

# Synthesis, Characterization, In-Vitro Antimicrobial Evaluation and Molecular Docking Studies of Aromatic Aldehydes Substituted Thiosemicarbazide Quinoxaline Derivatives

Bipin Bihari<sup>1\*</sup>, Girendra Kumar Gautam<sup>1,2</sup>, Akash Ved<sup>3</sup>

<sup>1</sup>Department of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India; <sup>2</sup>Shri Ram College of Pharmacy, Muzaffarnagar, U.P., India;

<sup>3</sup>Goel Institute of Pharmaceutical Sciences, Lucknow, U.P., India.

## ABSTRACT

**Background:** In the present research work a series of novel quinoxaline thiosemicarbazide derivatives were synthesized by substitution of some aromatic aldehydes and their antimicrobial evaluation against various microbial strains with molecular docking studies.

**Methods:** Lead molecule (1*E*, 4*E*)-1-(7-chloro-3-isopropyl quinoxaline-2(1*H*)-ylidene) thiosemicarbazide was synthesized and condensed with various aromatic aldehydes to synthesize derivatives. All derivatives (Va-Vf) were characterized by IR., NMR & Mass spectroscopy. The synthesized derivatives were evaluated in vitro for antibacterial and antifungal activities using the agar dilution method. Molecular docking studies of the derivatives 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.

**Results:** Compounds Vb, Vc, Ve&Vf exhibited potent antibacterial and antifungal activity. Compounds, Vb and Vc were found to exhibit more potent activity against Gram -Ve, bacterial strains at MIC 0.19 µg/ml whereas compound Ve and Vf showed potent activity against Gram +Ve bacterial strains and fungal strains at MIC 0.19 µg/ml and 0.78 µg/ml respectively. The docking studies revealed that all the compounds exhibit extensive binding to the active pockets of *E.coli* DNA gyrase B and topoisomerase IV. The compound Vb and Ve exhibit interactive binding energy -8.0 and -8.3 Kcal/mole to the active pocket site of *E.coli* DNA gyrase and -8.2 and 7.9 Kcal/mole to the active pocket site of topoisomerase IV respectively.

**Conclusion:** In terms of SAR study, it was revealed that the activity profile against microbial strains was altered with electronic effects like electron-withdrawing or donating substitutions in aromatic aldehydes substituted quinoxaline thiosemicarbazide derivatives.

**Key Words:** Antimicrobial, Aromatic aldehydes, *E.coli* DNA gyrase B, *E.coli* Topoisomerase IV, Quinoxaline, Thiosemicarbazide

## INTRODUCTION

Struggling for the development of an antimicrobial drug is a vital global issue due to the rapid development of resistance to currently used antimicrobial drugs, the emergence of new microbial infections and the existence of chronic microbial infections.<sup>1</sup> Bacteria may obtain resistance through a variety of mechanisms such as by spontaneous mutations or acquisition of genetic material from other resistant organisms or modifying binding sites or production of enzymes that inactivate antimicrobial agents or altering in outer membrane protein channel that the drugs require for cell entry.<sup>2</sup>

Quinoxaline and its derivatives are important nitrogen-containing benzo-hetero cyclic compounds.<sup>3</sup> Substituted quinoxaline derivatives are extensively employed in the building blocks of various pharmacologically active compounds. They exhibit a broad range of pharmacological activities such as antibacterial<sup>4,5,6</sup> antifungal<sup>7,8</sup>, antitubercular<sup>9</sup>, antimalarial<sup>10,11</sup>, antileishmanial<sup>12</sup>, anticancer<sup>13,14</sup> and antidepressant<sup>15,16</sup>. Also, quinoxaline derivatives reported for antioxidant<sup>17</sup>, antimycobacterial<sup>18</sup>, antithrombotic<sup>19</sup> and topoisomerase inhibition activity.<sup>20</sup>

Over the past few decades, an immense interest of researchers has been focused on thiosemicarbazide pharmacophore

### Corresponding Author:

Bipin Bihari, Ph.D. Scholar, Department of Pharmacy, Bhagwant University, Sikar Road, Ajmer-305004, Rajasthan, India.  
Tel: + 91- 8853177507; E-mail: bipin.project@gmail.com

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due to their wide range of synthetic and analytical applications and pharmacological activities<sup>21,22</sup>. Thiosemicarbazide derivatives were synthesized and studied for various pharmacological activities such as antiviral<sup>23,24</sup>, anticancer<sup>25</sup>, antitumor<sup>26,27</sup>, antiameobic<sup>28,29</sup>, antiproliferative<sup>30</sup>, antidiabetic<sup>31</sup>, anti-HIV<sup>32</sup>, anti-tubercular activities<sup>33</sup>. Recently, many thiosemicarbazide derivatives were synthesized and studied for their antibacterial and antifungal activity.<sup>34,35,36,37</sup>

DNA gyrase and topoisomerase IV are essential enzymes that display crucial roles in biological processes of bacterial growth such as replication, transcription, recombination repair and chromatin remodeling.<sup>38,39</sup> Nitrogen containing heterocyclic may be potential antibacterial drugs that inhibit bacterial topoisomerases<sup>40</sup> such as triazoles<sup>41</sup>, quinolones<sup>42</sup>, oxazopyridines, amino pyrazinamides and pyrazole.<sup>43</sup> In continuation of our search, it was found that certain thiosemicarbazide derivatives evaluated as inhibitors of DNA gyrase and topoisomerase II of *S. aureus* and *E. coli*.<sup>44,45</sup>

Based on the aforementioned facts, we decided to synthesize some novel quinoxaline hybrid thiosemicarbazide derivatives incorporated with different aromatic aldehydes through imine linkage in the thiosemicarbazide nucleus. All the synthesized compounds were screened in vitro for antibacterial and antifungal activities against various strains. In addition, 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<sup>46,47</sup>. Computational studies were performed to analyze binding and 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 synthetic reagent 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.

