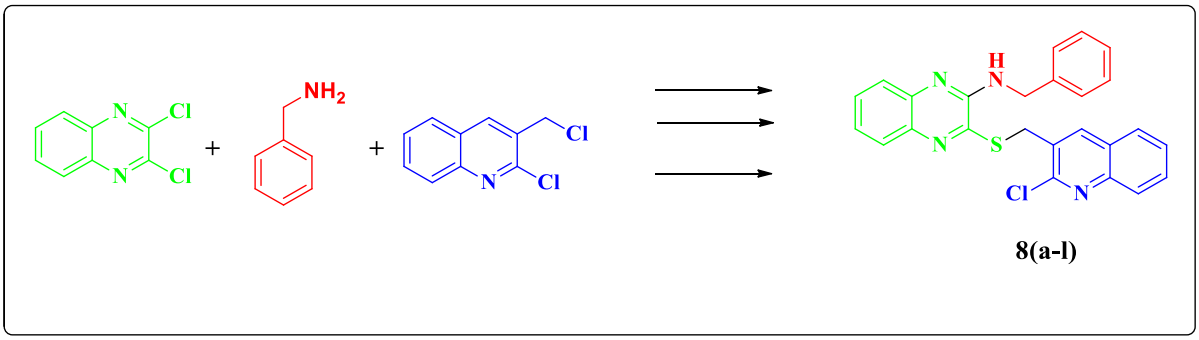
International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 23s, 2025
https://theaspd.com/index.php

Design and Synthesis of N-Benzyl-Quinoxaline Derivatives Linked with Quinoline Moiety for Antimicrobial Activity

Kuntla Krishnaveni[1], Praveen Kumar Rathod[2], Panaganti Leelavathi[2]
[1]Research Scholar, Department of Chemistry, RBVRR women's college, Hyderabad, Telangana-500027.
[2]Department of Chemistry, University College of Science, Osmania University, Hyderabad, Telangana-500007.
*Corresponding author's email ID: leelaou@gmail.com

ABSTRACT
In the present study, a novel series of N-benzyl-quinoxaline derivatives linked with a quinoline moiety through a thioether bridge were designed and synthesized with the objective of exploring their antimicrobial potential. The synthetic strategy involved the condensation of 2,3-dichloroquinoxaline with benzylamine to afford N-benzyl-3-chloroquinoxalin-2-amine, which was subsequently coupled with 2-chloroquinolin-3-ylmethyl intermediates to yield the target compounds. The structures of the synthesized derivatives were confirmed by spectroscopic techniques (^{1}H NMR, ^{13}C NMR). All the compounds were evaluated for their in vitro antimicrobial activity against selected Gram-positive and Gram-negative bacterial strains as well as fungal pathogens using the agar disc diffusion method, with gatifloxacin and clotrimazole as reference drugs. Several derivatives demonstrated promising antibacterial and antifungal activity, with some compounds exhibiting comparable or superior efficacy to the standard drugs. The findings suggest that molecular hybridization of quinoline and quinoxaline frameworks represents a valuable approach in the development of potent antimicrobial agents.

Keywords: *2,3-dichloroquinoxaline, Quinoline, benzylamine, Molecular hybridization, antimicrobial activity.*



Graphical Abstract

INTRODUCTION

Quinoxalines constitute a prominent class of nitrogen-containing heteroaromatic scaffolds with wide-ranging therapeutic potential. The fused benzopyrazine nucleus provides a rigid, electron-rich framework that enables interaction with multiple biological targets, including kinases, oxidoreductases, and nucleic acids.[1-3] Owing to this, quinoxaline derivatives have been widely investigated for their antimicrobial, anticancer, antiviral, anti-inflammatory, antitubercular, and antioxidant activities.[4-7] In medicinal chemistry, they are often regarded as privileged structures capable of generating potent bioactive compounds upon suitable substitution.[8]

Among this family, quinoxaline-2-thiol derivatives have received considerable attention due to the presence of the –SH moiety, which enhances biological interactions through hydrogen bonding, thiol metal coordination, nucleophilic addition, and redox modulation.[9,10] The thiol functionality has also been linked to the improvement of pharmacokinetic properties and target selectivity.[11] Importantly, substitution at the 3-position of the quinoxaline nucleus often leads to significant modulation of biological activity.[12,13] The incorporation of a benzylamino group at position-3 introduces steric bulk, lipophilicity, and electron-donating ability, which may favor enhanced membrane permeability, metabolic stability, and selective binding to hydrophobic enzyme pockets.[14] Previous reports on thio-quinoxaline and benzyl-substituted analogues demonstrate their strong potential as antimicrobial and anticancer agents [15,16] while several benzylated heteroaromatics have displayed potent cytotoxicity against breast, lung, and cervical cancer cell lines.[17,18] These findings strongly suggest that 3-(benzylamino)quinoxaline-

2-thiol may serve as a promising pharmacophore by combining the thiol and benzylamino functionalities in a single scaffold, thereby enabling dual interactions through polar and hydrophobic forces.

