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Design, synthesis, antimicrobial activity and molecular docking studies of some novel di-substituted sulfonylquinoxaline derivatives

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ABSTRACT

2,3-Dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride **1** was prepared via reaction of o-phenylene diamine with oxalic acid followed by chlorosulfonation with excess chlorosulfonic acid. A series of new sulfonylquinoxaline derivatives **2–6** were obtained upon reacting compound **1** with different types of amines. 2,3-Dichloro-6-morpholinosulfonylquinoxaline derivative **6** was subjected to further chemical reactions to afford many derivatives of 6-morpholino 2,3-disubstitutedquinoxalines, thus reaction of compound **6** with different secondary amines yielded mono and di secondary aminoquinoxaline derivatives **7–10** depending on the reactivity difference of the two chlorine atoms. Hydrazinolysis of compound **7** furnished hydrazino quinoxaline derivatives **11a-c**. Additionally triazolo and pyrazolyl quinoxaline derivatives **12–14** were obtained through the reaction of compound **11a** with phenyl isothiocyanate, formylpyrazole and ethyl acetoacetate. All the synthesized compounds were screened for their antibacterial and antifungal activities. Compounds **7a, 9b, 10a, 10c, 10f** and **11c** showed good to moderate antimicrobial activity against the tested Gram-positive, Gram-negative bacteria and fungi with MIC values ranging from 2.44 to 180.14 μM. Their MBC values were also evaluated using the same tested microorganisms. Moreover, screening against multi-drug resistant strains revealed the promiscuity of these new derivatives, especially compound **7a** that showed comparable antibacterial activity (MIC 4.91–9.82 μM) with Norfloxacin (MIC 2.44–9.80 μM). Furthermore, these compounds were evaluated as DNA Gyrase inhibitors and the obtained results were in the range of 15.69–23.72 μM. Immunomodulatory effect was also investigated and compounds **7a, 11c, 10f, 10c, 10a and 9b** showed high immunostimulatory action with ratio (142.6 ± 0.4, 135.7 ± 0.5, 117.8 ± 0. 39, 112.5 ± 0. 83, 86.4 ± 0. 47, 72.8 ± 0. 77) respectively. Molecular docking studies of the promising derivatives into DNA Gyrase binding site proved the usefulness of hybridizing quinoxaline scaffold with SO_2 and morpholine moieties as a hopeful strategy in designing new DNA Gyrase binding molecules.

1. Introduction

The upsurge and widespread of multi-drug resistant microorganisms such as *S. epidermidis* [1,2], *S. aureus* [3,4], *P. aeruginosa* [5,6], *E. coli* [7,8], *E. faecium* [9,10], *S. apiospernum* [11] had been reported as a major threats to human health. Abscess of the brain is a dreadful complication of *E. coli* infection [12]. Amoxicillin, Norfloxacin and Ciprofloxacin are the most common drugs used for the treatment of *E. coli* infections [13] but they are still associated with several side effects. Toxicity and drug resistance are important factors that can lead to treatment failure [14]. Therefore, there is an urgent need for the development of new antibacterial agents to fight against microbial infections. The demand for novel chemotherapeutic antibacterial agents represent an attractive strategy in the field of medicinal chemistry. After many years of extensive studies on structural modification of known antibacterial scaffolds, it is still difficult to deliver new leads, therefore, the focus of such antibacterial research has moved to the identification of novel chemical classes to target invading bacteria [15]. The study of quinoxaline derivatives has become of much interest in recent years on account of their antibacterial, antiviral, anti-cancer, anti-fungal, anti-helminthic and insecticidal [16–18] activities. The quinoxaline ring has frequently been used as a component of various

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antibiotic molecules, such as echinomycin, levomycin, and actindeutin, which inhibit the growth of Gram-positive bacteria and are active against various transplantable tumors. Triostin C (**I**) is an antibiotic that has quinoxaline core and exhibited activity against gram positive bacteria, its biological activity has been attributed to binding with the DNA of susceptible cells through bifunctional intercalation of the quinoxaline moiety [19–22]. Furthermore, Sanna and co-workers reported that quinoxaline derivatives bearing electron-withdrawing groups at the 6- or 7-positions have antibacterial, antifungal, and anticancer activities [23–26]. Hence, lipophilicity and the electronic properties of the substituents affect the biological activities of these compounds. The discovery of sulfonamides as antibacterial agents was one of the most fascinating area of chemotherapeutic agents and they were used successfully in the treatment of a variety of bacterial infections. The sulfonamide group is considered as a pharmacophore which is present in several biologically active molecules, in particular antimicrobial agents, for example, sulfacetamide (**II**) [27–29].

Also, numerous sulfonamide derivatives have been reported as carbonic anhydrase inhibitors [30], anticancer [31–34], and anti-inflammatory agents [35]. Furthermore, recent reports suggested that groups like morpholine, piperidine and piperazine can help in improving pharmacological properties. Linezolid (**III**) is a synthetic antibiotic used for the treatment of infections caused by multi-resistant bacteria as *streptococcus* and methicillin-resistant *S. aureus* (MRSA), by inhibiting bacterial protein synthesis [36–40]. In a continuation of our endeavors towards the development of potent and effective antimicrobial agents [41–44] and especially anti-bacterial agents derived from quinoxalines [45–49], this investigation deals with the rational design of new molecular hybrids as potential antimicrobial agents. These hybrids were designed to incorporate 6-morpholinosulfonylquinoxaline scaffold linked to various bioactive heterocyclic moieties at position-2, 3 through different atom spacers, as shown in Fig. 1, the reference drugs Triostin C (**I**), Sulfacetamide (**II**) and Linezolid (**III**) were lunched through fragment based drug design to help us in the design strategy of the new target compounds. The new compounds were evaluated for their antibacterial activity to investigate the effect of such structural modification on the biological effect.

2. Results and discussion:

2.1. Chemistry

The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in schemes 1-3. At first, *o*-phenylenediamine was treated with oxalic acid in the presence of 4 N HCl to afford 2,3-(1*H*,4*H*)-quinoxalinedione [25], subsequent treatment with chlorosulfonic acid to produce 2,3-quinoxalinedione-6-sulfonyl chloride (**1**) [50]. Interaction of the sulfonyl chloride derivative **1** with *m*-anisidine as a primary aromatic amine led to the formation of *N*-(3-methoxyphenyl)-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonamide (**2**). Its IR spectrum showed absorption bands at υ 3228 and 1693 and 1388, 1145 cm^{-1} representing NH, C=O and SO_2 groups respectively. The ^{1}H NMR spectrum showed one singlet signal at δ 3.65 ppm corresponding to methoxy group together with the aromatic protons as multiplet at δ 6.56–7.93 ppm and three exchangeable signals at δ 10.31, 12.06 and 12.12 ppm due to 3 NH protons. Its ^{13}C NMR spectrum showed signals at δ 55.44 for methoxy group of *m*-anisidine and two singlet signals at δ 155.35 and 160.14 ppm for carbonyl and the carbon attached to methoxy group in addition to the aromatic carbons between δ 105.88–139.29 ppm. The mass spectral data showed molecular ion peak at m/z = 347 (19%) which agreed with the molecular formula ($C_{15}H_{13}N_3O_5S$) while its base peak was observed at m/z = 116.

Similarly, interaction of quinoxaline derivative **1** with some selected bioactive heterocyclic secondary amines furnished the corresponding sulfonamide derivatives **3–5**. Structures of these compounds were elucidated by elemental analysis and spectroscopic data. IR spectrum of compound **3** showed absorption bands at υ 3497, 2260, 1673 cm^{-1} related to NH, C≡N and C=O groups respectively. ^{1}H NMR spectrum revealed the appearance of three singlet signals at δ 4.42, 11.92 and 11.94 ppm corresponding for CH_2 and two NH protons in addition to the aromatic protons which appeared in the region of 7.02–7.56 ppm. ^{13}C NMR spectrum showed signals at δ 15.45 for methylene group and three singlet signals at δ 143.99, 155.63 and 155.71 ppm corresponding to carbonyl and -C=N groups as well as the aromatic carbons. Its mass spectral data showed molecular ion peak at m/z = 381 (5%) which agreed with the molecular mass of the compound ($C_{17}H_{11}N_5O_4S$) as well as base peak was observed at m/z = 75. Also, compound **1** was treated with theophylline in dimethylformamide under reflux to obtain compound **4**. The IR spectrum of compound **4** displayed the presence of absorption bands at υ 3250, 1707&1662 cm^{-1} assignable to NH & 2 C=O groups, respectively. ^{1}H NMR spectrum displayed two singlet signals at δ 3.22 and 3.43 ppm corresponding to two methyl protons that attached to nitrogen of pyrimidine nucleus, multiplet signals at δ 7.02–7.42 ppm related to the four aromatic protons and another one singlet signal at δ 9.96 ppm due to NH proton. ^{13}C NMR spectrum showed signals at δ 34.85 for two methyl groups and two singlet signals at δ 155.71 and 155.78 ppm for carbonyl groups in addition to the aromatic carbons between 113.21 and 143.70 ppm. Mass spectrum displayed prominent molecular ion peak at m/z = 404 (16%) and base peak at m/z = 180.

The starting material 6-morpholinosulfonyl-2,3-dichloroquinoxaline (**6**)[51] was prepared in good yield through chlorination of compound **5c** with phosphorus oxychloride. The reactivity of dichloroquinoxaline derivative **6** towards some nitrogenous compounds as mono nucleophile was discussed. Thus, the interaction of dichloro derivative **6** with one mole of piperidine as a cyclic secondary amine in acetonitrile furnished a sole product which was formulated as 2-or 3-piperidino 6-morpholinosulfonylquinoxaline (**7**) or (**8**) respectively. According to the effect of the sulfonyl group, the authors favor isomer **7** due to the 2-position is presumed to be preferentially substituted due to the (-M) effect of the sulfonyl group in structure **7**. This means that the 2-carbon will be more susceptible to nucleophilic attack and the reaction proceed according to nucleophilic substitution through addition elimination mechanism. The supporting evidence of compound **7a** was confirmed by microanalyses and spectral data. The ^{1}H NMR spectra indicated the presence of one multiplet and three triplets at δ 1.68, 2.91, 3.58, 3.62 ppm corresponding for piperidinyl and morpholinyl protons besides to the aromatic protons. ^{13}C NMR spectra showed signals at δ 24.14, 25.63, 46.44, 50.04 and 65.73 ppm for the piperidinyl and morpholinyl carbons while the aromatic carbons of quinoxaline moiety were observed from δ 128.09 to 154.04 ppm.

The interaction of the dichloro derivative **6** with a cyclic secondary amines such as *N*-methylpiperazine and morpholine consumed one mole and produced a single product in each case, which was named as 2-*N*-methylpiprazinyl and 2-morpholinyl derivatives **7b,c** respectively (Scheme 2), based on elemental analyses and spectral data in experimental section. On the other hand, the interaction of compound **6** with two moles of the cyclic secondary amines yielded symmetrical 2,3-disubstitituted quinoxaline-6-morpholino-sulfonyl derivatives **9a-c**. The structure of compound **9b** was proven by IR analysis, which displayed absorption bands at υ 1603, 1354, 1156 cm^{-1} for C=N, SO_2 groups. ^{1}H NMR spectrum demonstrated new signals for two *N*-methyl piperazine moiety as well as morpholine protons at δ 2.22–3.62 ppm in addition to signals due to aromatic protons between δ 7.60 and 7.86 ppm that appeared as two doublet and one singlet signals. Moreover, ^{13}C NMR data showed signals at δ 44.75 ppm for two methyl carbons, δ 46.46, 46.87, 54.64 ppm for two piperazine rings, in addition to morpholine carbons at δ 46.15, 65.75 ppm and aromatic carbons in the range of δ 124.11–140.17 ppm and two signals at 148.81, 149.30 for two C=N. The structure of compound **9** was also, confirmed via the reaction of compound **7** with another mole of the same secondary cyclic amines.

