Evaluation of anti-bacterial activity of novel 2, 3-diaminoquinoxaline derivatives: design, synthesis, biological screening, and molecular modeling studies

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ABSTRACT
A series of nonsymmetrical 2,3-diaminoquinoxaline derivatives prepared straight-forwardly by the C-N coupling between chloro-quinoxaline (2,3-DCQ) and various amino substrates. Synthesized products (4a–z) with excellent purity (\textsuperscript{1}HNMR) without performing flash column chromatography purification. Investigated the antibacterial activity of selected nine compounds against Gram-positive, Gram-negative bacterial strains, and additional fungal strains. Overall, the selected screened compounds (4b, 4c, 4 h, and 4 n) presented significant antibacterial activity against bacterial strains. The resulting lead, displaying a novel quinoxaline compound (4c), showed antibacterial activity between 10.5 and 14.89 mm against all strains except A. Niger. Molecular docking studies revealed the binding poses and critical interactions of 2,3-diaminoquinoxaline analogues at the quinolone-binding site of S. aureus DNA gyrase. The docking results are in accordance with the antimicrobial testing data. Binding of these new analogues (as DNA intercalators) at quinolone-binding site involves the contribution from: I) Protein: Ser-1084, II) DNA: DG (G) 9 and DA (H) 13; and III) Mg\textsuperscript{2+} ion for their binding.

Introduction
Globally, WHO has documented antimicrobial resistance in many first and second-line medications. \cite{1} Lack of advanced medication, healthcare prescribed most expensive with serve toxicity and even less effective to treat chronic bacterial infection. Because of that, prolonged hospital stays and high cost of treatment with poor outcomes increase the tremendous burden on healthcare and may cause leading cause of death in the future. \cite{2} The most advanced second line, antimicrobial medication (vancomycin and methicillin) has developed resistance against \textit{Staphylococcus aureus} and a similar trend has been shown in the first line, fluconazole against candida fungal strain. \cite{3} Even though there are many medications existing for antimicrobial treatment, no one causes severe adversative effects \cite{4} such as local inflammation (penicillins), allergic reaction, phototoxicity (tetracyclines), effect on the liver, gray baby disorder, and myelosuppression (chloramphenicol) \cite{5}. The bacterial agent can undergo a mutation that causes resistance to

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corresponding medication or antibiotics, and finally less effectively binds with target sites like alteration in MOA, chemical alterations of the drug, or by other means. [6] Among that, major threatening is bacteria-produced biofilm to reduced penetration effect or neutralized antibiotics effect. [7,8] Quinoxaline motif is a key structural unit; it exists in various biologically active compounds. In recent research, many small molecules of quinoxaline have a tremendous demand in medicinal chemistry, broadly known as potent antiproliferative and antimicrobial agents. The quinoxaline-bearing antibiotics; including, Triostin A and similar kinds of levomycin and echinomycin to introduce into dsDNA and are advantageously effective toward the bacterial diseases caused by gram-positive bacteria. [9,10]

Quinoxaline has also documented to have promising anticancer activity in various research profiles and the U.S. FDA approved the potential anticancer drug for urothelial cancer and bladder cancer under the brand name Balversa (erdafitinib). [11] Quinoxaline scaffold is pharmacology impressive, displaying an array of interesting biological activities such as antibacterial [12–16], anti-tubercular [17–17], antimalarial [20–20], anti-viral [23,24], anti-HIV [25], anti-inflammatory [26–29], anti-fungal [30–33], anti-amoebic [34], anti-cancer [35–38], anti-proliferative [39,40], antitumor [41,42], antihypertensive [43], anticonvulsant [44–44], kinase inhibitor [47], antiepileptic [48,49], anti-HCV [50,51], analgesic [52,53] and also used in anthelmintic drugs. [54] Quinoxaline-based drugs presenting a broad range of diverse classes of biological activities as an outcome of diverse functionality on central structure Figure 1. Quinoxaline also used for crop protection in Agro-industries as a major component of insecticides [55], herbicides [56], and fungicides. [57] Besides their medicinal and crop protection applications these compounds widely used as dyes for solar cell applications, [58,59] fluorescent materials [60], organic semiconductors, [61–63], and corrosion inhibitors for metals. [64]

Because of the great call of antimicrobial therapy, continuous innovative research are going on quinoxaline scaffolds to hit novel candidates that may be, save the life for future prospects.

### Results 2.1 chemistry

The various steps during the synthesis of target compounds (4a-z) illustrated in Scheme 1. The reaction of benzene-1,2-diamine (OPD) with oxalic acid in refluxing 4 N HCl aqueous solution gave quinoxaline-2,3-dione (1). [65] Under reflux conditions, quinoxaline-2,3-dione (1) reacted with SOCl₂ in the presence of the catalytic amount of DMF in DCE solvent to afford 2,3-dichloro- quinoxaline (2). [65] Subsequently,

![Figure 1. The certain examples of Quinoxaline-based drugs against various targets.](image-url)
intermediate (2) treated with different alkyl amines (a-d) in the presence of K₂CO₃ to afford key intermediates (3a-d). Finally, treatment of these compounds (3a-d) with arylamine in refluxing ethanol furnished the target compounds of Series (4a-o), (4p-r), (4s-u), and (4 v-z) in Table 1.

K. S. Ahmed et al. (2019) designed and synthesized novel molecules based on N³-alkyl-6-nitro-N³-benzyl quinoxaline-derivatives. The nitro-group of quinoxaline alters the DNA structure of bacterial cells and inhibits the DNA synthesis and as a result, it presented an antibacterial profiles. [66] S. Paliwal et al. (2017) designed and synthesized small drug-like novel compounds of substituted Phenyl-3-Hydrazinyl-Quinoxaline-2-amines derivatives showed potent anti-microbial activities. From docking study of compounds revealed, it binds with the specific amino acid of dihydrofolate reductase protein of Staphylococcus aureus (PDB ID-4XE6). [67] From the above research work [66,67], we have designed a novel hybide scaffold (4) in Figure 2, which have combination effect and their binding site from 1,3 nitrogen interaction of quinoxaline ring with alkyl amine and similar interaction with aromatic amine. From the reported antimicrobial activities, [66,67] similar trends has been also adopted in selected screened compounds (4b, 4c, 4 h, 4 i, 4 m, 4 n, 4 w, 4x and 4z) in scaffold (4), among them, fluorinated compound (4b, 4c, 4 h and 4 n) presented broad and moderate activity on all bacterial strains.