Quinoline and its derivatives represent an important class of heteroaromatic compounds with well-documented biological and therapeutic applications.[19–21] The quinoline nucleus is widely found in naturally occurring alkaloids, synthetic drugs, and agrochemicals, and is considered a versatile scaffold in medicinal chemistry owing to its ability to modulate multiple biological targets.[22,23] Notably, the substitution pattern on the quinoline core greatly influences its pharmacological potential, often enhance lipophilicity, metabolic stability, and binding affinity.[24] The 2-chloroquinoline framework has received significant attention for its antimicrobial, anticancer, anti-inflammatory, and antiviral properties.[25,26] The introduction of a chlorine atom at the C-2 position increases electron density on the nitrogen atom, stabilizing interactions with biomolecular targets such as enzymes and DNA.[27] Moreover, the 3-(chloromethyl) substituent introduces a reactive electrophilic site capable of undergoing nucleophilic substitution or alkylation of biological macromolecules, thereby enhancing bioactivity.[28] This structural modification is particularly important in designing hybrid derivatives, as it allows coupling with pharmacophores through $-CH_2Cl$ reactivity, leading to the development of novel antibacterial and anticancer agents.[29]

Several 2-chloro-3-substituted quinoline analogues have been reported as potent antimicrobial agents against Gram-positive and Gram-negative bacteria.[30,31] The chloromethyl functionality has been implicated in improved bacterial cell penetration and disruption of metabolic pathways.[32] In addition, 2-chloro-3-(chloromethyl)quinoline derivatives have demonstrated cytotoxic potential by interfering with DNA topoisomerases and kinase pathways, which are critical in cancer progression.[33] They also display antimalarial activity, as quinoline-based halogenated analogues are known to inhibit heme polymerization in Plasmodium falciparum.[34]

Therefore, 2-chloro-3-(chloromethyl) quinoline serves as an important intermediate as well as a potential pharmacophore in drug discovery. Its dual reactive sites halogen substitution at C-2 and electrophilic chloromethyl group at C-3 make it an attractive scaffold for synthesizing biologically active hybrids.[35] The structural versatility of this compound provides opportunities to design novel therapeutic agents for microbial infections, inflammatory diseases, and cancer treatment.

Taken together, the quinoxaline and quinoline scaffolds remain among the most privileged heterocyclic systems in medicinal chemistry due to their broad spectrum of biological activities and ability to undergo diverse structural modifications. The incorporation of electron-withdrawing halogens and functional groups such as thiols and benzylamines further enhances their pharmacological profiles by improving lipophilicity, metabolic stability, and molecular interactions with biological targets. Consequently, the rational design, synthesis, and evaluation of such derivatives are highly warranted to expand the current library of bioactive heterocycles and to contribute to the ongoing search for new therapeutic agents.

MATERIALS AND METHODS

All chemicals and reagents used in the study were of laboratory grade and procured from Sigma-Aldrich. Melting points of the synthesized compounds were determined in open capillaries using Thiele's melting point apparatus and are reported uncorrected. The progress of reactions was monitored by thin layer chromatography (TLC) performed on silica gel G plates, pre-activated at 120 °C for 30 min. The developed spots were visualized by exposure to iodine vapours. ^{1}H NMR spectra were recorded on 400 MHz NMR spectrometer using DMSO-d_6 as solvent, with tetramethylsilane (TMS) as the internal standard (δ = 0 ppm). The ^{13}C-NMR spectra were recorded on 101 MHz spectrometer in DMSO-d_6.

N-benzyl-3-(((2-chloroquinolin-3-yl) methyl)thio)quinoxalin-2-amine (8a): Pale yellow solid, m.p.205-207 °C, Yield- 80 %. **^{1}H-NMR (400 MHz, DMSO):** δ 8.77 (s, 1H), 8.00 (s, 1H), 7.92 (s, 2H), 7.77 (s, 1H), 7.52 (dd, J = 45.1, 36.8 Hz, 4H), 7.35 (s, 3H), 7.26 (s, 2H), 7.17 (s, 1H), 4.84 (s, 2H), 4.66 (s, 2H); **^{13}C-NMR (101 MHz, DMSO-d_6):** δ 150.2, 148.2, 146.1, 145.5, 140.0, 139.7, 139.2, 136.2 (2C), 130.8 (2C), 129.2 (2C), 128.1 (2C), 127.5 (2C), 126.6 (2C), 125.5 (2C), 124.1, 79.2, 43.8, 31.1.

3-(((2-chloroquinolin-3-yl) methyl)thio)-N-(4-methylbenzyl)quinoxalin-2-amine (8b): Pale yellow solid, m.p.221-223°C, Yield- 81 %. **^{1}H NMR (400 MHz, DMSO-d6):** δ 8.78 (s, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.3 Hz, 1H), 7.81 (t, J = 7.8 Hz, 2H), 7.63 (t, J = 7.5 Hz, 1H), 7.45 – 7.38 (m, 1H), 7.31 (s, 1H), 7.24 (d, J = 7.9 Hz, 3H), 7.06 (d, J = 7.8 Hz, 3H), 4.83 (s, 2H), 4.59 (d, J = 5.8 Hz, 2H), 2.40 (s, 3H); **^{13}C-NMR (101 MHz, DMSO-d_6):** δ 168.4, 151.8, 138.6, 136.0, 131.3, 129.3 (4C), 128.3 (4C), 127.5, 125.9, 125.1, 124.2, 115.7, 79.5 (2C), 59.8, 43.7, 20.8, 14.1.

3-(((2-chloroquinolin-3-yl) methyl)thio)-N-(4-methoxybenzyl)quinoxalin-2-amine (8c): Pale yellow solid, m.p.215-217 °C, Yield 84 %. **^{1}H NMR (400 MHz, DMSO): δ** 8.78 (s, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 7.7 Hz, 2H), 7.92 (t, J = 7.6 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.46 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.4 Hz, 1H), 7.29 (d, J = 8.1 Hz, 2H), 6.83 (d, J = 8.2 Hz, 2H), 4.84 (s, 2H), 4.58 (d, J = 5.6 Hz, 2H), 3.67 (s, 3H); **^{13}C-NMR (101 MHz, DMSO-d_6): δ** 158.2, 150.3, 148.3, 146.2, 145.6, 140.2, 139.4, 136.3, 131.7, 131.0, 129.3, 129.0 (2C), 128.5, 127.9, 127.7, 127.6, 126.9, 125.6, 124.3, 113.6 (2C), 55.1, 43.4, 39.5, 31.2.