The Progress of reactions and purity of derivatives were monitored by ascending thin layer chromatography on pre-coated silica gel-G sheets (E. Merck and Co.). Column chromatography was performed over silica gel (60-120 Mesh) obtained from Qualigens<sup>TM</sup> (India). The percentage of yield, R<sub>f</sub> 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. <sup>1</sup>H NMR spectra were reported on a Bruker DPX-300 Spectrometer (300 MHz) using DMSO-D<sub>6</sub> 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. The structure of molecules was confirmed based on spectral data.

A series of aromatic aldehydes substituted (1*E*, 4*E*)-1-(7-chloro-3-isopropyl quinoxaline-2(1*H*)-ylidene) thiosemicarbazide derivatives were synthesized according to the respective scheme (Figure 1).

### Synthesis of 7-Chloro-3-isopropyl-1*H*-quinoxaline-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-1*H*-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<sub>11</sub>H<sub>11</sub>ClN<sub>2</sub>O; Mol. Wt: 222.67; IR (KBr, cm<sup>-1</sup>): 3465 (NH str.), 3102 (C-H sp<sup>2</sup> str.), 1659 (C=N str.), 1605 (C=C aromatic str.), 1372 (CH (CH<sub>3</sub>)<sub>2</sub> str.), 1042 (C-Cl str.), 1690 (C=O str.); <sup>1</sup>H- NMR (300 MHz, DMSO-d<sub>6</sub>) 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, -(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z [M+1]<sup>+</sup> 223.19; Anal. Calculated for (C<sub>11</sub>H<sub>11</sub>ClN<sub>2</sub>O): C, 59.33; H, 4.98; N, 12.58; Found: C, 59.29; H, 5.02; N, 12.52.

### Synthesis of (1*E*,4*E*)-1-(7-chloro-3-isopropylquinoxalin-2(1*H*)-ylidene) thiosemicarbazide(IV):

7-Chloro-3-isopropyl-1*H*-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 (1*E*, 4*E*)-1-(7-chloro-3-isopropyl quinoxaline-2(1*H*)-ylidene) thiosemicarbazide (IV). The completion of the reaction and purity of the compound was checked by a single spot TLC.

Yield: 85.5 %; m.p. 218-222 °C; Mol. Formula:

$C_{12}H_{14}ClN_5S$ ; Mol. Wt: 295.79; IR (KBr,  $cm^{-1}$ ): 3442(NH str.), 3066(C-H  $sp^2$  str.), 3002(N-H str.), 1654(C=N str.), 1602(C=C aromatic str.), 1361(CH(CH<sub>3</sub>)<sub>2</sub> str.), 1037(C-Cl str.); <sup>1</sup>H-NMR(300MHz,DMSO- $d_6$ ) $\delta$ (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,NH<sub>2</sub>), 2.18-2.64(m,1H,CH,i-pr.), 1.59(d,6H,-(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z[M+1]<sup>+</sup> 296.09; Anal. Calculated for  $C_{12}H_{14}ClN_5S$ : C, 48.73; H, 4.77; N, 23.68; S, 10.84; Found: C, 48.69; H, 4.72; N, 23.74.

### General procedure for the synthesis of a series of different aromatic aldehydes substituted quinoxaline thiosemicarbazide derivatives (Va-Vf):

A typical procedure is described here for the synthesis of a series of different aromatic aldehyde substituted quinoxaline thiosemicarbazide derivatives. In this step N<sup>7</sup>-(7-chloro-3-isopropyl-1*H*-quinoxaline-2-ylidene) thiosemicarbazide (IV) (0.01 mmol) was refluxed with different aromatic aldehydes (0.01 mol), in methanol (50 ml) and added glacial acetic acid (6-8 drops) for 4-5 hours. The progress of the reaction was monitored 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-Vf.

#### (1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-chlorophenyl) methylidene) thiosemicarbazide(Va):

Yield: 67.5 %; m.p.190-192 °C; Mol. Formula: $C_{19}H_{17}Cl_2N_5S$ ; Mol. Wt:418.34; IR (KBr,  $cm^{-1}$ ): 3215(N-H str.), 2933(C-H  $sp^2$  str. aromatic), 2875(C-H  $sp^2$  str. alkyl), 1599(C=N str.), 1572(C=C aromatic str.), 1337(CH(CH<sub>3</sub>)<sub>2</sub> str.), 1235(C=S str.), 785(C-Cl str.), 1110,1057,1005( aromatic C-H in plane bending), 668,614,519( aromatic C-H out plane bending); <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  10.89(s,1H, N-NH), 10.68(s,1H, NH), 9.15(s, 1H, N=CH), 7.28(s,2H, Ar. H), 7.24-7.27(d, 1H, Ar. H-8), 7.13-7.16(d, 1H, Ar. H-5), 7.01-7.04(d, 1H, ArH-6), 6.95-6.98(s,2H, Ar. H), 2.21-2.24(m, 1H, CH i-pr.), 1.95(s,6H,-(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z [M+1]<sup>+</sup> 419.07; Anal. Calculated. for  $C_{19}H_{17}Cl_2N_5S$ : C, 54.55; H, 4.10; N, 16.74; S, 7.66; Found: C, 54.52; H, 4.14; N, 16.73.

