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SYNTHESIS, *IN-VITRO* ANTIMICROBIAL EVALUATION AND MOLECULAR DOCKING STUDIES OF SOME NOVEL ACETOPHENONES SUBSTITUTED QUINOXALINE THIOSEMICARBAZIDE DERIVATIVES

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ABSTRACT: In the present research worked aims to increase the antimicrobial activity of quinoxaline thiosemicarbazide derivatives by substitution of some acetophenones and their antimicrobial evaluation against various antimicrobial strains with molecular docking studies. Lead molecule (1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene) thiosemicarbazide was synthesized and condensed with various substituted acetophenones to synthesize derivatives. All derivatives were characterized by IR., NMR & Mass spectroscopy. The synthesized derivatives were evaluated *in-vitro* for antibacterial and antifungal activities against various strains using the agar dilution method. Molecular docking studies of the derivatives (Va – Vg) were performed against *E. coli* DNA gyrase B and Topoisomerase IV to find out essential binding sites against target protein PDB: 1AJ6 and 1S14, respectively. Among all these compounds,Vf and Vg were found to exhibit more potent activity against Gram –Ve, Gram +Ve bacterial and fungal strains at MIC 0.19 µg/ml, 0.39µg/ml, and 0.78 µg/ml, respectively. The docking studies of all the compounds exhibit potent binding energy, but the compound Vg exhibit interactive binding energy -8.1 and -7.5 kcal/mol to the active pockets of *E.coli* DNA gyrase B and Topoisomerase IV against target protein PDB: 1AJ6 and 1S14, respectively. The compound Vg interacting with various active sites of amino acids of DNA gyrase B like ASN 46, ILE 94, ILE 78, PRO 79, ARG 76, THR 165, ASP 73 & VAL 71, and Topoisomerase IV like ASP 1133, ASP 1070, ARG 1159, ARG 1072, THR 1161 & HIST 1051.In terms of structure-activity relationship study, it is revealed that the activity profile against bacterial and fungal strains was altered by the formation of acetophenones substituted (1E, 4E)-1-(7-chloro-3-isopro-pylquinoxalin-2(1H)-ylidene) thiosemicarbazide derivatives.

INTRODUCTION: The emergence of new antimicrobial infections and antimicrobial resistance is a serious commination to public health globally due to widely disseminated and careless use of antimicrobials [1].

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The pathogens can develop resistance to mono drug therapy, multiple drug (MDR) therapies, and extensively used drug (XDR) therapy, which leads to the risk of developing pandemic illness [2]. Hence the demands a continuous effort to develop an effective and potent antimicrobial agent, effective against resistant pathogenic microorganisms [3-5].

Quinoxaline is an important nitrogen-containing benzo heterocyclic compound that has been used for the synthesis of a wide range of potent derivatives, and various investigation groups have

established their potential use in medical as well as in pharmacological applications [6]. Quinoxaline and its derivatives exhibit broad spectrum pharmacological activities such as antibacterial [7-9], antiviral [10], antifungal [11, 12], anticancer [13, 14], antitubercular [15], antileishmanial [16], antimalarial [17, 18], and antidepressant [19, 20] activities. Furthermore, quinoxaline-2-ones and quinoxaline-2, 3-diones were reported for their antimicrobial [21, 22], antithrombotics [23], analgesic, anti-inflammatory [24, 25] and SR protein-specific kinase-1 [26].

Thiosemicarbazide nucleus is the potent intermediate for the synthesis of pharmaceutically active compounds. Hence, it is widely used in pharmaceutical chemistry. The imine linkage (R_1R_2C=N-NH-CS-NHR_3), in the thiosemi-carbazide nucleus is useful for the synthesis of various potent derivatives [27]. Thiosemicarbazide derivatives exhibit a wide variety of pharmacological activities such as antiviral [28], anticancer [29], antitumor [30-32], anti-inflammatory, and antiamoebic [33-35], anti-tubercular activities [36]. Recently in continuation of our search, it was found that some thiosemi-carbazide derivatives significantly inhibit the activity of Staphylococcus aureus DNA gyrase and topoisomerase IV [37, 38].

On the rationality of these above facts and structure-activity relationship study, in the present research work aims to increase the antimicrobial activity of quinoxaline thiosemicarbazide derivatives by introducing a hydrophobic alkyl chain, electron releasing group, thiosemicarbazide nucleus in the quinoxaline ring, and substitution by various substituted acetophenones. In this present research work, we synthesized a series of novel acetophenones substituted quinoxaline thiosemicarbazide derivatives and evaluated *in-vitro* for antibacterial and antifungal activities against various strains. Furthermore, to understand the mechanism of action and binding activity, molecular docking studies were performed against two kinases, E.coli DNA gyrase B and *E. coli* Topoisomerase IV, against target protein PDB: 1AJ6 and 1S14 respectively [39, 40]. Computational studies were performed to analyze orientation patterns of the ligands with amino acids against target protein (PDB: 1AJ6 and 1S14).

EXPERIMENTAL: Materials and methods: All the reagents and the solvents used in the research work were of LR grade, but few were also of AR grade and obtained from Qualigens Ltd. (Fisher Scientific), Ranbaxy, and Fine Chemicals Ltd. India. Muller-Hinton and Sabouraud dextrose agar were obtained from Hi-Media Ltd. India. The bacterial and fungal strains were provided by the Department of Biotechnology of Saroj Institute of Technology & Management, Lucknow, India.

A series of derivatives were synthesized according to the respective scheme. Progress of reactions and purity of derivatives were monitored by ascending thin layer chromatography on precoated silica gel-G sheets (E. Merck and Co.). The spot was visualized by iodine vapors, and the purity of compounds was confirmed by a single spot on TLC plates. Column chromatography was performed over silica gel (60-120 Mesh) obtained from QualigensTM (India). The percentage of yield, Rf values, melting points, and spectral analysis are given for various purified compounds. Yields are presented for crude products. Log P values for synthesized compounds were calculated by using Chem Draw Ultra 10.0.

Melting points were determined by using the Digital Elico melting point apparatus. Infra-red spectra were measured on a Perkin-Elmer FT-IR RXI Spectrophotometer. 1HNMR spectra were reported on a Bruker DPX-300 Spectrometer (300 MHz) using DMSO-D6 as a solvent and tetramethylsilane (TMS) as an internal reference standard. Electron Spinning Ionization Mass spectra (ESI-MS) were obtained on the JEOL SX 102 spectrometer. Elemental analysis was determined on an Elemental Vario EL-III elemental analyzer.