3-(((2-chloro-6-methylquinolin-3-yl)methyl)thio)-N-(4-methoxybenzyl)quinoxalin-2-amine (8d): Pale yellow solid, m.p.212-214°C, Yield- 79 %. **^{1}H-NMR (400 MHz, DMS-d_6): δ** 7.74 (s, 1H),7.01 (d, J = 9.0 Hz, 1H), 6.92 (d, J = 8.6 Hz, 1H), 6.84 (s, 1H), 6.70 (d, J = 8.6 Hz, 1H), 6.62 (t, J = 7.6 Hz, 1H), 6.58 – 6.52 (m, 2H), 6.52 – 6.43 (m, 1H), 6.40 (d, J = 8.6 Hz, 2H), 5.92 (d, J = 8.7 Hz, 2H), 3.92 (s, 2H), 3.67 (d, J = 5.8 Hz, 2H), 2.77 (s, 3H), 1.60 (s, 3H); **^{13}C-NMR (126 MHz, DMSO-d_6)**: **δ** 158.1, 149.4, 148.3, 145.6, 144.8, 139.3, 137.5, 136.2, 133.0, 131.8, 129.2 (2C), 129.0, 128.4, 127.3, 126.8, 126.5, 125.6, 124.2 (2C), 113.8 (2C), 55.2 (2C), 43.5, 31.4, 21.1.

3-(((2-chloro-6,7-dimethylquinolin-3-yl)methyl)thio)-N-(4-methoxybenzyl)-7-methyl quinoxalin-2-amine (8e): Pale yellow solid, m.p.201-203°C**°C**, Yield 85 %. **^{1}H-NMR (400 MHz, DMSO-d_6): δ** 7.70 (s, 1H), 7.01 (d, J = 8.0 Hz, 1H), 6.65 (s, 1H), 6.60 (d, J = 8.2 Hz, 1H), 6.56 (t, J = 8.3 Hz, 2H), 6.47 (t, J = 7.5 Hz, 1H), 6.34 (d, J = 7.9 Hz, 2H), 6.16 (d, J = 7.8 Hz, 2H), 3.92 (s, 2H), 3.70 (d, J = 5.8 Hz, 2H), 1.69 (s, 3H), 1.50 (s, 3H), 1.31 (s, 3H); **^{13}C-NMR (101 MHz, DMSO-d_6)**: **δ** 148.3, 148.2, 145.5, 143.9, 139.6, 139.2, 136.9, 136.6, 136.2, 135.6, 135.0, 132.9, 128.9, 128.6 (3C), 128.3 (3C), 127.4, 126.8, 125.5, 124.2, 43.6, 30.9, 21.0, 20.6, 17.2.

3-(((2-chloroquinolin-3-yl)methyl)thio)-N-(cyclohexylmethyl)quinoxalin-2-amine (8f): Pale yellow solid, m.p.235-237 °C, Yield 78 %. **^{1}H-NMR (400 MHz, DMSO-d_6): δ** 7.93 (s, 1H), 7.13 (d, J = 32.9 Hz, 3H), 6.94 (s, 1H), 6.78 (s, 1H), 6.65 (d, J = 30.0 Hz, 2H), 6.51 (s, 1H), 5.96 (s, 1H), 3.99 (s, 2H), 1.67 (s, 2H), 0.83 (s, 6H); **^{13}C-NMR (101 MHz, DMSO-d_6)**: **δ** 150.8, 149.2, 146.7, 146.1, 140.7, 140.0, 136.5, 131.4, 129.8, 128.8, 128.6, 128.4, 128.2, 128.1, 127.3, 126.0, 124.4, 47.4, 37.1, 31.8 (2C), 31.2, 26.7 (2C), 26.0.

3-(((2-chloro-6-methylquinolin-3-yl)methyl)thio)-N-(2-fluorobenzyl)quinoxalin-2-amine (8g): Pale yellow solid, m.p.255-257 °C, Yield 82 %. **^{1}H-NMR (400 MHz, DMSO-d_6): δ** 7.80 (s, 1H), 7.10 (s, 1H), 6.95 (d, J = 7.8 Hz, 1H), 6.90 (s, 1H), 6.74 (s, 2H), 6.62 (s, 2H), 6.60 (s, 1H), 6.54 (s, 1H), 6.38 – 6.24 (m, 2H), 6.15 (s, 1H), 4.07 (s, 2H), 3.80 (s, 2H), 1.60 (s, 3H); **^{13}C-NMR (101 MHz, DMSO-d_6): δ** 150.4, 148.1, 146.3, 145.4, 144.6, 140.1, 139.0, 136.4, 131.0 (3C), 129.7 (3C), 129.2, 128.3, 127.5, 127.0, 125.5, 124.3, 79.0, 78.4, 43.4, 31.1.

