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Anti-gram-positive and anti-gram-negative pathogen-killing potential of some novel Quinoxaline compounds

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Abstract
Quinoxaline compounds were identified and synthesized based on biological data from multiple critical therapeutic scaffolds accessible from various medicinal chemistry sources. In a single of the current logical research, substituted phenyl-3-hydrazinyl-quinoxaline-2-amines were synthesized, characterized thoroughly by using a few sophisticated spectroscopic instruments which included FT-IR spectroscopy, Mass spectroscopy, and 1H-NMR spectroscopy, along with the CHN Analyzer, and subsequently evaluated for its anti-bacterial potentials against gram-negative microbes (*Escherichia coli*) and gram-positive microbial organisms (Staphylococcus aureus) using the conventional procedure. Sophisticated spectroscopic tools accurately identified the chemistry and structure of the suggested molecule. In an *in-vitro* anti-microbial screening assay, the chemical demonstrated amazing antibacterial activity against *E. coli* and fair bacterial activity against *S. aureus*, albeit with extremely low concentration. The chemical has been demonstrated to be more efficient against Gram-negative organisms. The research revealed multiple possibilities for the development of broad-spectrum antimicrobial medicines against resistant pathogenic strains by probing into an unknown family of quinoxaline compounds.

Keywords: Quinoxaline, *Escherichia coli*, *Staphylococcus aureus*, anti-fungal, anti-bacterial

1. Introduction
In rare circumstances, diazines are fused to benzene rings to form the quinoxaline. Quinoxaline is a heterocycle known as benzopyrazine because it combines benzene and pyrazine rings. The first nitrogen atom is positioned at position 1, while the second is at 4. The pyrazine ring system can be found in the fungal metabolite aspergillic acid and in dihydro form in the luciferin of several beetles, including the firefly. Methoxy pyrazines have a key role in the scent of many fruits and vegetables, including peas, capsicum, peppers, and several wines (Thiruvalluvar, 2007, Lu, *et al*. 2014) [2, 3]. Quinoxalines have a low melting point and exist as solids with 99% purity. It is miscible with water and has a melting point of 29-30 °C. It is a weekly basic (Pka - 0.56).
Microbes are omnipresent. The relationship between people and such microbes is usually positive, as seen by the microbial flora in the human stomach and skin. Despite this, the tissues of healthy animals and plants are essentially microbe-free. This is accomplished by the provision of both non-specific and specialized defensive mechanisms. Microbial infections can be established when germs violate these barriers by expressing virulence factors and adapting to a pathogenic way of life, or after sickness, unintentional trauma, or implantation of medical equipment. An infection is the invasion of a host organism by microorganisms, the growth of the invading organisms, and the host's reaction to those invaders, whereas an infectious illness is an infection that results in the emergence of clinical symptoms. Microorganisms commonly enter the body through the skin, respiratory system, digestive tract, urinogenital tract, and conjunctiva. Antibiotics are effective against many germs, and most infectious disorders. Despite medical advancements over the previous century, infectious illnesses continue to be the second greatest cause of mortality. Only six lethal infectious illnesses - pneumonia, TB, diarrheal disorders, malaria, measles, and, more recently, HIV/AIDS - cause for half of all premature deaths, primarily among children and young people. Every year, between 14 and 17 million people die as a result of infectious illnesses, the vast majority of whom reside in underdeveloped nations.

Since their discovery, antimicrobial medicines have been widely utilized and proved exceptionally successful in the treatment of bacterial illnesses. Antibacterial agents may be either bacteriostatic or bactericidal.

2. Materials and Methods

2.1 Chemicals

S. No.	Name of Materials	Name and Address of Manufacturer
1	Aniline	Qualigens Fine Chemicals, Navi Mumbai
2	Sodium bicarbonate	Himedia (Mumbai)
3	NaOH	Qualigens Fine Chemicals, Navi Mumbai
4	Hydrochloric Acid.	Qualigens Fine Chemicals, Navi Mumbai
5	Ethanol	Spectrochem pvt. ltd. Mumbai (India)
6	Chloroform	Spectrochem pvt. ltd. Mumbai (India)
7	Mathenol	Spectrochem pvt. ltd. Mumbai (India)
8	Ether	Himedia Laboratories pvt. ltd. Mumbai
9	Benzene	Central Drug House (P) ltd, New Delhi
10	Hydrazine hydrate	Himedia (Mumbai)
11	Glacial acetic acid	Qualigens Fine Chemicals, Navi Mumbai
12	Acetophenone	Qualigens Fine Chemicals, Navi Mumbai
13	Silica Gel G	Himedia (Mumbai)
14	Dimethyl Sulphoxide (DMSO)	Himedia (Mumbai)

2.2 Instrumentation

S. No.	Name of Instruments	Name and Address of Manufacturer
1.	Digital Balance	Parkar TH 500, India
2.	Digital Melting Point Apparatus	Jyoti Scientific Industries, India
3.	Electric Water Bath	Jyoti Scientific Industries, India
4.	Fourier Transform Infrared Spectrophotometer (FTIR-RXI)	Bruker, Japan
5.	^{1}H-NMR Spectrometer (AVANCE II-400)	Bruker, Japan
6.	Heating Mantle	Labtech Sunbim, India
7.	Hot Air Oven	Universal hot air oven
8.	Magnetic Stirrer	Remi 2 MLH
9.	pH Meter	Simtronics digital pH meter

