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Research Article

Synthetic Development of Antimicrobial Novel Quinoxaline Derivatives

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Abstract

A new approach to synthesize novel quinoxaline derivatives by a convenient and efficient route. All derivatives were synthesized from readily available benzil, ortho phenylene diamine and chlorosulfonic acid using different amines in ethanol as solvent. The reaction involves three steps which occurs in quick succession within appropriate time period to deliver the product with reasonably high yields.

All the synthesized derivatives were subjected for their spectral analysis by FT-IR, ^{1}H-NMR, HRMS and antimicrobial screening by disk diffusion method and determination MIC by tube dilution technique. It was found that quinoxaline derivatives of on suitable concentrations have pronounced effect as compared to antibiotic as a reference standard (Azithromycin) present in the market against both the gram positive and gram negative bacteria. The results obtained suggest potential new drugs for antimicrobial activity.

Keywords: Echinomycin, Quinoxaline, Antimicrobial, MIC, ^{1}H-NMR, HRMS

1. Introduction

Quinoxaline derivatives are the subject of considerable interest from both academic and industrial perspectives because they are significant intermediates for the manufacturing of pharmaceuticals and advanced materials[1-2]. Quinoxalines are becoming the attractive target for extensive research due to its inherent diverse properties. Various potential activities of the quinoxalines have been explored recently like antimicrobial[3-5], anticancer[6], antiviral[7], anti-inflammatory[8], anti-HIV[9], anti-tubercular, anxiolytic[10], antihelmintic[11], anticonvulsant[12], and antioxidant[13] etc. In the recent year, 2, 3 disubstituted quinoxalines reported to possess significant antimicrobial potential against bacteria, fungi and mycobacterium. Antimicrobial agent shows activity against bacteria, fungi, and mycobacterium species, called antibacterial, antifungal, antitubercular agents respectively. There are various quinoxaline derivatives showing antimicrobial activity. Quinoxaline core antibiotics like Echinomycin, Triostin- A showing antimicrobial activity by DNA cleaving property. It is believed that the antimicrobial potency of the quinoxaline due to the facilitate approach of the structure to prevent DNA directed RNA synthesis by virtue binding to CPG site on DNA[14]. The present study aims to synthesis, characterization and determination of antimicrobial susceptibility testing of various novel quinoxaline derivatives.

2. Material and Methods

Progress of all reaction was monitored by thin-layer chromatography using Merck precoated silica GF 254. Synthesized compounds were purified by column chromatography using silica gel 60 from Merck. Melting points were recorded on electrically heated melting point apparatus. All synthesized derivatives were characterized on a SHIMAZDU Infra Red Spectrometer (FTIR-8400S). The ^{1}H-NMR spectrums of synthesized derivatives were recorded on varian-VXR-300S at 300 MHz; Mass spectra of synthesized derivatives were recorded at MAT-120.

3. Experimental Details

3.1. Synthesis of 2,3-diphenylquinoxaline-6-sulfonylchloride: 2, 3-diphenylquinoxaline was treated with chlorosulfonic acid under ice-cold condition in fuming cupboard with constant stirring. The stirring was continued until the reaction reaches room temperature. The resultant mixture was poured into water to give sulfonylchloride derivative, product was filtered and recrystalized by ethanol.

3.2. General procedure for the synthesis of N-substituted-2, 3-diphenyl quinoxaline-6-sulfonamide: A primary amino group containing moiety was refluxed with 2, 3-diphenyl quinoxaline-6-sulfonyl chloride in 50 ml of 10% aqueous NaOH. The reaction mixture was poured into the crushed ice stirred until product solidifies; it was then filtered, washed with dilute NaOH, cold water solution and recrystalized from ethanol.