Synthesis of 7-Chloro-3-isopropyl-1H-quino-xaline-2-one(III): 4-Chlorobenzene-1, 2-diamine (I) (21.3 g, 0.15 M) was dissolved in n-butanol (300 ml) and warmed. Ethyl dimethyl pyruvate (II) (21.6 g, 0.15 M) was solubilized separately in n-butanol (150 ml) and added to the former solution with constant stirring. The reaction mixture was refluxed for about 1 hour 30 minutes on the water bath. The reaction mixture was allowed to cool, obtained crystals, which were allowed for filtration, washed and purified by recrystallization from ethanol to obtain the white crystals of 7-chloro-3-isopropyl-1H-quinoxaline-2-one (III).

The completion of the reaction and purity of the compound was checked by a single spot TLC.Yield: 89.5%; m.p. 225-228 ˚C; Mol. Formula: $C_{11}H_{11}C_lN_2O$; Mol. Wt: 222.67; IR (KBr, cm^{-1}): 3465 (NH str.), 3102 (C-H sp2 str.), 1659 (C=N str.), 1605 (C=C aromatic str.), 1372 (CH (CH_3)2str.), 1042 (C-Cl str.), 1690 (C=O str.);1H-NMR (300 MHz, DMSO-d6) δ (ppm): 10.15 (S, 1H, NH), 8.06 (S, 1H, Ar. H), 7.25 -7.28 (d, 1H, Ar. H), 6.97-7.05 (d, 1H, Ar. H), 2.25-2.43 (m, 1H, CH, i-pr.), 1.63 (s, 6H, -(CH3)2).ESI-MASS: m/z $[M+1]^+$ 223.19 Anal. Calculation for (:$C_{11}H_{11}C_lN_2O$): C, 59.33; H, 4.98; N, 12.58. Found: C, 59.29; H, 5.02; N, 12.52.

Synthesis of (1E, 4E)-1-(7-chloro-3-isopropylquinoxalin -2(1H)-ylidene) thiosemicarbazide(IV): 7-Chloro-3- isopropyl- 1H-quinoxaline-2-one (III) (22 g, 0.10 M) was dissolved in ethanol (350 ml) and added thiosemicarbazide (9 g, 0.10 M). The reaction mixture was stirred and refluxed for 4 hours. The reaction mixture was allowed to cool at room temperature, obtained crystals. The crystals were collected by filtration, washed and purified by recrystallization from ethanol to yield white crystals of (1E, 4E)-1-(7-chloro-3-isopropylquinoxalin -2(1H)-ylidene) thiosemicarbazide (IV). The completion of the reaction and purity of the compound was checked by a single spotTLC.Yield: 85.5 %; m.p. 218--222 ˚C; Mol. Formula: $C_{12}H_{14}C_lN_5S$; Mol. Wt: 295.79; IR (KBr, cm^{-1}): 3442 (NH str.), 3066 (C-H sp2 str.), 3002 (N-H2 str.), 1654 (C=N str.), 1602 (C=C aromatic str.), 1361 (CH (CH_3)2str.). 1037 (C-Cl str.);1H-NMR (300MHz, DMSO-d6)δ (ppm): 10.02 (S,1H,NH), 9.62(S,1H,NH),8.09(S,1H,Ar.H),7.26-7.34 (d,1H,Ar.H), 6.96-6.98(d,1H,Ar.H), 4.99 (S,2H,NH2), 2.18-2.64(m,1H,CH,i-pr.),1.59(d,6H,-(CH_3)2); ESI-MASS:m/z[M+1]+ 296.09; Anal. Calcd for $C_{12}H_{14}C_lN_5S$:C,48.73; H, 4.77; N, 23.68; S,10.84.Found: C,48.69;H,4.72;N,23.74;S, 10.15.

General procedure for the synthesis of a series of different acetophenones substituted quinoxaline thiosemicarbazide derivatives- (Va-Vg): A typical procedure is described here for the synthesis of a series of different acetophenone substituted quinoxaline thiosemicarbazide derivatives. The synthesized intermediate compound 1E,4E)-1-(7-chloro-3-isopro-pylquinoxalin- 2(1H)-ylidene) thiosemi-carbazide (IV) (0.01 mol) was refluxed with different substituted acetophenones (acetophenone Va, 4-methoxyacetophenone V_b, 4-bromo-acetophenone V_c, 2-bromoacetophenone V_d, 2, 4-dibromo-acetophenone V_e, 4- fluroaceto-phenone Vf and 4-nitroacetophenone Vg), (0.01 mol), in methanol (50 ml) and added glacial acetic acid (6-8 drops) for 5-8 h. The progress of the reaction was monitored at different time intervals by TLC on silica-gel 60 plates, until a distinct spot of the product was obtained. At the end of the reaction, the crude precipitate was filtered and recrystallized with methanol. The final product thus obtained was chromatographed on silica gel (60-120 mesh), using solvent system chloroform: methanol (3:1) as eluent to furnish pure compounds Va-Vg.

Spectral Data of Synthesized Derivatives(Va-Vg): (1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-phenyl ethylidene) thiosemi-carbazide (V_a):Yield: 67.5 %; m.p.187-190 ˚C; Mol. Formula:C20H20ClN5S; Mol. Wt: 397.92; IR (KBr, cm^{-1}): 3375(N-H str.), 3023 (C-H sp2 str. aromatic), 1618 (C=N str.), 1599 (C=C aromatic str.), 1331 (CH (CH_3)2 str.), 1251 (C=S str.), 757 (C-Cl str.), 1022,961,918 (aromatic C-H in plane bending), 839, 697,639,597 (aromatic C-H out of plane bending); 1H NMR (300 MHz,DMSO-d6): δ 10.55 (s,1H, N-NH), 10.16 (s,1H, NH), 7.95-7.99 (m,5H, Ar-H), 7.86-7.87(d, 1H, ArH-8), 7.23-7.27 (d, 1H, ArH-6), 6.75-6.78 (d, 1H, ArH-5), 2.52-2.58 (m, 1H, CH, i-pr.), 1.95 (s, 3H, -CH3), 1.22 (s, 6H, -CH_3); ESI-MASS : m/z [M+1]+ 399.09; Anal. Calculated for $C_{20}H_{20}C_lN_5S$: C, 60.37; H, 5.07; Cl, 8.91; N, 17.60; S, 8.06; Found: C, 60.42; H, 5.10; N, 17.56.