**Evaluation of biological activity**

**Antimicrobial and antifungal activities**

They bought the test microorganisms from the National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune (India). Nutrient agar, MGYP, and Potato Dextrose agar (25°C, 24 h) were used to cultivate bacteria and fungi, respectively. It measured the zone of inhibition in mm. Disc diffusion method [68,69] using sterile paper disk (diameter 6 mm), against bacterial strain in the concentration of 100 µg/disk screened for antibacterial activity and screened these compounds for antifungal activity against fungi Aspergillus Nigerr and Candida albicans by the disc diffusion method (Table 2) in the same concentration. The antibacterial activities compared with standard drugs viz. Chloramphenicol (10 µg/disk) and antifungal activity with Amphotericin B (100 µg/disk). The selected nine compounds were dissolved in DMSO, which were used as a control.

S. aureus; Staphylococcus aureus, B. subtilis; Bacillus subtilis, E. coli; Escherichia coli, K. pneumoniae; Klebsiella pneumoniae, S. typhi; Salmonella typhi, C. albicans; Candida albicans, A. Niger; Aspergillus Niger, Ch; Chloramphenicol, Am; Amphotericin B., (NA; Not applicable, Nt; Not tested).

**Structure–activity relationship study**

The above biological data (Table 2 or Figure 3) of the newly synthesized compounds revealed that all the nine novel derivatives adopted a significant antimicrobial and antifungal profile against the tested bacterial and fungal strains.

The results of antimicrobial screening showed that most of the studied compounds displayed variable growth inhibitory effects on the tested gram-positive, gram-negative bacterial and pathogenic fungal strains. As expected, we distinguish a vibrant dissimilarity in the activity between derivatives within the entire series, directing to the strengthening and diminishing effects of substitution at C-2 and C-3 of the quinoxaline scaffold. The diameter of (Zone of inhibitions) IZs of the antifungal activity has shown almost the same tendency as the antibacterial activities. Concerning the outcome of substitution at the C-3 position on aryl ring is clear that the change of such a substituent may have a remarkable effect on the antimicrobial activity, which may improve or decreased, depending on the electronic nature on the ring. Individually, aryl ring at C-3 having diverse group, fluorinated compound (4c, 4 h) [67] enhanced the activity because of an electron-withdrawing group and the same trend shown in the compound (4b, 4 n) because
of the CF$_3$ group [66] but directing CH$_3$ and –OCH$_3$ compound (4 m) decreases activity. Similar steric effect, diminished activities at C-2 position, cyclo-pentane series of compound (4x and 4z) presented inferior activities compare to the cyclo-propyl series of compound (4b and 4l). The zone of inhibition (mm) shown in Table 3 and 4 or Figure 3, revealed that compound (4c) could inhibit the growth of the gram-positive bacteria; *B. subtilis* and Gram-negative bacteria; *E. coli in vitro* having a zone of inhibition between 14.28 and 14.89 mm. Among the synthesized derivatives, compound 4c presented moderate activity on *C. albicans* is 11.33 mm. We do not exactly explain the SAR from few compounds but further, modification in structure can enhance the activity and may identify lead molecules from the same scaffolds.

**Molecular modeling study**

The synthesized and screened nine 2,3-diamino quinoxaline derivatives were subjected for physicochemical property estimation to understand

**Table 1.** Compounds of the scheme-1 (1–4 series) ($N^2$-alkyl-$N^3$-phenylquinoxaline-2,3-diamine derivatives): reaction time, yield, and M.P. of each compound.

| Entry | Compound | R$_1$ | R$_2$ | R$_3$ | Time (h) | Yield (%) | M.P. (°C) |
|-------|----------|-------|-------|-------|----------|-----------|-----------|
| 1.    | 4a       | H     | H     | H     | 12 h     | 87.00%    | 245–246   |
| 2.    | 4b       | H     | H     | CF$_3$ | 12 h     | 91.00%    | 237–238   |
| 3.    | 4c       | H     | H     | F     | 8 h      | 86.14%    | 254–256   |
| 4.    | 4d       | H     | H     | Cl    | 8 h      | 85.10%    | 238–240   |
| 5.    | 4e       | H     | H     | CH$_3$| 8 h      | 90.90%    | 245–246   |
| 6.    | 4f       | H     | H     | OCH$_3$ | 8 h   | 89.55%    | 135–136   |
| 7.    | 4g       | H     | H     | Br    | 8 h      | 83.83%    | 242–243   |
| 8.    | 4h       | H     | F     | H     | 6 h      | 89.55%    | 258–261   |
| 9.    | 4i       | H     | Cl    | H     | 6 h      | 86.87%    | 250–251   |
| 10.   | 4j       | H     | Br    | H     | 6 h      | 85.32%    | 246–247   |
| 11.   | 4k       | H     | CH$_3$| H     | 8 h      | 90.15%    | 250–251   |
| 12.   | 4l       | H     | OCH$_3$| H   | 6 h      | 87.45%    | 228–229   |
| 13.   | 4m       | CH$_3$| H     | H     | 12 h     | 45.45%    | 148–149   |
| 14.   | 4n       | H     | CF$_3$| H     | 12 h     | 83.06%    | 232–233   |
| 15.   | 4o       | H     | H     | CN    | 12 h     | 83.94%    | 231–233   |
| 16.   | 4p       | H     | H     | CF$_3$| 12 h     | 76.28%    | 276–278   |
| 17.   | 4q       | H     | H     | Cl    | 8 h      | 86.17%    | 275–277   |
| 18.   | 4r       | H     | H     | OCH$_3$| 8 h   | 92.08%    | 245–246   |
| 19.   | 4s       | H     | H     | CF$_3$| 12 h     | 75.01%    | 276–277   |
| 20.   | 4t       | H     | H     | Cl    | 8 h      | 86.33%    | 280–281   |
| 21.   | 4u       | H     | H     | OCH$_3$| 8 h   | 89.41%    | 248–250   |
| 22.   | 4v       | H     | H     | H     | 12 h     | 89.79%    | 265–267   |
| 23.   | 4w       | H     | H     | Cl    | 12 h     | 89.79%    | 275–276   |
| 24.   | 4x       | H     | H     | CF$_3$| 12 h     | 80.00%    | 276–277   |
| 25.   | 4y       | H     | H     | CH$_3$| 8 h      | 86.38%    | 280–281   |
| 26.   | 4z       | H     | H     | OCH$_3$| 8 h   | 92.59%    | 245–246   |
their drug-likeness. Next, molecular docking was employed for predicting the binding modes, interactions and binding affinities of these compounds.

**Physicochemical property estimation**

The estimated physicochemical properties of top nine compounds are shown in Table-3. Almost all the compounds possess nearly favorable drug-like properties with minor violations (acceptable) for Lipinski’s rule of five. These data of screened hit hints for their possibility as potential drug-like candidates.