3.2.1. N-(2-Nitrophenyl)-2, 3-diphenylquinoxaline-6-sulfonamide (a): 1.09 g of O-nitro aniline was refluxed with 1.9 g of 2, 3-diphenylquinoxaline-6-sulfonylchloride and 50 ml of 10% aq. NaOH solution for 2 hrs. Then reaction mixture was poured into 10 ml cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max}3417.12 (NH stretch); 3050.82 (CH stretch); 1668.41 (C=O stretch); 1592.62 (NH bend); 1542.18; 1352.66 (NO_2 stretch); 1337.76; 1168.86 (S=O) CM^{-1}, ^{1}H-NMR-δ ppm: 7.33-8.13 (m; 17H; Ar-H); 5.46 (s; 1H; NH), HRMS: m/z calcd for $C_{26}H_{18}N_4O_4S$: 482.1049; found 482.1044.

3.2.2. N-(2-Hydroxyphenyl)-2,3-diphenylquinoxaline-6-sulfonamide (b): 0.55 g of 2-Amino phenol was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml. of 10% aq. NaOH solution for 2.5 hrs. Then reaction mixture was poured into 10 ml cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max}3583.48 (OH stretch); 3050.85 (CH stretch); 3423.42 (NH stretch); 1668.16 (C=O stretch); 1572.62 (NH bend); 1334.70; 1168.86 (S=O) CM^{-1}, ^{1}H-NMR-δ ppm: 7.14-8.44 (m; 17H; Ar-H), 5.62 (s; 1H; OH), HRMS: m/z calcd for $C_{26}H_{19}N_3O_3S$: 453.1147; found: 453.1154.

3.2.3. N-(4-Chlorophenyl)-2,3-diphenylquinoxaline-6-sulfonamide (c): 0.65 g of 4-Chloro aniline was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml of 10% aq. NaOH solution for 1.30 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

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IR (KBr) ν_{max} 3429.83 (NH stretch); 3028.34 (CH stretch); 1672.58 (C=O stretch); 1588.65 (NH bend); 1350.67; 1180.24 (S=O); 763.42 (Cl) CM^{-1}, ^{1}H-NMR-δ ppm: 7.04-8.10 (m; 17H; Ar-H), 5.26 (s; 1H; NH), HRMS: m/z calcd for $C_{26}H_{18}ClN_3O_2S$: 471.0808; found: 471.0804.

3.2.4. N-(4-Hydroxyphenyl)-2,3-diphenylquinoxaline-6-sulfonamide (d): 0.55 g of 4-Amino phenol was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml. of 10% aq. NaOH solution for 3 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max} 3564.48 (OH stretch); 3050.81 (CH stretch); 3423.24 (NH stretch); 1668.24 (C=O stretch); 1578.24 (NH bend); 1332.23; 1168.57 (S=O) CM^{-1}, ^{1}H-NMR-δ ppm: 7.12-8.46 (m; 17H; Ar-H), 5.68 (s; 1H; OH), HRMS: m/z calcd for $C_{26}H_{19}N_3O_3S$: 453.1147; found: 453.1151.

3.2.5. N-(3-Chlorophenyl)-2,3-diphenylquinoxaline-6-sulfonamide (e): 0.65 g of 3-Chloro aniline was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml of 10% aq. NaOH solution for 2.5 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max} 3423.81 (NH stretch); 3032.36 (CH stretch); 1671.58 (C=O stretch); 1584.66 (NH bend); 1350.69; 1182.26 (S=O); 742.52 (Cl) CM^{-1}, ^{1}H-NMR-δ ppm: 7.06-8.13 (m; 17H; Ar-H), 5.12 (s; 1H; NH), HRMS: m/z calcd for $C_{26}H_{18}ClN_3O_2S$: 471.0808; found: 471.0811