(1E, 4E)- 1- (7- chloro- 3-isopropylquinoxalin-2(1H)- ylidene)-4- (1- (4- methoxyphenyl): ethylidene) thiosemicarbazide (Vb):Yield: 64.5 %; m.p.195-198 ˚C; Mol. Formula:$C_{21}H_{22}ClN_5OS$; Mol.Wt: 427.95; IR (KBr, cm^{-1}): 3273(N-H str.), 2977 (C-H sp2 str. aromatic), 2875 (C-H sp2 str. alkyl), 1602 (C=N str.), 1505 (C=C aromatic str.), 1373 (CH (CH3)2 str.), 1229 (C=S str.), 899 (C-Cl str.), 1143,1061,1005 (aromatic C-H in plane bending), 852,755,609 (aromatic C-H out plane bending); 1H NMR (300 MHz, DMSO-d6): δ 10.67 (s,1H, N-NH), 10.14 (s,1H, NH), 7.31-7.35 (m,4H, ArH), 7.26-7.30 (d, 1H, ArH-8), 6.94-6.96 (d, 1H,

ArH-6), 6.92-6.93 (d, 1H, ArH-5), 2.65-2.72 (m, 1H, CH, i-pr.), 2.17 (s,3H, -CH3), 2.06 (s, 3H, -CH_3), 1.28 (s,6H,-(CH3)2); ESI-MASS : m/z [M+1]+ 429.15; Anal. Calculated. for $C_{21}H_{22}C_lN_5OS$: C, 58.94; H, 5.18; Cl, 8.28; N, 16.36; S, 7.49; Found: C, 58.89; H, 5.21; N, 16.32.

(1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-bromophenyl) ethylidene) thiosemicarbazide (Vc): Yield: 67.5 %; m.p.205-207°C; Mol. Formula: $C_{20}H_{19}BrClN_5S$; Mol. Wt: 476.82; IR (KBr, cm^{-1}): 3362(N-H str.), 3010 (C-H sp2 str. aromatic), 2913 (C-H sp2 str. alkyl), 1644 (C=N str.), 1603 (C=C aromatic str.), 1378 (CH (CH_3)2 str.), 1217 (C=S str.), 768 (C-Cl str.), 1098,1001, (aromatic C-H in plane bending), 878,818,668 (aromatic C-H out plane bending); 1H NMR (300 MHz, DMSO-d6): δ 10.79 (s,1H, N-NH), 10.48 (s,1H, NH), 7.99 (s,2H, ArH), 7.79 (s,2H, ArH), 7.56-7.59 (d, 1H, ArH-8), 7.25-7.27 (d, 1H, ArH-6), 6.97-6.99 (d, 1H, ArH-5), 2.55-2.61 (m, 1H, CH, i-pr.), 2.25 (s,3H, -CH3), 1.95 (s,6H,-(CH_3)2); ESI-MASS : m/z [M+1]+ 478.05 Anal. Calculated for $C_{20}H_{19}BrClN_5S$: C, 50.38; H, 4.02; Cl, 7.44; N, 14.69; S, 6.72; Found: C, 50.42; H, 4.06; N, 14.65.

(1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene) -4-(1- (2-bromophenyl): ethylidene) thiosemicarbazide (Vd):Yield: 66 %; m.p; 208-210 ˚C; Mol. Formula: $C_{20}H_{19}BrClN_5S$; Mol. Wt: 476.82;IR (KBr, cm-1): 3354(N-H str.), 3015 (C-H sp2 str. aromatic), 2920 (C-H sp2 str. alkyl), 1649 (C=N str.), 1598 (C=C aromatic str.), 1372 (CH (CH_3)2 str.), 1223 (C=S str.), 774 (C-Cl str.), 1088, 1010,988 (aromatic C-H in plane bending), 874,809,658 (aromatic C-H out plane bending);1H NMR (300 MHz, DMSO-d6): δ 10.87 (s,1H, N-NH), 10.54 (s,1H, NH), 7.87-7.99 (m,4H, ArH), 7.53-7.56 (d, 1H, ArH-8), 7.34-7.37 (d, 1H, ArH-6), 6.91-6.95 (d, 1H, ArH-5), 2.45-2.49 (m, 1H, CH, i-pr.), 2.15 (s,3H, -CH3), 1.98 (s,6H,-(CH_3)2);ESI-MASS: m/z [M+1]+ 478.10; Anal. Calculated for $C_{20}H_{19}BrClN_5S$: C, 50.38; H, 4.02; Cl, 7.44; N, 14.69; S, 6.72; Found: C, 50.35; H, 4.05; N, 14.66.

(1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H) -ylidene) -4-(1-(2, 4-dibromophenyl): ethylidene) thiosemicarbazide (Ve): Yield: 64.5 %; m.p. 215-218 ˚C; Mol. Formula: $C_{20}H_{18}Br_2C_lN_5S$; Mol. Wt: 555.72; IR (KBr, cm-1): 3406 (N-H str.), 3019 (C-H sp2 str. aromatic), 2984 (C-H sp2 str. alkyl), 1637 (C=N str.), 1557 (C=C aromatic str.), 1362 (CH (CH_3)2 str.), 1339 (C-N str.), 1256 (C=S str.), 878 (C-Cl str.), 1179,1096,1042 (aromatic C-H in plane bending), 821,758,668 (aromatic C-H out plane bending);1H NMR (300 MHz, DMSO-d6): δ 10.69 (s,1H, N-NH), 10.40 (s,1H, NH), 7.42-7.46 (d,2H, ArH), 7.26 (s,1H, ArH), 6.99-7.01 (d, 1H, ArH-8), 6.94-6.97 (d, 1H, ArH-6), 6.74-6.76 (d, 1H, ArH-5), 2.49-2.56 (m, 1H, CH, i-pr.), 2.15 (s,3H, -CH_3), 1.59 (s,6H,-(CH_3)2); ESI-MASS: m/z [M+2]+ 557.89 Anal. Calculated for $C_{20}H_{18}Br_2ClN_5$: C, 43.23; H, 3.26; Cl, 6.38; Br, 28.76; N, 12.60; S, 5.77; Found: C, 43.28; H, 3.30; N, 12.56.