#### (1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-nitrophenyl) methylidene) thiosemicarbazide(Vb):

Yield: 68 %; m.p.197-200 °C; Mol. Formula: $C_{19}H_{17}ClN_5O_2S$ ; Mol. Wt:428.90; IR (KBr,  $cm^{-1}$ ):

3402(N-H str.), 2939(C-H  $sp^2$  str. aromatic), 2864(C-H  $sp^2$  str. alkyl), 1607(C=N str.), 1579(C=C aromatic str.), 1398(CH(CH<sub>3</sub>)<sub>2</sub> str.), 1231 (C=S str.), 747 (C-Cl str.), 1147,1105,1054 ( aromatic C-H in plane bending), 850,668,610,560 ( aromatic C-H out plane bending); <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  11.02 (s,1H, N-NH), 10.60 (s,1H, NH), 9.31 (s, 1H, N=CH), 7.86-7.89 (d,2H, Ar. H), 7.82-7.85 (d, 1H, Ar. H-8), 7.59-7.62 (d, 1H, Ar. H-5), 7.28-7.34 (d, 1H, Ar. H-6), 6.96-6.98 (d,2H, Ar. H), 2.17-2.23 (m, 1H, CH,i-pr.), 1.30 (s,6H,-(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z [M+2]<sup>+</sup> 430.15; Anal. Calculated. for  $C_{19}H_{17}ClN_5O_2S$ : C, 53.21; H, 4.00; N, 19.59; S, 7.46; Found: C, 53.24; H, 4.5; N, 19.56.

#### (1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-methoxyphenyl) methylidene) thiosemicarbazide(Vc):

Yield: 68.5 %; m.p.202-204°C; Mol. Formula:  $C_{20}H_{20}ClN_5OS$ ; Mol. Wt:413.92; IR (KBr,  $cm^{-1}$ ): 3380 (N-H str.), 3236 (C-H  $sp^2$  str. aromatic), 3097 (C-H  $sp^2$  str. alkyl), 1611 (C=N str.), 1576 (C=C aromatic str.), 1394 (CH(CH<sub>3</sub>)<sub>2</sub> str.), 1224(C=S str.), 779 (C-Cl str.), 1140,1052,979 ( aromatic C-H in plane bending), 904,668,556 ( aromatic C-H out plane bending); <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  10.60 (s,1H, N-NH), 10.51(s,1H, NH), 9.05 (s, 1H, N=CH), 7.71-7.74 (d,2H, Ar. H), 7.50-7.53 (d, 1H, Ar. H-8), 7.28-7.35 (d, 1H, Ar. H-5), 6.94-6.97 (d, 1H, ArH-6), 6.72-6.76 (d,2H, Ar. H), 2.46-2.53 (m, 1H, CH,i-pr.), 1.56 (s, 3H, -OCH<sub>3</sub>), 1.22 (s,6H,-(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z [M+1]<sup>+</sup> 415.02; Anal. Calculated. for  $C_{20}H_{20}ClN_5OS$ : C, 58.03; H, 4.87; N, 16.92; S, 7.75; Found: C, 58.07; H, 4.92; N, 16.88.

#### (1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-hydroxyphenyl) methylidene) thiosemicarbazide(Vd):

Yield: 70.5 %; m.p.195-197°C; Mol. Formula:  $C_{19}H_{18}ClN_5OS$ ; Mol. Wt:399.90; IR (KBr,  $cm^{-1}$ ):3430 (O-H str.), 3395 (N-H str.), 2924 (C-H  $sp^2$  str. aromatic), 2839 (C-H  $sp^2$  str. alkyl), 1601 (C=N str.), 1569 (C=C aromatic str.), 1372 (CH(CH<sub>3</sub>)<sub>2</sub> str.), 1222 (C=S str.), 738 (C-Cl str.), 1145,1115,1044 ( aromatic C-H in plane bending), 843,657,602,548 ( aromatic C-H out plane bending); <sup>1</sup>H NMR (DMSO- $d_6$ ):  $\delta$  10.97 (s,1H, N-NH), 10.56 (s,1H, NH), 10.05 (s, 1H, OH), 9.67 (s, 1H, N=CH), 7.83-7.87 (d,2H, Ar. H), 7.71-7.74 (d, 1H, Ar. H-8), 7.49-7.52 (d, 1H, Ar. H-5), 7.25-7.31 (d, 1H, Ar. H-6), 6.91-6.96 (d,2H, Ar. H), 2.19-2.26 (m, 1H, CH,i-pr.), 1.28 (s,6H,-(CH<sub>3</sub>)<sub>2</sub>); ESI-MASS: m/z [M+2]<sup>+</sup> 402.08; Anal. Calculated. for  $C_{19}H_{18}ClN_5OS$ : C, 57.07; H, 4.54; N, 17.51; S, 7.46; Found: C, 57.04; H, 4.57; N, 17.48.



