res.*, **137**(5), 963-971 (2013).

59- Maxwell, A.; Burton, N.P.; O'Hagan, N. High-throughput assays for DNA gyrase and other topoisomerases. *Nucleic Acids Res.*, **34**, e104 (2006). DOI: 10.1093/nar/gkl504

60- Burrell, M.R.; Burton, N.P.; Maxwell, A. A high-throughput assay for DNA topoisomerases and other enzymes, based on DNA triplex formation, in: K.R. Fox (Ed.). Drug-DNA Interaction Protocols, Humana Press, Totowa, NJ, 257–266 (2020).

61- Omar, F.A.; Abelrasoul, M.; Sheha, M.M.; Hassan, H.Y.; Musa, I.Y. Synthesis, antibacterial activity and molecular docking of substituted naphthyridines as potential DNA gyrase inhibitors. *ChemistrySelect*, **3**(9), 2604–2612 (2018). https://doi.org/10.1002/slct.201800108

62- Peng, H.; Marians, K.J. *Escherichia coli* topoisomerase IV. Purification, characterization, subunit structure, and subunit interactions. *J. Biol. Chem.*, **268**, 24481-24490 (1993).

63- Panetha, A.; Staczek, P.; Plech, T.; Strzelczyk, A.; Janowska, D.; Stefanska, J.; Dzitko, K.; Wujec, W.; Kosiek, S.; Panethe, P. Synthesis and antibacterial activity of 1,4-dibenzoylthiosemicarbazide derivatives. *J. Biomed. Pharmac.*, **88**, 1235–1242 (2017). http://dx.doi.org/10.1016/j.biopha.2017.02.001.

64- Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *J. Immunol. Methods*, **65**, 55–63 (1983). https://doi.org/10.1016/0022-1759(83)90303-4.

65- Scudiero, D.A.; Shoemaker, R.H.; Paull, K.D.; Monks, A.; Tierney, S.; Nofziger, T.H.; Currens, M.J.; Seniff, D.; Boyd, M.R. Evaluation of a soluble tetrazolium/ formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. *Cancer Res.*, **48**, 4827–4833 (1988).

66- Vaskin, P. Perimidine derivatives, US 3502647 A 1970-03-24 (1970).

67- Baraldi, P.G.; El-Kashef, H.; Farghaly, A.; Vanelle, P.; Fruttarolo, F. Synthesis of new pyrazolo[3,4-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines and related heterocycles. *Tetrahedron*, **60**(23), 5093-5104 (2004). https://doi.org/10.1016/j.tet.2004.04.010

68- Abo-Bakr, A.M. Synthesis and antibacterial activity of some new functionalized derivatives of 4-amino-5-benzyl-4*H*-[1,2,4]-traizole-3-thiol. *Int. J. Sci. Res.*, **3**(11), 15-23 (2014).

https://www.ijsr.net/search_index_results_paperid.php?id=OCT14282

69- El-Ansary, S.L.; Hussein, M.M.; Abdel Rahman, D.F.; Abdel Ghany, L.M.A. Synthesis, docking and *in vitro* anticancer evaluation of some new benzopyrone derivatives. *Bioorg. Chem.*, **53**, 50-66 (2014). DOI: 10.1016/j.bioorg.2014.02.003

70- El-Adl, K; Sakr, H.M.; Yousef, R.G.; Mehany, A.B.M.; Metwaly, A.M.; Elhendawy, M.A.; Radwan, M.M.; ElSohly, M.A.; Abulkhair, H.S.; Eissa, I.H. Discovery of new quinoxaline-2(1*H*)-one-based anticancer agents targeting VEGFR-2 as inhibitors: Design, synthesis, and anti-proliferative evaluation. *Bioorg. Chem.*, **114**, 105105 (2021). https://doi.org/10.1016/j.bioorg.2021.105105

71- Yousef, R.G.; Sakr, H.M.; Eissa, I.H.; Mehany, A.B.M.; Metwaly, A.M.; Elhendawy, M.A.; Radwan, M.M.; ElSohly, M.A.; Abulkhair, H.S.; El-Adl, K. New quinoxaline-2(1*H*)-ones as potential VEGFR-2 inhibitors: design, synthesis, molecular docking, ADMET profile and anti-proliferative evaluations. *New J. Chem.*, **45**, 16949-16964 (2021). DOI: 10.1039/d1nj02509k

72- Rida, S.M.; Ashour, F.A.; El-Hawash, S.A.M.; ElSemary, M.M.; Badr, M.H.; Shalaby, M.A. Synthesis of some novel benzoxazole derivatives as anticancer, anti-HIV-1 and antimicrobial agents. *Eur. J. Med. Chem.*, **40**, 949–959 (2005). DOI: 10.1016/j.ejmech.2005.03.023

73- Fadda, A.A.; Rabie, R. Cyanoacetylation of amines: recent advances in preparation methods and their synthetic uses in the formation of biologically active compounds. *Res. Chem. Intermed.*, **42**, 771–811 (2016).

74- Qingxiang, L.; Zeliang, H.; Zhixiang, Z. Tianjin Normal University, CN107793439-2018-A (2018).

75- Bondock, S.; Rabie, R.; Etman, H.A.; Fadda, A.A. Synthesis and antimicrobial activity of some new heterocycles incorporating antipyrine moiety. *Eur. J. Med. Chem.*, **43**, 2122-2129 (2008). DOI: 10.1016/j.ejmech.2007.12.009

76- Nasr, T.; Bondock, S.; Eid, S. Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridine derivatives bearing sulfisoxazole moiety. *Eur. J. Med. Chem.*, **84**, 491-504 (2014). DOI: 10.1016/j.ejmech.2014.07.052