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Synthesis and Characterization of 2,3-Diphenyl Quinoxaline 1,4-di-*N*-oxide Derivatives and Study of their Antimicrobial Activities

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ABSTRACT

The aim of the present study was to synthesize new compounds, 2,3-diphenyl,6-sub quinoxaline 1,4-di-*N*-oxide derivatives and to investigate the antimicrobial activities. 6-sub, 2,3-diphenyl quinoxalines (III a–f) have been prepared by the condensation of substituted o-phenylene diamines (I) with benzil (II). Six compounds (III a-f) have been synthesized and characterized. Compounds III a-f were further treated with m-Chloro per benzoic acid (m-CPBA)/dichloro Methylene (DCM) to form their corresponding 1,4-di-*N*-oxides IV a-f . Adopting the above procedure 2,3-diphenylpyrido[3,2-*b*]pyrazines (VII a-b) and their corresponding 1,4-di-*N*-oxides (VIII a-b) were synthesized and characterized. The structures of the above synthesized compounds were established on the basis of IR and ^{1}H NMR spectral data. These new compounds were screened for their antimicrobial activity. The results were presented.

Keywords: o-phenylene diamines, 1,4-di-*N*-oxide derivatives, Quinoxalines, benzil, m-CPBA, DCM.

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INTRODUCTION

Quinoxalines are a versatile class of nitrogen containing heterocyclic compounds and they constitute useful intermediates in organic synthesis [1] and also widely used in dyes [2], pharmaceuticals [3,4], and electrical/photochemical materials [5-10]. Quinoxaline ring moiety constitute part of the chemical structures of various antibiotics such as Echinomycin, Levomycin and Actinoleutin [11,12] that are known to inhibit growth of gram positive bacteria and are active against various transplantable tumors. Quinoxalines, including their fused-ring derivatives, display diverse pharmacological activities (antiviral, anticancer, and antibacterial) [13,14]. Oxidation of both nitrogens of the quinoxaline ring dramatically increases the diversity of certain biological properties, such as antibacterial activity [15-18] and hypoxia-selective anticancer activity [19]. Monge *et al*. [20-24] have reported the synthesis and biological evaluation of new agents derived from Quinoxalines 1,4-di-*N*-oxide (and related compounds) that have proved to be efficient cytotoxic agents for hypoxic cells in solid tumors. Recently, Sauvain *et al*. [25] reported that 3-(4′-chloro) phenylquinoxaline-2-carbonitrile-1,4-di-*N*-oxide had potent antimalarial activity particularly against a chloroquine resistant strain of *Plasmodium falciparum.* In continuation of our interest on the chemistry of quinoxalines, it is aimed to synthesize new quinoxaline derivatives and to examine their antimicrobial properties.

RESULTS AND DISCUSSIONS

Observing the results recorded in table –I it is evident that the formation of the products **III a-f** and **IV a-f** is directly depend on donating group or withdrawing group on aromatic ring. The fact is well recorded in case of **IIIb** (with drawing group) and **IIIc** (donating group) resulting the formation of **IVb** and **IVc** with long reaction time of 12hrs for **IIIb** and 2hrs in case of **IIIC.** Further the conformity of the synthesized 1,4-di-*N*-Oxides were identified by the IR spectral data (875-910 cm-1; N O stretching). The data (Table –II) indicated that compounds **IVa, IVe** and **VIIIb** shows excellent activity against *P.aeruginosa* and the compounds **IVb, IVc** and **IVd** shows moderately active against *P.aeruginosa.* The compounds **IVb, IVc, IVd** and **IVe** showed high activity against *B.subtilis,* **IVa,** and **VIIIb** shows moderately active against *B.subtilis.* The compounds **IVd, IVe** shows high activity against *E.coli* and **IVa, IVb, IVc, VIIIb** shows moderately active against *E.coli* and compound **VIIIb**, **IVe** showed high activity against *S.aureus*, **IVa, IVb, IVc, IVd** showed moderate activity against *S.aureus.* All the compounds showed minute activity against *B.cereus.* All these compounds are compared with the standard reference (streptomycin) for their antibacterial activities. Compounds **IVc, IVd** exhibited highest degree of antifungal activity and compounds **IVa, IVb, IVe, VIIIb** showed moderate antifungal activity when compared with standard reference Nystatin.