In order to obtain different cyclic secondary amines in the 2-and 3-

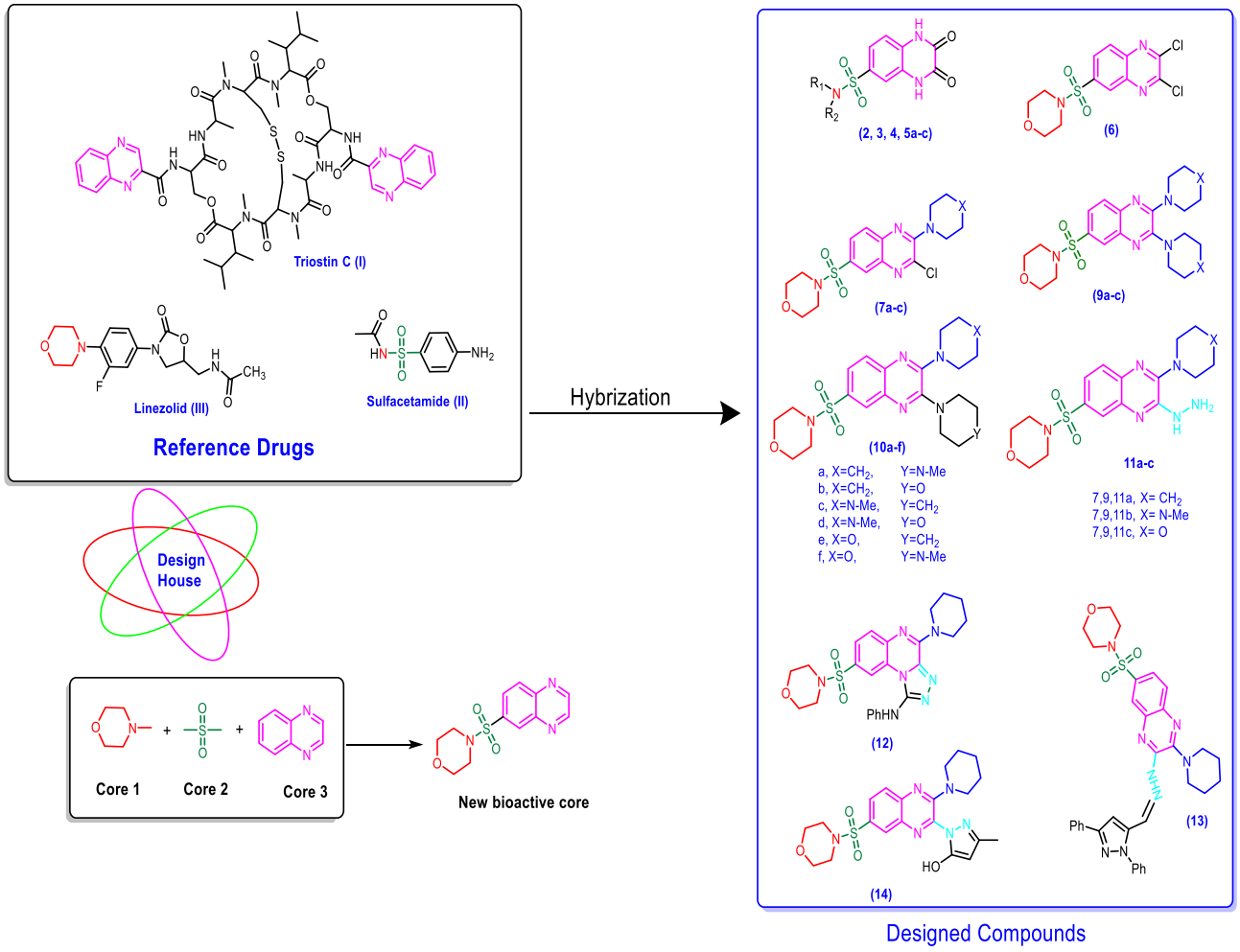
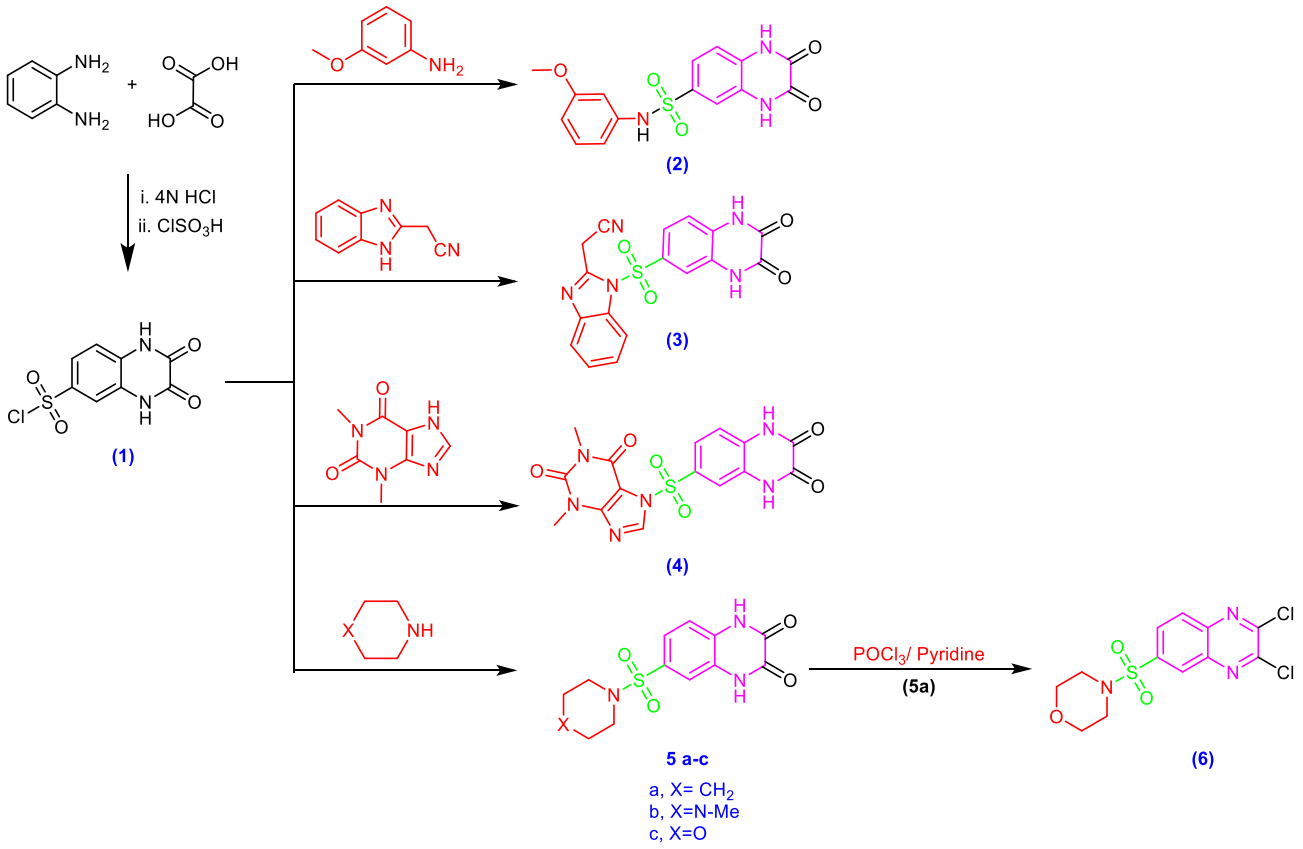


Fig. 1. Design strategy of the new quinoxaline derivatives based on the reference drugs (Triostin C (**I**), Sulfacetamide (**II**) and Linezolid (**III**) through fragment based drug design.

positions of quinoxaline to study the effect of type and position on the biological activity, compounds **7a-c** were reacted with another cyclic secondary amines where unsymmetrical 2,3-diamines were obtained. The structure elucidation of compounds **10a-f** were performed from elemental analyses and their spectroscopic data. IR spectra of compound **10f** showed absorption peaks at υ 1599, 1350, 1152 cm^{-1} for C=N & SO_2 moieties. ^1H NMR spectrum displayed singlet signal at δ 2.24 ppm for methyl protons, triplet signals at δ 2.51, 3.56 ppm for piperazine ring protons and triplet signals at δ 2.93, 3.47, 3.64 and 3.78 ppm for two morpholinyl moieties. Furthermore, ^{13}C NMR analysis demonstrated signals at δ 46.13 ppm for methyl carbon, δ 46.92, 54.54 ppm for piperazine moiety and δ 46.46, 47.49, 65.75 and 66.12 ppm for both morpholinyl carbons. Hydrazine hydrate was reacted with **7a-c** under reflux condition in acetonitrile and underwent hydrazinolysis to afford the corresponding 3-hydrazino quinoxaline derivatives **11a-c**. IR spectra of compound **11a** as an example, which displayed absorption bands at υ 3317, 3201 cm^{-1} for (NH_2, NH), υ 1608, 1346 and 1161 cm^{-1} due to C=N beside SO_2 respectively.^1H NMR data showed two new singlet signals exchangeable with D_2O at δ 4.63, 8.34 ppm related to NH_2 and NH protons respectively, in addition to signals due to morpholine, piperidine and aromatic protons. Moreover, ^{13}C NMR spectra demonstrated signals at δ 24.93, 26.32 & 48.15 ppm for piperidine carbons, δ 46.42, 65.77 ppm for morpholine carbons in addition to, aromatic carbons in the range of δ 111.80–137.45 ppm and 152.71 ppm for the C=N group.

Hydrazine derivatives are versatile reagents and have been extensively used as synthetic starting materials for the synthesis of several heterocyclic compounds of potential biological activity [52]. Thus, it was of our interest to study the reactivity of hydrazine derivative **11a** towards a variety of chemical reagents. Refluxing compound **11a** with phenyl isothiocyanate caused cyclization and the obtained product was 8-(morpholinosulfonyl)-*N*-phenyl-4-(piperidin-1-yl)-[1,2,4]triazolo [4,3-*a*]quinoxalin-1-amine (**12**). The structure of the prepared compounds was elucidated based on elemental analysis and spectral data. IR displayed the absence of NH_2 absorption band and the presence of absorption peaks at υ 3248, 1612 & 1374, 1172 cm^{-1} for NH, C=N & SO_2 groups. ^1H NMR data displayed signals at δ 1.18–1.22 ppm (as multiplet), 3.07 ppm (as a triplet) for piperidine protons, δ 3.02 and 3.56 ppm as triplet signals due to morpholine protons. In addition to aromatic protons in the range of δ 6.90–7.61 ppm and an exchangeable signal at δ 10.70 ppm due to NH proton. Furthermore, condensation of hydrazine quinoxaline derivative **11a** with 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde produced Schiff's base derivative **13**. IR spectrum of compound **13** displayed the presence of an absorption peaks at ν 3417, 1597 & 1350, 1165 cm^{-1} for NH, C=N, SO_2 groups. ^1H NMR analysis demonstrated a new singlet signal for pyrazole proton at δ 8.26 ppm and an exchangeable singlet signal at δ 9.04 ppm due to NH proton together with signals due to morpholine, piperidine and aromatic protons. The mass spectra displayed a molecular ion peak at m/z = 622 (21%) corresponding to a molecular formula ($C_{33}H_{34}N_8O_3S$).

Finally, the interaction of hydrazine derivative **11a** with ethyl acetoacetate afforded the pyrazolone derivative **14** through cyclization, which was found in tautomer with its hydroxyl derivative (Scheme 3). The IR spectrum demonstrated absorption bands at ν 3414, 1616 and



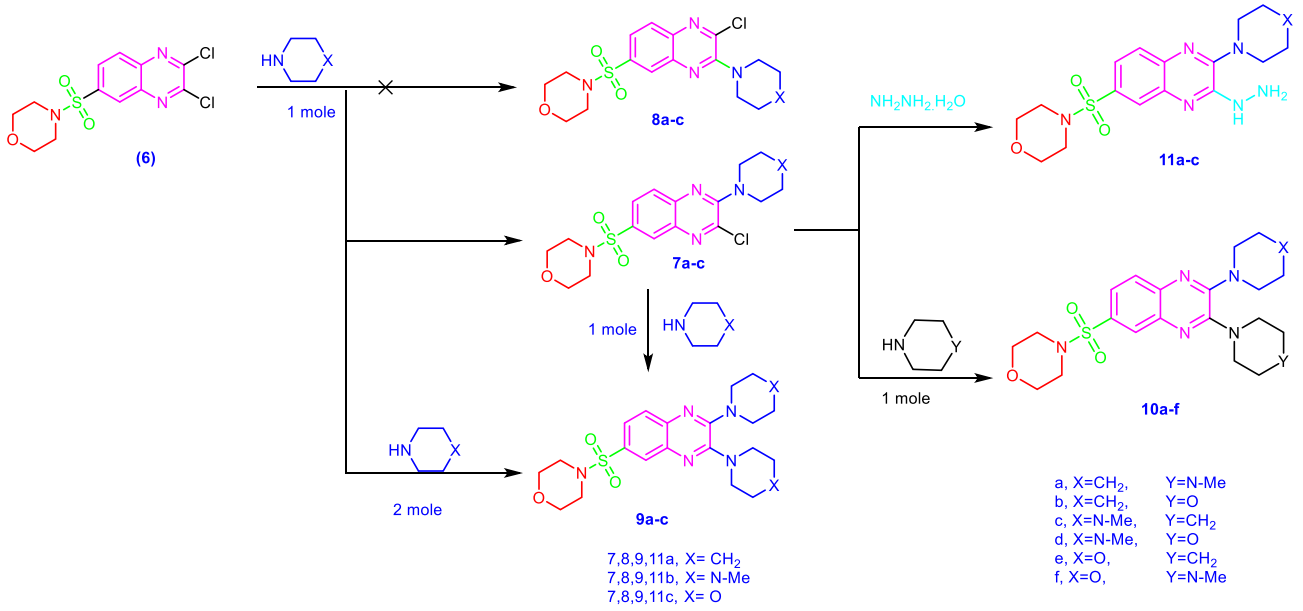
Scheme 1. Synthesis of new sulfonylquinoxaline derivatives **2–6**.

1342, 1156 for OH, C=N and SO_2 groups. Also, ^{1}H NMR spectra revealed a new exchangeable singlet signal at δ 12.41 ppm due to OH proton as well as singlet signal at δ 3.03 ppm for methyl group in pyrazole core. Its mass spectral data showed a molecular ion peak at m/z = 458 (21%) which agreed with the molecular mass of the compound ($C_{21}H_{26}N_6O_4S$) while the base peak was observed at m/z = 105.

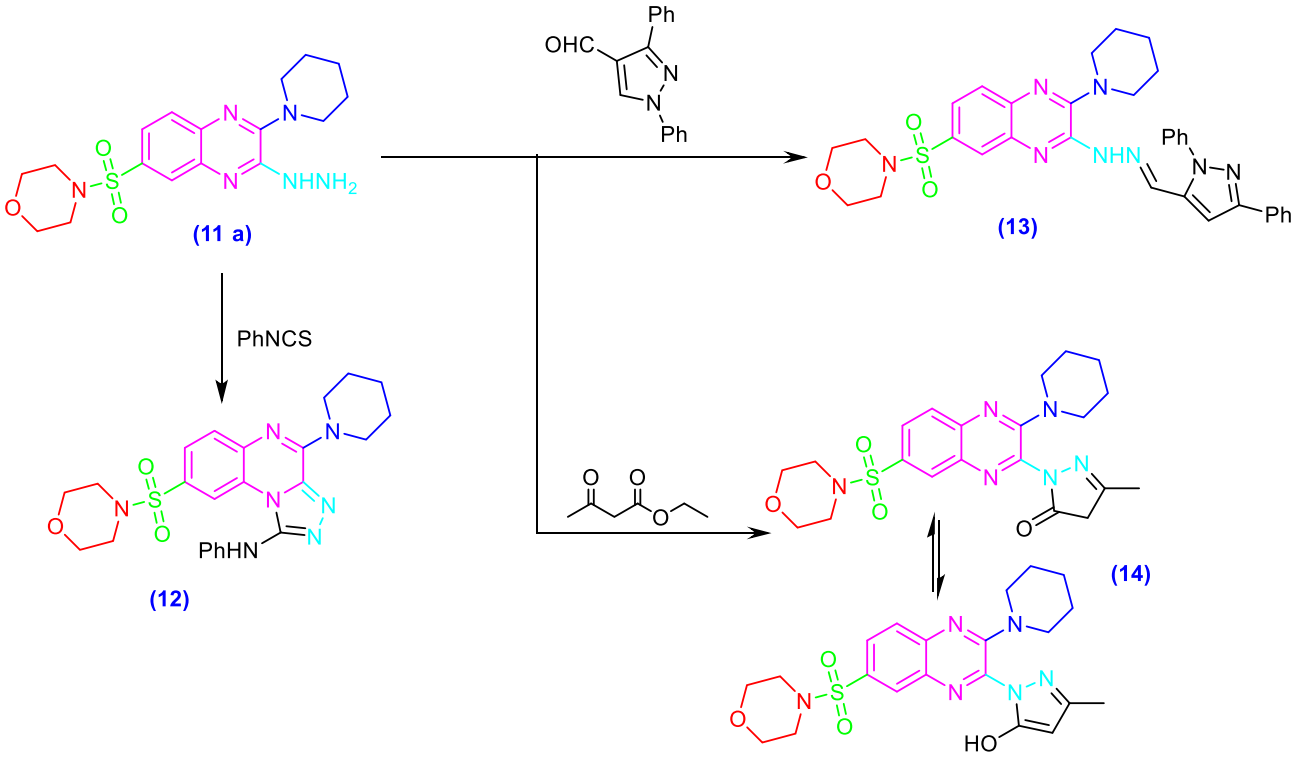
2.2. Biological activity evaluation

2.2.1. Antimicrobial activity evaluation

Anti-microbial activity of the newly synthesized quinoxaline derivatives **2–13** was evaluated against three Gram-positive strains (*B. subtilis* ATCC 6633, *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212), three Gram-negative (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922



Scheme 2. Synthesis of 2,3-disubstituted-6-(morpholinosulfonyl)quinoxaline derivatives **7a-11c.**



Scheme 3. Synthesis of 6-(morpholinosulfonyl)quinoxaline derivatives containing triazole or pyrazole **12–14.**

Table 1
Antimicrobial activity screening of the synthesized compounds (*In vitro*).

