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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL 2, 3-DIPHENYL QUINOXALINE-1, 4-DI-*N*-OXIDE DERIVATIVES

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ABSTRACT

A novel series of 2,3-diphenyl quinoxaline-1,4-di-*N*-oxide derivatives were synthesized and evaluated for their antibacterial and antifungal activities against gram negative viz *Escherichia coli*, *P.aeruginosa* and gram positive bacteria viz *staphylococcus aures*, *Bacillus subtilis and Bacillus cereus and pathogenic fungi* viz *Candida albicans* and *Saccharomyces cerevisiae*. Streptomycin and Nystatin used as standard drugs. All the compounds were characterized by physical and spectral data. All the compounds showed potent to moderately potent antimicrobial activity. These compounds can be further exploited to get the potent lead compound.

KEYWORDS: Quinoxaline, heterocycle, antibacterial activity, antifungal activity.

INTRODUCTION

In recent years, among the various classes of heterocyclic compounds, quinoxalines seemed as important component of pharmacologically active compounds. A quinoxaline, also called a benzopyrazine, in organic chemistry is a heterocyclic compound containing a ring complex made up of a benzene ring and a pyrazine ring. They are isomeric with quinozolines. Quinoxaline derivatives recently receive more attention of researchers. Several compounds containing quinoxaline ring are well known as drugs. For example Echinomycin, Levomycin, and Actinoleutin are known to inhibit growth of gram positive bacteria.

Quinoxalines and Quinoxaline 1, 4, di-N-Oxides are heterocyclic compounds that are often used in the synthesis of biologically active compounds. Quinoxalines are a large class of active chemical compounds exhibiting a broad spectrum of biological activities in animals as well as in humans. Literature studies on Quinoxalines have shown that these derivatives possess a wide variety of biological activities such as antimalarial[1], antispasmodial[2], antibacterial[3-5], anticonvulsant[6,7], anti-inflammatory[8,9], CNS depressant[10], antiplasomodial[11], antimicrobial[12,13], antifungal[14], antioxidant[15] anticancer[16], antinociceptive[17], antihistaminic [18], antipsychotic[19] . Quinoxaline 1,4-di-N-Oxides were first prepared as potential antagonists of vitamin-K activity molecules, but such antagonism has never been demonstrated.

EXPERIMENTAL

MATERIAL AND METHODS

The melting point of the compounds was determined in open capillary tube and values are uncorrected. IR spectra were recorded in KBr discs on a Brooker FTIR Spectrophotometer. The purity of the newly synthesized compounds was evidenced by HPLC (Agilent) and their elemental analysis was generally found to be in agreement with the structure. 1H-NMR spectra were recorded on a JOEL-JNM EX-90 FT-NMR, (90 MHZ) Spectrometer in $CDCl_3$/DMSO-d6 as a solvent, the chemical shifts(δ) are expressed in ppm using TMS as internal standard. TLC was carried out on a precoated plate (silica gel 60F-254, Merck) and spots were visualized with Iodine (or) UV light. All the solvents used were of analytical grade.

GENERAL PROCEDURE FOR THE SYNTHESIS

Synthesis of novel 2,3-diphenyl Quinoxalines (QX1-QX6)

An equimolar mixture of 5-substituted orthophenylene diamines and Benzil are taken in a round bottomed flask containing 25ml of methanol, under constant stirring. The reaction was further stirred. The process of the reaction was monitored by TLC in 254F silica gel plates with ethyl acetate. On continuous stirring for a period of time, a pale yellow precipitate was formed which indicated the completion of the reaction and the formation of the desired product. The reaction mixture was filtered and washed several times with ethanol. The yellow solid (QX1-QX6) was purified by column chromatography using silica gel (Merck) 60-120 mesh and a mixture of ethyl acetate and *n*-Hexane as the eluent. The compounds QX1-QX6 were crystallized from methanol.

Characterization of synthesized QX1-QX6 compounds

Compound QX1: 2,3-diphenyl quinoxaline: The sample was recrystallized using ethyl acetate. Yield 99%, M.P**:** 120-125^{0}C.

IR (KBr) cm^{-1}: 1672cm^{-1}, 1600cm^{-1}, 637cm^{-1}.

1HNMR ($CDCl_3$): δ7.39-δ8.2 (Ar-H).

Compound QX2: 2, 3-diphenylquinoxaline-6carboxylic acid: The sample was recrystallized using ethyl acetate. Yield**:** 99.7%, M.P: 282-285^{0}C

IR(KBr)cm^{-1} : 3689cm^{-1} , 3336cm^{-1}, 3061cm^{-1}, 1716cm^{-1}, 1600cm^{-1}.