**Binding mode analysis from docking studies**

Putative binding modes of both orthosteric or allosteric binders can be accurately predicted using molecular docking approach [70–72]. The molecular docking can also be effectively used for virtual-screening of compounds [73]. Here, we also used molecular docking approach to predict the binding modes of 2,3-diamino quinoxaline derivatives for their antibacterial activity via DNA intercalation. The results of binding mode analysis are summarized in Table-4. We used chloramphenicol as a reference compound to mimic our earlier experimental design. The binding modes of 2,3-diamino quinoxaline derivatives and reference compound corroborate quite well and is nearly comparable with antimicrobial testing data. The posing of our designs at quinolone-binding site of S. aureus DNA gyrase with DNA is shown in Figure 4 (A-H). The consistency in binding poses of our screened designs at quinolone-binding site is evident from the pose analysis as shown in Figure 4 (4). The interaction with Ser-1084 was seen for all the docked designs. Additionally, the intercalation through pi-pi stacking of quinoxaline moiety between DG (G) 9 and DA (H) 13 DNA base pairs were evident (2D ligand interaction maps in Supplementary Figure 4 figure 4). The important role of metal ion (Mg$^{+2}$ or Mn$^{+2}$) in mediating coordinated quinolone-metal-briding is well documented [74]. Similarly in our all docked poses the metal-coordination of pyrazine ring nitrogen with crystal bound Mg$^{+2}$ ion is observed (indicated in SI 2D ligand interaction maps). The Glide docking scores for nine tested designs were ranged from −5.06 to −7.18 kcal/mol. However the prime MM-GBSA scores of the selected poses ranged from 0.57 to −50.26 kcal/mol showing nearly comparable values to the values from antimicrobial testing data.

**Computational modeling**

Initially the physicochemical properties of most promising quinoxaline designs were calculated using Schrödinger [75] QikProp module [76]. Then their binding modes were estimated using GLIDE [76,77] docking module of Schrodinger Suite. The prime MM/GBSA [78,79] GΔ of binding was calculated for evaluating the binding affinities of new designs.

**Generation of 3D molecular structures**

The quinoxaline derivatives were modeled and minimized using Hyperchem7.5 [80] using MM+ force field. The output mol2 files were subjected for ligand preparation at physiological pH of 7.4 with LigPrep module.

**Protein structure selection and preparation**

The Quinoxaline derivatives binds as DNA intercalators [66] thereby shows antibacterial [12–16], activity. Accordingly, S. aureus DNA gyrase crystal structure (PDB ID: 2XCT) [74] complex with ciprofloxacin and DNA was selected for performing molecular docking studies. Fully automated Protein preparation wizard protocol was used to prepare the protein structure at ionization pH of 7.4. The H-bonding network was optimized and restrained minimization was performed using OPLS-2005 force field with the RMSD convergence criteria of 0.30 Å for all heavy atoms.

**Molecular docking study**

The fully prepared protein (containing DNA and ciprofloxacin) and ligand structures were considered for the molecular docking study. The GLIDE
**Figure 2.** Novel designed from the privileged structure (a) Antibacterial activity from DNA intercalators [66] (b) Hydrazine binding with specific amino acid of Dihydrofolate reductase protein. [67].

**Scheme 1.** Reagents and conditions: (a) (COOH)$_2$·2H$_2$O, 4 N HCl, 100°C, 6 h; (b) SOCl$_2$, DCE, Cat. DMF, 100°C, 6 h; (c) Alkyl amine (derivatives), K$_2$CO$_3$, DMF, rt, 12 h; (d) Aniline (derivatives), ethanol, reflux, 12 h.
Table 2. Antimicrobial and antifungal activity, zone of inhibition (mm).

| Comp. No. | S. aureus | B. subtilis | E. coli | K. pneumoniae | S. typhi | C. albicans | A. Niger |
|-----------|-----------|-------------|---------|---------------|---------|-------------|---------|
| 4b        | 10.88     | 10.56       | 10.66   | 10.56         | Nt      | 10.12       | 7.11    |
| 4c        | 10.56     | 14.89       | 14.28   | 10.11         | Nt      | 11.33       | 7.02    |
| 4h        | 11.16     | 14.11       | 11.51   | 10.30         | 12.91   | 7.11         | Nt      |
| 4l        | 9.33      | 10.12       | 10.92   | 11.22         | 7.92    | 8.14         | Nt      |
| 4m        | 7.12      | 9.33        | 9.74    | 7.00          | Nt      | 8.12         | 7.06    |
| 4n        | 12.72     | 12.53       | 10.11   | 9.85          | 12.53   | 7.25         | Nt      |
| 4w        | 9.94      | 10.27       | 9.89    | 8.56          | 11.15   | 8.17         | Nt      |
| 4x        | 9.81      | 10.54       | 9.20    | 7.12          | 9.9     | 7.24         | Nt      |
| 4z        | 7.92      | 8.32        | 7.12    | 7.14          | 7.15    | 7.1          | Nt      |
| Ch.       | 19.45     | 24.55       | 27.36   | 24.53         | 28.32   | NA          | NA      |
| Am.       | NA        | NA          | NA      | NA            | NA      | 18.00        | 22.13   |

Table 3. Physicochemical property estimation (QikProp properties) of new designs.

| Comp. No. | M.Wt | Donor HB | Acceptor HB | PSA | Rule of Five* |
|-----------|------|----------|-------------|-----|---------------|
| 4b        | 344.33 | 10.56    | 10.66       | 40.36 | 1             |
| 4c        | 294.33 | 14.89    | 14.28       | 40.37 | 0             |
| 4h        | 294.33 | 14.11    | 11.51       | 40.38 | 0             |
| 4l        | 306.36 | 10.12    | 10.92       | 48.53 | 0             |
| 4m        | 290.36 | 9.33     | 9.74        | 39.13 | 0             |
| 4n        | 344.33 | 12.53    | 10.11       | 40.43 | 1             |
| 4w        | 338.83 | 10.27    | 9.89        | 38.98 | 1             |
| 4x        | 372.39 | 10.54    | 0           | 38.99 | 1             |
| 4z        | 334.42 | 8.32     | 7.12        | 47.27 | 0             |
| Ch.       | 323.13 | 26.12    | 25.88       | 120.24| 0             |

M.Wt: molecular weight. Donor HB; estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (0.0–6.0). Acceptor HB; estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (2.0–20.0)*; violations of Lipinski’s rule of five. PSA; Van der Waals surface area of polar nitrogen, oxygen and carbonyl carbon atoms. Ch; Chloramphenical.

receptor grid was generated using the centroid of crystal-bound ciprofloxacin molecule within the search space of 14 Å box size for docking ligands 14 Å length at equally spaced 2 Å grids. The standard precision (SP) mode Glide docking was performed with a soft potential function at van der Waals radii scaling of 0.5 with applied core constraints for generating consistent poses of quinoxaline derivatives. The obtained ligand poses were finally selected based on the docking scores and presence of Ser1084 interaction. The images of the ligand poses were generated using Pymol (Schrödinger LLC, 2010) [81–82]. The 2D ligand interaction maps of poses were obtained using ligand-interaction tool from Schrödinger suite.