3.2.6. N-(3-Methoxyphenyl)-2,3-diphenylquinoxaline-6-sulfonamide (f): 0.62 g of 3-Methoxy aniline was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml. of 10% aq. NaOH solution for 2.30 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max} 3424.61 (NH stretch); 3042.26 (CH stretch); 1679.88 (C=O stretch); 1584.57 (NH bend); 1348.64; 1182.22 (S=O); ^{1}H-NMR-δ ppm: 7.36-8.19 (m; 17H; Ar-H), 5.32 (s; 1H; NH); 3.8 (s; 3H; OCH_3), HRMS: m/z calcd for $C_{27}H_{21}N_3O_3S$: 467.1304; found: 467.1308

3.2.7. N-(3-Hydroxyphenyl)-2,3-diphenylquinoxaline-6-sulfonamide (g): 0.55 g of 3-Amino phenol was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml. of 10% aq. NaOH solution for 2 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max} 3562.44 (OH stretch); 304.83 (CH stretch); 3443.18 (NH stretch); 1672.27 (C=O stretch); 1574.26 (NH bend); 1335.21; 1169.61 (S=O) CM^{-1}, ^{1}H-NMR-δ ppm: 7.14-8.38 (m; 17H; Ar-H), 5.65 (s; 1H; OH), HRMS: m/z calcd for $C_{26}H_{19}N_3O_3S$: 453.1147; found: 453.1149.

3.2.8. N-(4-Methoxyphenyl)-2,3-diphenylquinoxaline-6-sulfonamide (h): 0.62 g of 4-Methoxy aniline was refluxed with 1.9 g of 2, 3-Diphenylquinoxaline-6-sulfonylchloride and 50 ml. of 10% aq. NaOH solution for 3 hrs. Then reaction mixture was poured into 10 ml. cold water and stirred until product crystallized then product was filtered and recrystalized by ethanol.

IR (KBr) ν_{max} 3424.61 (NH stretch); 3042.26 (CH stretch); 1679.88 (C=O stretch); 1584.57 (NH bend); 1348.64; 1182.22 (S=O); ^{1}H-NMR-δ ppm: 7.36-8.19 (m; 17H; Ar-H), 5.32 (s; 1H; NH); 3.8 (s; 3H; OCH_3), HRMS: m/z calcd for $C_{27}H_{21}N_3O_3S$: 467.1304; found: 467.1309

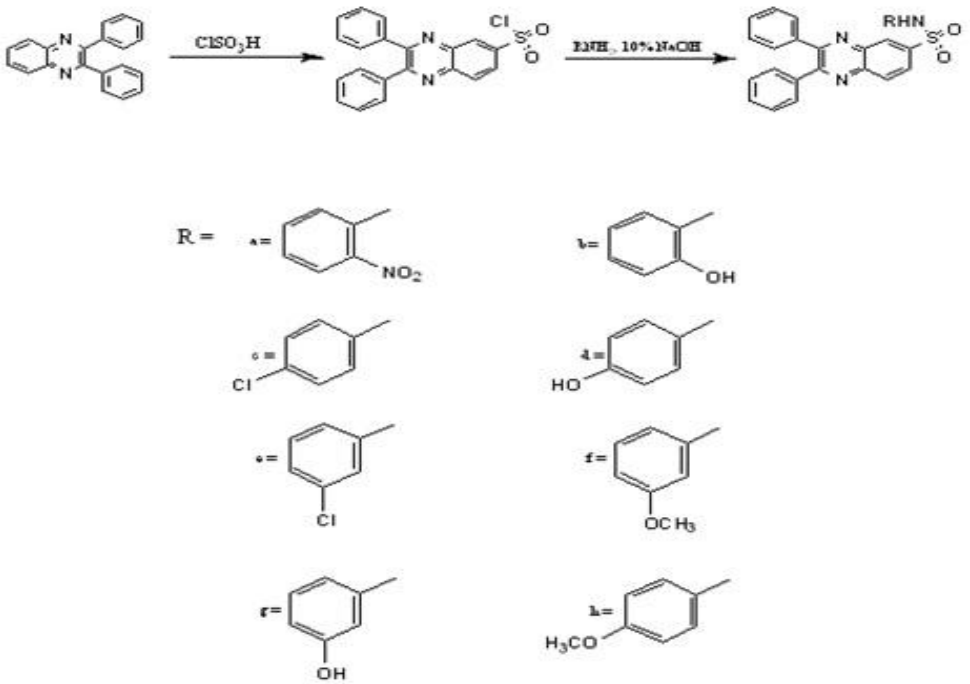
3.3. Antimicrobial activity: Zone of Inhibition: The ZOI was determined by disk diffusion method. Compounds (a-h) dilution was prepared in N, N-dimethyl formamide (DMF). After loading test sample in to plate kept it for incubation in incubator at 37^0 C for 24 hrs and the zone of inhibition was measured using vernier caliper in mm.