1HNMR ($CDCl_3$): δ7.25-δ8.35 (Ar-H), δ8.99 (COOH).

Compound QX3: 6-methoxy 2,3-diphenylquinoxaline: The sample was recrystallized using ethyl acetate. Yield 96.7 %, M.P: 160-165^{0}c.

IR(KBr)cm^{-1}: 2839cm^{-1},1600cm^{-1}, 1585cm^{-1}, 1185cm^{-1}.

1HNMR ($CDCl_3$): δ3.22-δ3.52 (-OCH_3), δ6.60-δ7.39 (Ar-H).

Compound QX4: 2,3-diphenylquinoxalin-6-yl)(phenyl)methanone: The sample was recrystallized using ethanol Yield 57 %, M.P: 100-110^{0}C.

IR (KBr) cm^{-1}: 3016cm^{-1}, 1600cm^{-1}, 1580cm^{-1}.

1HNMR ($CDCl_3$): δ3.52 (-OCH_3), δ6.60-δ7.39 (Ar-H).

Compound QX5: 6-chloro-2,3-diphenylquinoxaline1,4di-N-Oxide: The sample was recrystallized using ethyl acetate. Yield 68 %, M.P: 170-180^{0}C.

IR(KBr)cm^{-1}: 3336cm^{-1},1600cm^{-1}, 1584cm^{-1}, 1125cm^{-1}.

1HNMR ($CDCl_3$): δ7.24-δ8.38 (Ar-H).

Compound QX6: 5-methyl-2,3-diphenylquinoxaline1,4di-N-Oxide: The sample was recrystallized using ethyl acetate. Yield 83%, M.P: 113-115^{0}C.

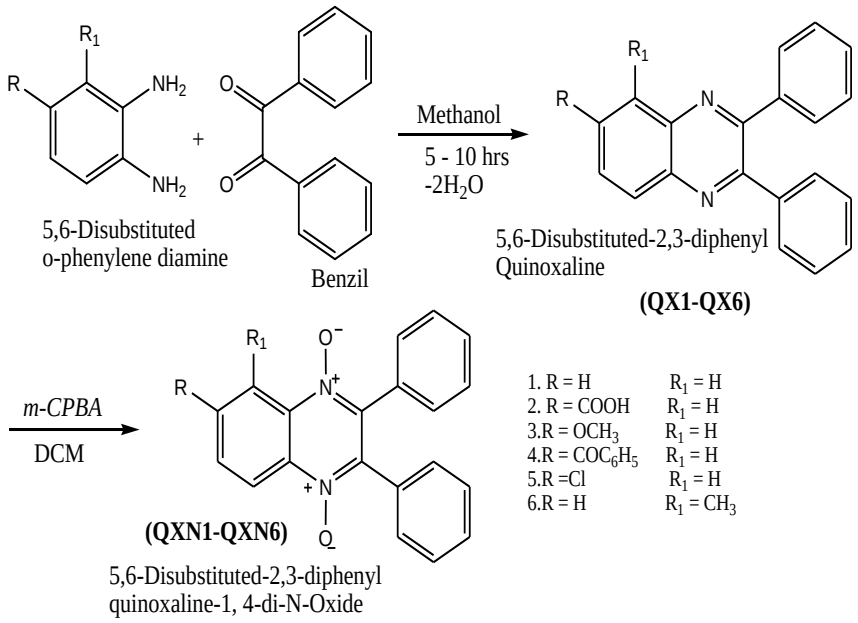
IR(KBr)cm^{-1}: 3689cm^{-1}, 3630cm^{-1}, 2850cm^{-1}, 1615cm^{-1}.

1HNMR ($CDCl_3$): δ2.31 (-CH_3), δ7.26-δ8.35 (Ar-H).

Synthesis of novel 2,3-diphenyl quinoxaline-1, 4-di-N-Oxides (QXN1-QXN6)

The crystallized compounds QX1-QX6 were treated with mCPBA in presence of DCM (50ml) and stirred for 2hrs and refluxed for 3hrs. After the completion of reaction, the solid product was washed with petroleum ether. The synthesized compounds viz., 6-substituted-

2,3-diphenyl quinoxaline-1,4-di-N-Oxide (QXN1-QXN6) were recrystallized from ethanol. The synthetic procedure is presented in following scheme.



Characterization of synthesized QXN 1-QXN 6 compounds

Compound QXN1: 2,3diphenyl quinoxaline 1, 4-di-N-Oxide: The sample was recrystallized using ethanol. Yield 93%, M.P: 82-85^{0}C.