**Experimental**

**Instruments and apparatus**

General reagents and solvents for the synthesis of compounds were analytical grade (AR) and used without further purification. Air-sensitive reactions were carried out

Table 4. Docking scores, residue contacts and ΔG of binding scores of proposed designs. Ch; Chloramphenicol.

| Comp. No. | Docking scores | Residue contacts | ΔG of binding |
|-----------|----------------|-----------------|---------------|
| 4b        | –6.45          | Ser-1084; DG (G):9; DA (H): 13 | –42.01 |
| 4c        | –6.11          | Ser-1084; DG (G):9; DA (H): 13 | –12.82 |
| 4h        | –6.06          | Ser-1084; DG (G):9; DA (H): 13 | –24.53 |
| 4l        | –6.21          | Ser-1084; DG (G):9; DA (H): 13 | –35.96 |
| 4m        | –5.06          | Ser-1084; DG (G):9 | –17.56 |
| 4n        | –5.93          | Ser-1084; DG (G):9; DA (H): 13 | 0.57 |
| 4w        | –6.55          | Ser-1084; DG (G):9; DA (H): 13; Arg-4548 | –11.74 |
| 4x        | –6.54          | Ser-1084; DG (G):9; DA (H): 13 | –26.27 |
| 4z        | –6.68          | Ser-1084; DG (G):9; DA (H): 13 | –40.71 |
| Ch.       | –7.18          | Ser-1084; DT (E):8; | –50.26 |
under dry nitrogen or argon atmosphere. Thin-layer chromatography was performed on silica gel plates (Merck Silica Gel 60, F254), and the spots were visualized under UV light (254 and 365 nm). All melting points were recorded on open glass capillary tubes using the Stuart Digital Melting Point SMP10 and are uncorrected. \(^1\)H NMR was recorded at 500 MHz (Bruker DPX) frequency and \(^13\)C NMR spectra were recorded at 150.85 MHz (Bruker DPX) in DMSO-\(d_6\) or CDCl\(_3\) solvent using tetramethylsilane (TMS) as the internal standard. Mass spectra were measured with ESI ionization in MSQ LCMS mass spectrometer. Flash column chromatography was carried out using silica gel (Merck, 230–240 mesh) and Key intermediate (3a-d) were eluted in n-pentane/ethyl acetate as a mobile phase and the pure product (4a-z) were purified from recrystallization (diether ether). The coupling constant was recorded in Hz. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, dt = doublet of triplets, t = triplet, q = quartet, m = multiplet.

**Chemistry**

*Synthesis of 1,4-dihydroquinoxaline-2,3-dione (1)*

It was prepared according to previously reported method. [65] The product was recrystallized from ethanol to give compound (1). White solid (13.5g, 90%). m.p. 360–362°C (lit. > 300°C). [65] \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 11.90 (br s, 2H), 7.11 (dd, \(J = 5.0\) Hz, \(10\) Hz, 2H), 7.06 (dd, \(J = 5.0\) Hz, \(10\) Hz, 2H). \(^13\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 155.36, 125.79, 123.18, 115.30

*Synthesis of 2,3-dichloroquinoxaline (2)*

According to reported method. [65] The product was recrystallized from ethanol to give compound (2). White needle (11.0 g, 90%). m.p. 110–115°C (lit. 100–102°C). [65] \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.98 (dd, \(J = 5.0, 5.0\) Hz, 2H), 7.77 (dd, \(J = 10.0, 5.0\) Hz, 2H). \(^13\)C NMR (150.85 MHz, 500 MHz, CDCl\(_3\)): \(\delta\) 145.31, 140.52, 131.18, 128.18

*General procedure for the synthesis of 3-chloro-N-alkylquinoxaline-2-amine (3a-d)*

To a stirred solution of 2,3-dichloroquinoxaline (4.0 g, 20 mmol) in DMF (40 mL). Alkyl amine derivative (a-d), (20 mmol) and anhydrous K\(_2\)
CO$_3$ (40 mmol) were added. The reaction mixture was stirred at room temperature, under nitrogen for 12 h. The completion of the reaction was monitored by TLC and spots were visualized under UV light. The reaction mixture was poured into ice-cold water (400 ml) and stirred for 15 min, then aqueous layer was extracted with ethyl acetate (50 ml x 2). The organic layers were combined, wash with brine solution (50 ml), dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under-reduced pressure which affords to give crude material that was purified by flash chromatography by using silica gel (230–240 mesh) using 15% ethyl acetate in n-pentane to obtain as white solid product (3a-d).

**3-chloro-N-cyclopropylquinoxaline-2-amine (3a).** White solid (3.49 g, 79.25%). m.p. 237–240°C. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 7.74 (dd, $J = 5.0$, 10 Hz, 2H), 7.68 (dd, $J = 5.0$ Hz, 1H), 7.63–7.57 (m, 1H), 7.53 (d, $J = 5.0$ Hz, 1H), 7.43–7.36 (m, 1H), 2.91 (dt, $J = 5.0$ Hz, 1H), 0.77 (td, $J = 5.0$ Hz, 2H), 0.67 (td, $J = 5.0$ Hz, 2H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ 149.58, 141.17, 137.80, 135.90,

**Figure 4.** Docking poses of our 2,3-diamino quinoxaline designs (A-H) at the quinolone-binding site of *S. aureus* DNA gyrase. Consistency in binding interactions with protein (Ser-1084) and DNA base pairs DG (G):9; DA (H): 13 and posing adjacent to Mg$^{2+}$ ion can be seen for all the new designs.
3-chloro-N-isopropylquinoxaline-2-amine (3b). White solid (3.48 g, 78.20%). m.p. 237–240°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 7.76 (dd, \(J = 5.0, 10\) Hz, 1H), 7.68 (dd, \(J = 5.0, 10\) Hz, 1H), 7.57–7.50 (m, 1H), 7.37–7.33 (m, 1H), 5.34 (d, \(J = 5.0\) Hz, 1H), 4.38 (dq, \(J = 5.0\) Hz, 1H), 1.32 (d, \(J = 5.0\) Hz, 6 H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 147.5, 141.0, 137.7, 135.3, 130.1, 127.4, 125.5, 124.3, 42.4, 21.7. MS (ESI) m/z Calcd. for C\(_{11}\)H\(_{10}\)ClN \([\text{M + H}]^+\) 221.69 Found 222.07.