(1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H) -ylidene)- 4- (1- (4-fluorophenyl): ethylidene) thiosemicarbazide (Vf): Yield: 66.5 %; m.p.197-199 ˚C; Mol. Formula: $C_{20}H_{19}ClFN_5S$; Mol. Wt: 415.91; IR (KBr, cm^{-1}): 3342(N-H str.), 3016 (C-H sp2 str. aromatic), 2903 (C-H sp2 str. alkyl), 1625 (C=N str.), 1599 (C=C aromatic str.), 1371 (CH (CH_3)2 str.), 1211 (C=S str.), 758 (C-Cl str.), 1088, 1011, (aromatic C-H in plane bending), 868, 812, 662 (aromatic C-H out plane bending); 1H NMR (300 MHz, DMSO-d6): δ 11.28 (s,1H, N-NH), 10.83 (s,1H, NH), 7.47-7.50 (d,2H, ArH), 7.32-7.34 (d,2H, ArH), 7.11-7.13 (d, 1H, ArH-8), 6.95-6.98 (d, 1H, ArH-6), 6.82-6.86 (d, 1H, ArH-5), 2.75-2.80 (m, 1H, CH, i-pr.), 1.72 (s,3H, -CH3), 1.28 (s,6H,-(CH_3)2);ESI-MASS: m/z [M+1]+ 417.09; Anal. Calculated for $C_{20}H_{19}ClFN_5S$: C, 57.76; H, 4.60; Cl, 8.52; N, 16.84; S, 7.71; F, 4.57; Found: C, 57.72; H, 4.64; N, 16.81.

(1E, 4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)- 4- (1- (4- nitrophenyl): ethylidene) thiosemicarbazide (Vg):Yield: 68 %; m.p.192-195 oC; Mol. Formula: $C_{20}H_{19}C_lN_6O_2S$; Mol. Wt: 442.92; IR (KBr, cm^{-1}): 3379(N-H str.), 3028 (C-H sp2 str. aromatic), 2923 (C-H sp2 str. alkyl), 1651 (C=N str.), 1610 (C=C aromatic str.), 1382 (CH (CH_3)2 str.), 1219 (C=S str.), 768 (C-Cl str.), 1087,1011, (aromatic C-H in plane bending), 880,823,678 (aromatic C-H out plane bending); 1H NMR (300 MHz, DMSO-d6): δ 11.28 (s,1H, N-NH), 10.98 (s,1H, NH), 7.58-7.61 (d,2H, ArH), 7.42-7.46 (d,2H, ArH), 7.25-7.28 (d, 1H, ArH-8), 6.99-7.13 (d, 1H, ArH-6), 6.85-6.89 (d, 1H, ArH-

5), 2.45-2.51 (m, 1H, CH,i-pr.), 1.79 (s,3H, -CH_3), 1.34 (s,6H,-(CH_3)2); ESI-MASS: m/z [M+1]+ 443.02; Anal. Calculated for $C_{20}H_{19}ClN_6O_2S$: C, 54.23; H, 4.32; Cl, 8.00; N, 18.97; O, 7.922; S, 7.24; Found: C, 54.27; H, 4.37; N, 18.94.

Antimicrobial Evaluation:

Microbial Strains: In the present research work, the synthesized derivatives (V_a-V_g), were evaluated *in-vitro* for their antibacterial activity against Gram-negative bacterial strains such as *Klebsiela pneumonie* (ATCC 15380), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27893), *Salmonella typhi* (MTCC 3216), *Helicobacter pylori* (ATCC 26695) and Gram-positive bacterial strains such as *Bacillus subtilis* (ATCC 6633), *Bacillus thuringiensis* (MTCC 714), *Staphylococcus aureus* (ATCC 25323), methicillin-resistant *Staphylococcus aureus* (ATCC 3591). Fungal strains used were *Penicillium chrysogenum* (ATCC 11709), *Aspergillus niger* (ATCC 9029), *Candida albicans* (ATCC 90028).

Antimicrobial Assay Methodology: Anti-microbial (Antibacterial and antifungal) activities of all the synthesized derivatives (Va-Vg), were assayed by using the agar dilution method to determine the minimum inhibitory concentrations (MICs) [41]. Ciprofloxacin (CFX) and Fluconazole (FCZ) were used as antibacterial and antifungal reference standards, respectively. The range of concentrations of synthesized agents being tested based on the two-fold dilution series (1 mg/L). The dilutions of the synthesized agents (Va-Vg) and reference drugs (CFX and FCZ) were prepared in Mueller-Hinton (MH) agar for bacteria and in Sabouraud dextrose agar for fungi. Each test derivatives (10 mg) were dissolved in 1mL of dimethylsulfoxide (DMSO) and the solution was diluted with water (9 ml). Twofold dilutions were made with melted Mueller-Hinton and Sabouraud dextrose agar to obtained the necessary concentrations of 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19, 0.098, 0.049,.0.025, 0.013 and 0.006 µg/ml. The microbial inoculums were prepared by emulsifying overnight colonies from Mueller-Hinton and Sabouraud dextrose agar media in 0.85% saline. The prepared inoculums suspension photometrically adjusted at 600 nm for a cell density comparable to approximately 0.5 McFarland standards (1.5×10^8 CFU/mL). The suspensions of microorganisms were diluted in 0.85% saline to give 10^7 CFU/mL for bacteria and 105 CFU/mL for fungi. The plate spot was inoculated with microbial suspensions about 1 µl each and incubated at 35-37 ˚C for 18-19 h for bacteria and 28-30 ˚C for 50-72 h for fungi. The minimum inhibitory concentration was observed and determined.

Antibacterial and Antifungal Study: The synthesized acetophenones substituted quinoxaline thiosemicarbazide derivatives (V_a-V_g) were evaluated for their antibacterial and antifungal activity. Most of the derivatives showed excellent to significant activity towards Gram-negative and Gram-positive bacterial and fungal strains. The minimum inhibitory concentration of derivatives along with Ciprofloxacin ranges from 0.19 – 0.78 µg/ mL for bacteria and with Fluconazole ranges 1.56 – 3.12 µg/ mL for fungal strains **Table 2**.