Antibacterial activity

The antimicrobial activities of the synthesized compounds **IVa, IVb, IVc, IVd, IVf, VIIIb** were determined by the agar well diffusion technique [26]. All the tested compounds along with standard streptomycin was screened *in vitro* for antibacterial activity against gram positive bacteria *Staphylococcus aureus* (MTCC 3160)*, Bacillus subtilis* (MTCC 441) and *Bacillus cereus* (MTCC 430), gram negative bacteria *Pseudomonas aeruginosa* (MTCC 424) and *Escherichia coli* (MTCC 443). 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 antibacterial 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°C for 24 hrs and the zone of inhibition was measured in mm. The results of the antibacterial activities are compared with standard reference and summarized in Table II.

Antifungal activity

All the synthesized compounds **IVa, IVb, IVc, IVd, IVf, VIIIb** were evaluated *in vitro* for antifungal activity by using agar well diffusion method, the test organisms are *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170) were used. They were grown on potato dextrose agar medium. The plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured in mm. Nystatin was used as a standard reference and DMSO was used as a solvent (control), which did not possess any inhibition zone. The results of the antifungal activities are summarized in Table II.

CONCLUSION

A new class of 1,4-di-*N*-oxide heterocyclic derivatives were synthesized and the results of antimicrobial data revealed that the compounds possess significant *in vitro* activity. The study would be a fruitful matrix for the development of 2,3-diphenyl quinoxaline 1,4-di-*N*-Oxide derivatives for further biological evaluation.

EXPERIMENTAL

General

The purity of the newly synthesized compounds was evidenced by HPLC and their elemental analysis was generally found to be in agreement with the structure. The melting point of the compounds was determined in open capillary tube on a Remi electric melting point

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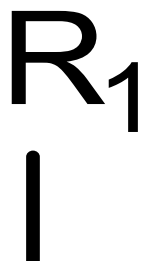
apparatus and values are uncorrected.IR spectra were recorded in KBr discs on a shimadzu IR Affinity Spectrophotometer.^{1}H-NMR spectra were recorded on a JOEL –JNM EX-90 FT- NMR, (90 MHZ) Spectrophotometer in $CDCl_3$ and DMSO - d_6 as a solvent, the chemical shifts (δ) are expressed in ppm using TMS 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

Synthesis of 6-substituted,2,3-diphenyl Quinoxaline (III a-f) and 6-substituted, 2,3-diphenyl quinoxaline 1,4 di-*N*-oxide (IVa-f):

The quinoxalines are prepared by condensation of 5-substituted, o-phenylene diamine (I) with benzil (II) in MeOH, stirred for 1hr at room temp, and further refluxed for 5-10 hrs. The reaction was monitored by TLC. The products (III a-f) are recrystallized with ethyl acetate. The structures were established by IR and ^{1}H NMR spectral data (see table-I).

The compounds (III a-f) are treated with MCPBA/DCM and stirred for 2hrs and refluxed for 3hrs. The products 6-substituted, 2,3-diphenyl quinoxaline1,4-di-*N*-Oxide (IV a-f) are recrystallized with ethanol. The synthetic procedure is presented in scheme –I



Scheme I

Synthesis of 2,3-diphenylpyrido[3,2-*b*]pyrazine (VIIa),7-bromo-2,3-diphenylpyrido[3,2-*b*]pyrazine (VIIb) and their1,4-*N*-dioxides (VIIIa &VIIIb):

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Pyridine-2,3-diamine(Va)/5-bromopyridine-2,3-diamine(Vb) was condensed with benzyl (VI) in MeOH, stirred for 5-10 hrs and refluxed for 4hrs. Reaction was monitored by TLC and the product (VII a-b) was recrystallized with Hexane and ethyl acetate.

The compounds (VII a-b) was further treated with MCPBA / DCM solvent and stirred for ½ hrs and reflux for 2hrs. The product formed (VIII a-b) are washed with ethyl acetate and recrystallized with hexane. All the above compounds were repeatedly crystallized and the structures of the above compounds were established by IR and ^{1}H NMR spectral data (see table-I). The synthetic procedure is presented in scheme-II.