3.2.3.3. 3-chloro-N-cyclobutylquinoxaline-2-amine (3c). White solid (3.95 g, 84.20%). m. p. 237–240°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 7.76 (dd, \(J = 5.0, 10\) Hz, 1H), 7.68 (dd, \(J = 5.0, 10\) Hz, 1H), 7.56–7.53 (m, 1H), 7.37–7.34 (m, 1H), 5.65 (d, \(J = 5.0\) Hz, 1H), 4.69–4.61 (m, 1H), 2.54–2.49 (m, 2H), 2.02–1.94 (m, 2H), 1.83 (dt, \(J = 5.0\) Hz, 2H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 147.4, 140.9, 137.6, 135.5, 130.1, 127.4, 125.5, 124.5, 46.2, 29.9, 14.8. MS (ESI) m/z Calcd. for C\(_{12}\)H\(_{12}\)ClN \([\text{M + H}]^+\) 233.70 Found 234.56.

3.2.3.4. 3-chloro-N-cyclopentylquinoxaline-2-amine (3d). White solid (3.4g, 80.20%). m.p. 237–240°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 7.76 (dd, \(J = 5.0, 10\) Hz, 1H), 7.69 (dd, \(J = 5.0, 10\) Hz, 1H), 7.54 (dd, \(J = 5.0, 10\) Hz, 1H), 7.36–7.33 (m, 1H), 5.48 (d, \(J = 5.0\) Hz, 1H), 4.50–4.43 (m, 1H), 2.18 (td, \(J = 5.0, 10\) Hz, 2H), 1.76 (dd, \(J = 5.0, 10\) Hz, 2H), 1.68 (dd, \(J = 5.0, 10\) Hz, 2H), 1.53 (dd, \(J = 5.0\) Hz, 2H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 148.0, 141.0, 137.7, 135.3, 130.1, 127.4, 125.5, 124.4, 52.6, 31.7, 23.7. MS (ESI) m/z Calcd. for C\(_{13}\)H\(_{14}\)ClN \([\text{M + H}]^+\) 247.73 Found 248.09.

General procedure for the synthesis of compounds (4a-z). A solution of 3-chloro-N-alkylquinoxaline-2-amine (200 mg, 0.91 mmol) and aniline derivatives (0.91 mmol) in ethanol (4 mL) were heated to reflux for 6 h until the reaction completed (monitored by TLC). Then the reaction mixture was cooled, filtered, and the solid was washed with petroleum ether to obtain a solid product (4a-z).

\(N^2\)-cyclopropyl-\(N^3\)-phenylquinoxaline-2,3-diamine (4a). Creamish white solid (0.22 g, 87.69%). m.p. 245–246°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 11.01 (br s, 1H), 10.36 (br s, 1H), 8.06 (d, \(J = 5.0\) Hz, 2H), 7.96 (dd, \(J = 10.0, 5.0\) Hz, 1H), 7.60 (dd, \(J = 10.0, 5.0\) Hz, 1H), 7.42 (dd, \(J = 10.0, 5.0\) Hz, 2H), 7.38 (t, \(J = 5.0\) Hz, 2H), 7.11 (t, \(J = 5.0\) Hz, 1H), 3.07 (s, 1H), 1.05 (d, \(J = 10.0\) Hz, 4H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 143.2, 141.2, 139.3, 133.9, 128.6, 126.0, 125.6, 123.5, 120.8, 24.8, 6.9. MS (ESI) m/z Calcd. for C\(_{17}\)H\(_{16}\)N\(_4\) \([\text{M + H}]^+\) 276.34 Found 277.14.

\(N^2\)-cyclopropyl-\(N^3\)-(4-(trifluoromethyl)phenyl)quinoxaline-2,3-diamine (4b). White solid (0.25 g, 79.87%). m.p. 237–238°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 10.61 (br s, 2H), 8.31 (d, \(J = 5.0\) Hz, 2H), 7.94 (d, \(J = 5.0\) Hz, 1H), 7.74 (d, \(J = 5.0\) Hz, 2H), 7.65 (d, \(J = 5.0\) Hz, 1H), 7.48–7.43 (m, 2H), 3.06 (d, \(J = 5.0\) Hz, 1H), 1.06–10.1 (m, 4H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 143.2, 141.2, 133.5, 126.8, 126.2, 126.0, 125.9, 125.8, 123.2, 123.1, 122.9, 120.3, 24.9, 6.9. MS (ESI) m/z Calcd. for C\(_{18}\)H\(_{15}\)F\(_3\)N\(_4\) \([\text{M + H}]^+\) 344.34 Found 345.14.

\(N^2\)-cyclopropyl-\(N^3\)-(4-fluorophenyl)quinoxaline-2,3-diamine (4c). Yellow solid (0.23 g, 86.14%). m.p. 254–256°C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)): \(\delta\) 11.01 (br s, 1H), 10.45 (br s, 1H), 8.08 (s, 2H), 7.94 (s, 1H), 7.59 (s, 1H), 7.42 (s, 2H), 7.23 (s, 2H), 3.06 (d, \(J = 5.0\) Hz, 1H), 1.06–10.2 (m, 4H). \(^{13}\)C NMR (150.85 MHz, DMSO-\(d_6\)) \(\delta\): 143.3, 133.8, 126.1, 125.6, 122.6, 122.5, 118.5, 115.4, 115.2, 24.8, 6.9. MS (ESI) m/z Calcd. for C\(_{17}\)H\(_{15}\)F\(_3\)N\(_4\) \([\text{M + H}]^+\) 294.33 Found 295.13.
**N²-(4-chlorophenyl)-N³-cyclopropylquinoxaline-2,3-diamine (4d).**
Yellow solid (0.24 g, 85.10%). m.p. 238–240°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.92 (br s, 1H), 10.43 (br s, 1H), 8.13 (d, J = 5.0 Hz, 2H), 7.92 (s, 1H), 7.62 (dd, J = 5.0 Hz, 1H), 7.43 (dd, J = 5.0 Hz, 4H), 3.05 (d, J = 5.0 Hz, 1H), 1.06–1.02 (m, 4H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 143.3, 141.1, 138.5, 136.7, 128.5, 126.9, 126.3, 126.1, 125.8, 122.1, 24.8, 6.9. MS (ESI) m/z Calcd. for C₁₇H₁₅ClN₄ [M + H]⁺ 310.79 Found 311.10

**N²-cyclopropyl-N³-(p-tolyl)quinoxaline-2,3-diamine (4e).**
Yellow solid (0.24 g, 90.90%). m.p. 245–246°C. ¹H NMR (500 MHz, DMSO-d₆): δ 11.06 (br s, 1H), 10.35 (br s, 1H), 7.93 (d, J = 5.0 Hz, 3H), 7.57 (d, J = 5.0 Hz, 1H), 7.41 (s, 2H), 7.18 (d, J = 5.0 Hz, 2H), 3.07 (s, 1H), 2.29 (s, 3H), 1.06–1.02 (m, 4H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 143.2, 141.2, 136.7, 132.6, 129.0, 126.0, 125.8, 125.5, 120.9, 24.7, 20.5, 6.9. MS (ESI) m/z Calcd. for C₁₈H₁₈N₄ [M + H]⁺ 290.37 Found 291.17

