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BIOLOGICALLY ACTIVE DIAZINES: SYNTHESIS, CHARACTER IZATION AND ANTIMICROBIAL ACTIVITY OF SOME 2, 3 DIPHENYLQUINOXALINE DERIVATIVES

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ABSTRACT

Benzopyrazines a versatile heterocyclic scaffold in 2, 3-diphenyl quinoxaline plays a significant role in the pharmaceutical field and present in many medicinally important derivatives used as antiviral, anticancer, antimicrobial agents, etc. In the present revision, a few 2, 3 diphenylquinoxaline derivatives have been synthesized and characterized. The synthesized compounds were screened for their *in-vitro* growth inhibiting activity against different strains of bacteria and fungi viz., *Staphylococcus aureus, Staphylococcus faecalis, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Salmonella typhi,* at 10 and 50 µg/ml concentration by using agar well diffusion technique. The results were compared with the standard antibiotics Ciprofloxacin (10 µg/ml). All the newly synthesized derivatives (A_{1-4} & B_{1-6}) were produced significant action against the bacterial strains at 10 µg/ml concentration. Meanwhile, no compounds have produced the promising activity against that of fungi strains even though while increasing concentrations.

KEY WORDS: 2, 3-diphenyl quinoxaline, Antimicrobial Evaluation, Agar well diffusion technique.

INTRODUCTION

Diazines are a group of organic compounds having the molecular formula $C_4H_4N_2$. Each contains a benzene ring in which two of the C-H fragments have been replaced by isolable nitrogen. There are three isomers: Pyrazine (1, 4-diazine), Pyrimidine (1,3-diazine), Pyridazine (1,2-diazine)[1]. Diazines (pyrazines) are fused to benzene ring to form quinoxalines to improve their chemical versatility by the resonance behavior of benzene and its basicity [1].

Quinoxaline derivatives are an important class of nitrogen containing benzo heterocyclic compounds consists a ring complex made up of a benzene ring and a pyrazine ring [2]. In the past decades this benzodiazines has extensive range of therapeutic uses and potential activities viz., acting as antimicrobial agents, cytotoxic agents, anti-tubercular, anxiolytic, anti-HIV, antioxidant, anti-inflammatory[7], antimalarial, anticancer, antidepressant, antibacterial, antifungal, antibiotics [2-8], such as echinomycin, levomycin and actinoleutin that are known to inhibit growth of gram positive bacteria and are active against various transplantable tumors [9], as well as rheumatoid arthritis, hemangioma and Kaposi's sarcoma, which are related to vasculogenesis and angiogenesis [10].

In addition to this, sulphonamide is a well-known moiety used in many pharmaceutical fields like antimicrobial, anti-inflammatory, antiproliferative, Carbonic anhydrase inhibitors, anti-tumor and radiosensitizing agents. Sulfonamide (also called sulphonamide, sulfa drugs or sulpha drugs) is the basic unit of several groups of drugs. Some sulfonamides are also devoid of antibacterial activity, e.g., sultiame which is used as anticonvulsant activity [11]. The sulfonylureas and thiazide diuretics are newer drug groups based on the antibacterial sulfonamides

Bacterial and fungal diseases are the most common all over the world. Though, many antibiotics are currently marketed, they have a tendency of becoming resistant and are prone to severe adverse effects after long term use. Hence, there is a never lasting demand in synthesis of novel antimicrobial agents having good potency, efficacy with lesser side effects. The current article is aimed to fuse

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diazine derivative like quinoxaline and sulfonamide analogs that are active against common pathogenic bacterial flora, especially to overcome the strains those are resistant with earlier quinoxaline derivatives by developing structural modifications [12].

EXPERIMENTAL

The melting points of the synthesized compounds (A_{1-4} & B_{1-6}) were determined using an open capillary tube using visual melting range apparatus and are uncorrected. IR spectra (KBr wafers) were taken on Shimadzu FT-IR Spectrophotometer, ortho phenylene diamine, Benzil, Chlorosulfonic acid was procured from sigma-Aldrich, India. All other chemicals used in the present studies were either of analytical reagent or synthetic grade quality.