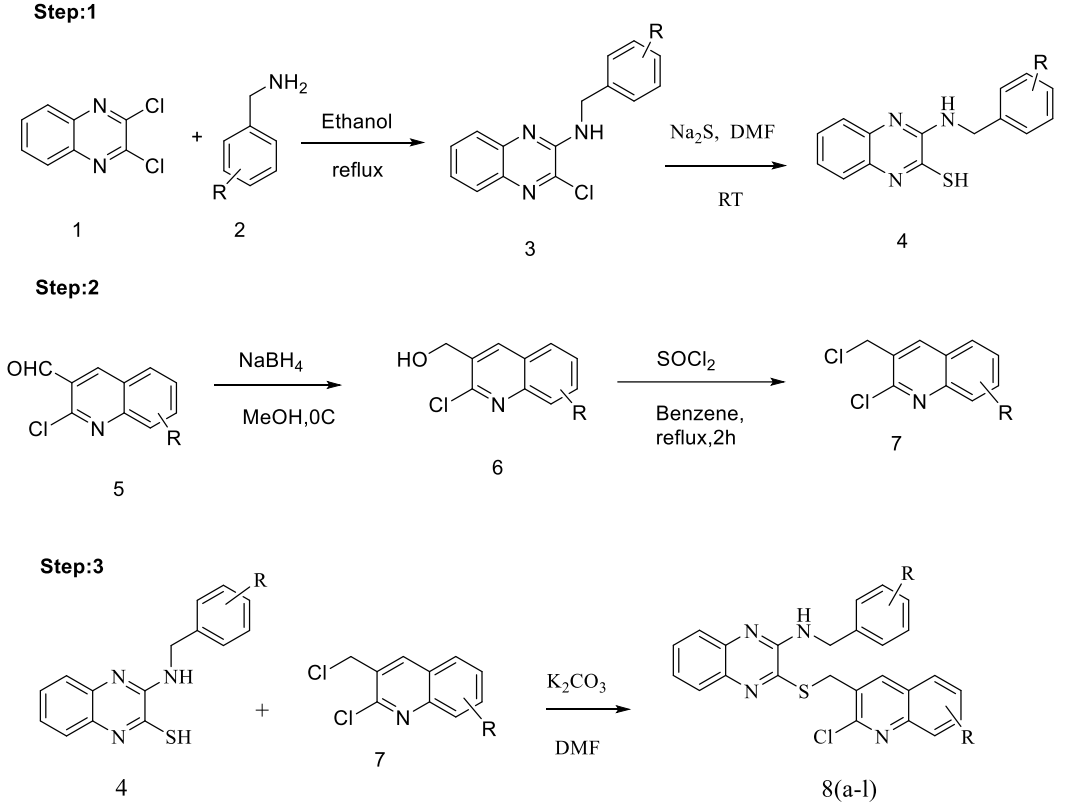
N-(4-bromobenzyl)-3-(((2-chloroquinolin-3-yl)methyl)thio)quinoxalin-2-amine (8h): Pale yellow solid,, m.p.231-233**°C**, Yield 84 %. **^{1}H NMR (400 MHz, DMSO-d_6): δ** 8.00 (s, 1H), 7.19 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 8.1 Hz, 2H), 7.00 (t, J = 7.7 Hz, 2H), 6.80 (dt, J = 11.8, 7.0 Hz, 2H), 6.70 (t, J = 6.7 Hz, 1H), 6.62 (d, J = 8.4 Hz, 1H), 6.60 (t, J = 7.4 Hz, 1H), 6.50 (s, 1H), 6.47(s,1H), 4.03 (s, 2H), 3.78 (d, J = 5.9 Hz, 2H); **^{13}C NMR (101 MHz, DMSO-d_6): δ** 148.1, 140.2, 139.1, 136.4 (2C), 131.0 (4C), 129.7 (4C), 129.2 (2C), 128.3 (2C), 127.6, 126.8, 125.5, 124.3, 79.0, 78.5, 43.4.

N-(4-bromobenzyl)-3-(((2-chloro-6-methylquinolin-yl)methyl)thio)quinoxalin-2-amine(8i): Pale yellow solid,, m.p.215-217**°C**, Yield 80 %. **^{1}H NMR (400 MHz, DMSO-d_6): δ** 7.64 (s, 1H), 6.91 (d, J = 8.1 Hz, 1H), 6.82 (d, J = 8.6 Hz, 1H), 6.74 (s, 1H), 6.65 – 6.55 (m, 2H), 6.49 (t, J = 6.9 Hz, 2H), 6.47 – 6.41 (m, 1H), 6.37 (t, J = 6.5 Hz, 1H), 6.36 (s, 1H), 6.30 (d, J = 8.4 Hz, 2H), 3.82 (s, 2H), 3.60 (d, J = 5.8 Hz, 2H), 1.45 (s, 3H); **^{13}C NMR (101 MHz, DMSO-d_6)**: **δ** 150.4, 148.1, 146.3, 145.4, 144.6, 140.1, 139.0, 136.4, 131.0 (3C), 129.7 (3C), 129.2 (2C), 128.3, 127.5, 127.0, 126.7, 125.5, 124.3, 79.0, 78.4, 43.4, 31.1.

3-(((2-chloroquinolin-3-yl)methyl)thio)-6,7-dimethyl-N-(4-methylbenzyl)quinoxalin-2-amine(8j): Pale yellow solid,, m.p.204-206**°C**, Yield 75 %. **^{1}H NMR (400 MHz, DMSO-d_6)**: **δ** 7.88 (s, 1H), 7.44 (s, 1H), 7.14 (d, J = 7.5 Hz, 1H), 7.07 (d, J = 7.9 Hz, 1H), 7.00 – 6.87 (m, 1H), 6.81 (s, 1H), 6.79 – 6.73 (m, 1H), 6.45 (s, 1H), 6.42 (d, J = 7.5 Hz, 2H), 5.95 (d, J = 7.7 Hz, 2H), 3.94 (s, 2H), 3.67 (s, 2H), 1.63 (s, 6H), 1.49 (s, 3H); **^{13}C NMR (101 MHz, DMSO-d_6): δ** 170.5, 150.2, 148.1, 146.3, 144.0, 140.1, 137.8, 136.9, 135.7, 135.1, 133.4, 131.0 (3C), 129.5 (3C), 126.9, 126.4, 125.3, 79.3 (2C), 59.8, 43.7, 31.2, 20.9, 19.8, 19.5,