Code	Table (1): *In vitro* Antimicrobial activity of the synthesized compoundswith mean diameter of inhibition zone (mm)							
	Gram-positive			Gram-negative			Fungi	
	B. subtilis	*S. aureus*	*E. faecalis*	*E. Coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*F. oxysporum*
2	14 ± 0. 31	12 ± 0. 78	13 ± 0. 21	15 ± 0. 24	17 ± 0. 65	14 ± 0. 24	12 ± 0. 25	14 ± 0. 37
3	21 ± 0. 32	15 ± 0. 42	11 ± 0. 52	24 ± 0. 32	15 ± 0. 65	14 ± 0. 96	16 ± 0. 32	13 ± 0. 65
4	15 ± 0. 12	21 ± 0. 35	14 ± 0. 45	18 ± 0. 52	14 ± 0. 54	13 ± 0. 45	18 ± 0. 45	11 ± 0. 35
5a	17 ± 0. 34	14 ± 0. 65	17 ± 0. 74	19 ± 0. 65	18 ± 0. 53	12 ± 0. 85	13 ± 0. 75	15 ± 0. 68
5b	20 ± 0. 58	18 ± 0. 75	13 ± 0. 85	18 ± 0. 14	17 ± 0. 44	13 ± 0. 83	15 ± 0. 65	17 ± 0. 75
5c	21 ± 0. 39	19 ± 0. 32	17 ± 0. 36	19 ± 0. 32	21 ± 0. 24	24 ± 0. 21	16 ± 0. 34	13 ± 0. 95
6	28 ± 0.22	25 ± 0.19	30 ± 0. 34	26 ± 0.62	17 ± 0. 34	28 ± 0. 21	23 ± 0.25	19 ± 0.15
7a	30 ± 0.21	27 ± 0.89	31 ± 0. 44	28 ± 0.65	22 ± 0. 14	27 ± 0. 46	24 ± 0.33	20 ± 0.79
7b	25 ± 0. 24	21 ± 0. 15	18 ± 0. 34	22 ± 0. 18	19 ± 0.2	20 ± 0. 29	22 ± 0. 14	18 ± 0. 5
7c	21 ± 0. 5	19 ± 0. 12	22 ± 0. 55	23 ± 0.81	12 ± 0. 2	19 ± 0. 16	18 ± 0.56	11 ± 0.15
9a	22 ± 0. 33	21 ± 0. 17	19 ± 0. 23	20 ± 0. 44	18 ± 0. 87	15 ± 0. 17	17 ± 0. 73	9 ± 0. 62
9b	25 ± 0. 21	21 ± 0. 27	23 ± 0. 34	26 ± 0. 37	23 ± 0. 77	19 ± 0. 2	18 ± 0. 58	14 ± 0. 94
10a	24 ± 0. 78	22 ± 0. 24	21 ± 0. 92	23 ± 0. 14	21 ± 0. 25	23 ± 0. 13	21 ± 0. 45	19 ± 0. 78
10b	12 ± 0.91	na	14 ± 0. 17	19 ± 0. 33	12 ± 0. 3	na	13.0 ± 0.2	na
10c	26 ± 0. 66	23 ± 0. 5	24 ± 0. 33	21 ± 0.14	20 ± 0. 61	22 ± 0.2	21 ± 0. 31	19 ± 0. 44
10d	21 ± 0.11	16 ± 0.88	24 ± 0. 44	14 ± 0.4	na	na	19 ± 0.16	na
10e	13 ± 0. 4	na	14 ± 0. 14	12 ± 0.21	na	15 ± 0. 29	12 ± 0. 64	na
10f	25 ± 0.17	21 ± 0.33	22 ± 0. 3	20 ± 0.2	18 ± 0.65	24 ± 0. 44	17 ± 0. 25	16 ± 0. 5
11a	16 ± 0. 42	15 ± 0. 75	17 ± 0.64	14 ± 0. 16	na	11 ± 0.62	13 ± 0.22	na
11b	14 ± 0. 5	12 ± 0. 13	na	13 ± 0.22	na	10 ± 0.42	9 ± 0. 53	na
11c	31 ± 0.17	28 ± 0.35	30 ± 0. 67	26 ± 0. 22	21 ± 0. 54	26 ± 0. 61	25 ± 0.36	22 ± 0.33
13	12 ± 0. 32	15 ± 0. 74	na	12 ± 0. 96	na	na	17 ± 0.2	13 ± 0. 38
S1	25 ± 0.62	25 ± 0.51	22 ± 0.24	23 ± 0.12	20 ± 0.15	21 ± 0.45	na	na
S2	na	na	na	na	na	na	22 ± 0.2	18 ± 0.32

*na: No activity, *S1 = Tetracycline, S2 = Amphotericin B

and *S. typhi* ATCC 6539), in addition to two fungal strains namely *C. albicans* (ATCC 10231) and *F. oxysporum* (RCMB 008002). The antimicrobial screening was determined by measuring inhibition zone (mm) via conventional paper disk diffusion method [53,54], data represented in Table 1, Tetracycline and the antifungal drug Amphotericin B were used as refrence drugs.

The results of antimicrobial activity screening revealed that compounds **6**, **7a**, **11c** showed better antibacterial activity than Tetracycline against the three tested Gram-positive strains. While compounds **6**, **10c** showed better activity against *B. subtilis* and *E. faecalis* strains, compounds **9b**, **10d** displayed good activity against *E. faecalis*. On the other hand, compounds **7b**, **9b** and **10f** showed

comparable activity against *B. subtilis*, and compounds **7c** & **10f** revealed comparable activity towards *E. faecalis*. Moreover, compounds **7a**, **11c** exhibited promising antibacterial activity than tetracycline against the three tested Gram-negative microorganisms. Compounds **3**, **6**, **9b** showed better activity than tetracycline against *E. coli,* compounds **5c, 9b, 10a** displayed good activity against *P. aeruginosa*, compounds **5c**, **10a**, **10c**, **10f** demonstrated good activity against *S. typhi*. On the other hand, compounds **7c**, **10a** and **10c** showed comparable activity against *E. coli* tested strain.

Compounds **7a** & **11c** revealed observed antifungal activity which is higher than that of the reference drug Amphotericin B against *C. albicans* and *F. oxysporum* microorganisms. On the other hand, compounds **6**, **10a**, **10c** showed better activity against *F. oxysporum*, while compound **7b** showed comparable activity to the reference standard Amphotericin B against both fungal strains *C. albicans* and F. oxysporum microorganisms.

2.2.2. Minimum Inhibitory/Bactericidal concentrations (MIC)/(MBC) and SAR study

Depending on the antimicrobial screening results for all compounds, previously represented in (Table 1), the minimum inhibitory concentrations (MIC) of the most active quinoxaline derivatives (**7a**, **9b**, **10a**, **10c**, **10f**, **11c**) were determined using the conventional paper disk diffusion method [53,54], data are represented in Table 2 & **Fig. S1**. Compound **7a** that contains piperidine and chlorine moieties as well as quinoxaline sulphonyl morpholine surprisingly showed very strong antibacterial potential on *B. subtilis* and *E. faecalis* (4.91, 2.44 μM) respectively, compared to the standard positive control tetracycline (MIC = 33.63 & 67.27 μM).

While compound **11c** with bis-morpholine moieties and 3-hydrazinoquinoxaline exhibited the most significant activity among all derivatives against *S. aureus* with MIC value 14.12 μM compared to the standard reference tetracycline (MIC = 67.27 μM). Interestingly, compounds **9b**, **10a**, **10c** and **10f** also displayed better antibacterial potential on *B. subtilis* (MIC of 32.84, 20.08, 16.95 and 16.88 μM) respectively, than the standard positive control Tetracycline that exhibited MIC value at 33.63 μM. Moreover, compounds **7a** & **10a** that have piperidinyl moiety in position two of the quinoxaline sulfonamide core showed better antibacterial potential on *S. aureus* (MIC: 19.67 & 60.29 μM) than Tetracycline (MIC of 67.27 μM), it is observed that the presence of chlorine atom in position three gave more potent effect than *N*-methyl piperazine.

Compounds **9b**, **10c**, **10f** & **11c** displayed better antibacterial potential on *E. faecalis* (MIC of 16.42, 8.46, 19.99 and 4.94 μM) than the standard positive control Tetracycline (MIC of 67.27 μM). On the other hand, compound **7a** exhibited very strong antibacterial potential on *E. coli*, *P. aeruginosa* and *S. typhi* (MIC of 19.67, 69.96 and 9.82 μM) compared to Tetracycline (MIC of 16.81, 67.27 and 33.63 μM). Furthermore, compounds **9b**, **11c** showed better antibacterial potential on *E. coli* (MIC of 19.44, 19.79 μM) when compared to tetracycline (MIC of 16.81 μM), whilst compounds **10a**, **10c**, **10f** and **11c** have displayed better antibacterial activity on *S. typhi* (MIC of 40.18, 60.29, 33.76, and 14.12 μM) than Tetracycline (MIC of 33.63 μM). Compound **11c** with bis-morpholine moiety as well as 3-hydrazinoquinaxoline derivatives exhibited antifungal potential with (MIC of 19.79, 39.59 μM) on *C. albicans* and *F. oxysporum* strains, additionally, compound **7a** has displayed the second-best antifungal activity among the tested compounds (MIC of 23.30, 46.63 μM) than the standard positive control Amphotericin B (MIC of 16.81, 33.63 μM).

For further exploration of the most active compounds **7a**, **9b**, **10a**, **10c**, **10f** and **11c**, minimum bactericidal concentrations (MBC) were determined using the conventional paper disk diffusion method [53,54]. The obtained results are represented in Table 3 & **Fig. S2**, bactericidal activity revealed that compounds **7a** and **11c** showed very strong bactericidal activity on all tested Gram-positive, Gram-negative and fungi strains compared to the standard positive control Tetracycline and Amphotericin B as shown from MBC values in Table 3**.** Interestingly, compounds **10a**, **10c** and **10f** displayed better bactericidal activity on *B. subtilis* with MBC values between 30.37 and 38.14 μM, while both **10c** and **10f** displayed good activity against *E. faecalis* with MBC values 16.93, 39.99 μM compared to tetracycline (MBC of 43.72, and 100.90 μM respectively).

Compounds **7a**, **10a** and **10c** exhibited better fungicidal activity on *C. albicans* (MBC of 44.26, 61.02, and 50.86 μM) than the standard positive control Amphotericin B (MIC of 37.26 μM), while compound **11c** showed to be the most promising derivative against the tested two fungal strains *C. albicans*, *F. oxysporum* with MBC values (31.66 and 67.30 μM) when compared to Amphotericin B (37.26 and 70.62 μM) respectively.

2.2.3. Drug resistance study

For further screening of the best active derivatives **7a**, **9b**, **10a**, **10c**, **10f** and **11c**, they were evaluated against a panel of drug-resistant Gram-positive bacterial strains *S. aureus* (ATCC 43300), *S. aureus* (ATCC 33591), multidrug-resistant Gram-negative *E. coli* (ATCC BAA-196) and *P. aeruginosa* (ATCC BAA-2111) [55,56]. As shown in Table 4 & **Fig. S3A, B** the selected compounds exhibited moderate to good potency against all the tested multi-drug resistant bacterial (MDRB) strains with MIC values of (4.91–32.84 μM), **Fig. S3A**. As for the tested strains *S. aureus, E. coli* and *P. aeruginosa*, it was found that compound **7a** has showed a comparable antibacterial activity (MIC 4.91, 9.82, 4.91, and 9.82 μM) to Norfloxacin (MIC 3.91, 2.44, 4.91, and 9.81 μM), however, Tetracycline as another positive control doesn't give any results against the tested resistant strains. Compound **7a** displayed the best results against (MDRB) compared with Norfloxacin, these may be due to the presence of chlorine atom in position three in addition to piperidinyl moiety in position two. The other five compounds also showed good activity against MDRB, near to that of the standard commercial drug Norfloxacin.

Next, we have evaluated the MBC for the most promising compounds against MDRS as shown in Table 4, **Fig. S3B.** Notably, almost all the tested derivatives **7a**, **9b**, **10a**, **10c**, **10f** and **11c** showed remarkable

Table 2
Minimal inhibitory concentrations (MIC) (μM) of the most active compounds.

Cpd. No.	Gram-positive			Gram-negative			*Fungi*	
	B. subtilis	*S. aureus*	*E. faecalis*	*E. Coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*F. oxysporum*
7a	4.91	19.67	2.44	19.67	69.96	9.82	23.30	46.63
9b	32.84	131.41	16.42	19.44	116.69	65.70	58.38	131.41
10a	20.08	60.29	135.69	67.84	135.69	40.186	33.91	67.84
10c	16.95	120.49	8.46	60.29	135.69	60.29	33.91	76.42
10f	16.88	135.11	19.99	135.11	180.14	33.76	67.55	119.98
11c	9.88	14.12	4.94	19.79	79.22	14.12	19.79	39.59
S1	33.63	67.27	67.27	16.81	67.27	33.63	–	–
S2	–	–	–	–	–	–	16.81	33.63

*S1 = Tetracycline, S2 = Amphotericin B.

Table 3
Minimum bactericidal concentrations (MBC) and minimum fungicidal concentration (μM) of the selected compounds against pathogenic microbes.

Cpd. No.	Gram-positive			Gram-negative			*Fungi*	
	***B. subtilis* ATCC 6633**	***S. aureus* ATCC29213**	***E. faecalis* ATCC 29212**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC27853**	***S. typhi* ATCC 6539**	***C. albicans* ATCC 10231**	***F. oxysporum* RCMB 008002**
7a	9.82	39.35	4.88	39.35	139.93	16.70	44.26	74.60
9b	52.54	249.67	32.84	38.89	186.70	118.26	87.57	183.97
10a	38.14	90.42	271.38	135.69	257.81	80.37	61.02	101.75
10c	33.91	240.99	16.93	120.58	271.38	90.42	50.86	145.20
10f	30.37	216.18	39.99	256.71	360.29	60.76	114.83	191.97
11c	16.80	26.82	9.38	35.61	150.51	26.82	31.66	67.30
Tetr.	43.72	94.17	100.90	20.17	94.17	47.08	–	–
Am. B	–	–	–	–	–	–	37.26	70.62

Table 4
Mean diameter of inhibition zone (mm), minimal inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) (μM) of the selected compounds against multidrug resistant bacteria (MDRB).