CHEMICAL SYNTHESIS

Synthesis of 2, 3- diphenyl quinoxaline (Step: 1):-

To a warm solution of 2.1g (0.01mol) of Benzil ($C_{14}H_{10}O_2$) in 8 ml of rectified spirit a solution of 1.1 g (0.01mol) of orthophenylene diamine($C_6H_8N_2$) in 8 ml of rectified spirit was added, warmed in water bath for 30 minutes, water was added until slight cloudiness persists and allowed to cool. Filtered and recrystallized from absolute alcohol.

Synthesis of 2, 3-diphenyl quinoxaline-6-sulfonyl chloride (Step: 2):-

To 2, 3-diphenyl quinoxaline($C_{20}H_{14}N_2$) (0.01mol), chlorosulfonic acid(HSO_3Cl) (0.015mol) was added and the reaction mixture was refluxed for a period of 5 hours. The mixture was poured slowly on ice-water mixture; white solid precipitated out was filtered, washed thoroughly with cold water to make it acid free and recrystallized by using absolute alcohol.

Synthesis of sulfonamide derivatives of 2, 3-diphenylquinoxaline (Step: 3):-

2, 3-diphenylquinoxaline-7-sulfonylchloride ($C_{20}H_{13}ClN_2O_2S$) was refluxed with 50 % (30 ml) ammonia solution for 1.5 hours. Then reaction mixture was cooled and poured into water to get sulfonamide derivatives of 2, 3-diphenylquinoxaline. The crude product was recrystalized from 90 % absolute alcohol.

The same procedures followed for the other derivatives of 2, 3-diphenylquinoxaline-7-sulfonylchloride with their corresponding amines. Thus the compounds A_{1-4} has acquired.

Synthesis of benzylidene derivatives of 2, 3-diphenylquinoxaline (Step: 4)

On the other hand in phase 2, the obtained derivative of step 3 product (A_1) 2, 3 diphenyl quinoxaline-7-sulfonamide was treated with equivalent amount of corresponding aldehyde in 25 ml of ethanol and reflux the mixture for 2 hours. Cool the reaction mixture and poured in to ice cold water to get a compounds B_{1-6}.

SCREENING FOR ANTIMICROBIAL ACTIVITY

The antimicrobial activity of all the newly synthesized compounds A_{1-4} and B_{1-6} were determined by well plate or agar diffusion technique. The medium used were double strength nutrient broth (Hi-Media) for antibacterial activity and double strength malt yeast extract (Hi- Media) for antifungal activity. The *in vitro* antimicrobial activity was carried out against 24 hours old cultures of bacterial and 72 hours old cultures of fungal strain. The different strains of bacteria and fungi viz., *Staphylococcus aureus* (NCIM 2122), *Bacillus subtilis* (NCIM 2193), *Streptococcus faecalis* (NCIM 5024) (Gram positive bacteria's), *Escherichia coli* (NCIM 2809), *Proteus vulgaris* (NCIM 2813), *Salmonella typhi* (NCIM 2501) (Gram negative bacteria's). Pure cultures of the test microorganisms were procured from the compounds were tested at the concentrations of 10 and 50 µg/ml and solutions were prepared by dissolving in dimethylsulfoxide (DMSO). The petri dishes used for antibacterial screening were incubated at 37±1 ºC for 24 hours. The results were compared to Ciprofloxacin (10 µg/ml) for antibacterial activity,respectively by measuring zone of inhibition in mm. The antibacterial screening results were presented in Table 2.

Table 1. Physico-chemical data of synthesized compounds

SL NO	Compound Code	R (Substituent)	Molecular Formula	Formula Weight	Yield (%)	Melting Point (^oC)
1	A_1	NH_2	$C_{20}H_{15}N_3O_2S$	361.41	81.89	110-112
2	A_2	C_6H_7N	$C_{26}H_{19}N_3O_2S$	437.51	75.32	120-122
3	A_3	$C_6H_7N_2$	$C_{26}H_{20}N_4O_2S$	452.52	80.08	095-098
4	A_4	CH_4N_3O	$C_{21}H_{17}N_5O_3S$	419.45	84.26	115-118
5	B_1	H	$C_{27}H_{19}N_3O_2S$	449.52	85.54	128-130
6	B_2	NO_2	$C_{27}H_{18}N_4O_4S$	494.52	80.15	125-127
7	B_3	Ortho Chloro	$C_{27}H_{18}ClN_3O_2S$	483.96	70.78	115-118
8	B_4	Para Chloro	$C_{27}H_{18}ClN_3O_2S$	483.96	69.36	119-120
9	B_5	Dimethylamino	$C_{29}H_{24}N_4O_2S$	492.59	71.28	105-108
10	B_6	Cinnamaldehyde	$C_{29}H_{21}N_3O_2S$	475.56	70.56	125-127