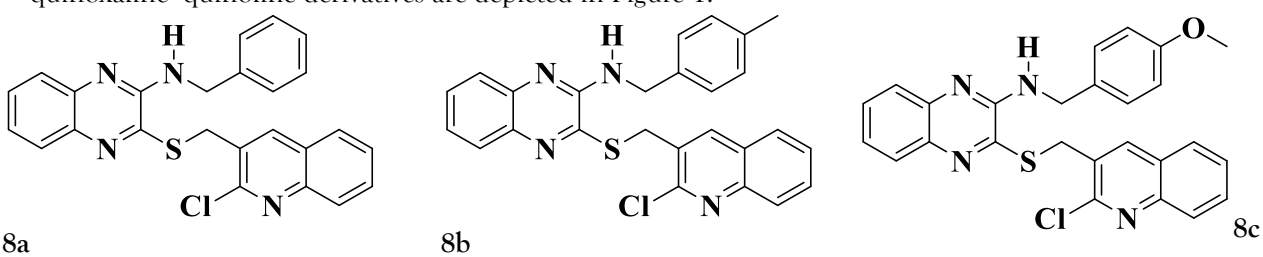
RESULTS AND DISCUSSIONS

In the present study, the design and synthetic pathway of the target N-benzyl-quinoxaline derivatives linked with a quinoline moiety is outlined in Scheme-1. In the step-1, A mixture of 2,3-dichloroquinoxaline (**1**) (0.5 mmol) and benzylamine (**2**) (0.75 mmol) was refluxed in ethanol (5 mL) at 78 °C for 5 h, with reaction progress monitored by TLC. After completion, the reaction mixture was concentrated to dryness, and the crude residue was purified by column chromatography using 2% ethyl acetate in hexane to yield N-benzyl-3-chloroquinoxalin-2-amine (**3**) as a pale yellow solid. In the subsequent step, N-benzyl-3-chloroquinoxalin-2-amine (0.37 mmol) was treated with sodium sulfide flakes (1.48 mmol) in DMF at room temperature, and the reaction was monitored by TLC. The resulting thick yellow solid was quenched by pouring onto crushed ice, collected by filtration, washed thoroughly with cold water, and dried under vacuum to afford 3-(benzylamino)quinoxaline-2-thiol (**4**) in good yield.
In the step-2, using the Vilsmeier–Haack reaction, the required precursors, 2-chloro-3-formylquinolines (**5**), were conveniently synthesized from acetanilides, and subsequent substitution of the chlorine atom with an aryloxy group afforded 2-aryloxy-3-formylquinolines. The obtained compounds were reduced with sodium borohydride in methanol to yield the corresponding alcohols (**6**), which were sufficiently pure and therefore used directly without further purification. These alcohols were then converted into the corresponding 2-chloro-3-(chloromethyl)quinoline (**7**) in good yield via reaction with thionyl chloride in benzene under reflux conditions.



Scheme-1: Synthesis of Novel N-Benzyl-3-(((2-Chloroquinolin-3-Yl) Methyl)Thio)Quinoxalin-2-Amine Derivatives (8a-j)

In the step-3, finally, the coupling of 3-(benzylamino)quinoxaline-2-thiol (**4**) with 2-chloro-3-(chloromethyl)quinoline (7) was carried out in DMF in the presence of potassium carbonate, and after 2 h, completion of the reaction was confirmed by TLC. The crude product was obtained by pouring the reaction mixture onto crushed ice, followed by filtration, drying, and purification with 10% ethyl acetate to furnish the desired products **8(a-j).** The chemical structures of all the synthesized N-benzyl-quinoxaline–quinoline derivatives are depicted in Figure 1.