Code	**Mean diameter of inhibition zone (mm) and minimal inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) (μM) of the most potent synthesized compounds against MDRB.**											
	***S. aureus* ATCC 43300**			***S. aureus* ATCC 33591**			***E. coli* *ATCC BAA*-196**			***P. aeruginosa* ATCC BAA-2111**		
	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC	IZ	MIC	MBC
7a	25 ± 0.16	4.91	9.82	23 ± 0.22	9.82	19.65	26 ± 0.66	4.91	9.82	25 ± 0. 24	9.82	19.65
9b	15 ± 0.4	32.84	65.70	22 ± 0.5	8.19	15.57	19 ± 0.11	32.84	65.70	23 ± 0. 99	13.14	24.95
10a	19 ± 0. 23	13.56	25.77	17 ± 0. 35	16.95	33.91	22 ± 0.15	8.46	16.08	20 ± 0. 66	19.27	34.69
10c	17 ± 0.77	16.95	32.19	21 ± 0.49	12.04	24.09	21 ± 0.23	16.95	32.19	22 ± 0.56	9.63	14.45
10f	18 ± 0.11	16.88	33.76	15 ± 0.91	19.19	34.54	20 ± 0.44	19.99	39.99	21 ± 0.33	16.88	33.76
11c	22 ± 0.65	11.25	22.51	25 ± 0.12	4.94	8.391	23 ± 0. 2	15.84	30.09	24 ± 0. 88	13.18	25.04
Tetr.	–	–	–	–	–	–	–	–	–	–	–	–
Nor.	25 ± 0.5	3.91	8.80	26 ± 0.5	2.44	4.88	27 ± 0.98	4.91	11.05	24 ± 0.47	9.80	14.68

activities against MDRS used in this study. Where the promising compounds exhibited MBC values range between 8.39 and 65.70 μM compared with Norfloxacin (4.88–14.68 μM). Compound **7a** showed MBC value 9.82 μM against *S. aureus* (ATCC 43300), however, Norfloxacin MBC value was 8.80 μM. The bactericidal activity of compounds **7a** and **10a** that having piperidinyl moiety in position two showed MBC values (9.82, and 16.08 μM) respectively against *E. coli* ATCC BAA-196 compared to 11.05 μM for Norfloxacin and this difference in activity for quinoxaline derivatives can be attributed to chloro and/or *N*-methyl piperazine moieties. Finally, for *P. aeruginosa* ATCC BAA-2111 compound **10c** with bis-piperidinyl beside 6-morpholinosulfonylquinoxaline derivatives showed the best MIC and MBC values 9.63 and 14.45 μM against 9.80 and 14.68 μM assigned for the positive control.

2.2.4. DNA Gyrase inhibition activity

Derivatives with promising activity **7a, 9b, 10a, 10c, 10f and 11c** were selected for screening as DNA gyrase inhibitors, as DNA Gyrase represents an important target for antimicrobial candidates.

Table 5
S. aureus DNA gyrase inhibitory activity of the investigated compounds.

Compound	DNA gyrase Supercoiling inhibition IC_{50} in μM
7a	17.10 ± 1.16
9b	19.21 ± 1.22
10a	15.69 ± 1.63
10c	23.72 ± 1.13
10f	18.85 ± 1.9
11c	17.69 ± 1.55
Ciprofloxacin	26.31 ± 1.64

Ciprofloxacin was used as a standard reference and the results were illustrated in (Table 5 **and Fig. S4**), IC_{50} values are expressed in (μM). 2-piperidinyl derivatives **7a**, **10a** and 2-morpholinyl derivative **11c** showed better activity (IC_{50} = 17.10, 15.69 & 17.69 μM) than the standard drug, Ciprofloxacin (IC_{50} = 26.31 μM), while compounds **10f** and **9b** exhibited DNA Gyrase inhibition at IC_{50} value equal to 18.85 and 19.21 μM respectively. The structure activity relationship showed that the most promising quinoxaline derivative **10a** that involving 2-pipridinyl in position two of quinoxaline nucleus as well as 4-*N*-methyl piperazinyl in position 3 in addition to 6-morpholinosulfonyl group in quinoxaline moiety followed by the quinoxaline derivatives **7a** and **11c** are more active than the other substituted analogs **9b**, **10c** & **10f**.

Furthermore, it was found that two quinoxaline derivatives **10a** and **10c** that contain the same substituents [(4-methylpiperazin-1-yl) and (2-piperidin-1-yl)] in position two and three to quinoxaline moiety showed nearly 8 μM differ in activity and that difference in activity illustrate that presence of piperidin-1-yl and methylpiperazin-1-yl in position 2 and 3 is preferred and caused higher activity against DNA gyrase. In the same way, quinoxaline derivative **10f** in which piperidinyl in position 2 was replaced with morpholine showed decrease in activity with nearly 3 μM. It is also observed that compounds **9b** and **10f** with 4-methylpiperazine moiety at position three revealed DNA Gyrase inhibition (IC_{50} = 19.21, and 18.85 μM) respectively, and this difference can be attributed to presence of morpholine core instead of *N*-methyl piperazine in position 2 in the quinoxaline scaffold.

2.2.5. Immunomodulatory activity for most active compounds

The promising compounds depending on the previous results were investigated *in vitro* to evaluate their immunomodulatory activity. The neutrophils play a major role as a killer cell for many types of infections [57]. The primary function of neutrophils is the intracellular killing of

Table 6
Intracellular killing activities of the tested compounds.

Compound	Intracellular killing activity %
7a	142.6 ± 0. 4
9b	72.8 ± 0.77
10a	86.4 ± 0. 47
10c	112.5 ± 0.83
10f	117.8 ± 0. 39
11c	135.7 ± 0. 5

microbes. The NBT reduction experiment was used to assess our selected compounds [53–54,58], the obtained results (Table 6 **and Fig. S5**) reflected the considerable effect of our compounds in killing ability toward neutrophils. Intracellular killing activities are presented by percentages (%). Compounds **7a, 9b, 10a, 10c, 10f and 11c** showed good potency as immunomodulatory agents, the highest immunostimulatory action was assigned to compounds **7a, 10c, 10f and 11c** with activity range between 117.8 ± 0. 39 and 142.6 ± 0.4.

2.3. Molecular docking study

The DNA Gyrase enzyme is one of the topoisomerases classes (topoisomerase II), these enzymes are involved in winding and unwinding of DNA during the process of replication and transcription. Gyrase enzyme affects the topological state of DNA, hence it is considered as an important intracellular target for antibacterial agents as a representative model for other DNA topoisomerases [59]. This fact encouraged us to investigate the binding mode, docking score energy and the expected type of interactions between the hopeful new molecules and binding site of the DNA Gyrase enzyme. Compounds **7a**, **9b**, **10a**, **10c**, **10f**, **11c**, that showed DNA Gyrase inhibition activity at a range from 15.69 to 23.72 μM in addition to Ciprofloxacin as a reference drug were docked into DNA Gyrase binding site, the obtained results are represented in **Table S1**, Figs. 2A-5B and the other figures are provided in the supplementary data files. The validation step was performed by removing the co-crystallized ligand and re-docking again into the Gyrase binding site at RMDS value equal 1.44 (Supp. Data). The obtained results revealed the promiscuity of the new quinoxalines to bind into the DNA Gyrease enzyme. The binding free energy and types of possible interactions in addition to, the interacting moiety of our docked compounds are represented in **Table S1.**

As shown from the obtained data quinoxaline core was able to form arene-cation interactions with different amino acids in the DNA Gyrase binding site as Lys 103 and Arg 76. Hybridizing quinoxaline scaffold with SO_2 and morpholine moieties proved to be a useful strategy in building up new molecules with good ability of the binding into DNA Gyrase binding site as morpholine and/or SO_2 showed hydrogen bonding interactions with Arg 76, Arg 136 and Gly 117. Further substitution with piperazine as compound **9b** and NH_2 group as compound **11c** also extended the ability of these compounds to form hydrogen bonding with Asp 73 and Arg 136 amino acids respectively.

3. Conclusion

New twenty-four quinoxaline derivatives were designed and synthesized, they were screened for their antimicrobial activity against six bacterial strains and two fungal species. Six compounds (**7a, 9b, 10a, 10c, 10f** and **11c**) revealed promising antibacterial activity with MIC value range of 2.44–180.14 μM. Additionally, these compounds showed good results against the tested multi-drug resistant strains, especially compound **7a** that showed comparable antibacterial activity (MIC 4-91-9.82 μM) to that of Norfloxacin (MIC 2.44–9.80 μM). Furthermore, compounds **7a, 10a** & **11c** exhibited potent DNA Gyrase inhibition (IC_{50} = 17.10, 15.69, and 17.69 μM) higher than the standard drug Ciprofloxacin (IC_{50} = 26.31 μM). Therefore, they can be considered as a hit for further optimization to obtain more active antibacterial agents. Molecular docking studies revealed that these compounds **(7a, 9b, 10a, 10c, 10f and 11c**) have binding mode and an affinity to DNA Gyrase binding site comparable to that of Ciprofloxacin. Also, it has shown the importance of hybridizing the quinoxaline scaffold with morpholine and SO_2 moieties for better binding with DNA Gyrase. Finally,

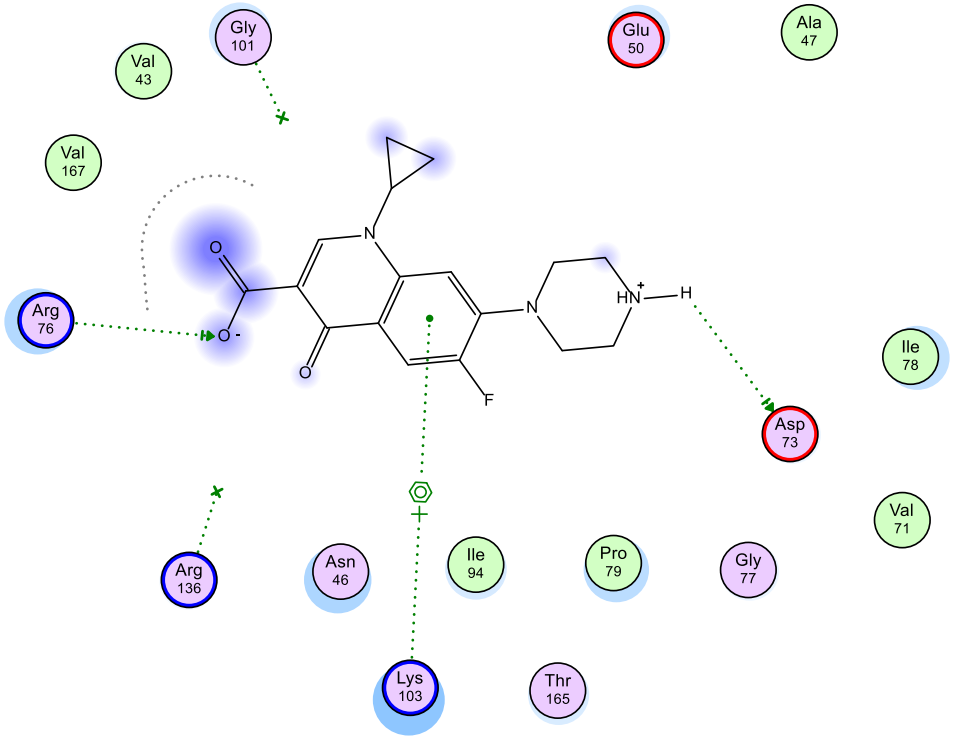


Fig. 2A. 2D for Ciprofloxacin docked into DNA Gyrase binding site.

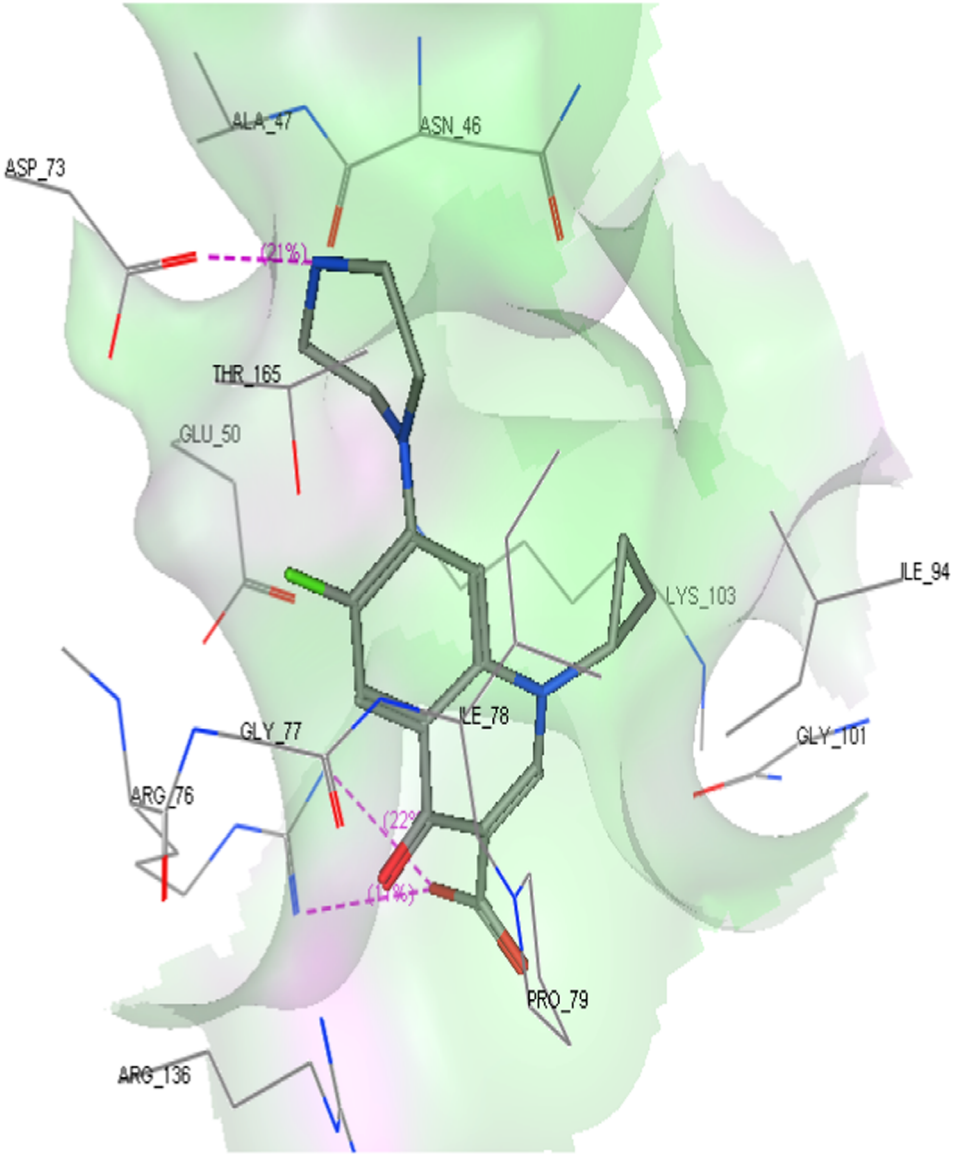


Fig. 2B. 3D for Ciprofloxacin docked into DNA Gyrase binding site.

compounds **7a, 10c, 10f and 11c** showed to have a good immunomodulating activity.