**(1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-methylphenyl) methylidene) thiosemicarbazide(Ve):**

Yield: 62.3 %; m.p.188-190°C; Mol. Formula:  $C_{20}H_{20}ClN_5S$ ; Mol. Wt: 397.92; IR (KBr,  $cm^{-1}$ ): 3354 (N-H str.), 2945 (C-H  $sp^2$  str. aromatic), 2840 (C-H  $sp^2$  str. alkyl), 1625 (C=N str.), 1529 (C=C aromatic str.), 1343 (CH  $(CH_3)_2$  str.), 1246 (C=S str.), 756 (C-Cl str.). 1173, 1044, 973 (aromatic C-H in plane bending), 835, 644, 593 (aromatic C-H out plane bending).  $^1H$  NMR(DMSO- $d_6$ ):  $\delta$  11.13 (s, 1H, N-NH), 10.70 (s, 1H, NH), 9.22 (s, 1H, N=CH), 7.40-7.43 (d, 2H, Ar. H), 7.36-7.39 (d, 1H, ArH-8), 6.79-6.81 (d, 1H, ArH-5), 6.58-6.62 (d, 1H, ArH-6), 6.17-6.21 (d, 2H, Ar. H), 2.61-2.69 (m, 1H, CH, i-pr.), 1.65 (s, 3H,  $-CH_3$ ), 1.27 (s, 6H,  $-(CH_3)_2$ ); ESI-MASS:  $m/z$   $[M+1]^+$  399.14; Anal. Calculated. for  $C_{20}H_{20}ClN_5S$ : C, 60.37; H, 5.07; N, 17.60; S, 8.06; Found: C, 60.41; H, 5.11; N, 17.57.

**(1E,4E)-1-(7-chloro-3-isopropylquinoxalin-2(1H)-ylidene)-4-(1-(4-isopropylphenyl) methylidene) thiosemicarbazide(Vf):**

Yield: 69.5 %; m.p.210-212°C; Mol. Formula:  $C_{22}H_{24}ClN_5S$ ; Mol. Wt: 425.98; IR (KBr,  $cm^{-1}$ ): 3316 (NH str.), 2982 (C-H  $sp^2$  aromatic str.), 2808 (C-H  $sp^2$  aliphatic str.), 1668 (C=N aromatic str.), 1512 (C=C str.), 1372 (CH  $(CH_3)_2$  str.), 1342 (C-N str.), 1272 (C=S str.), 789 (C-Cl str.), 1164, 1069, 978 (aromatic C-H in plane bending), 841, 698, 594 (aromatic C-H in out of plane bending).  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  11.15 (s, 1H, N-NH), 10.35 (s, 1H, NH), 8.61 (s, 1H, N=CH), 7.85-7.88 (d, 2H, Ar. H), 7.43-7.47 (d, 1H, Ar. H-8), 7.25-7.25 (d, 1H, Ar. H-5), 6.97-7.01 (d, 1H, ArH-6), 6.71-6.75 (d, 2H, Ar. H), 2.25-2.40 (m, 2H, CH, i-pr.), 1.34 (s, 6H,  $(CH_3)_2$ ), 1.30 (s, 6H,  $-(CH_3)_2$ ); ESI-MASS :  $m/z$   $[M+1]^+$  427.12 Anal. Calculated for  $C_{22}H_{24}ClN_5S$ : C, 62.03; H, 5.68; N, 16.44; S, 7.53; Found: C, 62.06; H, 5.72; N, 16.41.

## ANTI-MICROBIAL EVALUATION

**Microbial Strains:** All the synthesized compounds (Va-Vf), were evaluated *in vitro* for their antibacterial and antifungal activity against Gram-negative bacterial strains such as *Klebsiella pneumoniae* (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:** Antimicrobial evaluation of all the synthesized derivatives (Va-Vf), were assayed by using the agar dilution method to determine the

minimum inhibitory concentrations (MICs).<sup>48</sup> 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 and reference drugs were prepared in Mueller-Hinton (MH) agar for bacteria and in Sabouraud dextrose agar for fungi. Each test derivatives (10 mg) were dissolved in 1 mL of dimethyl sulfoxide (DMSO) and the solution was diluted with water (9 mL). Two-fold dilutions were made with melted Mueller-Hinton and Sabouraud dextrose agar to obtain 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  $\mu$ g/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 ( $1.5 \times 10^8$  CFU/mL). The suspensions of microorganisms were diluted in 0.85% saline to give  $10^7$  CFU/mL for bacteria and  $10^5$  CFU/mL for fungi. The plate spot was inoculated with microbial suspensions about 1  $\mu$ L each and incubated at 35-37°C for 18-19 hours for bacteria and 28-30°C for 50-72 hours for fungi. The minimum inhibitory concentration was observed and determined.