**N²-cyclopropyl-N³-(4-methoxyphenyl)quinoxaline-2,3-diamine (4 f).** Brown solid (0.24 g, 89.55%). m.p. 135–136°C. ¹H NMR (500 MHz, DMSO-d₆): δ 8.89 (br s, 1H), 8.79 (br s, 1H), 7.81 (d, J = 10.0 Hz, 2H), 7.51 (d, J = 10.0 Hz, 1H), 7.47 (d, J = 10.0 Hz, 1H), 7.27 (t, J = 5.0 Hz, 2H), 6.97 (d, J = 10.0 Hz, 2H), 3.77 (s, 3H), 3.02 (s, 1H), 0.88 (d, J = 5.0 Hz, 2H), 0.68 (d, J = 5.0 Hz, 2H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 162.1, 155.0, 140.9, 125.2, 124.6, 124.1, 122.1, 113.9, 55.2, 24.3, 6.5. MS (ESI) m/z Calcd. for C₁₈H₁₈N₄O [M + H]⁺ 306.37 Found 307.15

**N²-(4-bromophenyl)-N³-cyclopropylquinoxaline-2,3-diamine (4g).**
Brown solid (0.27 g, 83.83%). m.p. 242–243°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.85 (br s, 1H), 10.36 (br s, 1H), 8.08 (d, J = 10.0 Hz, 2H), 7.92 (s, 1H), 7.63 (s, 1H), 7.58 (d, J = 10.0 Hz, 2H), 7.45 (s, 2H), 3.05 (s, 1H), 1.03 (d, J = 10.0 Hz, 2H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 141.1, 140.2, 138.9, 136.4, 133.8, 131.4, 126.3, 126.0, 125.8, 125.7, 122.6, 122.5, 115, 24.8, 6.9. MS (ESI) m/z Calcd. for C₁₇H₁₅BrN₄ [M + H]⁺ 355.24 Found 355.05

**N²-cyclopropyl-N³-(3-fluorophenyl)quinoxaline-2,3-diamine (4 h).**
Off-white solid (0.24 g, 89.55%). m.p. 258–261°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.85 (br s, 1H), 10.45 (br s, 1H), 8.12 (d, J = 10.0 Hz, 1H), 7.91 (d, J = 5.0 Hz, 1H), 7.87 (d, J = 5.0 Hz, 1H), 7.66 (d, J = 5.0 Hz, 1H), 7.48–7.38 (m, 3H), 6.92 (t, J = 10.0 Hz, 1H), 3.04 (s, 1H), 1.03–1.01 (m, 4H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 163.2, 160.8, 143.2, 141.4, 141.2, 133.6, 130.2, 126.3 (d, C-F), 125.9, 116.3, 109.7 (d), 107.1 (d), 24.8, 6.9. MS (ESI) m/z Calcd. for C₁₇H₁₅FN₄ [M + H]⁺ 294.33 Found 295.15

**N²-(3-chlorophenyl)-N³-cyclopropylquinoxaline-2,3-diamine (4i).**
Off-white solid (0.25 g, 86.87%). m.p. 250–251°C. ¹H NMR (500 MHz, DMSO-d₆): δ 11.09 (br s, 1H), 10.60 (br s, 1H), 8.28 (s, 1H), 8.08 (d, J = 10.0 Hz, 1H), 7.97 (d, J = 5.0 Hz, 1H), 7.62 (d, J = 10.0 Hz, 1H), 7.44 (dd, J = 5.0 Hz, 2H), 7.40 (t, J = 5.0 Hz, 1H), 7.14 (d, J = 5.0 Hz, 1H), 3.07 (d, J = 5.0 Hz, 1H), 1.06–1.02 (m, 4H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 143.2, 141.2, 134.0, 132.9, 130.2, 126.5, 125.8, 125.0, 119.8, 118.9, 118.2, 24.9, 6.9. MS (ESI) m/z Calcd. for C₁₇H₁₅ClN₄ [M + H]⁺ 310.79 Found 311.10

**N²-(3-bromophenyl)-N³-cyclopropylquinoxaline-2,3-diamine (4j).**
Yellow solid (0.27 g, 85.32%). m.p. 246–247°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.94 (br s, 1H), 10.49 (br s, 1H), 8.40 (d, J = 5.0 Hz, 1H), 8.11 (d, J = 10.0 Hz, 1H), 7.94 (d, J = 5.0 Hz, 1H), 7.61 (dd, J = 5.0 Hz, 1H), 7.45 (t, J = 5.0 Hz, 2H), 7.34 (t, J = 5.0 Hz, 1H), 7.28 (d, J = 5.0 Hz, 1H), 3.05 (d, J = 5.0 Hz, 1H), 1.03 (t, J = 5.0 Hz, 4H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 143.3, 141.2, 130.6, 126.5, 126.1, 125.8, 122.8, 122.3, 119.2, 24.8, 6.9. MS (ESI) m/z Calcd. for C₁₇H₁₅BrN₄ [M + H]⁺ 355.24 Found 355.05
**N²-cyclopropyl-N³-(m-tolyl)quinoxaline-2,3-diamine (4k).** Yellow solid (0.24 g, 90.15%). m.p. 250–251°C. ¹H NMR (500 MHz, DMSO-d₆): δ 11.06 (br s, 1H), 10.28 (br s, 1H), 7.95 (d, J = 5.0 Hz, 1H), 7.89–7.86 (m, 2 H), 7.60 (dd, J = 10.0 Hz, 1H), 7.42 (dd, J = 5.0 Hz, 2 H), 7.26 (t, J = 10.0 Hz, 1H), 6.93 (d, J = 10.0 Hz, 1H), 3.06 (d, J = 5.0 Hz, 1H), 2.33 (s, 3 H), 1.05–1.02 (m, 2 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 143.1, 141.3, 139.2, 137.8, 133.8, 128.5, 126.1, 125.6, 124.3, 121.3, 118.1, 24.8, 21.3, 6.9. MS (ESI) m/z Calcd. for C₁₈H₁₈N₄ [M + H]⁺ 290.37 Found 291.15

**N²-cyclopropyl-N³-(3-methoxyphenyl)quinoxaline-2,3-diamine (4 l).** Yellow solid (0.24 g, 87.45%). m.p. 228–229°C. ¹H NMR (500 MHz, DMSO-d₆): δ 11.11 (br s, 1H), 10.38 (br s, 1H), 7.97 (s, 1H), 7.85 (s, 1H), 7.66 (d, J = 5.0 Hz, 1H), 7.61 (dt, J = 5.0 Hz, 1H), 7.43 (dd, J = 5.0 Hz, 2 H), 7.27 (t, J = 5.0 Hz, 1H), 6.69 (dd, J = 5.0 Hz, 1H), 3.77 (s, 3 H), 3.06 (t, J = 5.0 Hz, 1H), 1.05 (d, J = 5.0 Hz, 4 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 159.3, 143.1, 141.2, 140.5, 133.7, 129.3, 126.2, 125.7, 113.0, 109.0, 106.5, 55.0, 24.9, 7.0. MS (ESI) m/z Calcd. for C₁₈H₁₈N₄O [M + H]⁺ 306.37 Found 307.15