2.3 Synthesis

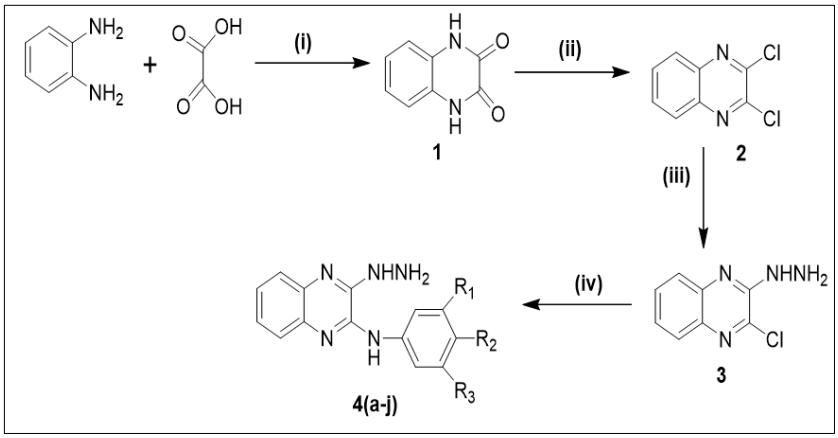
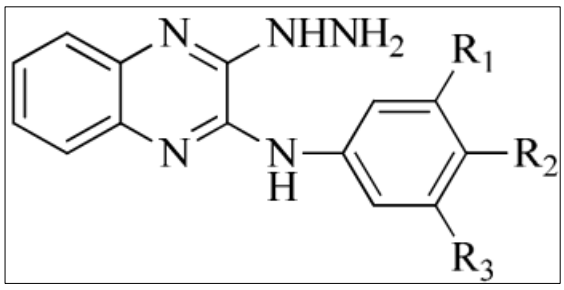


Fig 1: Synthesis of substituted phenyl-3-hydrazinyl-quinoxalin-2-amine derivatives 4(a-j). (i) 4N HCl; Reflux for 1 hr; (ii) $POCl_3$/ $SOCl_2$; DMF; (iii) NH_2-NH_2.H_2O; and (iv) Ethanol, substituted anilines.

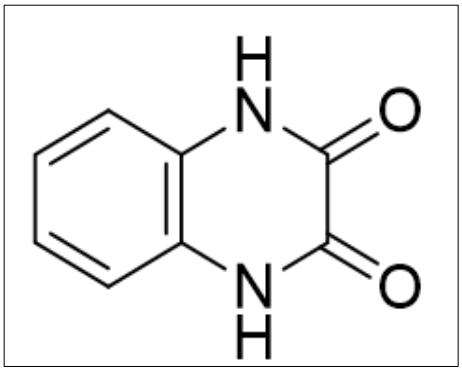
2.3.1 Synthesis of substituted phenyl-3-hydrazinyl-quinoxalin-2-amine derivatives 4(a-j)



Derivatives	R_1	R_2	R_3
4a	H	H	H
4b	O-CH_3	H	O-CH_3
4c	O-CH_3	O-CH_3	O-CH_3
4d	H	O-CH_3	H
4e	H	Cl	H
4f	H	Br	H
4g	H	F	H
4h	H	NO_2	H
4i	Cl	H	H
4j	Cl	Cl	H

2.3.1.1 Synthesis of 1, 4-dihydro quinoxaline-2, 3-dione (1)

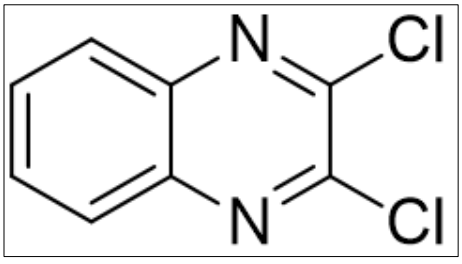
An aqueous solution of oxalic acid dihydrate (0.238 mole, 15 g) and 4.5 mL of concentrated HCl was heated to 100°C before adding O-phenylenediamine (0.204 mole, 11 g) while stirring continuously. The temperature was maintained at 100 °C for 30 minutes. Precipitate was successfully obtained after adding the reaction mixture to the crushed ice. Following filtering, the residue was washed with water, and the resultant product was recrystallized with entirely ethanol. (Romer, 2009) [27].



IR Vmax (KBr, in cm-1): 3123 (Ar-C-H, str.), 1668 (C=O str.), 1568 (C=N str), 3327 (N-H str.); 1HNMR (300 MHz, DMSO-d6, δ): 7.03- 8.02 (4H, m, Ar-C-H), 7.74 (2H, d, -NH of quinoxaline); FAB-MS (m/z): 162 [M+]; Anal. Calcd for C8H16N2O2: C, 59.26; H, 3.73; N, 17.28, O, 19.73. Found: C = 59.23; H = 3.71; N = 17.27; O = 19.70.

2.3.1.2 Synthesis of 2, 3-dichloro quinoxaline (2)

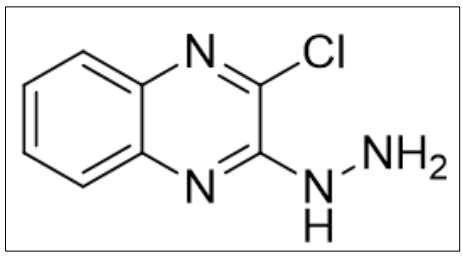
A solution of quinoxaline-2,3-dione (1) (0.1 mole, 8.1 g) and freshly distilled phosphorous oxy tri-chloride (POCl3, 60 mL) was refluxed with N, N-dimethylformamide (DMF, 5 mL) for 2 hours. The resulting reaction mixture was allowed for 30 minutes. The reaction mixture was carefully put into frozen water while stirring. The reddish precipitate was filtered, washed, and recrystallized using a combination of chloroform and n-hexane. (Sarges *et al*., 1990) [28].



IR Vmax (KBr, in cm-1): 3051 (Ar-C-H, str.), 1613 (C=N str), 759 (Cl str); 1H-NMR (300 MHz, DMSO-d6, δ): 7.9-7.0 (4H, m, Ar-C-H); FAB-MS (m/z): 197 [M+], 199 [M+2], 201 [M+4] (9:6:1); Anal. Calcd for C8H4Cl2N2: C, 48.28; H, 2.03; Cl, 35.62, N, 14.07 Found: C, 48.26; H, 2.04; Cl, 35.64, N, 14.10.

2.3.1.3 Synthesis of 2-chloro-3-hydrazinyl quinoxaline (3)

Compound 2 (4.6 g, 0.01 mole) and hydrazine hydrate (0.64 g, 0.01 mole) were dissolved in 100% ethanol and refluxed for 16 hours over a water bath. After the reaction was completed, the fluid was cooled and poured over the crushed ice. The solid product was separated and crystallized, yielding compound 3. (Könnecke, 1978) [29].