Molecular Docking Studies: In today's world, the structure based drug design, molecular docking study is the most important tool which has been widely used ever since the early 1980s [42]. In this research work, docking studies were performed of all the synthesized derivatives and elucidate the mode of binding between the enzyme active binding site and the novelbioactive compounds, in addition also find out possible interactions between ligand and receptor with docking score.

To understand the interaction of all the synthesized derivatives (Va- Vg) were docked into active site of *E. coli* DNA gyrase B kinase and *E. coli* Topoisomerase IV. Crystal structure model of the target (PDB: 1AJ6 and 1S14) were downloaded from worldwide protein data bank (http://www.rcsb.org) and molecular docking studies were performed using the AutoDock Tool 1.5.6 (ADT) 2011 software (Molecular Graphic Laboratory, The Script Research Institute, U. S. A.), To analyze the docking result and execute the protocol, The Discovery Studio® v17.2.0.16349 software (Client, U. S. A) was employed.

Protein Preparation: The Crystal structure model of the target *E.coli* DNA gyrase B (PDB code: 1AJ6) and *E. coli* Topoisomerase IV (PDB code: 1S14), with their native ligand Novobiocin were downloaded from Protein Data Bank and prepared

by the multistep process through the protein preparation menu of the AutoDock (version 1.5.6). The protocol was especially used to obtain the optimized and minimized energy conformation of the protein. Firstly, the bond order in the protein was assigned; hydrogen atoms were added, and the water molecules which did not participate in interactions were removed. Following the above steps of preparation, the protein was subjected to assign AD4 and Kollman charges.

Ligand Preparation: The ChemDraw® Ultra 8.0 (Cambridgesoft, USA) software was used to draw the various chemical structures of the ligand molecules, while the Chem3D® Ultra 8.0 (Cambridgesoft, U. S. A.) was used to convert the sketched two-dimensional structures to three dimensional (3D) structures.

Active Site Prediction: The Sitemap applies theoretical methods and predicts the most accurate binding site. The grid was generated via a selection of grid box force fields as center on ligand, possible for ligand interaction within the protein. The binding site was recognized by specifying the atoms of a co-crystallized ligand (Novobiocin) presented in the pocket while the native ligand atoms were ignored by the software during the docking procedure. The scores were then calculated as free energy of binding (ΔGb), and the final ten highest-scoring poses (conformations) for each molecule along with their scores and binding energies (ΔGb) were collated into a database. The database file generated from the docking procedure was further analyzed, with the binding mode (interactions) of the highest ten conformations for each docked molecule in the active site visualized and studied with the help of the Discovery studio visualization window. Among the visualization of the conformation generated from the docking for each molecule, the conformation with the best binding mode (interactions) with the lowest binding energy(ΔGb) was selected for further analysis.

As described earlier that native ligand novobiocin was shown to have antibacterial activity. The native ligand novobiocin binds to *E. coli* DNA gyrase B kinase through two hydrogen bonds with the essential amino acids Asp 46 and Asp 73 and arene-cation interaction with Arg 7643,44. All derivatives synthesized in this research work consist of large no. of functional groups like NH_2, C=S and phenyl ring, which exhibit promising binding interactions with receptors like native ligand novobiocin. Based on these facts, we choose the target *E. coli* DNA gyrase B (PDB code: 1AJ6) and *E. coli* Topoisomerase IV (PDB code: 1S14) for docking study.

RESULTS AND DISCUSSION:

Chemistry: A series of various acetophenones substituted quinoxaline thiosemicarbazide derivatives (Va-Vg) were synthesized with a good percentage yield as per scheme **Fig.** 1. Structure and physicochemical data of the final compounds are represented in **Table 1**. The chemical structures of the compounds were confirmed by elemental analysis, IR, NMR & Mass spectroscopy.

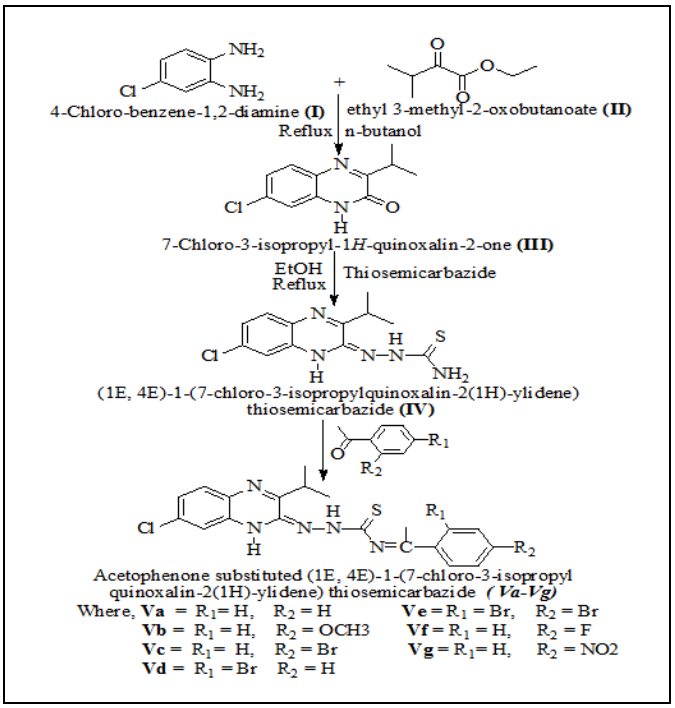

FIG. 1: SYNTHETIC SCHEME

The IR spectra of the compounds exhibit absorption bands due to N-H, C-H, C=C, C=N, (CH $(CH_3)_2$, C=S, and C-Cl stretching. The IR of synthesized compounds showed absorption bands near ranges 3406-3273, 3023-2977 and 2920- 2875 correlated with N-H stretching, C-H sp2 aromatic stretching and C-H sp2 aliphatic stretching respectively. Also the stretching absorption bands near ranges 1649-1602 , 1378-1331, and 1256-1217 correlated C=N, -$CH(CH_3)_2$, and C=S groups, respectively. A sharp stretching absorption band ranges between 899-757 indicated C-Cl group.

The1H-NMR at 300 MHz, solvent used DMSO-d6 of all the derivatives showed sharp singlet peak near about δ 1.34-1.98 ppm indicated isopropyl CH_3 protons and δ 1.72-2.13 ppm indicated protons of acetophenone CH_3. Multiple peaks ranges between δ 2.54-2.98 due to protons of isopropyl C-H. A broad set of singlet and doublet peak ranges δ 6.00-8.00 correlated to aromatic hydrogens and sharp singlet peak range between δ10.40-11.28 indicatedhydrogens of N-H group. The mass spectrum analysis of the compounds displayed characteristic peaks normally with [M+1]+ value. The elemental analysis outcomes of the compounds almost ranged within ± 0.4% of the calculated values.