Table 2. Evaluation of *in vitro* antibacterial activity of 2,3 diphenyl quinoxalines

Test Compounds	Zone of inhibition (mm)											
	Gram Positive						Gram Negative					
	Staphylococcus aureus		*Bacillus subtilis*		*Staphylococcus faecalis*		*Escherichia coli*		*Salmonella typhi*		*Proteus vulgaris*	
	10^a	50^a	10^a	50^a	10^a	50^a	10^a	50^a	10^a	50^a	10^a	50^a
A_1	18	21	11	18	21	27	18	22	17	23	16	22
A_2	22	32	15	29	27	33	20	26	23	28	20	27
A_3	23	28	13	26	26	27	18	25	22	26	19	24
A_4	21	22	15	22	22	24	15	22	21	24	18	23
B_1	18	22	20	23	21	23	18	24	22	23	22	23
B_2	24	33	16	30	26	33	22	26	23	30	24	27
B_3	23	23	15	23	22	25	21	25	21	23	23	25
B_4	22	24	14	24	21	24	21	24	20	25	24	26
B_5	21	29	16	27	28	32	20	27	24	28	25	27
B_6	23	31	18	31	29	32	21	26	25	29	25	28
Ciprofloxacin[b]	32		28		34		27		30		28	

[a] µg/ml, [b] Ciprofloxacin (10µg/ml) was used as positive reference standard antibiotic.

Fig 1. Effect of prototypes (10µg/ml) and Ciprofloxacin (50µg/ml) on *in Vitro* antimicrobial Activity(gram positive)

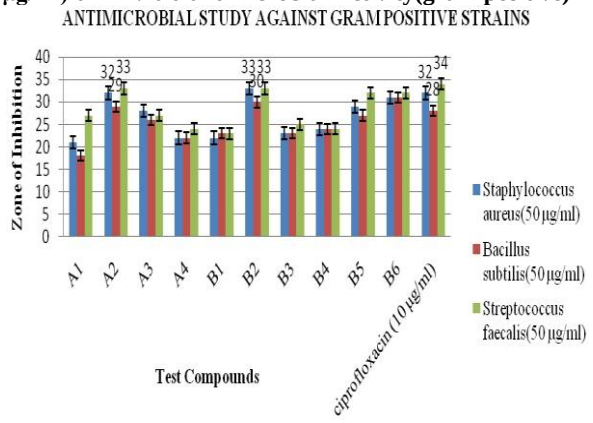
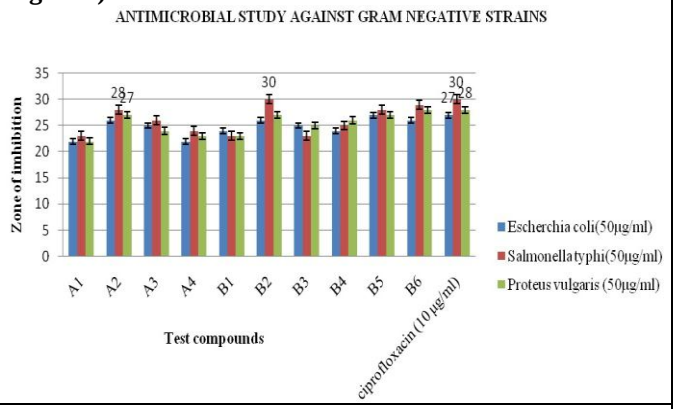
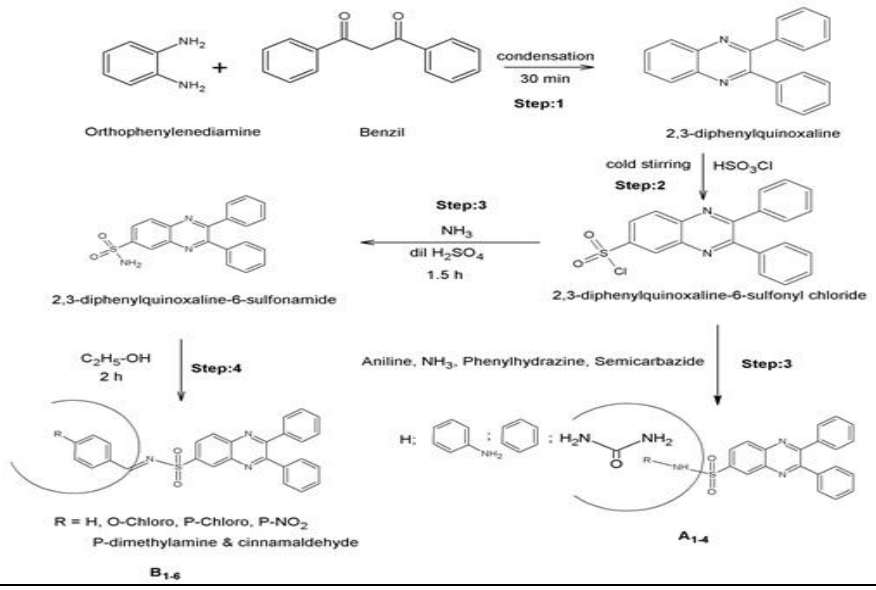