**Antibacterial and Antifungal Study:** The synthesized aromatic aldehydes substituted quinoxaline thiosemicarbazide derivatives (Va-Vf) were evaluated for their antibacterial and antifungal activity. Most of the compounds showed excellent to significant activity towards Gram-negative, Gram-positive bacterial and fungal strains. The minimum inhibitory concentration of more active compounds along with Ciprofloxacin ranges from 0.19 – 0.78  $\mu$ g/mL for bacteria and with Fluconazole ranges 0.78 – 3.12  $\mu$ g/mL for fungal strains. The results of the antibacterial and antifungal activity evaluation are summarized in **Table 2**.

## MOLECULAR DOCKING

**Molecular Docking Studies:** In today's globalized world, the molecular docking technique is one of the largely acclaimed structure-based drug design approaches, widely used ever since the early 1980s.<sup>49</sup> To understand the binding interactions of all the synthesized derivatives were docked into the 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 Auto Dock 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 and ligand 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 ChemDraw® Ultra 8.0 (Cambridge soft, USA) software was used to draw the various chemical structures of the ligand molecules.

**Active Site Prediction:** The Sitemap applies theoretical methods and predicts the most accurate binding site. A receptor grid was generated via the selection of the grid box. The binding site was recognized by specifying the atoms of a co-crystallized ligand (novobiocin). The scores were then calculated as the free energy of binding ( $\Delta G_b$ ) and the final ten highest-scoring poses (conformations) for each molecule along with their scores and binding energies ( $\Delta G_b$ ) 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

## RESULT AND DISCUSSION

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

The IR spectra of the compounds exhibit absorption bands due to OH, N-H, C-H, C=C, C=N, (CH (CH<sub>3</sub>)<sub>2</sub>, C=S and C-Cl stretching. The IR of synthesized compounds showed absorption bands near ranges 3215-3395 cm<sup>-1</sup>, 2932-3236 cm<sup>-1</sup> and 2839- 3097 cm<sup>-1</sup> correlated with N-H stretching, C-H sp<sup>2</sup> aromatic stretching and C-H sp<sup>2</sup> aliphatic stretching respectively. Also, the stretching absorption bands near ranges 1599-1668 cm<sup>-1</sup>, 1337-1398 cm<sup>-1</sup> and 1222-1272 cm<sup>-1</sup> correlated with C=N, -CH(CH<sub>3</sub>)<sub>2</sub>, and C=S groups respectively. IR displayed a characteristic broad absorption band at 3430 cm<sup>-1</sup> for the OH group in compound Vd. The <sup>1</sup>H-NMR at 300 MHz, the solvent used DMSO-d<sub>6</sub> of all the derivatives showed a sharp singlet peak near range  $\delta$  1.27-1.95 ppm indicated isopropyl CH<sub>3</sub> (6H) protons and  $\delta$  8.61-9.67 ppm indicated protons of Schiff bridge (N=CH). Multiple peaks appeared in all compounds ranges between  $\delta$  2.17-2.64 due to protons of isopropyl C-H. A broad set of singlet and doublet peak ranges  $\delta$  6.62-7.82 correlated to quinoxaline moiety aromatic hydrogens and sharp singlet peak range between  $\delta$  10.40-11.28 indicated hydrogens of N-H group. A sharp

singlet peak in compound Vd at  $\delta$  10.05 indicated hydroxyl group (OH) proton. The mass spectrum analysis of the compounds displayed characteristic peaks normally with [M+1]<sup>+</sup> value and [M+2]<sup>+</sup> value in compound Vd. The elemental analysis outcomes of the compounds almost ranged within  $\pm$  0.4% of the calculated values.

**Antimicrobial activity:** The synthesized derivatives exhibited significant activity against Gram-negative and Gram-positive bacteria when compared with standard Ciprofloxacin antibacterial drug (**Table 2**). The compound Vb showed more potent activity against Gram-negative strains. *aeruginosa* (0.39  $\mu$ g / mL) and *H. pylori* (0.39  $\mu$ g / mL) and equipotent activity against *K. pneumonia* (0.19  $\mu$ g / mL), *E. coli* (0.19  $\mu$ g / mL), and less active against *S. Typhi* (0.19  $\mu$ g / mL). The compound Vc exhibited good activity against *H. pylori* (0.39  $\mu$ g / mL) but equipotent activity *K. pneumoniae* (0.19  $\mu$ g / mL), *E. coli* (0.19  $\mu$ g / mL) and *P. aeruginosa* (0.78  $\mu$ g / mL). Whereas no compounds showed significant activity against *S. typhi*.

On the study of Gram-positive strains, a more excellent two-fold activity was observed of compound Ve and Vf against *B. subtilis* (0.39  $\mu$ g / mL), *S. aureus* (0.19  $\mu$ g / mL) and *MRSA* (0.78  $\mu$ g / mL) and equipotent activity against *B. thuringiensis* (0.39  $\mu$ g / mL). The compound Vc also showed twofold activity against *B. subtilis* (0.39  $\mu$ g / mL) and equipotent activity against *B. thuringiensis* (0.39  $\mu$ g / mL) and *MRSA* (1.56  $\mu$ g / mL). Thus, it was found that compound B exhibited more potent activity against Gram-negative bacterial strains but less active against Gram-positive bacterial strains whereas compound Ve and Vf showed more potent activity against Gram-positive bacterial strains rather than Gram-negative bacterial strains. The study revealed that the compound Vc showed good activity against some Gram-negative strains as well as some Gram-positive strains when compared with reference standard drug ciprofloxacin.