**N²-cyclopropyl-N³-(o-tolyl)quinoxaline-2,3-diamine (4 m).** Brown solid (0.12 g, 45.45%). m.p. 148–149°C. ¹H NMR (500 MHz, DMSO-d₆): δ 8.15 (s, 1H), 7.50 (dd, J = 5.0 Hz, 1H), 7.35 (dd, J = 5.0 Hz, 1H), 7.30–7.26 (m, 3 H), 7.26–7.20 (m, 2 H), 7.14 (td, J = 5.0 Hz, 20.0 Hz, 2 H), 3.02 (dd, J = 5.0 Hz, 1H), 2.15 (s, 3 H), 0.83 (dt, J = 5.0 Hz, 2 H), 0.61–0.58 (m, 2 H). MS (ESI) m/z Calcd. for C₁₈H₁₈N₄ [M + H]⁺ 290.37 Found 291.05

**N²-cyclopropyl-N³-(3-trifluormethylphenyl)quinoxaline-2,3-diamine (4 n).** Off white solid (0.26 g, 83.06%). m.p. 232–233°C. ¹H NMR (500 MHz, DMSO-d₆): δ 11.10 (br s, 1H), 10.79 (br s, 1H), 8.60 (d, J = 5.0 Hz, 1H), 8.38 (d, J = 10.0 Hz, 1H), 7.98 (d, J = 5.0 Hz, 1H), 7.60 (dd, J = 10 Hz, 5.0 Hz, 2 H), 7.50–7.35 (m, 3 H), 3.08 (s, 1H), 1.06–10.1 (m, 4 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 143.2, 129.8, 129.5, 127.0 (q, CF₃), 126.6, 126.3, 125.8, 125.6, 123.9, 119.5, 116.5, 24.9, 6.9. MS (ESI) m/z Calcd. for C₁₈H₁₅F₃N₄ [M + H]⁺ 344.34 Found 345.15

**3.2.4.15. 4-((3-(cyclopropylamino)quinoxalin-2-yl)amino)benzonitrile (4o).** White solid (0.23 g, 83.94%). m.p. 231–233°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.64 (br s, 2 H), 8.57 (br s, 1H), 8.36 (d, J = 10.0 Hz, 1H), 7.94 (d, J = 5.0 Hz, 1H), 7.63–7.59 (m, 2 H), 7.44 (dd, J = 10 Hz, 5.0 Hz, 3 H), 3.07 (d, J = 5.0 Hz, 1H), 1.03 (t, J = 10 Hz, 4 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 143.4, 141.2, 140.4, 133.6, 129.8, 126.5, 126.1, 125.8, 125.1, 123.9, 119.4, 116.5, 24.8, 6.9. MS (ESI) m/z Calcd. for C₁₈H₁₅N₅ [M + H]⁺ 301.35 Found 302.13

**N²-isopropyl-N³-(4-(trifluormethyl)phenyl)quinoxaline-2,3-diamine (4p).** White solid (0.24 g, 76.28%). m.p. 276–278°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.56 (br s, 2 H), 8.27 (d, J = 5.0 Hz, 2 H), 7.84 (s, 1H), 7.73 (d, J = 5.0 Hz, 2 H), 7.60 (d, J = 10.0 Hz, 1H), 7.43–7.37 (m, 2 H), 4.54 (s, 1H), 1.41 (d, J = 6.0 Hz, 6 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 143.3, 140.9, 133.1, 126.5, 125.8, 125.7, 125.4, 124.5 (q, CF₃), 123.6, 120.5, 44.9, 21.5. MS (ESI) m/z Calcd. for C₁₈H₁₇F₃N₄ [M + H]⁺ 346.36 Found 347.14

**N²-(4-chlorophenyl)-N³-isopropylquinoxaline-2,3-diamine (4q).** Yellow solid (0.27 g, 86.17%). m.p. 275–277°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.43 (br s, 2 H), 8.07 (d, J = 5.0 Hz, 2 H), 7.83 (d, J = 5.0 Hz, 1H), 7.55 (d, J = 5.0 Hz, 1H), 7.44 (d, J = 10.0 Hz, 2 H), 7.40–7.34 (m, 2 H), 4.53 (s, 1H), 1.41 (d, J = 5.0 Hz, 6 H). ¹³C NMR (150.85 MHz, DMSO-d₆): δ: 141.0, 138.5, 128.5, 127.0, 125.6, 125.4, 122.5, 45.1, 21.6. MS (ESI) m/z Calcd. for C₁₇H₁₇ClN₄ [M + H]⁺ 312.80 Found 313.13

**N²-isopropyl-N³-(4-methoxyphenyl)quinoxaline-2,3-diamine (4 r).** Brown solid (0.25 g, 92.08%). m.p. 245–246°C. ¹H NMR (500 MHz,
DMSO-$d_6$: δ 10.37 (br s, 2 H), 7.83 (d, J = 10.0 Hz, 3 H), 7.51 (dd, J = 5.0 Hz, 1H), 7.34 (t, J = 5.0 Hz, 2 H), 6.99 (d, J = 5.0 Hz, 2 H), 4.53 (s, 1H), 3.77 (s, 3 H), 1.40 (d, J = 5.0 Hz, 6 H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ: 156.0, 141.2, 141.1, 140.2, 140.1, 134.9, 125.8, 125.6, 123.2, 114.0, 55.3 (OCH$_3$), 47.4, 28.9, 15.2. MS (ESI) m/z Calcd. for C$_{19}$H$_{20}$N$_4$O [M + H]$^+$ 320.40 Found 321.14

$N^2$-cyclopentyl-$N^3$-(4-(trifluoromethyl)phenyl)quinoxaline-2,3-diamine (4s). Yellow solid (0.22 g, 89.79%). m.p. 265–267°C. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 10.46 (br s, 2 H), 8.00 (d, J = 10.0 Hz, 2 H), 7.88 (s, 1H), 7.57–7.48 (m, 1H), 7.45–7.30 (m, 4 H), 7.12 (t, J = 5.0 Hz, 1H), 4.58 (dt, J = 5.0 Hz, 1H), 2.13 (td, J = 5.0 Hz, 2 H), 1.93 (d, J = 5.0 Hz, 2 H), 1.87–1.75 (m, 2 H), 1.70–1.55 (m, 2 H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ: 141.2, 140.9, 139.2, 133.1, 128.6, 125.9, 125.8, 125.2, 123.6, 121.3, 54.4, 31.4, 23.9. MS (ESI) m/z Calcd. for C$_{19}$H$_{20}$F$_4$N$_4$ [M + H]$^+$ 304.40 Found 305.19