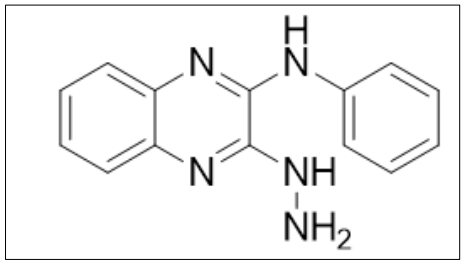
IR Vmax (KBr, in cm-1): 3123 (Ar-C-H, str.), 3367 (N-H str.), 1571 (N-N str.) 766 (Cl str); 1H- NMR (300 MHz, DMSO-d6, δ): 7.63- 7.78 (4H, m, Ar-C-H), 3.84 (1H, s, -NH of hydrazine), 2.13 (2H, s, -NH of hydrazine); FAB-MS (m/z): 194 [M+], 196 [M+2] (3:1); Anal. Calcd for C8H4ClN4: C, 49.37; H, 3.63; Cl, 18.22, N, 28.79 Found: C, 49.34; H, 3.66; Cl, 18.23, N, 28.76.

2.3.2 Synthesis of substituted phenyl-3-hydrazinyl-quinoxaline-2-amine 4(a-j)

Compound 3 (1.94 g, 0.01 mole) and substituted aniline were suspended in ethanol (10 mL) and agitated at reflux for 8 hours. The contents were cooled to room temperature, and the precipitates of the compound were filtered, washed, and recrystallized with alcohol.

2.3.2.1 3-hydrazinyl-*N*-phenylquinoxalin-2-amine 4(a)

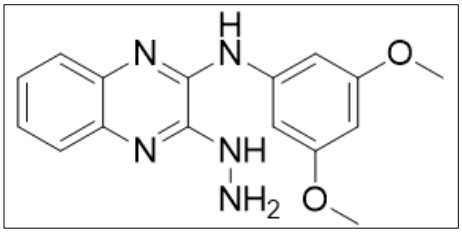
IR Vmax (KBr, in cm-1): 3106 (Ar-C-H, str.), 3166 (N-H str.), 1567 (C=N str.); 1H-NMR (300 MHz, DMSO-d6, δ): 7.74-7.35 (9H, m, Ar-C-H), 3.74 (1H, s-NH of hydrazine), 2.07 (2H, s,-NH of hydrazine), 3.58 (1H, s,-NH of 2˙amine); FAB-MS (m/z): 251 [M+]; Anal. Cal.



2.3.2.2 *N*-(3, 5-dimethoxyphenyl)-3hydrazinylquinoxalin-2-amine 4(b)

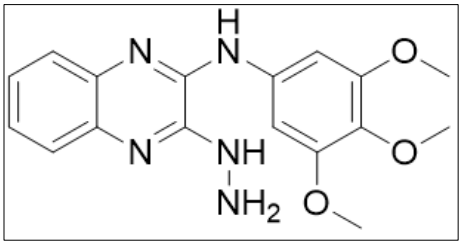
IR Vmax (KBr, in cm-1): 3347 (Ar-C-H, str.), 3318 (N-H str.), 1573 (C=N str), 1035 (C-O-C str.); 1H-NMR (300 MHz, DMSO-d6, δ): 7.74-7.35 (6H, m, Ar-C-H), 3.77 (1H, s,-NH of hydrazine), 2.17 (2H, s,-NH of hydrazine), 3.48 (1H, s,-NH of 2˙amine), 4.17 (6H, s, -OCH3); FAB- MS (m/z): 311 [M+]; Anal. Calcd for C16H17N5O2: C, 61.72;

H, 5.50; N, 22.49; O, 10.28 Found: C, 60.76; H, 5.47; N, 22.48; O, 10.27.



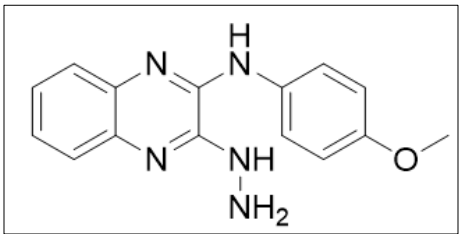
2.3.2.3 3-hydrazinyl-*N*-(3, 4, 5-trimethoxyphenyl) quinoxalin-2-amine 4(c)

IR Vmax (KBr, in cm-1): 3048 (Ar-C-H, str.), 3162 (N-H str.), 1614 (C=N str), 1123 (C-O-C str); 1H-NMR (300 MHz, DMSO-d6, δ): 7.71-7.25 (7H, m, Ar-C-), 3.48 (1H, s -NH of hydrazine), 2.17 (2H, s, -NH of hydrazine), 3.77 (1H, s-NH of 2˙ amine), 4.35 (3H, s, OCH3); FAB- MS (m/z): 281[M+]; Anal. Calcd for C15H15N5O: C, 64.04; H, 5.37; N, 24.90; O, 5.69 Found: C, 64.07; H, 5.37; N, 24.88; O, 5.68IR Vmax (KBr, in cm-1): 3105 (Ar-C-H, str.), 3179 (N-H str.), 1566 (C=N str), 1180 (C-O-C str); 1H-NMR (300 MHz, DMSO-d6, δ): 5.52-5.61 (2H, m, Ar-C-H), 7.65-7.79 (4H, m, -CH of quinoxaline), 3.53 (1H, s-NH of hydrazine), 2.21 (2H, s,-NH of hydrazine), 3.21 (1H, s,-NH of 2˙amine), 3.81 (9H, s, -OCH3); FAB-MS (m/z): 341 [M+]; Anal. Calcd C17H19N5O3: C, 59.81; H, 5.60; N, 20.52; O, 14.06 Found: C, 59.83; H, 5.57; N, 22.54; O, 14.02.



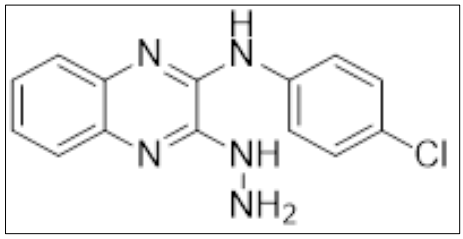
2.3.2.4. 3-hydrazinyl-*N*-(4-methoxyphenyl) quinoxalin-2-amine 4 (d)

R Vmax (KBr, in cm-1): 3048 (Ar-C-H, str.), 3162 (N-H str.),1614 (C=N str), 1123 (C-O-C str); 1H-NMR (300 MHz, DMSO-d6, δ): 7.71-7.25 (7H, m, Ar-C-H), 3.48 (1H, s, -NH of hydrazine), 2.17 (2H, s, -NH of hydrazine), 3.77 (1H, s-NH of 2˙ amine), 4.35 (3H, s, OCH3); FAB- MS (m/z): 281 [M+]; Anal. Calcd for C15H15N5O: C, 64.04; H, 5.37; N, 24.90; O, 5.69 Found: C, 64.07; H, 5.37; N, 24.88; O, 5.68.