4. Experimental

All solvents and reagents were freshly distilled and purified according to standard procedures. All melting points are recorded on digital Gallen Kamp MFB-595 instrument and are uncorrected. The IR spectra (KBr) (cm^{-1}) were detected on a Shimadzu 440 spectrophotometer ^{1}H NMR and ^{13}C NMR spectra (δ, ppm) were performed at the Main Chemical Warfare Laboratories, Chemical Warfare Department, Ministry of Defense, Cairo, Egypt, on a Varian Gemini 500 (400 MHz and 101 MHz) spectrometer, deuterated dimethyl sulfoxide (DMSO-d_6) was used as a solvent and TMS as an internal standard; chemical shifts are expressed in δ ppm. The data were presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent), coupling constant (s) in Hertz (Hz), and integration. Mass spectra were recorded on Thermo Scientific ISQLT mass spectrometer at the Regional Center for Mycology and Biotechnology, Al-Azhar University. Elemental analyses were carried out at Micro Analytical Unit, Cairo University.

4.1. Chemistry

4.1.1. 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride (1)

It was prepared according to previously reported method [50]. Yield: 88%; as White crystals from ethanol/DMF mixture; m.p.: 348–350 °C; IR: ν/cm^{-1}: 3354, 3169 (NH), 3038 (CH-Ar), 2942, 2841 (CH-aliph), 1674 (C=O), 1387, 1161 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 7.02 (d, J = 8.3 Hz, 1H), 7.29 (dd, J = 8.3, 1.6 Hz, 1H), 7.42 (s, 1H), 11.91 (s, 1H, NH, exchangeable with D_2O), 11.93 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 113.21, 114.73, 121.07, 125.13, 126.05, 143.86, 155.67 (C=O), 155.75 (C=O); Anal. Calcd. for $C_8H_5ClN_2O_4S$ (260.65): C, 36.87; H, 1.93; N, 10.75; Found: C, 36.83; H, 1.89; N, 10.71

4.1.2. N-(3-methoxyphenyl)-2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonamide (2)

2,3-dioxoquinoxaline derivatives **1** (10 mmol, 2.6 g) was dissolved in dry dimethylformamide (20 mL), followed by addition of *m*-anisidine (10 mmol, 1.23 g) and the resulting mixture was kept under stirring at room temperature for 10 h. The reaction mixture was then poured into water (200 mL) and the solid formed was recrystallized from DMF to give desired product **2** as pale brown powder.

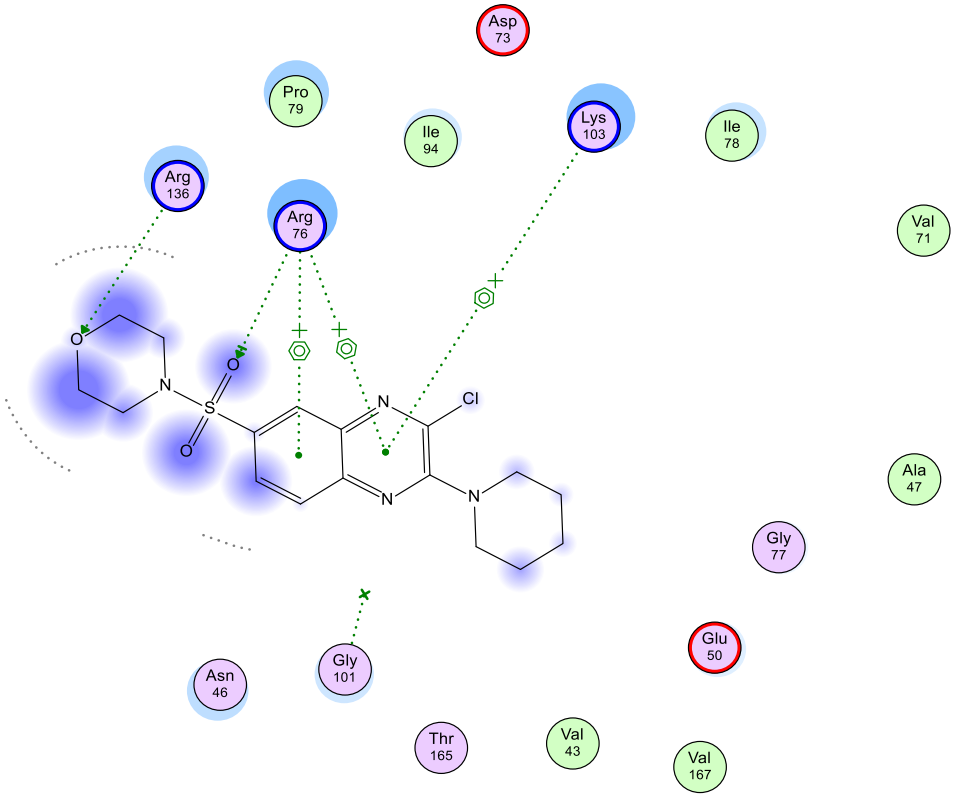


Fig. 3A. 2D for compound **7a** docked into DNA Gyrase binding site.

Yield: 75%; m.p.: 308–310 °C; IR: ν/cm^{-1}: 3228 (NH), 3059(CH-Ar), 2947, 2835 (CH-aliph.), 1693 (C═O), 1388, 1145 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 3.65 (s, 3H, –OCH_3), 6.56 (d, J = 8.2, 1.8 Hz, 1H, Ar-H), 6.65 (d, J = 7.4 Hz, 1H, Ar-H), 7.08–7.10 (m, 1H, Ar-H), 7.17 (d, J = 8.5 Hz, 1H, Ar-H), 7.44 (dd, J = 8.4, 1.7 Hz, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 10.31, 12.06, 12.12 (3 s, 3H, 3NH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 55.44 (CH_3), 105.88, 109.44, 112.18, 114.19, 115.97, 121.94, 126.26, 129.81, 130.46, 133.88, 139.29, 155.35 (2C═O), 160.14 (═C–O); MS (m/z, %): 56(65%), 90 (42%), 109 (44%), 116 (100%), 121 (59%), 154 (45%), 161 (86%), 235 (40%), 262 (66%), 303 (49%), 325 (41%), 332 (56%), 346 (M^+ − 1, 17%), 347 (M^+, 19%), 348 (M^+ + 1, 17%), 349 (M^+ + 2, 10%); Anal. Calcd. for $C_{15}H_{13}N_3O_5S$ (347.35): C, 51.87; H, 3.77; N, 12.10; Found: C, 51.85; H, 3.73; N, 12.06

4.1.3. 2-(1-((2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-6-yl)sulfonyl)-1H-benzo[d]imida-zol-2-yl)acetonitrile (3)

A mixture of 2,3-dioxoquinoxaline derivatives **1** (10 mmol, 2.6 g) and 2-(1*H*-benzo[*d*]imidazol-2-yl)acetonitrile (10 mmol, 1.57 g) in dioxane (20 mL), containing 3 drops of TEA, was heated under reflux for 1 h and then stirred at room temp. till completion of the reaction which was monitored by TLC. The solid obtained after completion was

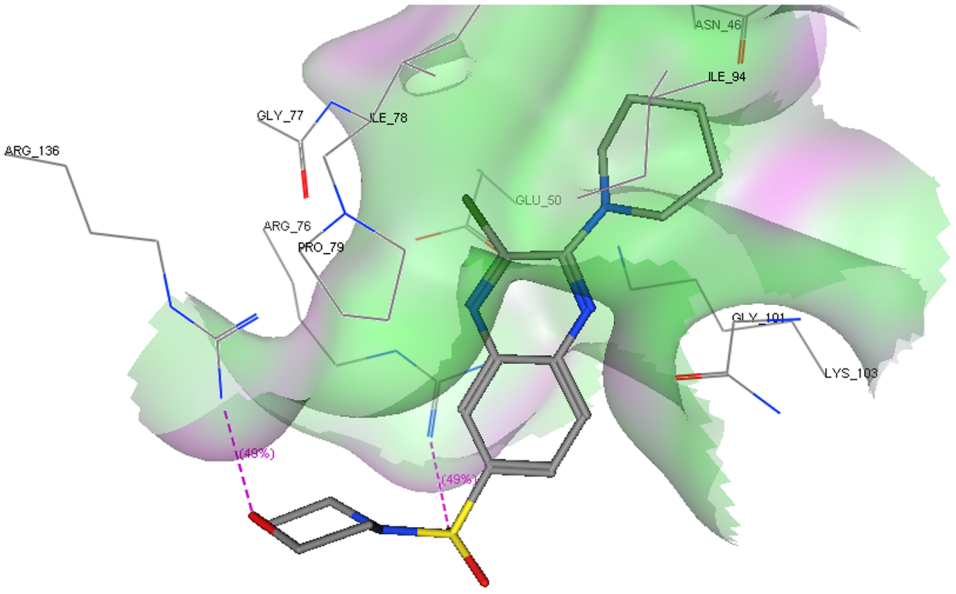


Fig. 3B. 3D for compound **7a** docked into DNA Gyrase binding site.

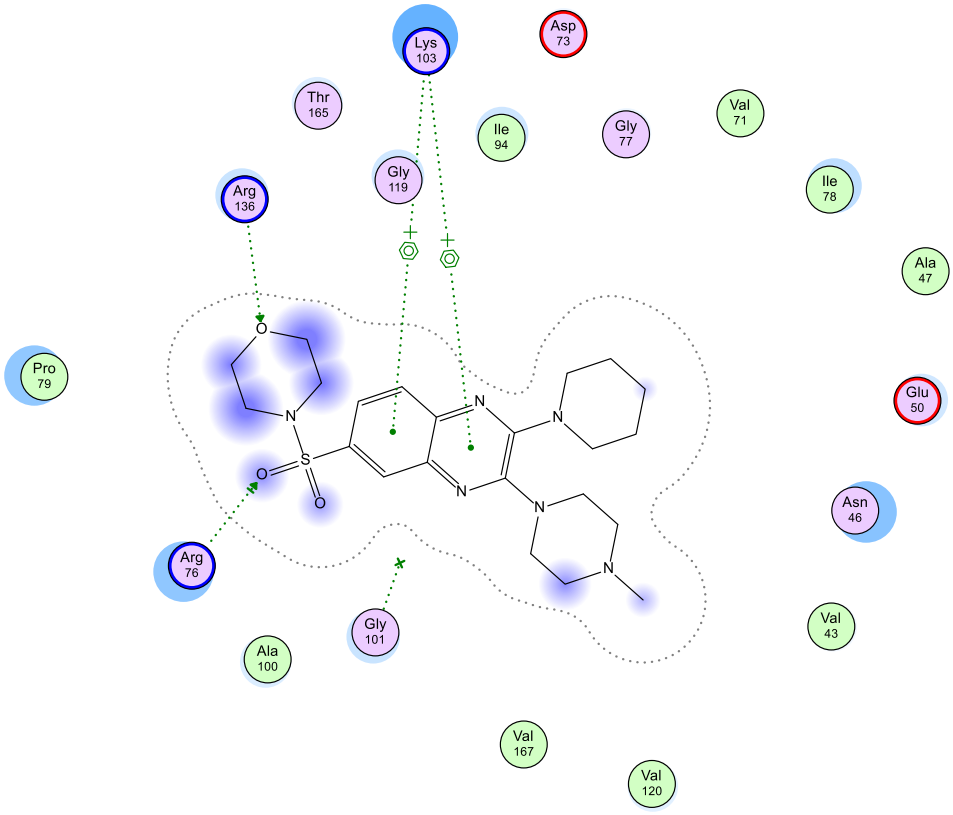


Fig. 4A. 2D for compound **10a** docked into DNA Gyrase binding site.

filtered, washed with ethanol and recrystallized from DMF to give compound **3** as pale brown powder.

Yield: 70%; m.p.:>360 °C; IR: ν/cm^{-1}: 3497 (NH), 3052(CH-Ar), 2943, 2840 (CH-aliph.), 2260 (C≡N), 1673 (C═O), 1387, 1195 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 4.42 (s, 2H, CH_2), 7.02 (d, J = 8.3 Hz, 2H, Ar-H), 7.22 (d, J = 6.0 Hz, 1H, Ar-H), 7.30 (d, J = 8.3 Hz, 2H, Ar-H), 7.43 (s, 1H, Ar-H), 7.56 (d, J = 6.0 Hz, 1H, Ar-H), 11.92, 11.94 (2 s, 2H, 2NH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 15.45 (CH_2), 113.20, 114.67, 117.16, 121.06, 122.06, 123.97, 125.08, 125.96, 127.20, 128.87, 131.96, 134.12, 138.04, 143.99 (C═N-imidazole), 155.63 (C═O), 155.71 (C═O); MS (m/z, %): 45(40%), 65 (40%), 75 (100%), 89 (69%), 103 (64%), 116 (92%), 120 (51%), 264 (49%), 381 (M^+, 5%); Anal. Calcd. for $C_{17}H_{11}N_5O_4S$ (381.37): C, 53.54; H, 2.91; N, 18.36; Found: C, 53.50; H, 2.85; N, 18.31.

4.1.4. 6-((1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl) sulfonyl)-1,4-di-hydroquino-xaline-2,3-dione (4)

2,3-dioxoquinoxaline derivatives **1** (10 mmol, 2.6 g) was dissolved in dry dimethylformamide (20 mL), followed by addition of 1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (10 mmol, 1.8 g) and the resulting mixture was heated under reflux for 1 h. The precipitate formed

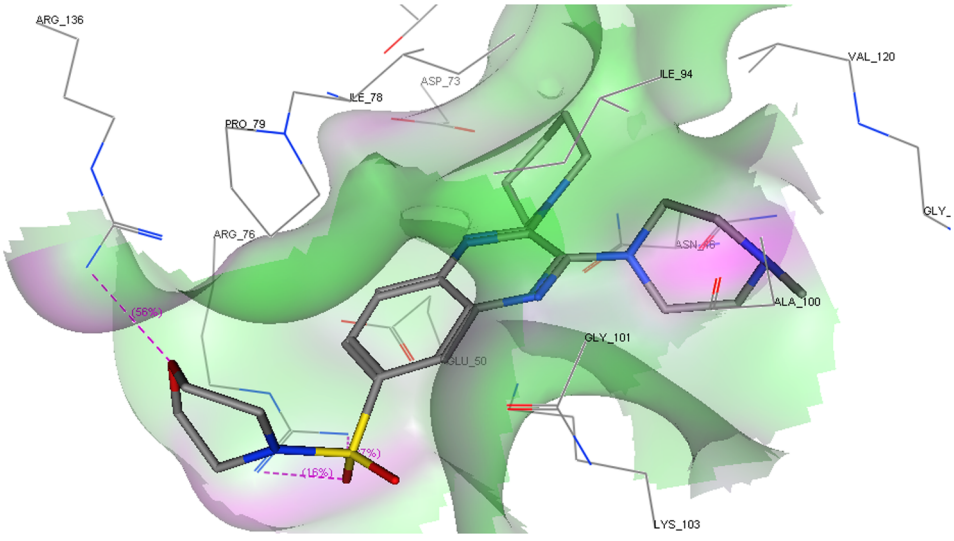


Fig. 4B. 3D for compound **10a** docked into DNA Gyrase binding site.