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 Vc and Vf exhibit a twofold amplified activity against *A. niger* (3.12  $\mu$ g / mL) and *C. Albicans* (1.56  $\mu$ g / mL), whereas equipotent activity against *P. Chrysogenum* (0.78  $\mu$ g / mL). The compound Ve exhibit equipotent activity against *Chrysogenum* (0.78  $\mu$ g / mL) and *A. niger* (6.25  $\mu$ g / mL), but less active against *C. Albicans* (6.25  $\mu$ g / mL). The overall study revealed that compound Vc, Vf and some extent compound Ve explored the best potential activity against fungal strains in comparison with that of the standard compounds.

**Molecular docking results:** The docking of ligand molecules within the active pocket site of *E. coli* DNA gyrase B revealed that all the inhibitor compounds were exhibited the bonding with no. of amino acids which are showed in **Figure 2**. Theoretically, all the synthesized compounds showed very

good docking energy ranging from -8.0 to -8.3 kcal/mol for PDB:1AJ6 and -7.6 to -8.2 for PDB:1S14 (Table 3).

We concentrated our attention on the more potent compounds **Vb**, **Vc**, **Ve** and **Vf** embedded nicely within the active pocket of *E. coli* DNA gyrase B (PDB: 1AJ6) with the binding energy of -8.0, -8.0, -8.3 and -8.1 Kcal/mol respectively. The interactions of the compounds revealed that the methyl groups of isopropyl present on the quinoxaline ring was involved in hydrophobic interaction with **ILE94** and **VAL20** that may explain the observation that isopropyl substitution on the quinoxaline ring enhances *E. Coli* DNA gyrase B inhibitory potency. The quinoxaline ring displayed hydrophobic interaction (arene-cation interaction) with **ASN46** and **THR165**. Furthermore, the secondary amine group of the thiosemicarbazide moiety showed hydrogen linkage with **GLU50** and thio group linked with **THR165** and **GLY177**. The nitro and methoxy group in compound **Vb** and **Vc** showed binding with **VAL43**, **VAL71** and **VAL 167** (Figure 2).

The docking study of the synthesized compounds against *E. coli* Topoisomerase IV using PDB code: 1S14 reveals that all the compounds exhibited good binding energy ranging from -7.6 to -8.2 kcal/mol (Table 4). The more potent compound **Vb** showed good docking energy (-8.2 kcal/mol) and docked effectively in the active pocket site of *E. coli* Topoisomerase IV. The ligand-protein complexes showed that the quinoxaline ring of each compound binds extensively through hydrophobic interactions with **GLU1046** and **MET1074**. The **NH** of the quinoxaline ring displayed hydrogen bond linkage with **GLY1073**. The nitrogen of thiosemicarbazide moiety exhibit hydrogen and hydrophobic linkage with **ARG1132** and **ARG1072** (Figure 3).

## CONCLUSION

In conclusion, the present research reports the successful synthesis of different benzaldehydes substituted (1*E*, 4*E*)-1-(7-chloro-3-isopropyl quinoxaline- 2(1*H*)-ylidene) thiosemicarbazide derivatives and their in-vitro antimicrobial evaluation with molecular docking studies. Most of the evaluated compounds showed slightly more significant antimicrobial activity in comparison to reference compound. The structure-activity relationship study affirmed that the substitution by para-nitro benzaldehyde (**Vb**) enhance the activity against Gram-negative bacteria whereas substitution by para-methyl benzaldehyde (**Ve**) and para-isopropyl benzaldehyde (**Vf**) enhance the spectrum of activity against Gram-positive bacteria as well as fungal strains. Substitution by para-methoxy benzaldehyde as in compound **Vc** enhances the spectrum of activity against both Gram-negative as well as Gram-positive bacterial strains and also against fungal strains in comparison to reference compound.

The electron-withdrawing and donating groups substituted in the para position of benzaldehydes exhibited well binding with amino acids in the active pocket site of DNA gyrase B and *E. coli* Topoisomerase IV. Thus, following this research, the synthesized 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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**Table 1: Structure and Physico-chemical data of the synthesized compounds (Va-Vg).**

Comp. Code	Structure (Z)	Mol. Formula	Mol. wt.	MP (°C)	% Yield	Rf Value	Log P
Va	-Cl	C <sub>19</sub> H <sub>17</sub> C <sub>12</sub> N <sub>5</sub> S	418.34	190-192	67.5	0.52	6.10
Vb	-NO <sub>2</sub>	C <sub>19</sub> H <sub>17</sub> ClN <sub>6</sub> O <sub>2</sub> S	428.90	197-200	68.0	0.49	3.75
Vc	OCH <sub>3</sub>	C <sub>20</sub> H <sub>20</sub> ClN <sub>5</sub> OS	413.92	202-204	68.5	0.56	5.42
Vd	-OH	C <sub>19</sub> H <sub>18</sub> ClN <sub>5</sub> OS	399.90	195-197	70.5	0.47	5.16
Ve	-CH <sub>3</sub>	C <sub>20</sub> H <sub>20</sub> ClN <sub>5</sub> S	397.92	188-190	62.3	0.55	6.03
Vf	-CH(CH <sub>3</sub> ) <sub>2</sub>	C <sub>22</sub> H <sub>24</sub> ClN <sub>5</sub> S	425.98	210-212	69.5	0.54	6.78