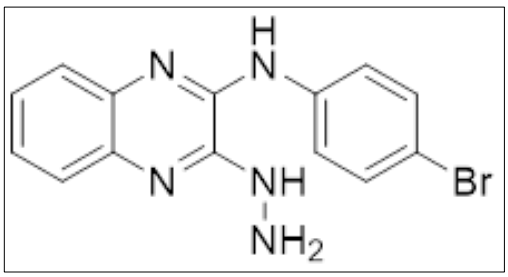
2.3.2.5. *N*-(4-chlorophenyl)-3-hydrazinylquinoxalin-2-amine 4(e)

IR Vmax (KBr, in cm-1): 3048 (Ar-C-H, str.), 3162 (N-H str.),1614 (C=N str), 1123 (C-O-C str); 1H-NMR (300 MHz, DMSO-d6, δ): 7.71-7.25 (7H, m, Ar-C-H), 3.48 (1H, s, -NH of hydrazine), 2.17 (2H, s, -NH of hydrazine), 3.77 (1H, s-NH of 2˙ amine), 4.35 (3H, s, OCH3); FAB-MS (m/z): 281 [M+]; Anal. Calcd for C15H15N5O: C, 64.04; H, 5.37; N, 24.90; O, 5.69 Found: C, 64.07; H, 5.37; N, 24.88; O, 5.68IR Vmax (KBr, in cm-1): 3464 (Ar-C-H, str.), 3314 (N-H str.), 1536 (C=N str), 709 (C-Cl str.); 1H-NMR (300 MHz, DMSO-d6, δ): 7.22-7.66 (4H, m, Ar-C-H), 7.67-7.79 (4H, m, -CH of quinoxaline), 3.70 (1H, s, -NH of hydrazine), 2.07 (2H, s, -NH of hydrazine), 3.53 (1H, s-NH of 2˙ amine); FAB-MS (m/z): 285 [M+]; Anal. Calcd for C14H12ClN5: C, 58.85; H, 4.23; Cl, 12.41; 24.51; Found: C, 88.67; H, 4.27; Cl, 12.42; N, 24.53.



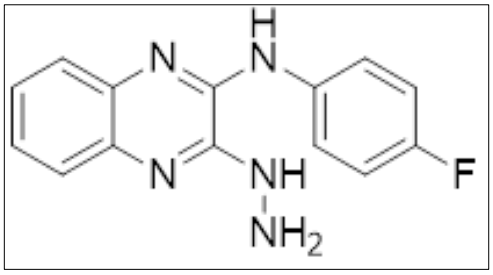
2.3.2.6. *N*-(4-bromophenyl)-3-hydrazinylquinoxalin-2-amine 4(f)

IR Vmax (KBr, in cm-1): 3048 (Ar-C-H, str.), 3162 (N-H str.), 1614 (C=N str), 1123 (C-O-C str); 1H-NMR (300 MHz,DMSO-d6, δ): 7.71-7.25 (7H, m, Ar-C-H), 3.48 (1H, s, -NH of hydrazine), 2.17 (2H, s, -NH of hydrazine), 3.77 (1H, s-NH of 2˙ amine), 4.35 (3H, s, OCH3); FAB- MS (m/z):281 [M+]; Anal. Calcd for C15H15N5O: C, 64.04; H,5.37; N, 24.90; O, 5.69 Found: C, 64.07; H, 5.37; N, 24.88; O, 5.68IR Vmax (KBr, cm-1): 3262 (Ar-C-H, str.), 3339 (N-H str.), 1521 (C=N str.); 1H-NMR (300 MHz, DMSO-d6, δ): 7.02-7.36 (4H, m, Ar-C-H), 3.41 (1H, s, -NH of hydrazine), 2.0 (2H, s, -NH of hydrazine), 3.27 (1H, s-NH of 2˙ amine); FAB-MS (m/z): 329 [M+]; Anal. Cal.



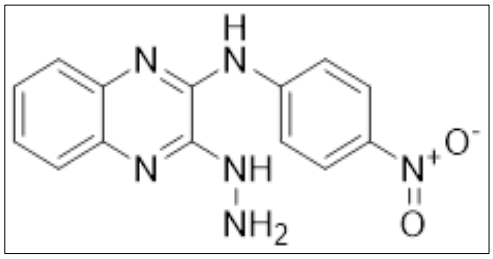
2.3.2.7. *N*-(4-flurophenyl)-3-hydrazinylquinoxalin-2-amine 4(g)

IR Vmax (KBr, in cm^{-1}): 3378 (Ar-C-H, str.), 3327 (N-H str.), 1587 (C=N str); ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 7.31-7.46 (8H, m, Ar-C-H), 3.83 (1H, s, -NH of hydrazine), 1.87 (2H, s, -NH of hydrazine), 3.68 (1H, s-NH of 2˙ amine); FAB-MS (m/z): 269 [M^+]; Anal. Calcd for $C_{14}H_{12}FN_5$: C, 62.44; H, 4.49; F, 7.06; N, 26.01; Found: C, 62.47; H, 4.49; F, 7.07; N, 26.03.



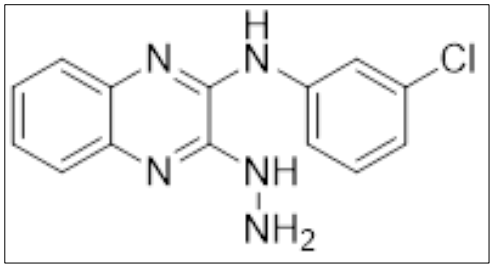
2.3.2.8. 3-hydrazinyl-*N*-(4-nitrophenyl) quinoxalin-2-amine 4(h)

IR Vmax (KBr, in cm^{-1}): 3394 (Ar-C-H, str.), 3317 (N-H str.), 1594 (C=N str), 1372 (NO_2 str); ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 6.81-8.16 (4H, m, Ar-C-H), 7.64-7.81 (4H, m, -CH of quinoxaline), 3.92 (1H, s, -NH of hydrazine), 1.91 (2H, s, -NH of hydrazine), 3.73 (1H, s,-NH of 2˙ amine); FAB-MS (m/z): 296 [M^+]; Anal. Calcd for $C_{14}H_{12}N_6O_2$: C, 56.75; H, 4.09; N, 28.36; O, 10.80 Found: C, 56.78; H, 4.07; N, 28.38; O, 10.76.