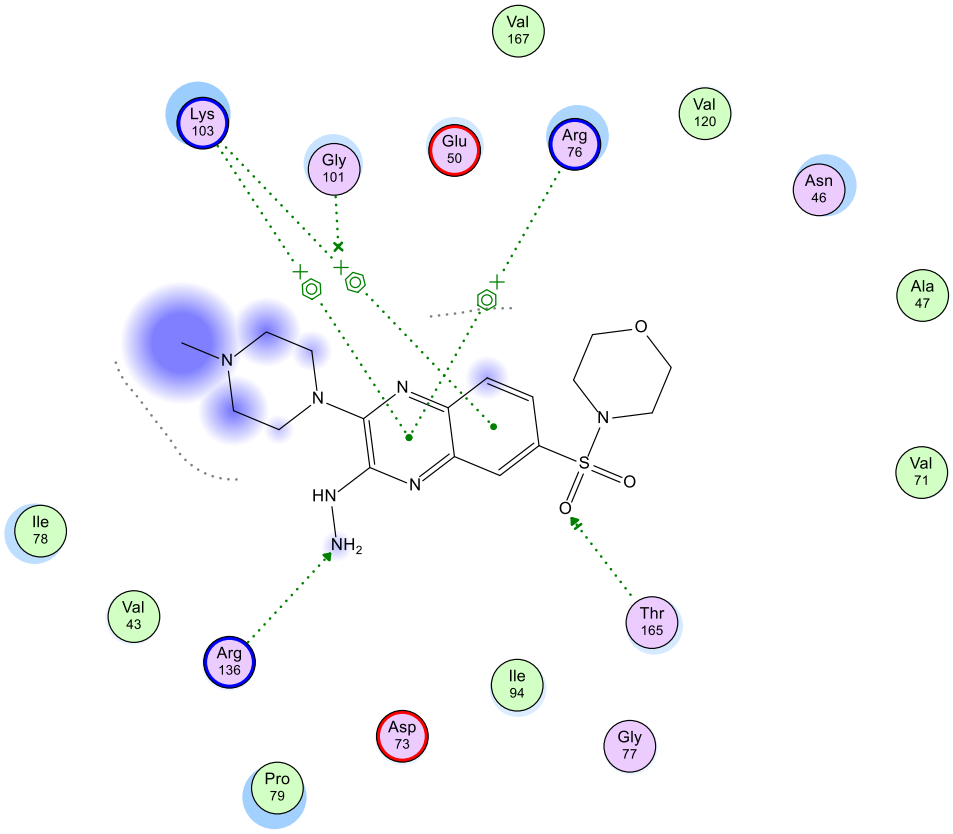


Fig. 5A. 2D for compound **11c** docked into DNA Gyrase binding site.

while hot was filtered, washed with ethanol and recrystallized from DMF to give compound **4** as white crystals.

Yield: 88%; m.p.: 320–322 °C; IR: ν/cm^{-1}: 3250 (NH), 3025(CH-Ar), 2943, 2823 (CH-aliph.), 1707, 1662 (C═O), 1381, 1174 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 3.22 (s, 3H, CH_3), 3.43 (s, 3H, CH_3), 7.02 (d, J = 8.3 Hz, 1H, Ar-H), 7.30 (dd, J = 8.3, 1.7 Hz, 1H, Ar-H), 7.42 (s, 2H, Ar-H), 9.96 (s, 2H, NH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 34.85 (2(CH_3)), 113.21, 114.79, 121.07(2C), 125.16, 126.12 (2C), 141.00, 143.70, 155.71 (2C = O), 155.78 (2C = O); MS (m/z, %): 102(44%), 122 (82%), 180 (100%), 323 (62%), 325 (49%), 339 (50%), 404 (M^+, 16%); Anal. Calcd. for $C_{15}H_{12}N_6O_6S$ (404.36): C, 44.56; H, 2.99; N, 20.78; Found: C, 44.51; H, 2.81; N, 20.66.

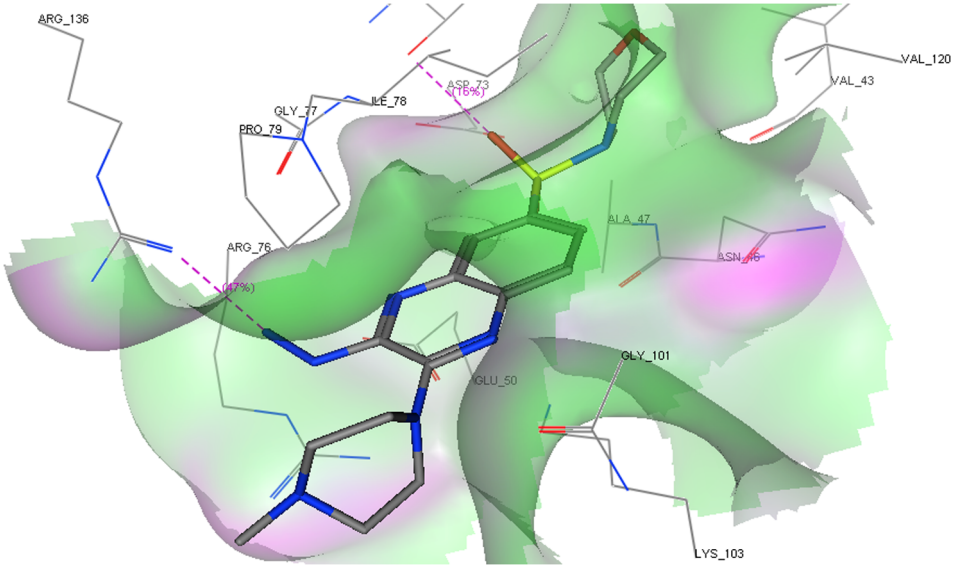


Fig. 5B. 3D for compound **11c** docked into DNA Gyrase binding site.

4.1.5. Synthesis of 6-(alk-1-ylsulfonyl)-1,4-dihydroquinoxaline-2,3-dione (5a,b)

A mixture of 2,3-dioxoquinoxaline derivatives **1** (10 mmol, 2.6 g) and piperidine or 1-methyl piperazine (20 mmol) in dimethylformamide (20 mL) was refluxed for 5 h. The solid obtained was precipitated while hot, filtered, dried and recrystallized from DMF to give desired product **5a**,**b**.

4.1.6. 6-(piperidin-1-ylsulfonyl)-1,4-dihydroquinoxaline-2,3-dione (5a)

As white crystals; yield: 73%; m.p.: 317–320 °C; IR: ν/cm^{-1}:3142 (NH), 3040(CH-Ar), 2951, 2831 (CH-aliph.), 1684 (C═O), 1405, 1169 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.53 (m, 2H, CH_2), 1.66 (m, 4H, C-$(CH_2)_2$), 2.98 (t, 4H, N$(CH_2)_2$), 7.03 (d, J = 8.3 Hz, 1H, Ar-H), 7.28 (dd, J = 8.3, 1.6 Hz, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 8.22, 11.91 (2 s, 2H, 2NH exchangeable with D_2O); Anal. Calcd. for $C_{13}H_{15}N_3O_4S$ (309.34): C, 50.48; H, 4.89; N, 13.58; Found: C, 50.35; H, 4.76; N, 13.85.

4.1.7. 6-((4-methylpiperazin-1-yl)sulfonyl)-1,4-dihydroquinoxaline-2,3-dione (5b)

As light rose crystals; yield: 70%; m.p.: 290–292 °C; IR: ν/cm^{-1}:3182 (NH), 3047(CH-Ar), 2939, 2835 (CH-aliph.), 1708 (C═O), 1384, 1161(SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.54 (s, 3H, −N−CH_3), 2.88 (t, 4H, $N^4(CH_2)_2$), 3.10 (t, 4H, $N^1(CH_2)_2$), 7.31 (d, J = 8.4 Hz, 1H, Ar-H), 7.44 (dd, J = 8.4, 1.9 Hz, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 12.07, 12.26 (2 s, 2H, 2NH exchangeable with D_2O); Anal. Calcd. for $C_{13}H_{16}N_4O_4S$ (324.36): C, 48.14; H, 4.97; N, 17.27; Found: C, 48.06; H, 4.93; N, 17.15

4.1.8. 6-(morpholine-4-sulfonyl)-1,4-dihydroquinoxaline-2,3-dione (5c)

A solution of 2,3-dioxo-1,2,3,4-tetrahydroquinoxaline-6-sulfonyl chloride **(1)** (10 mmol, 2.6 g) in dioxane (20 mL), was treated with (20 mmol, 1.74 mL) morpholine and the resulting mixture was kept under stirring at room temperature for 2 h. The precipitate formed was collected by filtration and recrystallized from ethanol/DMF mixture to give **5c** as white crystals.

Yield: 90%; m.p.:338–340 °C; IR: ν/cm^{-1}:3253, 3148 (NH), 3069(CH-Ar), 2955, 2863 (CH-aliph.), 1678 (C═O), 1379, 1154 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.84 (t, 4H, N$(CH_2)_2$), 3.62 (t, 4H, O$(CH_2)_2$), 7.30 (d, J = 8.4 Hz, 1H, Ar-H), 7.41 (dd, J = 8.4, 1.9 Hz, 1H, Ar-H), 7.47 (s, 1H, Ar-H), 12.05, 12.20 (2 s, 2H, 2NH exchangeable with D_2O), ^{13}C NMR (101 MHz, DMSO) δ/ppm: 46.26 (-N$(CH_2)_2$), 65.71 (-O$(CH_2)_2$), 114.92, 116.12, 122.82, 126.56, 128.65, 130.23, 155.26, 155.63 (2C = O); MS (*m/z*, %):46(100%), 63 (86%), 73 (50%), 75 (90%), 76 (44%), 77 (50%), 129 (50%), 132 (41%), 309 (M^+-2, 10%), 311 (M^+, 13%), 312 (M^+ +1, 24%), 314 (M^+ +3, 6%); Anal. Calcd. for $C_{12}H_{13}N_3O_5S$ (311.31): C, 46.30; H, 4.21; N, 13.50; Found: C, 46.25; H, 4.15; N, 13.43

4.1.9. ***2,3-dichloro-6-(morpholinosulfonyl)quinoxaline (6)*** *according to reported method [51]*

As off-white needles from acetonitrile; yield: 85%;; m.p.:183–185 °C; IR: ν/cm^{-1}: 3074(CH-Ar), 2962, 2918, 2856 (CH-aliph.), 1600 (C═N), 1350, 1154 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.99 (t, 4H, N$(CH_2)_2$), 3.62 (t, 4H, O$(CH_2)_2$), 8.15 (dd, J = 8.8, 2.0 Hz, 1H, Ar-H), 8.33 (d, J = 8.8 Hz, 1H, Ar-H), 8.39 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 46.36 (-N$(CH_2)_2$), 65.73 (-O$(CH_2)_2$), 128.55, 129.04, 130.24, 137.56, 139.78, 142.18, 147.35, 148.15 (2C-Cl); MS (*m/z*, %): 49 (53%), 63 (41%), 73 (44%), 74 (57%), 75 (63%), 78 (58%), 104 (69%), 116 (100%), 264 (67%), 265 (52%), 324 (55%), 345 (M^+-3, 13%), 348 (M^+, 10%), 350 (M^+ +2, 4%); Anal. Calcd. for $C_{12}H_{11}Cl_2N_3O_3S$ (348.20): C, 41.39; H, 3.18; N, 12.07; Found: C, 41.35; H, 3.11; N, 11.96.

4.1.10. 3-Chloro-6-morpholinosulfonyl-2-(substitutedamine)quinoxaline (7a-c)

A solution 2,3-dichloroquinoxaline derivatives **6** (10 mmol, 3.48 g) and the requisite cyclic secondary amine (10 mmol) in 30 mL of acetonitrile was refluxed for 8 h. The reaction mixture was cooled and then quenched onto crushed ice and stirred until the product precipitated out. The precipitate was filtered, washed with water, dried and recrystallized from the proper solvent.

4.1.11. 3-Chloro-6-morpholinosulfonyl-2-(piperidin-1-yl)quinoxaline (7a):

As yellow crystals from ethanol; yield: 75%; m.p.:148–150 °C; IR: ν/cm^{-1}: 3048 (CH-Ar), 2952, 2907, 2878, (CH-aliph.), 1559 (C═N), 1338, 1166 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.68 (m, 6H, 3(CH_2)-pip), 2.91 (t, 4H, -N$(CH_2)_2$-morph), 3.58 (t, 4H, N$(CH_2)_2$-pip), 3.62(t, 4H, O$(CH_2)_2$), 7.88 (dd, J = 8.7, 2.0 Hz, 1H, Ar-H), 7.92 (d, J = 8.7 Hz, 1H, Ar-H), 8.12 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.14 (CH_2-pip), 25.63(C-$(CH_2)_2$-pip), 46.44 (N$(CH_2)_2$-morph), 50.04 (N$(CH_2)_2$-pip), 65.73 (O$(CH_2)_2$), 128.09, 128.14, 128.31, 132.06, 136.40, 142.54, 143.20 (Cl−C═N), 154.04 (*N*−C═N); Anal. Calcd. for $C_{17}H_{21}ClN_4O_3S$ (396.89): C, 51.45; H, 5.33; N, 14.12; Found: C, 51.41; H, 5.24; N, 14.03.

4.1.12. 3-Chloro-2-(4-methylpiperazin-1-yl)-6-morpholinosulfonylquinoxaline (7b):

As shiny light brown crystals from acetonitrile/DMF; yield: 76%; m.p.:208–210 °C; IR: ν/cm^{-1}: 3054 (CH-Ar), 2901, 2864 (CH-aliph.), 1610 (C═N), 1345, 1164 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.24 (s, 3H, −N−CH_3), 2.53 (t, 4H, $N^4(CH_2)_2$-piperazine), 2.93 (t, 4H, N$(CH_2)_2$-morph), 3.62 [(t, 8H, O$(CH_2)_2$ + $N^1(CH_2)_2$-piperazine), 7.90 (dd, J = 8.8, 1.9 Hz, 1H, Ar-H), 7.94 (d, J = 8.7 Hz, 1H, Ar-H), 8.13 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 46.44 [(CH_3), ($N^1(CH_2)_2$-piperazine)], 48.60 (N$(CH_2)_2$-morph), 54.35 ($N^4(CH_2)_2$-piperazine), 65.73 (O$(CH_2)_2$), 128.12, 128.33, 128.38, 132.49, 136.61, 142.33, 143.07 (Cl−C═N), 153.68 (*N*−C═N); Anal. Calcd. for $C_{17}H_{22}ClN_5O_3S$ (411.91): C, 49.57; H, 5.38; N, 17.00; Found: C, 49.51; H, 5.34; N, 16.85.

4.1.13. 3-Chloro-2-morpholino-6-morpholinosulfonylquinoxaline (7c)

As yellow crystals from acetonitrile; yield: 60%; m.p.:163–165 °C; IR: ν/cm^{-1}: 3079(CH-Ar), 2964, 2920, 2898, 2859 (CH-aliph.), 1603 (C═N), 1348, 1156 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.92 (t, 4H, N$(CH_2)_2$), 2.98 (t, 4H, N$(CH_2)_2$), 3.64 (t, 4H, O$(CH_2)_2$), 3.80 (t, 4H, O$(CH_2)_2$), 8.16 (dd, J = 8.9,1.9 Hz, 1H, Ar-H), 8.34 (d, J = 8.8 Hz, 1H, Ar-H), 8.42 (s, 1H, Ar-H); Anal. Calcd. for $C_{16}H_{19}ClN_4O_4S$ (398.86): C, 48.18; H, 4.80; N, 14.05; Found: C, 48.13; H, 4.76; N, 14.01.