IR(KBr)cm^{-1}: 1600cm^{-1},1672cm^{-1}, 977cm^{-1}, 637cm^{-1}.

1HNMR ($CDCl_3$): δ7.26-δ8.24 (Ar-H).

Compound QXN2: 6-carboxylic-2,3-diphenylquinoxaline 1,4-N-dioxide: The sample was recrystallized using ethanol. Yield 92-95%, M.P: 92-95^{0}C.

IR(KBr)cm^{-1}: 3689cm^{-1}, 3336cm^{-1}, 3061cm^{-1}, 1716cm^{-1}, 1600cm^{-1}, 902cm^{-1}.

1HNMR ($CDCl_3$): δ7.25-δ8.35 (Ar-H) δ9.02 (COOH).

Compound QXN3: 6-methoxy 2,3-diphenylquinoxaline 1,4-N-dioxide: The sample was recrystallized using ethanol Yield 81 %, M.P: 140-145^{0}C.

1HNMR ($CDCl_3$): δ3.22-δ3.52 (-OCH3), δ6.60-δ7.39 (Ar-H).

IR(KBr)cm^{-1}: 3016cm^{-1}, 2964cm^{-1}, 2839cm^{-1}, 1600cm^{-1}, 1585cm^{-1}, 1185cm^{-1}, 898cm^{-1} .

Compound QXN4: **2, 3-diphenyl 6-benzoylquinoxaline 1,4-N-dioxide:** The sample was recrystallized using ethanol Yield 43 %, M.P: 118-120^{0}C.

1HNMR ($CDCl_3$): δ7.35-δ8.38 (Ar-H),

IR(KBr)cm^{-1}:, 3336 cm^{-1}, 1615cm^{-1}, 1580cm^{-1} , 909cm^{-1}.

Compound QXN5: 6-chloro2,3-diphenylquinoxaline1,4di-N-Oxide: The sample was recrystallized using ethanol. Yield 72 %, M.P: 92-93^{0}C,

1HNMR ($CDCl_3$): δ7.24-δ8.38 (Ar-H).

IR(KBr)cm^{-1}: 3336cm^{-1},1600cm^{-1}, 1584cm^{-1}, 1125cm^{-1}, 899cm^{-1}.

Compound QXN6: 5-methyl-2,3-diphenylquinoxaline1,4-N-dioxide: The sample was recrystallized using ethanol. Yield 47%, M.P: 130-140^{0}C

1HNMR ($CDCl_3$): δ7.26-δ8.35 (Ar-H), δ2.31 (-CH_3).

IR(KBr)cm^{-1}: 3689cm^{-1}, 3630cm^{-1}, 2850cm^{-1}, 1615cm^{-1}, 910cm^{-1}.

BIOLOGICAL EVALUATION

After characterization of the synthesized compounds it is proposed to evaluate their antimicrobial activity. The antimicrobial activities of the synthesized compounds QXN1, QXN2, QXN3, QXN4, QXN5 and QXN6 were determined by the agar well diffusion technique.

Antimicrobial Activity

All the tested compounds along with standard streptomycin and nystatin were screened *in-vitro* for antimicrobial activity against gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), gram negative bacteria *Peudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443) and pathogenic fungi *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). The solutions of each tested compound were dissolved in dimethyl sulphoxide (DMSO). The different concentrations (200µg/ml, 100µg/ml, 50µg/ml and 25µg/ml) were used for testing antimicrobial activities. The sterile nutrient agar medium was inoculated with test organism. The inoculation has to be completed under aseptic conditions and when the medium was in molten state. The inoculated medium was transferred to sterile petri dishes, evenly distributed and allowed to solidify. The cups (6 mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. Into each of these cups, 0.05mL (50µg) of the test compound/reference standard/control was added by using a micropipette. DMSO was used as a control (solvent) which did not possess any inhibition zone. The plates were incubated at 37^{0}C for 24hrs and the zone of inhibition was measured in mm. The results of the antimicrobial activities are summarized in Table 1.