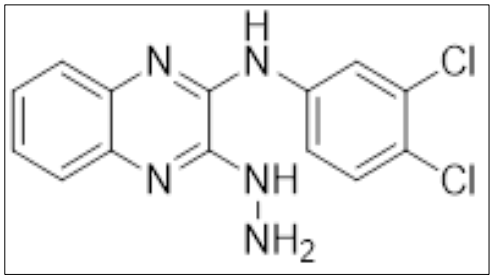
2.3.2.9. *N*-(3-chlorophenyl)-3-hydrazinylquinoxalin-2-amine 4(i)

IR Vmax (KBr, in cm^{-1}): 3430 (Ar-C-H, str.), 3210 (N-H str.), 1526 (C=N str), 767 (C-Cl str.); ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 7.22-7.66 (4H, m, Ar-C-H), 7.67-7.79 (4H, m, -CH of quinoxaline), 3.70 (1H, s, -NH of hydrazine), 2.07 (2H, s, -NH of hydrazine), 3.53 (1H, s,-NH of 2˙ amine); FAB-MS (m/z): 285 [M^+]; Anal. Calcd for $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; Cl, 12.41; 24.51; Found: C, 88.61; H, 4.21; Cl, 12.47; N, 24.51.



2.3.2.10 *N*-(3, 4-dichlorophenyl)-3-hydrazinylquinoxalin-2-amine 4(j)

IR Vmax (KBr, in cm^{-1}): 3466 (Ar-C-H, str.), 3328 (N-H str.), 1537 (C=N str); ^{1}H-NMR (300 MHz, DMSO-d_6, δ): 7.22-7.66 (4H, m, Ar-C-H), 7.67-7.79 (4H, m, -CH of quinoxaline), 3.70 (1H, s, -NH of hydrazine), 2.07 (2H, s, -NH of hydrazine), 3.53 (1H, s,-NH of 2˙ amine); FAB-MS (m/z): 285 [M^+]; Anal. Calcd for $C_{14}H_{12}ClN_5$: C, 58.85; H, 4.23; Cl, 12.41; N, 24.51; Found: C, 88.68; H, 4.27; Cl, 12.41; N, 24.45.



2.4 *In-vitro* antimicrobial screening

2.4.1 Test organisms: Standard drugs cultures of bacteria *Escherichia coli* (ATCC 9637) and *Bacillus subtilis* (ATCC 9372) and fungi *Candida albicans* (ATCC 10231) and *Aspergillus niger* were obtained from the Microbiology Laboratory, NIPRD.

2.4.2 Antibiotic susceptibility testing

Antibacterial activity of novel synthesized quinoxalines derivatives were evaluated by the paper disk diffusion method on Mueller-Hinton agar (MHA) plates (Against bacteria) and Sabroud dextrose agar (SDA) culture media plate (Against fungi). The cultures were adjusted to 0.5 McFarland turbidity standard drugs and inoculated onto MHA plates (15 cm diameter). Sterile filter paper disks (diameter 6 mm) soaked in a known concentration of compounds (100 µg/mL per disk) in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland and 10^6 CFU/mL cultures of bacteria. The cultured plates were then incubated at 37±1 °C for 18 hr. Paper disks soaked in a known concentration (50 µL) of ciprofloxacin (standard bacterial compound) / itraconazole (standard antifungal compound) in distilled water were used as positive control. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disk. For each quinoxalines derivatives, three replicate trials were conducted against each organism.

2.5 Statistical Analysis

All the values are expressed as mean standard drugs error of mean (S.E.M.) and analyzed by one way ANOVA and post hoc Tukey multiple comparison test by employing statistical software, Graph Pad in Stat 3. Differences between groups were considered significant at $p<0.05$ level.

3. Results and Discussion

3.1 Chemistry

Quinoxaline derivatives were synthesized according to the principle 4(a-j) (Figure 1). 2, 3-Dihydroxyquinoxaline (1) is prepared by treating 1, 2-diaminobenzene with oxalic acid in aqueous hydrochloric acid solution. Chlorination of 2,3-dihydroxyquinoxaline (1) yields dichloroquinoxaline (2). The resulting compound (2) is treated with hydrazine hydrate to obtain 3-hydrazino compound (3). Finally, the substituted aniline reacts with compound (3) and refluxes in ethanol to yield compound 4(a-j).

Table 1: Physical characterization of compounds 1, 2, 3, 4(a-j).

Compound	M. P. (ºC)	Yield (%)	Colour	Rf
1	360-362	80	Gray	0.8
2	150-152	70	brick red	0.7
3	294-296	75	Yellow	0.8
4a	198-200	68	Brown	0.7
4b	187-190	72	faint gray	0.8
4c	202-204	64	light gray	0.8
4d	192-184	72	dark brown	0.7
4e	204-206	67	light yellow	0.8
4f	200-202	64	Yellow	0.8
4g	193-195	69	light brown	0.6
4h	189-191	73	creamy white	0.8
4i	198-201	64	light yellow	0.7
4j	191-193	70	Yellow	0.8