8a **8b** **8c**

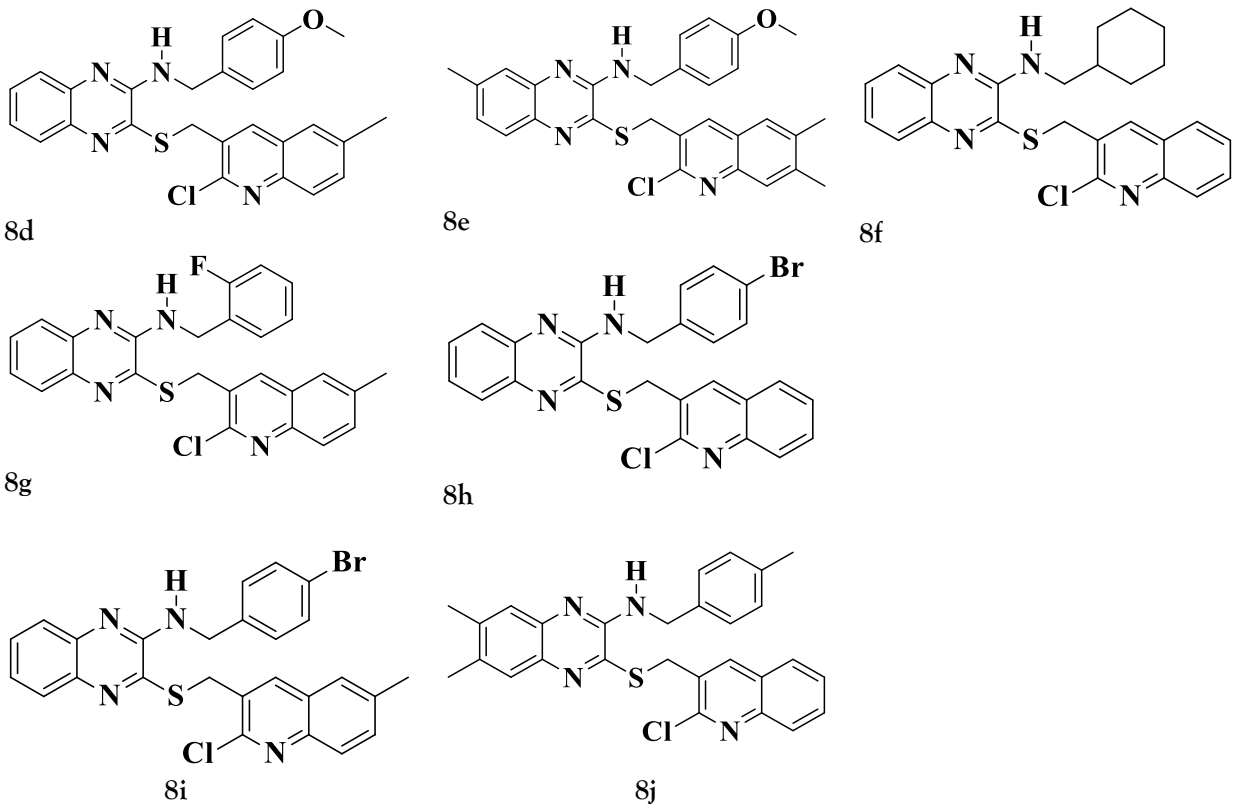


Figure: 1 Structures of designed target Novel N-Benzyl-3-(((2-Chloroquinolin-3-Yl) Methyl)Thio)Quinoxalin-2-Amine Derivatives 8(a-j)

ANTIMICROBIAL EVALUATION

Antibacterial activity:

The freshly synthesized compounds 8(a–j) were evaluated for their in vitro antibacterial activity against representative Gram-positive strains (Staphylococcus aureus and Bacillus subtilis) and Gram-negative strains (Escherichia coli and Klebsiella pneumoniae) at two different concentrations (10 µg/mL and 20 µg/mL) using the agar disc diffusion method. The zone of inhibition was measured in millimeters, with gatifloxacin serving as the standard reference drug (Table 1).

Table 1 Antibacterial activity of compound **8 (a-j)** at different 10, 20 µg/mL concentrations

Compound	Zone of inhibition (mm)							
	Gram positive bacteria				Gram negative bacteria			
	Stapylococus aeureus		Bacillus subtilis		Escherichia coli		Klebsiella pneumonia	
	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL	10 µg/mL	20 µg/mL
8a	13.2	22.4	14.5	22.1	18.5	19.6	12.8	18.7
8b	10.5	12.4	14.7	21.4	16.1	21.6	14.4	20.1
8c	19.4	27.9	19.4	34.3	14.0	19.7	09.9	16.7
8d	23.1	28.8	22.1	31.2	18.5	23.5	13.8	20.1
8e	24.7	27.6	22.4	32.2	17.6	24.4	12.8	22.1
8f	13.5	15.6	19.4	18.5	15.6	14.7	09.9	11.5
8g	17.5	24.5	16.8	28.1	14.5	17.8	06.9	13.4
8h	21.6	26.4	21.4	30.5	10.8	14.8	10.4	11.4
8i	19.5	28.6	20.4	31.2	12.8	16.8	08.9	13.4
8j	14.2	20.7	15.7	19.4	11.2	14.4	09.6	12.8
Gatifloxacin	21.2	28.4	19.7	28.8	15.7	19.4	10.1	17.2

The biological evaluation revealed that compounds 8c, 8d, and 8e exhibited the highest antibacterial activity, which can be attributed to the presence of a methoxy substituent on the benzyl moiety, likely enhancing electronic effects and improving binding interactions with bacterial targets. Similarly, compounds, 8g displayed good inhibitory activity across the tested strains, which may be ascribed to the presence of a fluoro atom on the benzyl ring, known to enhance lipophilicity and facilitate stronger penetration through bacterial cell membranes. Compounds 8h and 8i showed comparatively better activity against Gram-positive bacteria than Gram-negative strains, whereas compounds 8a and 8b exhibited the reverse trend, being more effective against Gram-positives organisms. Structure activity relationship (SAR), indicating that the nature and position of substituents on the benzyl moiety play a decisive role in modulating antibacterial potency. Moreover, the antibacterial activity was found to be concentration-dependent, as larger zones of inhibition were consistently observed at 20 µg/mL compared to 10 µg/mL, confirming a dose-responsive effect. Among the tested organisms, S. aureus (Gram-positive) and E. coli (Gram-negative) were the most susceptible, while K. pneumoniae exhibited comparatively lower sensitivity to the synthesized compounds. These findings strongly suggest that selective substitution patterns on the quinoxaline scaffold can significantly enhance antibacterial efficacy and guide the rational design of future derivatives.

Antifungal activity:

All the synthesized compounds 8(a–j) were screened for their in vitro antifungal activity against three pathogenic fungi, namely Aspergillus niger, Aspergillus flavus, and Fusarium oxysporum, at a concentration of 50 µg/mL using the agar disc diffusion method. The results were compared with clotrimazole as the standard reference drug (Table 2).