Minimum Inhibitory Concentration: The MIC was determined according to dilution method. Compounds (a-h) were dissolved in N, N-dimethyl formamide (DMF) next, the solution was diluted and suspensions of each bacterial species were prepared. After incubation at 37^0C for 24 hrs, the turbidity was observed. The MIC was determined as the lowest concentration of compound that completely inhibited organism growth.

4. Results and Discussion

The present article describes a synthesis and characterization of novel quinoxaline derivatives by addition-elimination reaction mechanism which is given in scheme I (Figure I).

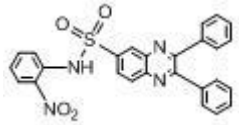
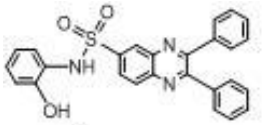
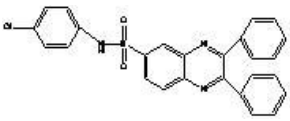
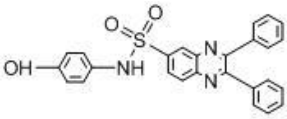
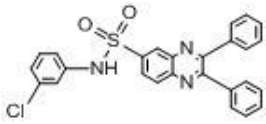
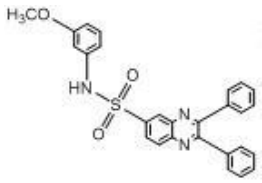
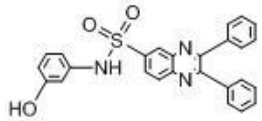
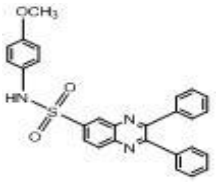
Figure I. Scheme for Synthesis of Novel Quinoxaline Derivatives



Scheme 1. Reaction scheme for the synthesis of novel quinoxaline derivatives (a-h)

As reported ortho Phenylene diamine and benzil in presence of ethanol gives 2,3-diphenylquinoxaline[15] which was treated with chlorosulfonic acid under ice cold condition to give 6-sulfonyl chloride 2,3-diphenylquinoxaline [16]. It was further refluxed with various aromatic primary amines by using 10% aqueous sodium hydroxide to yield novel quinoxaline derivatives by nucleophilic substitution reaction. Physical data of the synthesized compounds is shown in table I.

Table I. Physical Data of Synthesized Compounds

Sr. No.	Compounds	Molecular formula	Melting range (^{0}C)	Percentage yield (%)
a		$C_{26}H_{18}N_4O_4S$	198-202	83
b		$C_{26}H_{19}N_3O_3S$	186-190	79
c		$C_{26}H_{18}ClN_3O_2S$	222-226	74
d		$C_{26}H_{19}N_3O_3S$	202-204	74
e		$C_{26}H_{18}ClN_3O_2S$	216-218	78
f		$C_{27}H_{21}N_3O_3S$	206-208	69
g		$C_{26}H_{19}N_3O_3S$	192-194	81
h		$C_{27}H_{21}N_3O_3S$	212-214	72

The synthesized derivatives were characterized by different spectral studies using FT-IR, ^{1}H-NMR and HRMS. The structure of 2,3-diphenylquinoxaline-6-sulfonylchloride was confirmed by the FT-IR absorption peak at 1350, 1150 CM^{-1} (S=O) and 700 CM^{-1} (Cl). In case of N-substituted-2,3-diphenyl quinoxaline-6-sulfonamide presence of NH stretch band and HRMS peak confirmed synthesis of novel quinoxaline compounds.