All compounds were determined by IR, 1H-NMR and mass spectrometry. The infrared spectra of all synthesized compounds showed some characteristic peaks indicating the presence of groups. In the infrared spectrum of the compound, the presence of absorption bands of the C=O group and NH of quinoxaline at 1650 cm-1 and 3226 cm-1 further confirms the formation of compound 1. 7.03-8.02 ppm due to the four aromatic protons and 7.74 ppm due to the NH proton of the quinoxaline ring. The disappearance of the strong absorption peak of C=O and the appearance of the absorption peak at 730 cm-1 due to C-Cl confirmed the formation of compound 2. It was therefore confirmed that the hydrazine-forming N-H group of compound 3 was known. The appearance of an absorption peak at 3367 cm-1 with other peaks occurring at 3.84 ppm (one proton) and 2.13 ppm (two protons) in the 1H-NMR spectrum. The presence of peaks at 3062-2910 cm-1 in transmission spectrum 4(a-j) confirms the presence of aromatic compounds (Ar C-H). The C-H multiple peak near 6.29-7.69 ppm of the aromatic ring indicates protons of the aromatic ring. A peak around 10.21 ppm and two single peaks around 3.93 ppm and 4.60 ppm are protons of the secondary and primary amines in quinoxaline. Compound 4a shows the same peak as compound 3 and some additional peaks at 6.18-7.51 ppm due to aromatic protons and a singlet at 3.94 ppm assigned to the N-H of the secondary amine. Similarly, compound 4(b-j) shows peaks in the same region as 4a; In addition, the compound shows an IR peak at 1035 cm-1 (symmetric str. 1) and some additional peaks around 3.78 ppm (OCH3). While the values of (C-O-C), (C-OCH3) at 1175 cm-1 (sem. str. C-O-C) and 1121 cm-1 (sem. str. C-O-C) confirm the formation of 4b, 4c, 4d, compounds 4e and 4f are The peak at 1356 cm-1 is marked by the -OCH3 group and compound 4h (sem. throat infrared spectrum of Ar-NO2). The molecular weight of the synthesized compounds was confirmed by GC-MS. The molecular ion peaks of compounds 2 and 4e [M+] can be clearly seen at m/z 263 and 428, which ultimately helps predict the molecular structure of compounds such as C11H9N3O5 and C20H20N4O7, respectively.

3.2 Antimicrobial activity

Ciprofloxacin and ketoconazole derivatives were used as standard drugs to study their antibacterial and antifungal properties. Ciprofloxacin showed the best activity against Staphylococcus aureus. The inhibition ranges were 19 mm, 17 mm, 20 mm and 20 mm against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and E. coli, while ketoconazole showed the highest resistance against M. nigra and E. coli. The inhibition zone of C. albicans was 22 mm and 22 mm (Table 2 and Figures 2 - 4).

Table 2: Antibacterial and antifungal activity of synthesized compounds (4a-4j).

Compound Code	Zone of inhibition in mm					
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***	***A. Niger***	***C. albinos***
4a	09	09	08	10	10	12
4b	10	10	08	11	15	16
4c	13	14	10	12	19	16
4d	12	14	13	12	16	15
4e	18	16	19	16	19	17
4f	12	13	15	12	14	13
4g	15	15	16	15	11	13
4h	11	13	12	11	09	12
4i	16	15	16	15	16	16
4j	12	14	13	12	14	15
Control	00	00	00	00	00	00
Ciprofloxacin	19	17	20	20	-	-
Ketoconazole	-	-	-	-	22	22

Note: The zone of inhibition was measured in mm from the one end to another end of inhibition zone at three different diagonals and the average value is recorded. '-' denotes no activity, 6-11 mm poor activity, 12-15 mm moderate activity, 16-19 mm and above good activity.

According to the information obtained from the use of antibiotics, all of the synthesized compounds showed very little antibacterial activity. It has good anti-inflammatory activity. Among the synthesized compounds, compounds 4e, 4g and 4i showed good antibacterial activity (>14mm inhibition zone), and compounds 4c, 4f and 4j showed moderate activity (10-13 mm inhibition zone). Antifungal activity, compounds 4e, 4c, and 4i showed good antifungal activity, and compounds 4b, 4d, and 4j showed moderate antifungal activity. Overall, compound 4e is the best anti-bacterial and anti-fungal agent.

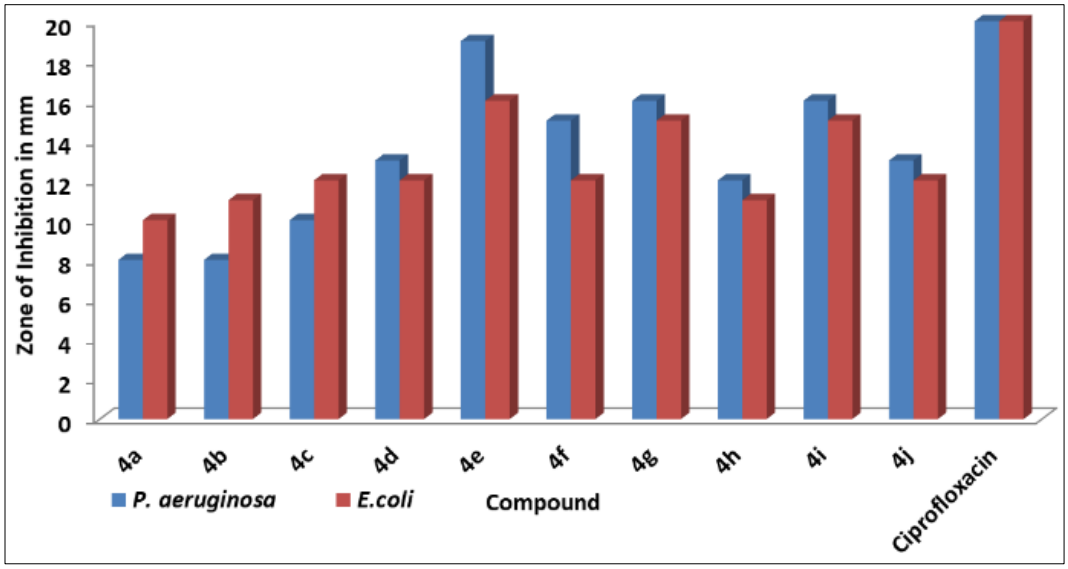


Fig 2: Representation of zone of inhibition of the derivatives against Gram negative organisms.