TABLE 1: STRUCTURE AND PHYSICO-CHEMICAL DATA OF THE SYNTHESIZED COMPOUNDS VA-VG

Comp. Code	Structure		Mol. Formula	Mol. Wt.	MP (^{0}C)	% Yield	Rf Value
	R_1	R_2					
Va	H	H	$C_{20}H_{20}ClN_5S$	397.92	187-190	67.5	0.53
Vb	H	OCH_3	$C_{21}H_{22}ClN_5OS$	427.95	195-198	64.5	0.48
Vc	H	Br	$C_{20}H_{19}BrClNS$	476.82	205-207	67.5	0.55
Vd	Br	H	$C_{20}H_{19}BrClNS$	476.82	208-210	66	0.54
Ve	Br	Br	$C_{19}H_{16}Br_2ClN_5S$	555.72	215-218	64.5	0.56
Vf	H	F	$C_{20}H_{19}ClFN_5S$	415.91	197-199	66.5	0.54
Vg	H	NO_2	$C_{20}H_{19}ClN_6O_2S$	442.92	192-195	68.5	0.47

Antimicrobial Activity: The synthesized derivatives exhibited significant activity against Gram-negative and Gram-positive bacteria, when compared with standard Ciprofloxacin antibacterial drug **Table 2**. It was found that compound V_g exhibits an equipotent activity against *K. pneumoniae* (0.19 µg/ mL), *E. coli* (0.19 µg / mL), *P. aeruginosa* (0.0.78 µg/ mL) and less active against *S. typhi* (0.19 µg/ mL). The compound V_e and Vf also showed good activity against these species but less active when compare to V_g, Whereas no other compounds showed significant activity against *S. typhi*. Compounds V_f and V_g also displayed very good two fold activity against *H. Pylori* (0.39 µg / mL).On the study of Gram-positive strains a more excellent twofold activity was observed of compound V_g against *B. subtilis* (0.39 µg / mL) and MRSA (0.78 µg / mL) and equipotent activity against B. thuringiensis (0.39 µg / mL)andS. aureus (0.39 µg / mL). The compound V_f also showed equipotent activity against *B. subtilis* (0.78 µg / mL), *S. aureus* (0.39 µg / mL), and MRSA (1.56 µg / mL). The compound V_b was less potent against Gram-negative strains but showed good activity against Gram-positive strains, equipotent against *B. subtilis* (0.78 µg / mL) and MRSA (1.56 µg / mL). The two compounds Vf and V_g exhibit equipotent activity against *E. coli* (0.19 µg / mL). Thus the compound V_f and V_g were more active against both Gram-negative as well as Gram-positive strains, and compound Ve, was also showed good activity against both strains but less active when compared to V_f and V_g. The compound V_b was less potent against Gram-negative strains but showed good activity against Gram-positive strains.

TABLE 2: *IN-VITRO* ANTIMICROBIAL EVALUATION OF COMPOUNDS (Va-Vg) EXPRESSED*MIC (µg/ml)

Comp. Code	Antibacterial Activity									Antifungal Activity		
	Gram-negative strains					Gram-positive strains						
	K.p	E.c	P.a	S.t	H.p	B.s	B.t	S.a	MRSA	P.c	A.n	C.a
Va	1.56	1.56	6.25	0.78	1.56	3.12	6.25	3.12	3.12	3.12	6.25	12.5
Vb	1.56	0.78	3.12	0.39	0.78	0.78	0.78	0.78	1.56	0.78	6.25	3.12
Vc	0.78	0.78	3.12	1.56	0.78	1.56	3.12	1.56	1.56	1.56	12.5	6.25
Vd	0.78	0.78	3.12	1.56	0.78	1.56	3.12	1.56	1.56	1.56	6.26	6.25
Ve	0.38	0.38	1.56	0.39	0.78	0.78	1.56	0.78	1.56	1.56	6.25	6.26
Vf	0.19	0.38	0.78	0.19	0.39	0.78	0.78	0.39	1.56	0.78	6.25	3.12
Vg	0.19	0.19	0.78	0.19	0.39	0.39	0.39	0.39	0.78	1.56	3.12	3.12
CFX	0.19	0.19	0.78	0.09	0.78	0.78	0.39	0.39	1.56	-	-	-
FCZ	-	-	-	-	-	-	-	-	-	0.78	6.25	3.12

K.p = *Klebsiela pneumonie*; E.c = *Escherichia coli*; P.a = *Pseudomonas aeruginosa*, S.t = *Salmonella typhi*, H.p = *Helicobacter pylori*, B.s = *Bacillus subtilis*, B.t = *Bacillus thuringiensis*, S.a = *Staphylococcus aureus*, P.c = *Penicillium chrysogenum*, A.s = *Aspergillus niger*, C.a = *Candida albicans*, MRSA = Methicillin-resistant *Staphylococcus aureu* *MIC: Minimum inhibitory concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period ofincubation

The study of antifungal activity was tested against strains such as *P. chrysogenum*, *A. niger* and *C. albicans* using fluconazole as a standard drug **Table 2**. Compound V_g exhibits a twofold amplified activity against *A. niger* (3.12 µg / mL) and equipotent activity against C. albicans (3.12 µg / mL), but less active against *P. chrysogenum* (1.56 µg / mL). The compound V_b and V_f also exhibit equipotent activity against *C. albicans* (3.12 µg / mL), *P. chrysogenum* (1.56 µg / mL) and *A. niger* (6.25 µg / mL). Other compounds exhibited less potentactivity against all fungal strains.Overall study revealed that compound V_g and V_f explored the best potential activity in comparison with that of the standard compounds, followed by V_e, V_b, V_d, V_c and V_a in decreasing order exhibit excellent to good activity against various bacterial and fungal strains.

Molecular Docking Results: The *in-vitro* antimicrobial results of the synthesized compounds in hand which prompted to do *in-silico* studies to support the *in-vitro* activity. To correlate the observed potencies and structure-activity relationship of our newly synthesized compounds, automated docking was used to determine the possible binding modes and interactions with in the active site of *E. coli* DNA gyrase B (PDB code: 1AJ6) and *E. coli* Topoisomerase IV (PDB code: 1S14).