**Table 2: In vitro antimicrobial evaluation of synthesized compounds (Va-Vf) expressed as <sup>a</sup>MIC (µg/ml).**

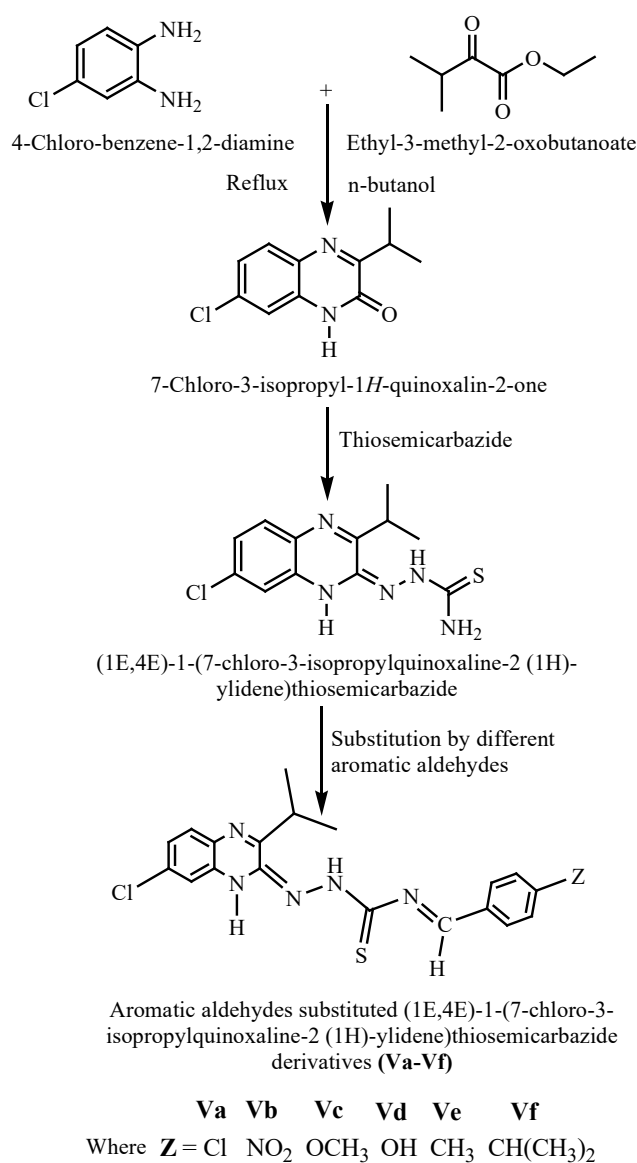
Comp. code	Antibacterial Activity								Antifungal Activity			
	Gram-negative strains						Gram-positive strains					
	K. p	E. c	P. a	S. t	H. p	B. s	B. t	S. a	MRSA	P. c	A. n	C. a
Va	0.78	0.78	0.78	0.19	0.78	0.78	0.78	1.56	1.56	1.56	6.25	6.25
Vb	0.19	0.19	0.39	0.19	0.39	1.56	0.78	1.56	3.12	1.56	6.25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

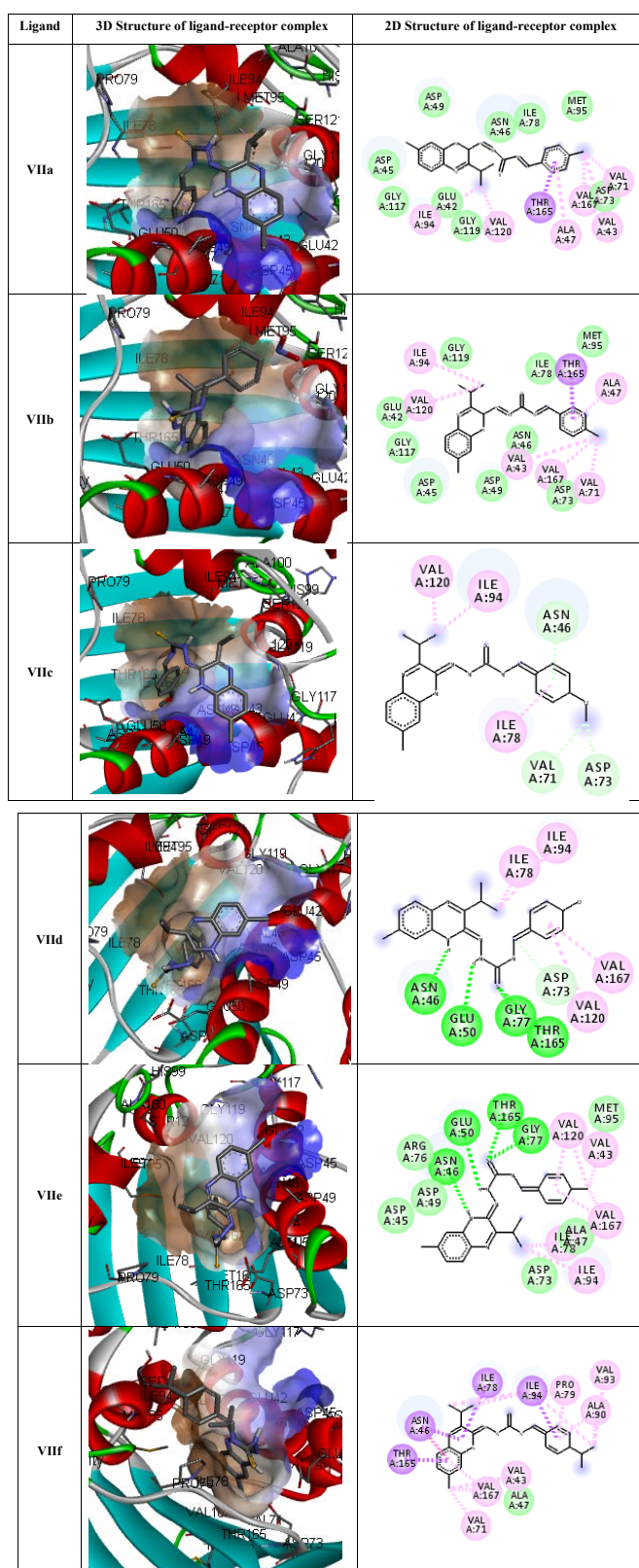
<sup>a</sup>MIC: Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of a microorganism after a period of incubation.