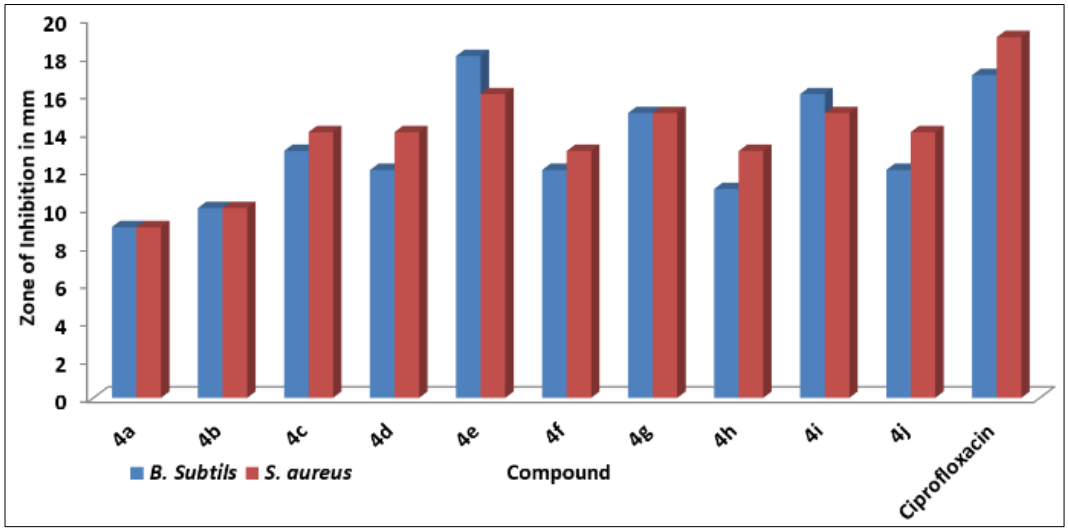


Fig 3: Representation of zone of inhibition of the derivatives against Gram positive organisms.

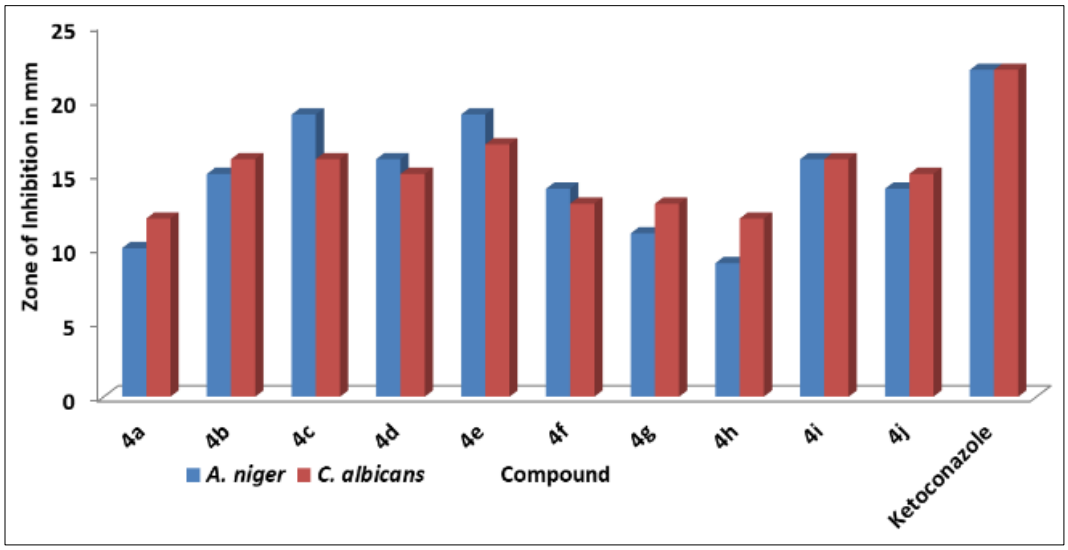


Fig 4: Representation of zone of inhibition of the derivatives against fungal organisms.

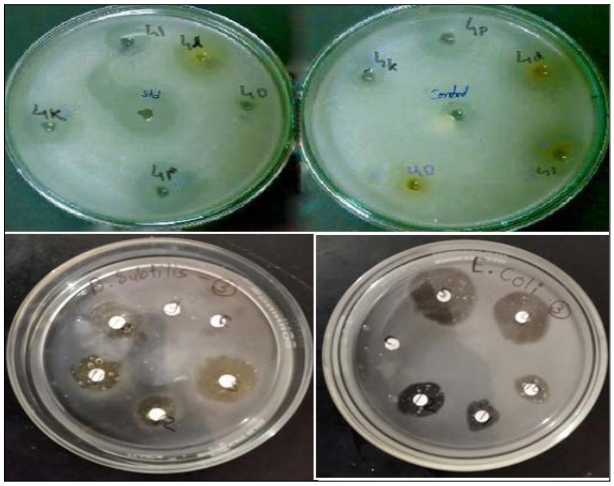


Fig 5: Zone of inhibition of standard drugs and test compounds.

However, further research into the activities and classes of these drugs are known to have anti-inflammatory potential. The antibacterial properties of synthetic analogues can be further verified by testing them in different models.

4. Conclusion

Novel quinoxaline derivatives are an expanding class of heterocyclic compounds with diverse chemical functions. There are many uses such as antibiotics, antibiotics, antibiotics, antivirals, anti-HIV drugs, antibiotics for pain, and antibiotics. Published pharmaceutical models based on drug formulation also mention the quinoxaline moiety. On this basis, new quinoxaline derivatives have been developed and their antibacterial properties have been tested. Complementary modeling can be an alternative method to help discover new antibiotics compared to chemical models, and these synthetic models offer a good opportunity to discover the ideal precursor for antimicrobial activity.