The docking of ligand molecules within the active pocket site of *E. coli* DNA gyrase B reveals that all the inhibitor compounds are exhibiting the bonding with no. of amino acids which is shown in **Fig. 2**. Theoretically, all the seven molecules showed very good binding energy ranging from -7.3 to -8.1 kcal/mol **Table 3**. To understand the atomistic level details of chemical moieties and their binding mode (interactions) within the active site of *E. coli* DNA gyrase B, they were visualized in two (2D) and three dimensions (3D) and analyzed with the help of PyMOL (Schrodinger) and Discovery studio. We focused our attention on the more potent compound Vg 43 embedded nicely within the active pocket of *E. coli* DNA gyrase B with a binding energy of -8.1 Kcal/mol. The interactions of the compound Vg revealed that the methyl groups of isopropyl present on quinoxaline ring were involved in hydrophobic interaction with ILE94 that may explain the observation that isopropyl substitution on the quinoxaline enhances *E. coli* DNA gyrase B inhibitory potency. The quinoxaline ring displayed hydrophobic interaction (arene-cation interaction) with ASN46, THR165, and ILE78. The substituted phenyl ring was involved in cation-arene interactions with PRO79. Moreover, the nitrogen of the thiosemicarbazide moiety showed linkage with ARG76 and chlorine atom substituted at position 7 on quinoxaline ring exhibited hydrophobic interactions with VAL43, VAL71 and VAL167. Similarly, the second Vf (Docking score -7.5) and third Ve (Docking score -7.5) potent compound also displayed binding as compound Vg such as hydrophobic interaction with ILE94, ASN46, THR165, cation-arene interaction with ILE78, PRO79, and hydrophobic interactions of chlorine atom with VAL43, VAL71 and VAL167 **Fig. 2**.

The docking study of the synthesized compounds against *E. coli* TopoisomeraseIV using PDB code: 1S14 reveals that all the compounds exhibited good binding energy ranging from -6.7 to -7.5 **Table 4**. The more potent compound Vg showed good docking energy (-7.5) and docked effectively in the active pocket site of *E. coli* Topoisomerase IV. The ligand-protein complexes showed that the quinoxaline ring bind through hydrophobic interactions with ASP1070 and ARG1054. The NH of the quinoxaline ring displayed hydrogen bond linkage with ARG1159, THR1161, and ASP1070.

The NH and C=S of thiosemicarbazide moiety exhibit hydrogen bond linkage with HIS1051. Furthermore, the phenyl ring showed hydrophobic interactions with ARG1072 through an arene-cation interaction. The NO_2 substituted on the para position of phenyl ring showed hydrogen bonding with ASP1133.

Similarly, the other active compound but lees than V_g like V_f (docking score: -7.2) and V_b (docking score: -7.3), also displayed hydrophobic interaction with ASP 1070, ARG1054, arene-cation interaction with ARG 1072, and Hydrogen bonding with ARG1159, THR1161, ASP 1133, HIS 1051 and ASP1070 **Fig. 3**. The molecular docking study of all the seven compounds (V_a-V_g) reveals that all the compounds occupied nicely in the active pocket site of the *E. coli* DNA gyrase B (PDB code: 1AJ6) and *E. coli* Topoisomerase IV (PDB code: 1S14)

and bonded with the variety of amino acid residues. The compounds were stabilized in the pocket of proteins by hydrogen bond interactions, hydro-phobic bond interactions, arene-cation interactions, and π-π interactions.

TABLE 3: MOLECULAR DOCKING BINDING ENERGY OF SYNTHESIZED COMPOUNDS (V_a-V_g)

Comp. Code	Target site: PDB: 1AJ6	Target site: PDB: 1S14
	Binding energy (Kcal/mol)	Binding energy (Kcal/mol)
Va	-7.4	-7.2
Vb	-7.3	-7.3
Vc	-7.4	-6.7
Vd	-7.4	-7.3
Ve	-7.5	-6.9
Vf	-7.5	-7.2
Vg	-8.1	-7.5