Table 2 Antifungal activities of the compound **8 (a-j)** at concentration 50µg/mL

Compound	Zone of Inhibition (mm) at 50µg/mL concentration		
	Aspergillus niger	Aspergillus flavus	Fusariumoxy sporum
8a	12.7	15.2	12.8
8b	13.4	15.4	14.5
8c	18.8	17.6	18.6
8d	17.9	16.9	17.4
8e	18.5	17.5	16.8
8f	14.2	14.8	12.4
8g	18.3	10.5	16.1
8h	12.4	16.8	14.8
8i	15.9	16.2	12.8
8j	12.5	13.4	14.2
Clotrimazole	17.3	16.4	15.9

The antifungal evaluation revealed that compounds 8c, 8d, and 8e exhibited the highest activity against all three fungal strains, which can be attributed to the presence of a methoxy substituent on the benzyl moiety, enhancing lipophilicity and thereby improving their interaction with fungal targets. Compounds 8g, 8h, and 8i displayed notable inhibition against A. niger and F. oxysporum, likely due to the electron-withdrawing fluoro group on the benzyl ring, which may increase binding affinity and stability within the fungal microenvironment. Interestingly, compound 8j was found to be particularly effective against A. flavus, which may be correlated with the presence of bromo substituents on the benzyl group, imparting enhanced antifungal potency. Overall, all the synthesized compounds demonstrated promising antifungal potential, with certain derivatives showing activity comparable to clotrimazole, highlighting the significance of specific substituents in governing antifungal efficacy and suggesting their potential as lead scaffolds for further optimization.

CONCLUSION

In the present study, a novel series of N-benzyl-quinoxaline derivatives linked with a quinoline moiety through a thioether bridge were successfully designed and synthesized. The structures of the compounds 8(a–j) were confirmed by spectral and analytical techniques. The synthesized derivatives were evaluated

for their antimicrobial activity against selected bacterial and fungal strains. Several compounds demonstrated promising antibacterial and antifungal activities, with some showing comparable efficacy to the standard reference drugs. These findings highlight the potential of quinoxaline–quinoline hybrid scaffolds as a valuable structural framework for the development of new antimicrobial agents.

Acknowledgement

We thank Department of Chemistry, OU for laboratory infrastructure under UGC-SAP (DRS-II) and DST-FIST programmes. We also thanks to CFRD, OU for analytical support.

Funding

The authors declare that no financial support was received from any funding agencies, institutions, or universities for the present research work.

REFERENCES

1. Bansal, Y.; Silakari, O. The therapeutic journey of quinoxaline derivatives: A review. Bioorg. Med. Chem. **2014**, 22(15), 3806–3815. https://doi.org/10.1016/j.bmc.2014.06.018
2. Rawal, R. K.; Tripathi, R.; Katiyar, S. B.; Chhonker, Y. S. Emerging role of quinoxaline derivatives in anticancer drug discovery. J. Mol. Struct. **2020**, 1200, 127092. https://doi.org/10.1016/j.molstruc.2019.127092
3. Nainwal, L. M.; Singh, J.; Malik, M. Y. Quinoxaline as a promising scaffold for novel drug design. Mini-Rev. Med. Chem. **2019**, 19(6), 478–495. https://doi.org/10.2174/1389557519666181112152809
4. Patel, R. V.; Kumari, P.; Rajani, D. P. Quinoxaline-based derivatives: Biological activities and recent developments. Curr. Med. Chem. **2012**, 19(25), 4451–4476. https://doi.org/10.2174/092986712802884311
5. Basu, D.; Mondal, P.; Ghosh, S. Quinoxaline-based hybrids in medicinal chemistry: Recent advances and perspectives. Eur. J. Med. Chem. **2021**, 219, 113430. https://doi.org/10.1016/j.ejmech.2021.113430
6. Gupta, S.; Kumari, P.; Jain, R. Biological activities of quinoxaline derivatives: A review. Int. J. Pharm. Sci. Res. **2013**, 4(9), 3293–3300.
7. Kamble, R. R.; Patil, P. S.; Masti, S. P. Quinoxaline derivatives as potential therapeutic agents: Structural modifications and biological profile. Med. Chem. Res. **2016**, 25(5), 1043–1056. https://doi.org/10.1007/s00044-016-1552-4
8. Sharma, P.; Singh, D.; Kumar, V. Advances in the design and synthesis of bioactive quinoxaline-thio hybrids. J. Heterocycl. Chem. **2022**, 59(7), 1219–1234. https://doi.org/10.1002/jhet.4451
9. Singh, A.; Rani, P.; Verma, P. K. Synthesis and pharmacological evaluation of novel thio-quinoxaline analogues. Chem.-Biol. Interact. **2018**, 295, 12–20. https://doi.org/10.1016/j.cbi.2018.09.006
10. Oguro, Y.; Miyamoto, N.; Okada, K.; Takagi, T.; Iwata, H.; Awazu, Y.; Miki, H.; Hori, A.; Kamiyama, K.; Imamura, S. Design, synthesis and biological evaluation of quinoxaline derivatives as kinase inhibitors. Bioorg. Med. Chem. **2010**, 18(20), 7260–7272. https://doi.org/10.1016/j.bmc.2010.08.017
11. Abdel-Rahman, A. A.-H.; Abdel-Maksoud, M. S. Quinoxaline-based bioactive molecules: Synthetic approaches and medicinal prospects. Arab. J. Chem. **2017**, 10(2), 197–214. https://doi.org/10.1016/j.arabjc.2014.05.006
12. Thakur, A.; Sharma, S.; Kumar, V. Structural modifications on quinoxaline ring: A strategy towards enhanced biological potential. Future Med. Chem. **2019**, 11(6), 629–650. https://doi.org/10.4155/fmc-2018-0401
13. Abdel-Aziz, H. A.; El-Zahabi, H. S. Biological evaluation of substituted quinoxalines as antimicrobial agents. Molecules **2014**, 19(8), 11704–11722. https://doi.org/10.3390/molecules190811704
14. Kharb, R.; Sharma, P. C.; Yar, M. S. Pharmacological significance of benzyl-substituted heterocycles: Recent updates. Eur. J. Pharm. Sci. **2011**, 43(3), 133–146. https://doi.org/10.1016/j.ejps.2011.05.013
15. Khan, A.; Siddiqui, N.; Ahsan, W. Quinoxaline hybrids: Recent advances in synthesis and pharmacological applications. Med. Chem. Commun. **2017**, 8(6), 1005–1022. https://doi.org/10.1039/C7MD00077A
16. El-Sayed, W. A.; El-Bendary, E. R. Quinoxaline derivatives as potential antimicrobial and anticancer agents. Arch. Pharm. Chem. Life Sci. **2015**, 348(9), 647–660. https://doi.org/10.1002/ardp.201500147
17. Zhang, L.; Li, Y.; Xu, Z.; Wang, J. Antimicrobial potential of thio-quinoxaline derivatives. Eur. J. Pharmacol. **2018**, 820, 123–132. https://doi.org/10.1016/j.ejphar.2017.12.034
18. Li, J.; Sun, H.; Xu, Y. Benzylated heterocycles as anticancer scaffolds: Recent developments. Bioorg. Chem. **2020**, 96, 103593. https://doi.org/10.1016/j.bioorg.2019.103593
19. De, A.; Ganguly, S.; Banerjee, S. Quinoline and its derivatives: A versatile scaffold for medicinal chemistry. Eur. J. Med. Chem. **2019**, 166, 361–385. https://doi.org/10.1016/j.ejmech.2019.01.004
20. Michael, J. P. Quinoline, quinazoline and acridone alkaloids. Nat. Prod. Rep. **2008**, 25(1), 166–187. https://doi.org/10.1039/b613652n
21. Kumar, S.; Bawa, S.; Gupta, H. Biological activities of quinoline derivatives. Mini-Rev. Med. Chem. **2009**, 9(14), 1648–1654. https://doi.org/10.2174/138955709789878098
22. Wang, Y.; Li, Y.; Liu, X. Quinoline-containing hybrids: Recent advances in structural modifications and biological potential. Bioorg. Med. Chem. **2021**, 46, 116372. https://doi.org/10.1016/j.bmc.2021.116372
23. Karrouchi, K.; Radi, S.; Ramli, Y.; Taoufik, J.; Mabkhot, Y. N.; Al-Aizari, F. A.; Ansar, M. Quinoline derivatives: Synthesis and pharmacological applications. Molecules **2018**, 23(1), 134. https://doi.org/10.3390/molecules23010134
24. Kaur, R.; Dwivedi, A. R.; Kumar, B. Design, synthesis and biological evaluation of halogenated quinoline derivatives. Med. Chem. Res. **2017**, 26(9), 2189–2200. https://doi.org/10.1007/s00044-017-1921-y
25. Raj, R.; Singh, P.; Gupta, A. Quinoline derivatives as emerging antimicrobial agents. Future Med. Chem. **2017**, 9(5), 485–505. https://doi.org/10.4155/fmc-2016-0217
26. Singh, K.; Kaur, R.; Arora, R. 2-Chloroquinoline derivatives: Synthesis and pharmacological potential. Chem. Biol. Drug Des. **2020**, 96(5), 1125–1135. https://doi.org/10.1111/cbdd.13749