4.1.14. 2,3-sym.bis(sec-amino)-6-morpholinosulfonylquinoxaline (9a-c):

Method A:

A solution of compound **6** (10 mmol, 3.48 g) and cyclic secondary amines (20 mmol) in 30 mL of acetonitrile was refluxed for 8 h. Then, the solvent was evaporated under reduced pressure. The resulting precipitate was filtered, dried and recrystallized from the proper solvent.

Method B:

A mixture of compound **7a-c** (10 mmol) and the same secondary amines (10 mmol) in 30 mL of acetonitrile was refluxed for 6 h. After the reaction time, the solvent was evaporated, and the resulting precipitate was washed with H_2O, filtered, dried and recrystallized from the proper solvent.

4.1.15. 6-morpholinosulfonyl-2,3-bispiperidinoquinoxaline (9a)

As pale yellow crystals from dioxane; yield: 70%; m.p.:173–175 °C; IR: ν/cm^{-1}: 3061(CH-Ar), 2965, 2921, 2859 (CH-aliph.), 1637 (C═N), 1348, 1162 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.52 (m, 6H, 3(CH_2)-pip), 1.64 (m, 6H, 3(CH_2)-pip), 2.95 (t, 4H, N$(CH_2)_2$-morph), 3.45 (t, 4H, N$(CH_2)_2$-pip), 3.54 (t, 4H, N$(CH_2)_2$-pip), 3.61 (t, 4H, O

($CH_2)_2$), 7.55 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.71 (d, J = 8.5 Hz, 1H, Ar-H), 7.81 (s, 1H, Ar-H), ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.50 (2(CH_2)-pip), 25.65 (2($CH_2)_2$-pip), 25.70 (2($CH_2)_2$-pip), 46.46 (N($CH_2)_2$-morph), 47.72 (N($CH_2)_2$-pip), 47.81 (N($CH_2)_2$-pip), 65.75 (O($CH_2)_2$), 123.77, 126.05, 126.85, 130.38, 136.54, 140.25, 149.11 (C═N), 149.53 (*N*-C═N); Anal. Calcd. for $C_{22}H_{31}N_5O_3S$ (445.58): C, 59.30; H, 7.01; N, 15.72; Found: C, 59.23; H, 6.92; N, 15.59.

4.1.16. 2,3-bis(4-methylpiperazine-1-yl)-6-morpholinosulfonylquinoxaline (9b)

As pale yellow powder from acetonitrile; yield: 61%; m.p.:148–150 °C; IR: ν/cm^{-1}: 3059(CH-Ar), 2906, 2853 (CH-aliph.), 1603 (C═N), 1354, 1156 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.22 (s, 6H, 2(CH_3)), 2.48 (t, 8H, 2[N^4($CH_2)_2$-piperazine]), 2.89 (t, 4H, N($CH_2)_2$-morph), 3.48 (t, 4H, N^1($CH_2)_2$-piperazine), 3.55 (t, 4H, N^1($CH_2)_2$-piperazine), 3.62 (t, 4H, O($CH_2)_2$), 7.60 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.75 (d, J = 8.6 Hz, 1H, Ar-H), 7.86 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 44.75 (2(CH_3)), 46.15 (N($CH_2)_2$-morph), 46.46 (N^1($CH_2)_2$-piperazine), 46.87 (N^1($CH_2)_2$-piperazine), 54.64 (2[N^4($CH_2)_2$-piperazine]), 65.75 (O($CH_2)_2$), 124.11, 126.28, 127.18, 130.94, 136.59, 140.17, 148.81 (C═N), 149.30 (C═N); Anal. Calcd. for $C_{22}H_{33}N_7O_3S$ (475.61): C, 55.56; H, 6.99; N, 20.62; Found: C, 55.52; H, 6.95; N, 20.78

4.1.17. 2,3-bis(morpholino)-6-morpholinosulfonylquinoxaline (9c)

As yellow crystals from acetonitrile; yield: 70%; m.p.:198–200 °C; IR: ν/cm^{-1}: 3062(CH-Ar), 2947, 2873, 2856 (CH-aliph.), 1620 (C═N), 1344, 1161 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.87 (t, 4H, N($CH_2)_2$), 3.51 (t, 4H, N($CH_2)_2$), 3.58 (t, 4H, N($CH_2)_2$), 3.62 (t, 4H, O($CH_2)_2$), 3.76 (t, 8H, 2O($CH_2)_2$), 7.64 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.78 (d, J = 8.6 Hz, 1H, Ar-H), 7.88 (s, 1H, Ar-H); MS (*m/z*, %): 119 (54%), 151 (100%), 214 (64%), 381 (43%), 447 (M$^+$ − 2, 33%), 449 (M$^+$, 31%); Anal. Calcd. for $C_{20}H_{27}N_5O_5S$ (449.53): C, 53.44; H, 6.05; N, 15.58; Found: C, 53.40; H, 5.97; N, 15.52

4.1.18. 2,3-unsym.bis(sec-amino)-6-morpholinosulfonylquinoxaline (10a-f):

A mixture of compound **7a-c** (10 mmol) and requisite cyclic secondary amine (10 mmol) in 30 mL of acetonitrile was refluxed for 6 h. The reaction mixture was cooled and solid obtained after cooling was filtered, washed with acetonitrile, dried and recrystallized from the proper solvent.

4.1.19. 3-(4-methylpiperazin-1-yl)-2-(piperidin-1-yl)-6-morpholinosulfonylquinoxaline (10a):

As yellow crystals from ethanol/acetonitrile; yield: 80%; m.p.:213–215 °C; IR: ν/cm^{-1}: 3064(CH-Ar), 2957, 2894, 2854 (CH-aliph.), 1617 (C═N), 1344, 1160 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.65 (m, 6H, 3(CH_2)-pip), 2.24 (s, 3H, CH_3), 2.50 (t, 4H, N^4($CH_2)_2$-piperazine), 2.90 (t, 4H, N($CH_2)_2$-morph), 3.50 (t, 4H, N^1($CH_2)_2$-piperazine), 3.55 (t, 4H, N($CH_2)_2$-pip), 3.64 (t, 4H, O($CH_2)_2$), 7.60 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.74 (d, J = 8.6 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H); MS (*m/z*, %): 58 (32%), 73 (32%), 90 (34%), 117 (66%), 144 (100%), 159 (92%), 318 (28%), 460 (M$^+$, 8%); Anal. Calcd. for $C_{22}H_{32}N_6O_3S$ (460.60): C, 57.37; H, 7.00; N, 18.25; Found: C, 57.33; H, 6.94; N, 18.20.

4.1.20. 3-morpholino-2-(piperidin-1-yl)-6-morpholinosulfonylquinoxaline (10b)

As yellow crystals from acetonitrile; yield: 73%; m.p.:113–115 °C; IR: ν/cm^{-1}: 3035 (CH-Ar), 2988, 2958, 2894, 2853 (CH-aliph.), 1628 (C═N), 1344, 1161 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.62 (m, 6H, 3(CH_2)-pip), 2.87 (t, 4H, N($CH_2)_2$-morph), 3.49 (t, 4H, N($CH_2)_2$-morph), 3.55 (t, 4H, O($CH_2)_2$), 3.60 (t, 4H, N($CH_2)_2$-pip), 3.77 (t, 4H, O($CH_2)_2$), 7.59 (dd, J = 8.6, 2.0 Hz, 1H, Ar-H), 7.74 (d, J = 8.6 Hz, 1H, Ar-H), 7.85 (s, 1H, Ar-H), ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.47 ((CH_2) -pip), 25.50 (2(CH_2)-pip), 43.28 (N($CH_2)_2$-pip), 46.44 (N($CH_2)_2$-morph), 47.46 (N($CH_2)_2$-morph), 47.86, 63.72, 65.74 (O($CH_2)_2$), 66.20, 66.79 (O($CH_2)_2$), 124.10, 126.21, 127.03, 130.73, 136.38, 140.38, 148.68 (C═N), 149.52 (C═N); Anal. Calcd. for $C_{21}H_{29}N_5O_4S$ (447.55): C, 56.36; H, 6.53; N, 15.65; Found: C, 56.32; H, 6.49; N, 15.59.

4.1.21. 2-(4-methylpiperazin-1-yl)-3-(piperidin-1-yl)-6-morpholinosulfonylquinoxaline (10c)

As off-white powder from acetonitrile; yield: 75%; m.p.:220–222 °C; IR: ν/cm^{-1}: 3054(CH-Ar), 2920, 2852 (CH-aliph.), 1595 (C═N), 1337, 1160 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.64 (m, 6H, 3(CH_2)-pip), 2.26 (s, 3H, CH_3), 2.48 (t, 4H, N^4($CH_2)_2$-piperazine), 2.89 (t, 4H, N($CH_2)_2$-morph), 2.98 (t, 4H, N^1($CH_2)_2$-piperazine), 3.46 (t, 4H, N($CH_2)_2$-pip), 3.62 (t, 4H, O($CH_2)_2$), 7.58 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.73 (d, J = 8.6 Hz, 1H, Ar-H), 7.84 (s, 1H, Ar-H); MS (*m/z*, %): 42 (34%), 53 (32%), 63 (100%), 75 (58%), 88 (94%), 143 (61%), 460 (M$^+$, 9%); Anal. Calcd. for $C_{22}H_{32}N_6O_3S$ (460.60): C, 57.37; H, 7.00; N, 18.25; Found: C, 57.34; H, 6.98; N, 18.20.

4.1.22. 2-(4-methylpiperazin-1-yl)-3-morpholino-6-morpholinosulfonylquinoxaline (10d)

As off-white needles from acetonitrile; yield: 76%; m.p.:140–142 °C; IR: ν/cm^{-1}: 3048(CH-Ar), 2918, 2862 (CH-aliph.), 1610 (C═N), 1349, 1165 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.27 (s, 3H, CH_3), 2.54 (t, 4H, N^4($CH_2)_2$-piperazine), 2.91 (t, 4H, N($CH_2)_2$-morph), 3.51 (t, 4H, N($CH_2)_2$-morph), 3.64 (t, 8H, 2[O($CH_2)_2$]), 3.80 (t, 4H, N^1($CH_2)_2$-piperazine), 7.63 (dd, J = 8.6, 2.1 Hz, 1H, Ar-H), 7.78 (d, J = 8.6 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 43.56 (-N-CH_3), 45.87, 46.43 (N^1($CH_2)_2$-piperazine), 46.66 (N($CH_2)_2$-morph), 47.54 (N($CH_2)_2$-morph), 54.37 (N^4($CH_2)_2$-piperazine), 65.73 (O($CH_2)_2$), 66.08 (O($CH_2)_2$), 124.21, 126.31, 127.22, 131.10, 136.54, 140.19, 148.68 (C═N), 149.20 (C═N); Anal. Calcd. for $C_{21}H_{30}N_6O_4S$ (462.57): C, 54.53; H, 6.54; N, 18.17; Found: C, 54.46; H, 6.47; N, 18.11.

4.1.23. 2-Mopholino-6-morpholinosulfonyl-3-piperidinoquinoxaline (10e)

As yellow crystals from acetonitrile; yield: 80%; m.p.:128–130 °C; IR: ν/cm^{-1}: 3048(CH-Ar), 2918, 2862 (CH-aliph.), 1610 (C═N), 1349, 1165 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.62 (m, 6H, 3(CH_2)-pip), 2.98 (t, 4H, N($CH_2)_2$-morph), 3.45 (t, 4H, N($CH_2)_2$-morph), 3.53(t, 4H, N($CH_2)_2$-pip), 3.64 (t, 4H, O($CH_2)_2$), 3.80 (t, 4H, O($CH_2)_2$), 7.61 (dd, J = 8.6, 2.0 Hz, 1H, Ar-H), 7.77 (d, J = 8.6 Hz, 1H, Ar-H), 7.87 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.48, 25.47, 25.70 (C-($CH_2)_3$-pip), 46.47 (N($CH_2)_2$-morph), 47.40, 47.72 (N($CH_2)_2$-morph), 47.81, 47.95 (N($CH_2)_2$-pip), 65.75 (O($CH_2)_2$), 66.24 (O($CH_2)_2$), 123.94, 126.20, 127.13, 130.92, 136.79, 140.04, 149.17 (C═N), 149.22 (C═N); Anal. Calcd. for $C_{21}H_{29}N_5O_4S$ (447.55): C, 56.36; H, 6.53; N, 15.65; Found: C, 56.30; H, 6.57; N, 15.71

4.1.24. 3-(4-Methylpiperazin-1-yl)-2-morpholino-6-morpholinosulfonylquinoxaline (10f)

As yellow crystals from ethanol; yield: 77%; m.p.:180–182 °C; IR: ν/cm^{-1}: 3042(CH-Ar), 2959, 2920, 2854 (CH-aliph.), 1599 (C═N), 1350, 1152 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.24 (s, 3H, -N-CH_3), 2.51 (t, 4H, N^4($CH_2)_2$-piperazine), 2.93 (t, 4H, N($CH_2)_2$-morph), 3.47 (t, 4H, N($CH_2)_2$-morph), 3.56(t, 4H, N^1($CH_2)_2$-piperazine), 3.64 (t, 4H, O($CH_2)_2$), 3.78(t, 4H, O($CH_2)_2$), 7.63 (dd, J = 8.5, 2.0 Hz, 1H, Ar-H), 7.81 (d, J = 8.6 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 46.13 (-N-CH_3), 46.46 (N($CH_2)_2$-morph), 46.92 (N^1($CH_2)_2$-piperazine), 47.49 (N($CH_2)_2$-morph), 54.54 (N^4($CH_2)_2$-piperazine), 65.75 (O($CH_2)_2$), 66.12 (O($CH_2)_2$), 124.17, 126.32, 127.27, 131.10, 136.69, 140.12, 148.81 (C═N), 149.19 (C═N); Anal. Calcd. for $C_{21}H_{30}N_6O_4S$ (462.57): C, 54.53; H, 6.54; N, 18.17; Found: C, 54.48; H, 6.50; N, 18.12

4.1.25. 3-Hydrazino-2-sec.amino-6-morpholinosulfonylquinoxalines (11a-c):

To a mixture of compound **7a-c** (10 mmol), hydrazine hydrate (20 mmol), 25 mL of acetonitrile was added and refluxed for 6 h. After the reaction time, the solvent was evaporated under reduced pressure, and the resultant solid was washed with H_2O and dried. The obtained precipitate was recrystallized from the proper solvent.