Scheme – II

Table -1 Physical and Spectral data of synthesized compounds:

Comp	Molecular formula	Reaction time (hrs)	M.P (^{O}C)	Yield (%)	^{1}HNMR	IR
IIIa	$C_{20}H_{14}N_2$	1	120-125	99	7.39-8.2(Ar-H)	1600, 1672,637
IVa	$C_{20}H_{14}N_2O_2$	2	82 - 85	93	7.26-8.24(Ar-H)	1600, 1672, 977, 637
IIIb	$C_{21}H_{14}N_2O_2$	12	282 - 285	99.7	7.25-8.35(Ar-H) 8.99(-COOH)	3336,3689,3061,1716, 1600

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IVb	$C_{21}H_{14}N_2O_4$	5	92 – 95	91.2	7.25-8.35(Ar-H) 9.02(-COOH)	3336,3689,3061,1716, 1600,909
IIIc	$C_{21}H_{16}N_2O$	2	160 - 165	96.7	3.22-3.52(-OCH_3) 6.60-7.39(Ar-H)	1185,1585,1600,2839
IVc	$C_{21}H_{16}N_2O_3$	1	140 – 145	81	3.22-3.52(-OCH_3) 6.60-7.39(Ar-H)	898,1185,1585,1600,2839, 2964,3016
IIId	$C_{27}H_{18}N_2O$	3	100 - 110	57	7.35-8.38(Ar-H)	1615,1580,3336
IVd	$C_{27}H_{18}N_2O_3$	2	118-120	43	7.35-8.38(Ar-H)	909,1615,1580, 3336
IIIe	$C_{20}H_{13}Cl\ N_2$	1	170-180	68	7.24-8.38(Ar-H)	1125,1584,1600,3336.
IVe	$C_{20}H_{13}Cl\ N_2O_2$	1	92-93	72	7.24-8.38(Ar-H)	899.3,1125,1584,1600, 3336
IIIf	$C_{21}H_{16}N_2$	½	113-115	83	2.31(-CH_3) 7.26-8.35(Ar-H)	1615,2850,3630,3689
IVf	$C_{21}H_{16}N_2O_2$	1	130-140	47	2.31(-CH_3) 7.26-8.35(Ar-H)	910,1615,2850,3630,3689
VII a	$C_{19}H_{13}N_3$	2	103-105	59	7.26-8.24(Ar-H)	768,1661,1594, 3060
VIIIa	$C_{19}H_{13}N_3O_2$	3	70-80	69	7.26-8.54(Ar-H)	768,876,910,976,1661, 1594,3060
VII b	$C_{19}H_{12}$ Br N_3	1	91-93	48	7.28-8.54(Ar-H)	787,1126,1661,1612
VIIIb	$C_{19}H_{12}$ Br N_3O_2	½	80-85	57	7.28-8.54(Ar-H)	786,837,876,910,976,1126,1612,1661

Table II: Antimicrobial activities of the synthesized 1,4- di - *N*-oxides:

				Bacteria			Fungi	
Compound	**Conc. of Compound**	***E.coli***	***P.aeruginosa***	***S.aureus***	***B.subtilis***	***B.cereus***	***C.albicans***	***S.cerevisiae***
Compound IVa	200µg/ml	15	19	14	15	12	12	9
	100µg/ml	12	16	9	11	9	9	-
	50µg/ml	9	13	-	-	--	-	-
	25µg/ml	-	9	-	-	-	-	-
Compound IVb	200µg/ml	15	15	12	18	14	13	12
	100µg/ml	9	11	9	16	9	9	9
	50µg/ml	-	9	-	9	-	-	-
	25µg/ml	-	-	-	-	-	-	-
Compound IVc	200µg/ml	15	16	13	18	13	17	14
	100µg/ml	12	14	11	15	9	15	10
	50µg/ml	9	11	9	9	-	12	-

	25µg/ml	-	9	-	-	-	9	-
Compound IVd	200µg/ml	18	15	13	18	12	14	13
	100µg/ml	15	12	11	14	9	11	10
	50µg/ml	10	10	9	11	-	9	-
	25µg/ml	-	-	-	-	-	-	-
Compound IVe	200µg/ml	18	19	16	18	9	12	13
	100µg/ml	15	15	13	12	-	9	9
	50µg/ml	9	12	9	9	-	-	-
	25µg/ml	-	9	-	-	-	-	-
Compound VIIIb	200µg/ml	14	20	19	14	12	13	12
	100µg/ml	10	15	15	10	9	9	9
	50µg/ml	-	11	12	-	-	-	-
	25µg/ml	-	9	9	-	-	-	-
Streptomycin	(200µg/ml)	21	22	23	20	25	-	-
Nystatin	(200µg/ml)	-	-	-	-	-	20	18

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