27. Chen, J.; Liu, Z.; Xu, H. Halogen-substituted quinolines as bioactive scaffolds. Curr. Org. Chem. **2018**, 22(6), 593–608. https://doi.org/10.2174/1385272822666171222144744
28. O'Connor, C. J.; Beckmann, H. S. G. Electrophilic quinoline derivatives in medicinal chemistry. Chem. Rev. **2019**, 119(6), 5595–5648. https://doi.org/10.1021/acs.chemrev.8b00512
29. Patel, H. M.; Patel, R. V. Functionalized quinoline derivatives: Emerging significance in drug discovery. RSC Adv. **2014**, 4(93), 51608–51625. https://doi.org/10.1039/c4ra08315e
30. Ali, I.; Lone, M. Y.; Aboul-Enein, H. Y. Design and biological evaluation of novel quinoline derivatives as antibacterial agents. Eur. J. Med. Chem. **2018**, 148, 249–263. https://doi.org/10.1016/j.ejmech.2018.02.026
31. Vekariya, R. H.; Patel, R. V. Quinolines as potent antibacterial scaffolds. Med. Chem. Commun. **2016**, 7(6), 1014–1034. https://doi.org/10.1039/C6MD00054B
32. Zhang, J.; Zhou, H.; Li, X. Role of chloromethyl substitution in enhancing antibacterial potency of quinoline derivatives. Chem.-Biol. Interact. **2019**, 310, 108728. https://doi.org/10.1016/j.cbi.2019.06.012
33. Wu, X.; Liu, Q.; Chen, Y. Anticancer activities of halogenated quinoline derivatives: Mechanistic insights. Bioorg. Chem. **2017**, 75, 289–297. https://doi.org/10.1016/j.bioorg.2017.10.011
34. Rathore, D.; McCutchan, T. F.; Sullivan, M. Quinoline-based antimalarials: Mechanisms of action and resistance. Drug Resist. Updates **2005**, 8(1–2), 41–54. https://doi.org/10.1016/j.drup.2005.01.001
35. Yadav, P.; Ghosh, S.; Kumar, R. Synthetic and biological aspects of chloromethyl-quinoline derivatives. J. Heterocycl. Chem. **2021**, 58(2), 499–511. https://doi.org/10.1002/jhet.4143