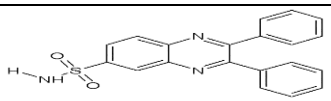
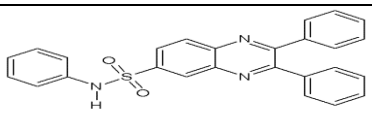
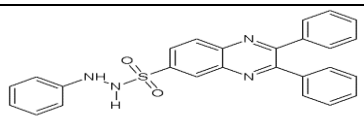
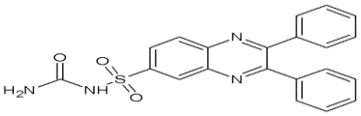
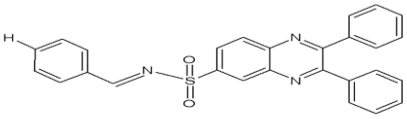
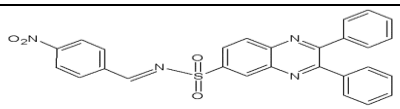
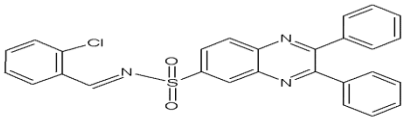
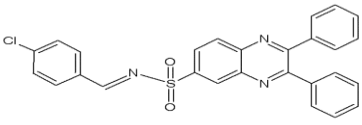
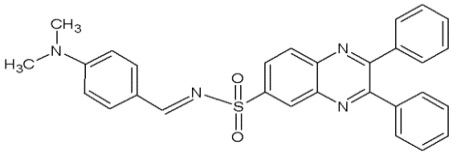
Fig 2. Effect of prototypes (10 µg/ml) and Ciprofloxacin (50µg/ml on *in Vitro* antimicrobial Activity(gram negative)