4.1.26. -Hydrazino-2-piperidino-6-morpholinosulfonylquinoxaline (11a)

As yellow crystals from acetonitrile; yield: 85%; m.p.: 280–282 °C; IR: ν/cm^{-1}: 3317, 3201 (NH_2, NH), 3070(CH-Ar), 2931, 2854 (CH-aliph.), 1608 (C═N), 1346, 1161 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.55–1.71 (m, 6H, 3(CH_2)-pip), 2.86 (t, 4H, N(CH_2)$_2$-morph), 3.64(t, 4H, N(CH_2)$_2$-pip), 3.88 (t, 4H, O(CH_2)$_2$), 4.63 (s, 2H, NH_2 exchangeable with D_2O), 7.10 (d, J = 8.2 Hz, 1H, Ar-H), 7.35 (s, 1H, Ar-H), 7.72 (d, J = 8.8 Hz, 1H, Ar-H), 8.34 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.93 ((CH_2) -pip), 26.32 (2(CH_2) -pip), 46.42 (N(CH_2)$_2$-morph), 48.15 (N(CH_2)$_2$-pip), 65.77 (O(CH_2)$_2$), 111.80, 120.37, 124.22, 127.64, 130.99, 131.88, 137.45, 152.71(C═N); Anal. Calcd. for $C_{17}H_{24}N_6O_3S$ (392.48): C, 52.03; H, 6.16; N, 21.41; Found: C, 51.97; H, 6.11; N, 21.38

4.1.27. 3-Hydrazino-2-(4-methylpiperazine)-6-morpholinosulfonylquinoxaline (11b)

As pale yellow crystals from acetonitrile; yield: 65%; m.p.: 265–267 °C; IR: ν/cm^{-1}: 3338, 3228 (NH_2, NH), 3068(CH-Ar), 2912, 2864 (CH-aliph.), 1601 (C═N), 1353, 1158 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.22 (s, 3H, -N-CH_3), 2.49 (t, 4H, N^4(CH_2)$_2$-piperazine), 2.93 (t, 4H, N(CH_2)$_2$), 3.04 (t, 4H, N^1(CH_2)$_2$-piperazine), 3.63 (t, 4H, O(CH_2)$_2$), 4.39 (s, 2H, NH_2 exchangeable with D_2O), 5.70 (s, 1H, NH exchangeable with D_2O), 7.73 (d, 2H, Ar-H), 8.27 (s, 1H, Ar-H); MS (*m/z*, %): 41 (59%), 55 (100%), 69 (53%), 83 (85%), 136 (82%), 143 (51%), 194 (52%), 245 (57%), 258 (60%), 262 (53%), 387 (54%), 406 (M^+-1, 28%); Anal. Calcd. for $C_{17}H_{25}N_7O_3S$ (407.49): C, 50.11; H, 6.18; N, 24.06; Found: C, 50.05H, 6.14; N, 23.95

4.1.28. 3-Hydrazino-2-(morpholino)-6-morpholinosulfonylquinoxaline (11c)

As yellow crystals from acetonitrile; yield: 72%; m.p.: 273–275 °C; IR: ν/cm^{-1}: 3331, 3211 (NH_2, NH), 3052(CH-Ar), 2969, 2901, 2857 (CH-aliph.), 1594 (C═N), 1340, 1150 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 2.93 (t, 4H, N(CH_2)$_2$), 3.06 (t, 4H, N(CH_2)$_2$), 3.64 (t, 4H, O(CH_2)$_2$), 3.80 (t, 4H, O(CH_2)$_2$), 4.41 (s, 2H, NH_2 exchangeable with D_2O), 5.73 (s, 1H, NH exchangeable with D_2O), 7.76 (d, 2H, Ar-H), 8.29 (s, 1H, Ar-H); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 46.36 (N(CH_2)$_2$), 47.38 (N(CH_2)$_2$), 65.76 (O(CH_2)$_2$), 66.67 (O(CH_2)$_2$), 126.41, 126.60, 126.99, 128.04, 140.90, 147.30, 148.93(C═N); MS (*m/z*, %): 137 (50%), 149 (44%), 171 (67%), 178 (100%), 183 (93%), 216 (49%), 223 (59%), 248 (66%), 178 (100%), 258 (45%), 269 (58%), 283 (55%), 296 (83%), 324 (93%), 359 (62%), 368 (53%), 369 (60%), 374 (43%), 394 (M^+, 21%); Anal. Calcd. for $C_{16}H_{22}N_6O_4S$ (394.45): C, 48.72; H, 5.62; N, 21.31; Found: C, 48.65; H, 5.57; N, 21.26.

4.1.29. 8-(morpholinosulfonyl)-N-phenyl-4-(piperidin-1-yl)-[1,2,4]triazolo[4,3-a]quinoxalin-1-amine (12)

To a mixture of 3-hydrazino quinoxaline derivatives **11a** (10 mmol, 3.92 g) dissolved in (20 mL) of acetonitrile and few drops of TEA, (10 mmol, 1.35 mL) of phenyl isothiocyanate was added drop wise with stirring. The mixture was refluxed for 6 h, then cooled and the resulting solid was filtered, dried and recrystallized from dioxane to give white powder of product **12**.

Yield: 65%; m.p.: 185 °C (deco.); IR: ν/cm^{-1}: 3248 (NH), 3045 (CH-Ar), 2978, 2939, 2881 (CH-aliph), 1612 (C═N), 1374, 1172 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.18–1.20 (m, 6H, 3(CH_2)-pip), 3.02 (t, 4H, N(CH_2)$_2$-morph), 3.07 (t, 4H, N(CH_2)$_2$-pip), 3.56 (t, 4H, O(CH_2)$_2$), 6.90 – 6.94 (m, 1H, Ar-H), 7.27 – 7.31 (m, 2H, Ar-H), 7.39 (d, J = 7.3 Hz, 2H, Ar-H), 7.47 (d, J = 7.5 Hz, 1H, Ar-H), 7.50 (s, 1H, Ar-H), 7.61 (d, J = 7.9 Hz, 1H, Ar-H), 10.70 (s, 1H, NH, exchangeable with D_2O); Anal. Calcd. for $C_{24}H_{27}N_7O_3S$ (493.59): C, 58.40; H, 5.51; N, 19.86; Found: C, 58.34; H, 5.45; N, 19.82.

4.1.30. 4-((3-(2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)hydrazinyl)-2-(piperidin-1-yl)-6-morpholinosulfonylquinoxaline (13)

A mixture of 3-hydrazino quinoxaline derivatives **11a** (10 mmol, 3.92 g) and 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (10 mmol, 2.48 g) was refluxed in acetonitrile (20 mL) in presence of few drops of acetic acid for 8 h. The progress of reaction was monitored by TLC. Upon completion of reaction, the reaction mixture was cooled. The product precipitated out was filtered, washed with acetonitrile and purified by recrystallization from acetonitrile to give compound **13** as yellow crystals.

Yield: 57%; mp: 223–225 °C; IR: ν/cm^{-1}: 3417 (NH), 3047 (CH-Ar), 2931, 2850 (CH-aliph), 1597 (C═N), 1350, 1165 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.69 (m, 2H, CH_2-pip), 1.74–1.81 (m, 4H, C-(CH_2)$_2$-pip), 2.93 (t, 4H, N(CH_2)$_2$-morph), 3.27 (t, 4H, N(CH_2)$_2$-pip), 3.63 (t, 4H, O(CH_2)$_2$-morph),7.29 (s, 2H, Ar-H), 7.43–7.45 (m, 3H, Ar-H), 7.50 (t, J = 6.4 Hz, 2H, Ar-H), 7.59 (t, J = 5.0 Hz, 2H, Ar-H), 7.64 (s, 1H), 7.69 (s, 2H, Ar-H), 7.93 (s, 1H), 7.98 (d, J = 7.8 Hz, 1H, Ar-H), 8.26 (s, 1H, Ar-H), 9.04 (s, 1H, NH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 24.58 (CH_2-pip), 26.46 ((CH_2)$_2$-pip), 46.36 (N(CH_2)$_2$-morph), 57.27 (N(CH_2)$_2$-pip), 65.76 (O(CH_2)$_2$-morph), 116.00, 118.82, 119.43, 122.95, 123.66, 126.37, 126.66, 126.74, 126.92, 127.31, 127.81, 129.01, 129.20, 129.40, 130.04, 130.30, 130.75, 131.56, 140.83, 141.32, 146.99(C═N), 147.06(C═N), 148.89(C═N), 150.78 (C═N); MS (*m/z*, %): 45 (100%), 64 (62%), 69 (44%), 80 (48%), 93 (55%), 130 (86%), 301 (43%), 622 (M^+, 13%); Anal. Calcd. For $C_{33}H_{34}N_8O_3S$ (622.75): C, 63.65; H, 5.50; N, 17.99; Found: C, 63.61; H, 5.48; N, 17.94.

4.1.31. 3-(3-Methyl-2,4-dihydro-5H-pyrazol-5-one-1-yl)-6-morpholinosulfonyl-2-piperidinoquino xaline (14)

A solution of 3-hydrazino quinoxaline derivatives **11a** (10 mmol, 3.92 g) and ethyl acetoacetate (10 mmol, 1.3 mL) was heated on a boiling water bath in a fume cupboard for 15 min. with occasional stirring. The heavy syrup was allowed to cool, and 15–20 mL of ether was added and stirred the mixture vigorously to get crystalline the desired product. The precipitate was filtered, washed thoroughly with ether and then recrystallized from ethanol / dioxane to get yellow crystals of (**14**).

Yield: 48%; m.p.: 290–292 °C; IR: ν/cm^{-1}: 3414 (OH), 3070 (CH-Ar), 2924, 2854 (CH-aliph), 1616 (C═N), 1342, 1156 (SO_2); ^{1}H NMR (400 MHz, DMSO) δ/ppm: 1.69 (m, 6H, 3(CH_2)-pip), 2.93 (t, 4H, N(CH_2)$_2$-morph), 3.03 (s, 3H, CH_3), 3.63 (t, 8H, [O(CH_2)$_2$] + [N(CH_2)$_2$-pip]), 7.58 (d, J = 8.6 Hz, 1H, Ar-H), 7.71 (s, 1H, CH), 7.81 (dd, J = 8.6,1.8 Hz, 1H, Ar-H), 8.25 (s, 1H, Ar-H), 12.41 (s, 1H, OH exchangeable with D_2O); ^{13}C NMR (101 MHz, DMSO) δ/ppm: 14.17 (CH_3), 23.63 (CH_2-pip), 25.37 ((CH_2)$_2$-pip), 45.40 (N(CH_2)$_2$-morph), 64.80 (N(CH_2)$_2$-pip), 65.76 (O(CH_2)$_2$-morph), 115.05, 122.72, 125.41, 126.37, 127.69, 132.72, 139.88, 146.04, 147.95, 149.05, 151.31(═C−OH); MS (*m/z*, %): 43 (92%), 105 (100%), 135 (83%), 178 (53%), 202 (47%), 350 (43%), 439 (41%), 458 (M^+, 21%), 461 (M^+ +3, 48%); Anal. Calcd. for $C_{21}H_{26}N_6O_4S$ (458.54): C, 55.01; H, 5.72; N, 18.33; Found: C, 54.96; H, 5.68; N, 18.30.

4.2. Biological activity

4.2.1. Antimicrobial activity

Gram-positive (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212), Gram-negative (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 6539), *Candida albicans* (ATCC 10231) and *Fusarium oxysporum* (RCMB 008002) were used in our *In vitro*

antimicrobial activity evaluations at the bacteriology laboratory, Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. The antimicrobial potential of newly synthesized organic compounds was investigated towards the tested microorganisms and expressed as the diameter of the inhibition zones according to the agar plate diffusion method [55]. Briefly, 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5 cells/mL for fungi. One mL of each sample (at 0.5 mg/mL) was added to each well (10 mm diameter holes cut in the agar gel). The plates were incubated for 24 h at 37 °C (for bacteria and yeast) and for 72 h at 27 °C (for filamentous fungi), each test was repeated three times. After incubation, the microorganism's growth was observed. Tetracycline was used as standard antibacterial drugs while Amphotericin B was used as standard antifungal drug. The resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. Solvent controls (DMSO) were included in every experiment as negative control. DMSO was used for dissolving the tested compounds and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms.

4.2.2. Evaluating the minimal inhibition concentration (MIC)

A conventional technique termed paper disk diffusion was used to investigate the MIC of the active compounds [56,60–62] through employing a 12.7 mm diameter filter paper (Whatman, Germany). Bacteria were grown in a media of nutrient agar, while fungi and yeasts were grown in a media of Sabouraud́s agar. The synthesized compounds were dissolved and loaded on paper disks with different concentrations. Loading the drying disks over the agar plates' surface inoculated with the selected microorganisms was carried out, then growth inhibition was tested when incubated (at 37 °C for a day) for the bacterial strains and yeasts and fungi (at 27 °C for three days); Also, MIC confirmed by using the broth micro-dilution procedure described in the Clinical and Laboratory Standards Institute (CLSI) guidelines [63]. Where repeating of every experiment for three-times was performed for reproducibility.

4.2.3. Multidrug resistant bacteria (MDRB) assay

The test microorganisms that were used in the present study are multidrug resistant Gram-positive Strains, (Staphylococcus aureus ATCC 43300 and Staphylococcus aureus ATCC 33591), multidrug resistant Gram-negative (Escherichia coli ATCC BAA-196 and Pseudomonas aeruginosa ATCC BAA-2111), they were used to define the antimicrobial activity of the newly synthesized organic compounds. Antimicrobial screening and MICs were performed by the same method mention before [55–56,64–65] except we used both Norfloxacin, and Tetracycline as the positive control.

4.2.4. DNA gyrase

The *In vitro* enzyme inhibition determination for the most active compounds, were carried out in the confirmatory diagnostic unit, Vacsera, Egypt. The evaluation performed through profiling of the tested compounds against *S. aureus* DNA gyrase according to the reported method [66].

4.2.5. Studying the activity of the intracellular killing

The activity of the intracellular killing was studied through a nitro-blue tetrazolium (NBT) reduction method through the developed *Baehner and Nathan technique* [67]. In HBSS, the isolated neutrophils and the tested compound were incubated at 37 °C (for half an hour) followed by extracting the blue formazon (reduced dye) through pyridine and the spectrophotometric measurements were carried out at 515 nm. A negative control sample (which includes all the reagents without the neutrophil suspension) was used for comparative studies. Absorbance variation between the negative control and the cell cultures which actively phagocyte latex particles express the index of the neutrophils' intracellular killing activity.

4.3. Molecular docking study

PDB code: 4DUH was obtained from Protein Data Bank. 4DUH represents the crystal structure for a complex between a small molecule and DNA gyrase of E. coli, 24 kDa domain [68]. Refinement process was achieved by removal of water chains, then protonation step and energy minimization were done using Merck Molecular force field (MMff 94x), finally detecting the binding site was attained by Molecular Operating Environment docking tool 10.2008 (MOE). The selected compounds were drawn as 2D by Chem Draw, saved as mol files, retrieved by MOE, protonated and subjected to energy minimization then saved as mdb file to be ready for docking simulation. First, we started with verification step by re-docking the co-crystallized ligand into DNA Gyrase binding site at RMDS value equal 1.44, the docking score energy is −15.77 Kcal/mol. 2D and 3D figures for the verification process are provided in the supplementary data file. After that the saved mdb file for the selected compounds was used for docking simulation into DNA Gyrase binding site, the obtained data are represented in **Table S1** and Figs. 2A-5B, in addition to the other figures that are supplied in supplementary data files.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104164.

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