RESULTS AND DISCUSSIONS

Table 1: Antimicrobial activities of novel 2,3-diphenyl quinoxaline-1,4-di-*n*-oxide derivatives

Name of The Compound	Conc. of Compound µg/ml	Zone of Inhibition in mm						
		Gram positive bacteria			Gram Negative bacteria		Fungi	
		S.aureus	*B.subtilis*	*B.cereus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*S.cerevisiae*
QXN1	200 µg/ml	14	15	12	15	19	12	9
	100 µg/ml	9	11	9	12	16	9	NI
	50 µg/ml	NI	NI	NI	9	13	NI	NI
	25 µg/ml	NI	NI	NI	NI	9	NI	NI
QXN2	200 µg/ml	12	18	14	15	15	13	12
	100 µg/ml	9	16	9	9	11	9	9
	50 µg/ml	NI	NI	NI	NI	9	NI	NI
	25 µg/ml	NI	NI	NI	NI	NI	NI	NI
QXN3	200 µg/ml	13	18	13	15	16	17	14
	100 µg/ml	11	15	9	12	14	15	10
	50 µg/ml	9	9	NI	9	11	12	NI
	25 µg/ml	NI	NI	NI	NI	9	9	NI
QXN4	200 µg/ml	13	18	12	18	15	14	13
	100 µg/ml	11	14	9	15	12	11	10
	50 µg/ml	9	11	NI	10	10	9	NI
	25 µg/ml	NI	NI	NI	NI	NI	NI	NI
QXN5	200 µg/ml	16	18	12	18	19	12	13
	100 µg/ml	13	12	NI	15	15	9	9
	50 µg/ml	9	9	NI	9	12	NI	NI
	25 µg/ml	NI	NI	NI	NI	9	NI	NI
QXN6	200 µg/ml	19	14	12	14	20	13	12
	100 µg/ml	15	10	9	10	15	9	9
	50 µg/ml	12	NI	NI	NI	11	NI	NI
	25 µg/ml	9	NI	NI	NI	9	NI	NI
Streptomycin		23	20	25	21	22		
Nystatin							20	18

The data recorded in Table-III indicated that compounds QXN1, QXN5 and QXN6 showed potent activity against *P.aeruginosa* and the compounds QXN2, QXN3 and QXN4 show moderate active against *P.aeruginosa.* Compounds QXN2, QXN3, QXN4 and QXN5 exhibited high activity against *B.subtillis* (QXN1, and QXN6 show moderate active against *B.subtillis*). The compounds QXN4, QXN5 show high activity against *E.coli* and QXN1, QXN2, QXN3 and QXN6 show moderate activity against *E.coli*. Compound QXN2, QXN6 show high activity against *S.aureus;* while QXN1, QXN2, QXN3 and QXN4 showed moderate activity. All the compounds showed minute activity against *B.cereus*. All these

compounds are compared with the standard reference (streptomycin) for their antibacterial activities. Compounds QXN2, QXN3, QXN5 and QXN6 showed moderate antifungal activity when compared with standard reference "Nystatin".

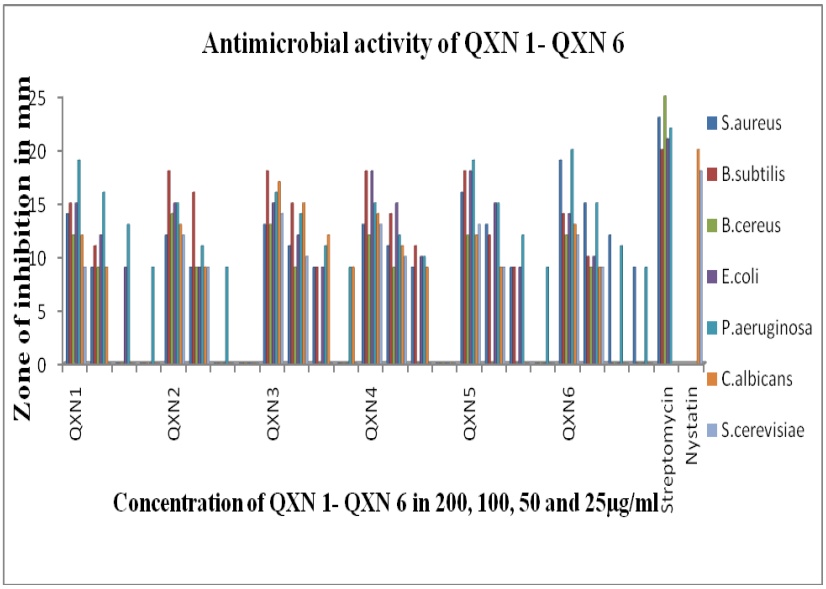


Fig 1: Antimicrobial activity of novel 2,3-diphenyl quinoxaline-1,4-di-*n*-oxide derivatives

CONCLUSION

In this study, certain novel quinoxaline 1,4-di-N-oxide derivatives were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds possess significant *in-vitro* activity. The study would be a fruitful matrix for the development of novel quinoxaline 1,4-di-N-oxide derivatives for further bio-evaluation.

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