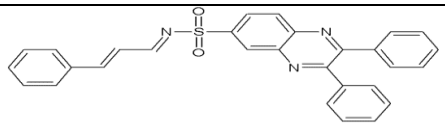


SCHEME



LIST OF NEWLY SYNTHESIZED COMPOUNDS

SL NO	COMPOUND NAME	STRUCTURE
1	2,3 diphenylquinoxaline-6-sulfonamide	 **A_1**
2	N, 2,3-triphenylquinoxaline-6-sulfonamide	 **A_2**
3	N,2,3-triphenylquinoxaline-6-sulfohydrazide	 **A_3**
4	1-(2,3-diphenylquinoxaline-6-yl sulfonyl)semicarbazide	 **A_4**
5	(E) -N-benzylidene-2,3-diphenylquinoxaline-6-sulfonamide	 **B_1**
6	(E) -N (4-nitrobenzylidene) -2,3-diphenylquinoxaline-6-sulfonamide	 **B_2**
7	(E)-N-(2-chlorobenzylidene) -2,3-diphenylquinoxaline-6-sulfonamide	 **B_3**
8	(E)-N-(4-chlorobenzylidene) -2,3-diphenylquinoxaline-6-sulfonamide	 **B_4**
9	(E) -N-(dimethylamino) benzylidene) -2,3-diphenylquinoxaline-6-sulfonamide	 **B_5**

10	(E) -2,3-diphenyl-N-((E) -3-phenylallylidene) quinoxaline-6-sulfonamide	 **B_6**

RESULTS AND DISCUSSION

The results of antimicrobial activity indicated that all the newly synthesized compounds A_{1-4} and B_{1-6} were found to be almost as active against all bacterial strains used in this *in vitro* bioassay at the concentration range of 10-50 µg/ml. The results of the antimicrobial studies were compared with the standard antibiotic Ciprofloxacin at 10 µg/ml concentrations. The test compounds A_2, B_2, B_5 & B_6 exhibited significant activity against all the bacterial strains *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Salmonella typhi* at 50 µg/ml concentrations. In fact, these compounds exhibited comparatively equipotent activity at 50 µg/ml concentration with that of standard Ciprofloxacin (10 µg/ml) which has a core 2, 3-diphenylquinoxaline moiety. However,on the other hand the test compounds A_3, A_4, B_3 & B_4 showed moderate activity against the same strains at 10 µg/ml concentration but there is a likeness at 50 µg/ml concentration.

Relatively the test compounds A_1 & B_1 showed comparatively mild activity than other compounds.

It was noticed that benzylidene derivatives of substituted quinoxaline (B_{1-6}) were found to possess comparatively equipotent activity than sulphonamido quinoxaline derivatives (A_{1-6}) due to the presence of an additional phenyl moiety with imine nitrogen as a substituent with the parent scaffold 2, 3-diphenyl quinoxaline at 7^{th} position.

Introduction of benzylidene itself have improved the potency of the compound in antimicrobial action with this sulphomoyl group have potentiate the antimicrobial action. Similarly the sulphonamido quinoxalines and the followers of phenyl hydrazine and semicarbazide too had shown the significant antimicrobial action in the present study.

Moreover, from a perusal of the results, it was evident that the compounds substituted with cinnamaldehyde, dimethylamine, aniline and para nitro phenyl at seventh position of the 2, 3-diphenylquinoxaline nucleus showed higher activity against all the tested bacterial strains *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Salmonella typhi* at 50 µg/ml concentrations. In fact, these compounds exhibited comparatively equipotent activity. Meanwhile, no compounds have produced the promising activity against that of fungi strains even though while increasing concentrations.

CONCLUSION

In the present attempt, all the newly synthesized compounds A_{1-4} [sulphonamido quinoxalines] and B_{1-6} [quinoxaline substituted with benzyidene] were found to be almost active against all bacterial strains used in this *in vitro* bioassay at the concentration range of 10-50 µg/ml. In this study the both the set of compounds A_{1-4} [sulphonamido quinoxalines] and B_{1-6} [quinoxaline substituted with benzyidene] are containing a heterocyclic scaffold, diazine. Therefore the probable mechanism of antibacterial activity is to inhibit the enzyme dihydropteroate synthase, DHPS. DHPS catalyses the conversion of PABA (para-aminobenzoate) to dihydropteroate, a key step in folate synthesis. Folate is necessary for the cell to synthesize nucleic acids (nucleic acids are essential building blocks of DNA and RNA), and in its absence cells will be unable to divide. Hence the sulfonamide antibacterials exhibit a bacteriostatic rather than bactericidal effect which is evident in the antibacterial sulfonamides act as competitive inhibitors in the same dihydropteroate synthase enzymne. However, further revision is necessary to ascertain the molecular mechanism of antibacterial activity of test compounds.

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CONFLICT OF INTEREST

No interest.

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