The present work reports the study of the antimicrobial activity of synthetic quinoxaline derivatives (a-h) against bacterial strains by disk diffusion method against gram positive (*S. aureus*) and gram negative bacteria (*E. coli*) and determination MIC by tube dilution technique against *S. aureus* and *E. coli*. The turbidity was measured colorimetrically at about 420 nm. The prokaryotic strains used were *S. aureus* (ATCC2079), *E. coli* (ATCC2685). The results are summarized in table II and III. Eight compounds shown antimicrobial activity indicating that the synthesis quinoxaline derivatives is the structural unit that exerts antimicrobial action. Among these three compounds (a, c, e) has a strong electron withdrawing group. These results suggest that electron withdrawing group is need for high activity. The results obtained suggest potential new drugs for antimicrobial activity.

Table II. Antimicrobial Activity Data of Synthesized Compounds (ZOI)

Quinoxaline derivatives	Concentration (mcg)	Zone of Inhibition (mm)	
		Gram Positive Bacteria (*S. aureus*)	Gram Negative Bacteria (*E. coli*)
a	100	10	09
	200	13	12
	400	26	22
b	100	07	05
	200	12	09
	400	22	16
c	100	09	08
	200	15	13
	400	29	23
d	100	06	07
	200	10	14
	400	18	24
e	100	10	08
	200	17	15
	400	32	27
f	100	09	02
	200	14	06
	400	27	14
g	100	07	06
	200	12	10
	400	25	21
h	100	11	04
	200	16	07
	400	28	15
Azithromycin	100	12	10
	200	19	14
	400	39	29

Table III. Antimicrobial Activity Data of Synthesized Compounds (MIC)

Quinoxaline derivatives	Concentration (mcg/ml)	% Transmittance at 420 nm	
		Gram Positive Bacteria	Gram Negative Bacteria
		S. aureus	*E. coli*
a	1000	97	89
	500	53	54
	250	43	51
	125	**40**	**42**
	62.5	23	36
b	1000	70	61
	500	65	65
	250	**52**	71
	125	35	**70**
	62.5	32	35
c	1000	80	60
	500	74	68
	250	69	67
	125	**62**	**62**
	62.5	50	40
d	1000	75	72
	500	68	75
	250	65	72
	125	**62**	**75**
	62.5	12	67
e	1000	70	73
	500	61	64
	250	66	41
	125	65	45
	62.5	35	25
f	1000	76	79
	500	75	76
	250	72	75
	125	**70**	**73**
	62.5	19	21
g	1000	72	78
	500	70	74
	250	67	71
	125	**64**	**72**
	62.5	22	27
h	1000	77	72
	500	76	75
	250	70	73
	125	**69**	**72**
	62.5	24	29

5. Conclusion

We synthesized novel quinoxaline derivatives, evaluated their antimicrobial activity against various bacterial strains and antimicrobial data of novel quinoxaline derivatives was obtained. It was found that quinoxaline derivatives of on suitable concentrations have pronounced effect as compared to antibiotic as a reference standard (Azithromycin) present in the market against both the gram positive and gram negative bacteria. Among these three compounds (a, c, e) has a strong electron withdrawing group. These results suggest that electron withdrawing group is need for high antimicrobial activity. The results obtained suggest potential new drugs for antimicrobial activity. The derivatives were confirmed by FT-IR, ^{1}H-NMR and HRMS. The spectral characterization reveals the formation of proposed quinoxaline derivatives. These results demonstrate that quinoxaline framework with electron withdrawing substituent is a good candidate for novel industrial antimicrobial agents and opens the new doors in future for global area of synthetic research in a field of chemistry.

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