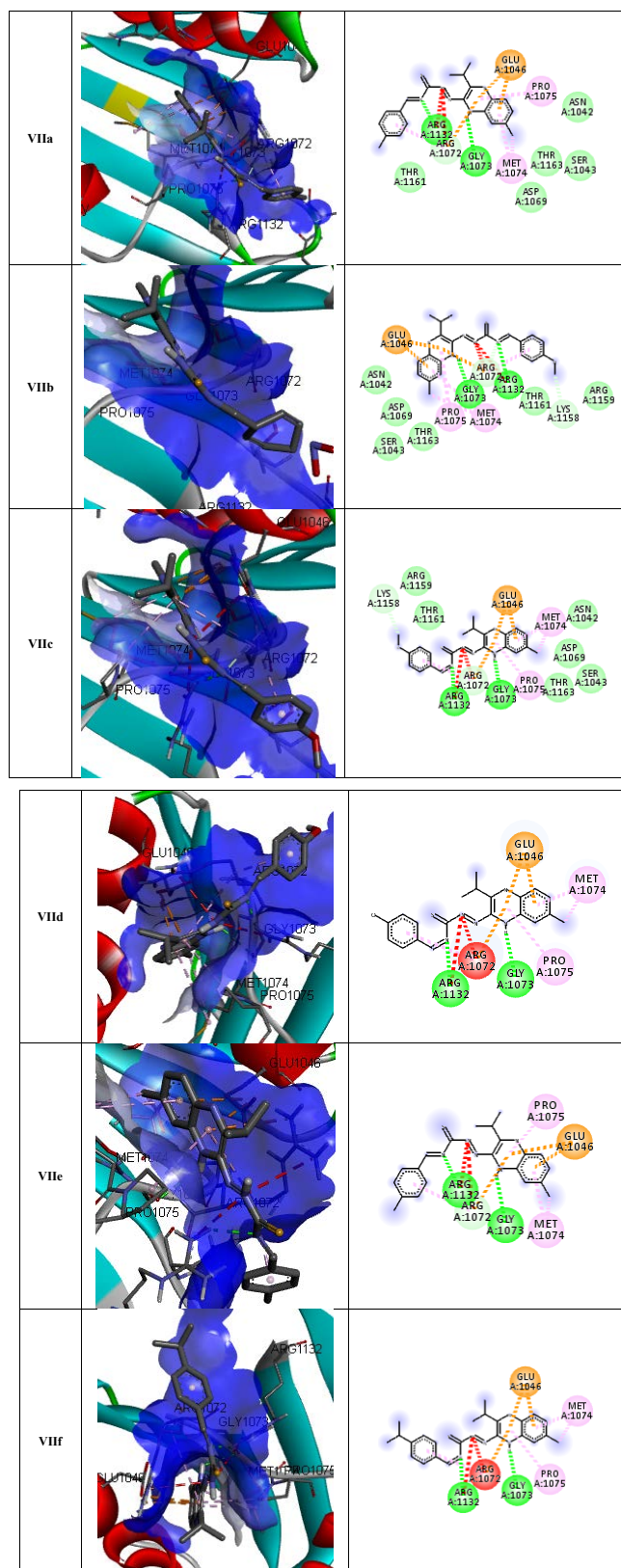
**Table 3: Molecular docking binding energy of the synthesized compounds (Va-Vf).**

Ligands	Target site: PDB:1AJ6	Target Site: PDB:1S14
	Binding energy (Kcal/mol)	Binding energy (Kcal/mol)
Va	-8.0	-7.8
Vb	-8.0	-8.2
Vc	-8.0	-7.6
Vd	-8.1	-7.6
Ve	-8.3	-7.9
Vf	-8.1	-7.9

**Figure 1:** Synthetic scheme for the synthesis of compounds (Va-Vf).



**Figure 2:** Two & three-dimensional docking studies showing binding interactions of compounds with active site of *E. Coli* DNA Gyrase B (PDB ID: 1AJ6).



**Figure 3:** Two & three-dimensional docking studies showing binding interactions of compounds with active site of *E. Coli* DNA Topoisomerase IV (PDB ID: 1S14).