5. References

1. Krchnak V, Holladay MW, Krchn V. Solid Phase Heterocyclic Chemistry. Chemical. 2002;102(II):61-91.
2. Thiruvalluvar A, Subramanyam M, Butcher RJ, Adhikari AV, Wagle S. 2-Chloro-3-Methyl-Quinoxaline. Acta Crystallographica Section E: Structure Reports Online; c2007, 63(12).
3. Lu X, Fan S, Wu J, Jia X, Wang ZS, Zhou G. Controlling the Charge Transfer in D-A-D Chromophores Based on Pyrazine Derivatives. Journal of Organic Chemistry. 2014;79(14):6480-6489.
4. Finar L. Organic chemistry. 5th ed. Wesley Lowman Ltd; c2006.
5. Bansal RK. Heterocyclic chemistry. 3rd ed. New Age International Pvt. Ltd; c2000.
6. Cai JJ, Zou JP, Pan XQ, Zhang W. Gallium triflate catalyzed synthesis of quinoxaline derivatives. Tetrahedron Lett. 2008;49:7386-7390.
7. Kirubavathy S, Jone R, Velmurugan K, Parameswary K, Chitra S. Synthesis, Characterization and antimicrobial screening of CU(II) and VO(IV) complexes of 2,3-diphenyl quinoxaline. International Journal of recent scientific research. 2014;5(1):289-294.
8. Thakuria HG, Das GP. One-pot efficient green synthesis of 1,4-dihydro-quinoxaline-2,3-dione derivatives. J Chem Sci. 2006;118(5):425-428.
9. Kurasawa Y, Muramatsu M, Yamazaki K. Synthesis of 3-(α-chlorophenyl hydrazono) heteroarylmethy1-2-oxo-1,2 dihydroquinoxalines with antimicrobial Activity. J Heterocyclic Chem. 1986a;23:1387-1390.
10. Kurasawa Y, Muramatsu M, Yamazaki K, Setsuko S. A convenient synthesis and antifungal activity of 1-aryl-1H- and 1-aryl-3-heteroary1-1H-pyrazolo[3,4-b]quinoxalines. J Heterocyclic Chem. 1986b;23:1379-1382.
11. Ibrahim MA. Studies in Vilsmeier-Haack reaction: Part VIII-application to quinoxaline-2-thiones and their antibacterial activities. Indian J Chem. 1991;30B:89-92.
12. Bansal OP, Srinivas JS, Singh PP, Junnarkar AY. Synthesis and pharmacology of some new 2-arylamino-4-oxo [1,3]thiazino [5,6-b] quinoxalines. Indian J Chem. 2003;32B:901-902.
13. Venugopalan BE, Pinto de Souza KM, Sathe DK, Chatterjee NI. Synthesis and antimalarial activity of pyrido [3,2-f] quinoxalines and their Noxides. Indian J Chem. 1999;34B:778-790.
14. Campiani G, Aiello F, Fabbrini M, Morelli E. Quinoxalinyl ethyl pyridyl thioureas (qxpts) as potent non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors. Further SAR studies and identification of a novel orally bioavailable hydrazine-based antiviral agent. J Med Chem. 2001;44:305-350.
15. Lainne E, Seitz JS, William C, Robert R. Synthesis and anti-mycobacterial activity of pyrazine and quinoxaline derivatives. J Med Chem. 2002;45:5604-5606.
16. Chen F, Arthr M, Norris D, Henry H, Das J. Imidazoquinoxalinesrc-family kinase inhibitors: SAR, QSAR, and the discovery of (S)-N-(2-Chloro-6-methylphenyl)-2-(3-methyl-1-piperazinyl)imidazo[1,5-α]pyrazin-6-amine (BMS-279700) as a potent and orally active inhibitor with excellent *in vivo* antiinflammatory activity. J Med Chem. 2004;47:4517-4529.
17. Stuart TH, Lisa P, Juiwanna K, Jennifer P, Kathryn W, Matthew E. Design, synhesis and biological evaluation of analogues of the antitumor agent, 2-{4-[7-chloro-2-quinoxalinyl]phenoxy}propionicacid. J Med Chem. 2001;44:1758-1776.
18. Nagarajan K, Parthasarathy S, Shenoy J. 2,3-Dichloro-6-nitroquinoxaline synthesis of s-triazolo [3, 4-a]quinoxaline. Indian J Chem. 1986;25B:739-740.
19. Parke DR. Synthesis of perhydro-2 (1H)-quinoxalinones and perhydropyrrolo [1, 2-a] quinoxaline-4(5H)-one derivatives. J Heterocyclic Chem. 1987;24:1297.
20. Cohen FL, Tartasky D. Microbial resistance to drug therapy: A review. Am J Infect Control. 1997;25(1):51-63.
21. Bari SB, Mahajan B, Surana SJ. Resistance to Antibiotics: A Challenge in Chemotherapy. Indian J Pharm Educ Res. 2008;42(1):3-11.
22. Wright GD. Bacterial resistance to antibiotics: Enzymatic degradation and modification. Adv Drug Deliv Rev. 2005;57:1451-1470.
23. Block JH, Beale JM. Antifungal Agents. In: Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry. 12th ed. Lippincott Williams and Wilkins; c2010. p. 191-205.
24. Lemke TL, Williams DA, Roche VF, Zito SW. Antibiotics and Antimicrobial agents. In: Foye's

Principles of Medicinal Chemistry. 6th ed. Lippincott Williams and Wilkins; c2008. p. 1028-1083.
25. Sithambaram Karthikeyan M, Jagadesh Prasad D, Poojary B, Subramanya Bhat K. Bioorg. Med. Chem. 2006;14:7482.
26. Pandeya SN, Sriram D, Nath G, Decelercq E. Eur J Pharmacol. 1999;9:25.
27. Romer DR. Synthesis of 2,3-dichloroquinoxalines via Vilsmeier reagent chlorination. J Heterocycl Chem. 2009;46:317-319.
28. Sarges R, Howard HR, Browne RG, Lebel LA, Seymour PA, Koe BK. 4-Amino [1, 2, 4] triazolo [4, 3-a] quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. J Med Chem. 1990;33:2240-2254.
29. Könnecke A, Dörre R, Lippmann E. Synthesen mit Tetrazolo[1,5-a]chinoxalinen. Z Für Chemie. 1978;18(7):257-258.
30. Fang B, Zhou CH, Rao XC. Eur J Med Chem. 2010;45:4388-4398.
31. Khan SA, Yusuf M. Eur J Med Chem. 2009;44:2597-2600.
32. Bala AV, Ramalakshmi N, Sankari E, Arunkumar S. Comprehensive review on versatile pharmacology of quinoxaline derivative. Russ J Bioorg Chem. 2022;48:657-677.
33. Sridevi CH, Balaji K, Naidu A, Sudhakaran R. E-J Chem. 2010;7:234-238.
34. Jorgensen JH, Turnidge JD, Washington JA. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Mannual of Clinical Microbiology, Murray PR, Pfaller MA, Tenover FC, Baron EJ, Yolker RH, Eds. ASM Press, Washington, DC, USA. 7th edition; c1999. p. 1526-1543.