$N^2$-cyclobutyl-$N^3$-(4-(trifluoromethyl)phenyl)quinoxaline-2,3-diamine (4t). Ellow solid (0.23 g, 86.33%). m.p. 280–281°C. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 10.14 (br s, 2 H), 8.10 (d, J = 5.0 Hz, 2 H), 7.82 (d, J = 5.0 Hz, 1H), 7.57–7.55 (m, 1H), 7.45–7.41 (m, 2 H), 7.40–7.35 (m, 2 H), 4.75–4.63 (m, 1H), 2.47–2.32 (m, 4 H), 1.90–1.75 (m, 2 H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ: 123.6, 126.6, 126.5, 125.9, 125.8 (s), 125.6 (d), 123.2, 120.3, 47.3, 29.0, 15.2. MS (ESI) m/z Calcd. for C$_{19}$H$_{17}$F$_3$N$_4$ [M + H]$^+$ 358.37 Found 359.14

$N^2$-(3-chlorophenyl)-$N^3$-cyclobutylquinoxaline-2,3-diamine (4u). Yellow solid (0.24 g, 87.59%). m.p. 275–276°C. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 10.52 (br s, 2 H), 8.08 (d, J = 10.0 Hz, 2 H), 7.85 (s, 1H), 7.58–7.52 (m, 1H), 7.45–7.33 (m, 4 H), 4.61–4.52 (m, 1H), 2.11 (dt, J = 5.0 Hz, 2 H), 1.92 (s, 2 H), 1.83 (dd, J = 5.0 Hz, 2 H), 1.63 (dd, J = 5.0 Hz, 2 H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ: 141.1, 138.5, 138.4, 134.3, 133.2, 128.5, 127.0, 126.1, 125.7, 122.6, 54.3, 31.4, 23.9. MS (ESI) m/z Calcd. for C$_{19}$H$_{17}$ClN$_4$ [M + H]$^+$ 338.84 Found 339.13

$N^2$-cyclobutyl-$N^3$-(4-methoxyphenyl)quinoxaline-2,3-diamine (4v). Yellow solid (0.24 g, 89.41%). m.p. 248–250°C. $^1$H NMR (500 MHz, DMSO-$d_6$): δ 10.44 (br s, 2 H), 8.10 (d, J = 10.0 Hz, 2 H), 7.82 (d, J = 5.0 Hz, 1H), 7.51 (dd, J = 5.0 Hz, 1H), 7.34 (dd, J = 5.0 Hz, 2 H), 6.99 (d, J = 10.0 Hz, 2 H), 4.74–4.65 (m, 1H), 3.77 (s, 3 H), 2.48–2.45 (m, 4 H), 1.90–1.75 (m, 2 H). $^{13}$C NMR (150.85 MHz, DMSO-$d_6$): δ: 156.0, 141.2, 141.1, 140.2, 140.1, 134.9, 125.8, 125.6, 123.2, 114.0, 55.3 (OCH$_3$), 47.4, 28.9, 15.2. MS (ESI) m/z Calcd. for C$_{19}$H$_{20}$O$_4$N$_4$ [M + H]$^+$ 372.40 Found 373.16
**N²-cyclopentyl-N³-(p-tolyl)quinoxaline-2,3-diamine (4y).** Yellow solid (0.22 g, 86.38%). m.p. 280–281°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.43 (br s, 2 H), 7.85 (d, J = 5.0 Hz, 3 H), 7.55–7.48 (m, 1H), 7.40–7.32 (m, 2 H), 7.20 (d, J = 10.0 Hz, 2 H), 4.58 (s, 1H), 2.30 (s, 3 H), 2.12 (td, J = 5.0 Hz, 2 H), 1.91 (d, J = 5.0 Hz, 2 H), 1.86–1.82 (m, 2 H), 1.66–1.59 (m, 2 H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 141.2, 136.5, 132.9, 129.0, 125.7, 125.0, 121.5, 54.3, 31.4, 23.9 (CH₃), 20.5. MS (ESI) m/z Calcd. for C₂₀H₂₂FN₄ [M + H]^+ 318.42 Found 319.18

**N²-cyclopentyl-N³-(4-methoxyphenyl)quinoxaline-2,3-diamine (4z).** Yellow solid (0.25 g, 92.59%). m.p. 245–246°C. ¹H NMR (500 MHz, DMSO-d₆): δ 10.54 (br s, 2 H), 8.25–7.70 (m, 3 H), 7.53 (dd, J = 5.0 Hz, 1 H), 7.40–7.30 (m, 2 H), 7.00 (d, J = 10.0 Hz, 2 H), 4.61 (s, 1 H), 3.79 (s, 3 H), 2.14 (td, J = 10.0 Hz, 2 H), 1.94 (d, J = 5.0 Hz, 2 H), 1.88–1.81 (m, 2 H), 1.69–1.60 (m, 2 H). ¹³C NMR (150.85 MHz, DMSO-d₆) δ: 158.4, 156.0, 141.2, 140.8, 125.7, 125.6, 123.4, 123.3, 116.1, 113.9, 55.3 (OCH₃), 54.4, 31.5, 23.9. MS (ESI) m/z Calcd. for C₂₀H₂₂N₄O [M + H]^+ 334.42 Found 335.18

**Conclusions**

The aim of the work to design and synthesize novel diamino-quinoxaline scaffolds and products were screened against bacterial and fungal strains. Quinoxaline scaffold presented the preliminary significant antimicrobial activity against a panel of pathogenic microbes including two Gram-positive bacteria (S. aureus and B. subtilis), three Gram-negative bacteria (E. coli, K. pneumoniae and Salmonella typhi), and two fungal strains (C. albicans and A. niger). From the quantitative analysis, the compound (4c) presented a broad-spectrum (Zone of inhibitions) on all the strains between 10.11 and 14.89 mm. Our docking-based binding mode analysis results are in accordance with the antibacterial testing data. In summary, docking predicts our diamino-quinoxaline analogues as DNA intercalators where they bind at microbial DNA gyrase quinolone-binding site with critical Mg²⁺ ion metal-coordination, thereby showing their antibacterial activity.

**Acknowledgments**

The authors extend their appreciation to the Department of Chemistry, Bhupal Nobles’ University, Udaipur, Rajasthan, India, for providing technical support in this project (materials used for experiments).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

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S.K.S. designed the research and performed the experimental works; Y.K.J. wrote the manuscript; J.M.P. wrote the biological part, P.V.P. done molecular modeling study; N.S.C. and G.P.S. revised the manuscript.

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