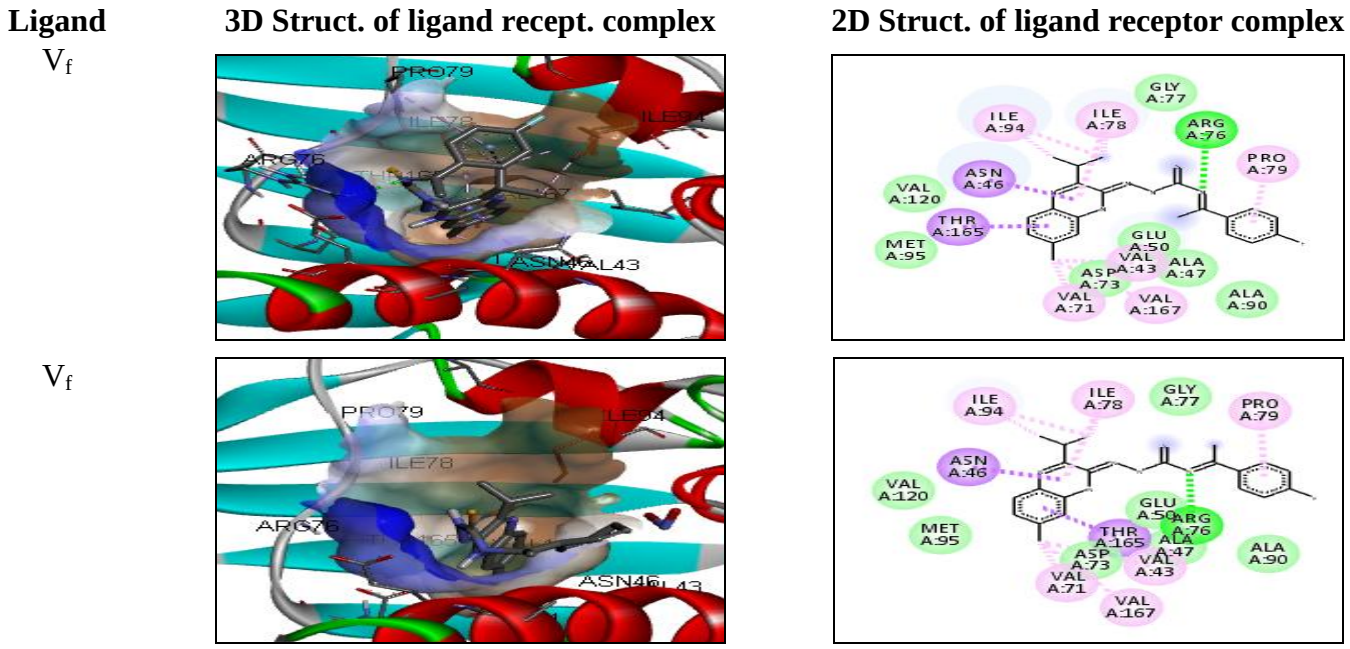

FIG. 2: TWO & THREE DIMENSIONAL BINDING INTERACTIONS OF MOST ACTIVE COMPOUNDS VF AND VG IN THE ACTIVE SITE OF E. COLI DNA GYRASE B (PDB ID: 1AJ6)

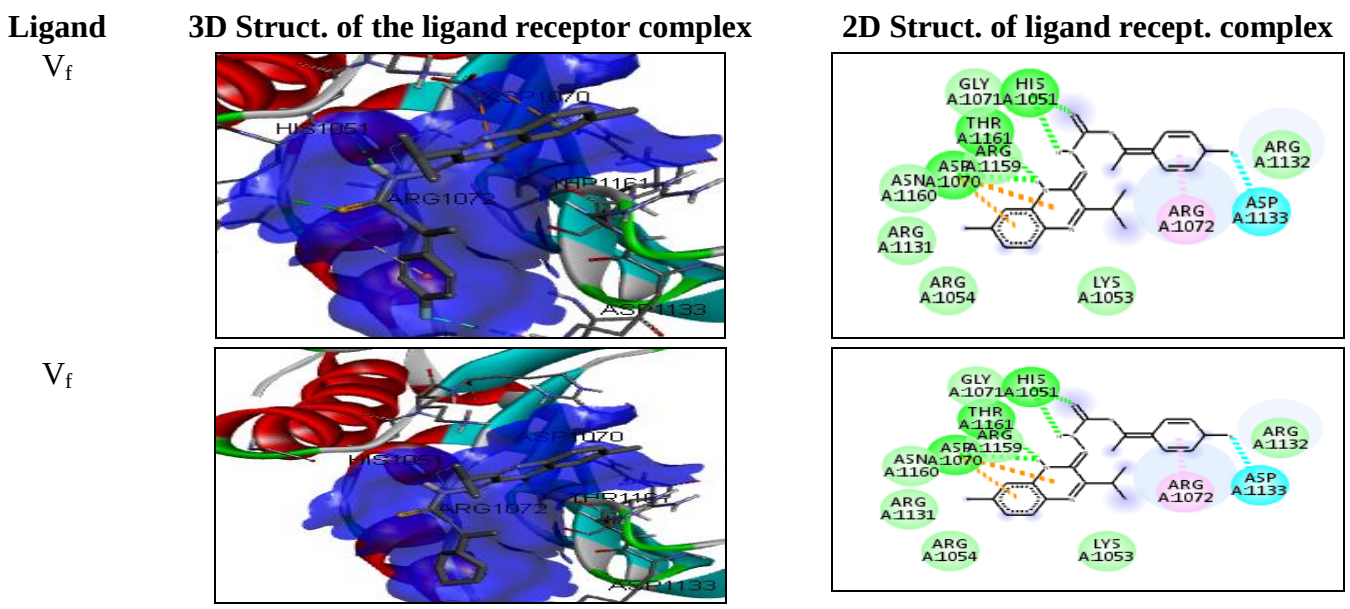

FIG. 3: TWO & THREE DIMENSIONAL BINDING INTERACTIONS OF MOST ACTIVE COMPOUNDS Vf AND Vg IN THE ACTIVE SITE OF *E. Coli* DNA TOPOISOMERASE IV (PDB ID: 1S14)

CONCLUSION: In order to find out of new structural design and looking promising as powerful antibacterial and antifungal agents, we have demonstrated the multistep synthesis of seven new acetophenones substituted (1E, 4E)-1-(7-chloro-3-isopropylquinoxalin- 2(1H)-ylidene) thio-semicarbazide derivatives bearing a quinoxaline thiosemicarbazide nucleus. In terms of structural activity relationship study revealed that the activity profile against bacterial and fungal strains were altered by the formation of acetophenones substituted (1E, 4E)-1- (7-chloro-3-isopro-pylquinoxalin-2(1H)-ylidene) thiosemicarbazide derivatives. The substitution by 4- nitroaceto-phenone and 4-fluoroacetophenone as in compound V_g and V_f respectively, enhance the spectrum of activity for Gram- negative to Gram-positive bacterial strains and fungal strains as well as substitution by 4-methoxyacetophenone as in compound Vb enhance the spectrum of activity against Gram-positive bacterial and fungal strains. Substitution by chlorine and isopropyl group in quinoxaline also enhances the activity.

The molecular docking studies also showed that all the compounds exhibit good docking energy to bind and inhibit the *E. coli* DNA gyrase B kinase and *E. coli* Topoisomerase IV. Based on the biological evaluation and molecular docking study, we conclude that the compound V_g, V_f, and V_b exhibit good activity against microbial strains and good docking energy. The most active compound V_g; 4-nitrocetophenone substituted quinoxaline thiosemicarbazide derivative showed the interactions (hydrogen bond acceptor, donor, hydrophobic bond interactions, arene-cation interactions, and π-π interactions) with amino acids ILE94, ASN46, THR165, ILE78. PRO79. ARG76VAL43, VAL71, and VAL167 in the binding pocket of *E. coli* DNA gyrase B. On the other hand, compound Vg displayed interactions with amino acids ASP1070, ARG1054. ARG1159, THR1161. HIS1051. ASP1133 in the binding pocket of *E.coli* Topoisomerase IV.

Thus by *in-vitro* and docking studies, it is revealed that the compound Vg and Vf are potent antimicrobial molecules. In accordance with this research, lead molecules could be considered as candidates for more clinically relevant researches in the future to overcome this type of antimicrobial resistance.

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HUMAN AND ANIMAL RIGHTS: No humans and animals were used for studies in the present research work.

CONFLICTS OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

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