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Comparative study of antimicrobial activities of azines and 1,4-diazabutadienes to establish the pharmacophore in the lead

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Abstract: **There have been many recent reports of biological activity of derivatives of acyclic aldazines and ketazines against microorganisms. Current work has been carried out to elucidate the importance of the azine framework (>C=N-N=C<) in exhibition of biological activity and to hence establish the pharmacophore in the lead compound. The scope of this paper is to compare the activity of 1,4-diazabutadiene or 1,4-DAD (-N=CH-CH=N-) which is a structural isomer of azine and is only different in the arrangement of atoms. The biological activities of two isomeric compounds 4-hydroxybenzalazine and N,N'-bis(4-hydroxyphenyl)-1,4-diazabutadiene were tested against two bacterial species - E.coli, S.aureus. The azine was found to be significantly more active against both the Gram positive bacteria S.aureus as well as the Gram negative E.coli indicating not only its enhanced potency as against its structural isomer but also its broad spectrum mode of action.**

Keywords: **Azine, antibacterial, *E.coli, S.aureus,* broad spectrum.**

I. INTRODUCTION

The term azine could refer to cyclic 6 membered ring structure containing nitrogen or adiimine framework which is formedon condensation of 2 molecules of a carbonyl compound with one molecule of hydrazine. The azines which have been taken up for current study are N-N linked diimines which are 2,3-diaza derivatives of 1,3-butadiene.[1] The synthesis of these compounds have been reported extensively in literature using methods ranging from room temperature stirring of carbonyl compounds with hydrazine sulphate[2] to solventless synthesis by grinding[3]to newer techniques like microwave synthesis[4].

Organic chemists are faced with a constant quest for synthesis or isolation of new antibiotics due to an increase in microbial resistance to existing ones. This coupled with an ease in functionalising carbonyl group led to the synthesis of many potent antimicrobial schiff's bases and their metal complexes.[5] An azine framework is a bis-schiff's base and hence exhibits good biological activities against many microorganisms. There are many unstructured reports of biological activities of azines. Most of the literature reports are based on random hits of molecules which have antimicrobial activities. However there a few reports on the pivotal role played by theazine functional group in exhibiting biological activity. 5-nitrofurfurylidene azines with aldehydes and ketones were claimed to possess good antimicrobial activity[6]and the published work of *Kidd &Johnston* regards the azine side chain as a "requisite" for the activity of nitrofuran antimicrobials.[7] The importance of azine moiety was reinstated by *Bharatam et al* when they established that the biological activity of a guanide anti-hypertensive drug – guanabenz is primarily due to the azine and not its hydrazone tautomer, contrary to what was previously known.[8]

Kurteva et al provided a semi structured report on the activity of azines. Substituent effect on the biological activity of acyclic benzalazines was studied with respect to both the position and type of substituents on the benzene ring of symmetrical aldazines.[9] It was found that introduction of substituents, in general, led to a loss in activity, however the hydroxy derivatives were seen to be more biological active against bacterial as well as fungal strains.

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1,4-Diazabutadienes which are structural isomers of azines (Fig.1) have been probed for their applications as liquid crystals.[10] Some 1,4-DAD complexes of nickel and cobalt complexes have been reported to have moderate to good antimicrobial activities.[11]However, there are no reported antimicrobial activities of 1,4-DAD's or their derivatives.

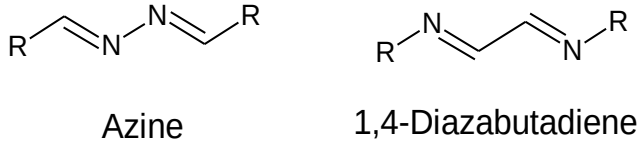

Fig. 1

The current study has been undertaken to compare the antimicrobial activities of azine and 1,4-DAD to get an idea of the pharmacophore in the molecule. The molecular models selected for the study are hydroxyazine [1,4-Bis(4-hydroxyphenyl)-2,3-diaza-1,3-butadiene] and a hydroxyl DAD [1,4-Bis(4-hydroxyphenyl)-1,4-diaza-1,3-butadiene] (Fig.2). The two compounds are isomeric and only differ in the position of nitrogen. 4-hydroxy derivative was chosen as the hydroxyazine derivatives were seen to be more potent than other substituents.

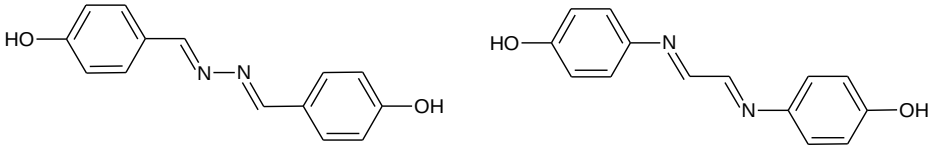

Fig. 2

II. MATERIALS & METHODS

All the chemicals were procured from Merck India. Melting points were determined in open capillaries using Veego silicone oil based apparatus VMP-DS. Reaction progress was monitored by TLC using redistilled solvents.

Procedure for the preparation of azine: The azine, 1,4-Bis(4-hydroxybenzylidene)hydrazine; was prepared by the literature protocol[12] as follows: 23.5 mmol of 4-hydroxybenzaldehyde was mixed with 10mmol of hydrazine sulphate along with 10mmol of triethylamine and ground together. The reaction progress was monitored through TLC and on completion, water was added, crude product filtered and recrystallized from alcohol. An orange coloured crystalline solid was obtained with yield of 85% and melting point of 268^{o}C [lit. 268^{o}C[9]].

Procedure for the preparation of 1,4-DAD: The 1,4-DAD, 1,4-Bis(4-hydroxyphenyl)-1,4-diaza-1,3-butadiene; was prepared by the literature protocol[13] as follows: 6 mmol of 4-aminophenol, 1 mmol of glyoxaltrimerdihydrate and 10 mol % of p-toluenesulphonic acid were placed in a mortar and ground till the reaction was complete indicated by TLC. Product was recrystallized from alcohol to give yellow needle like crystals with yield of 80% and melting point of 195^{o}C [lit. 196^{o}C[14]]

Antimicrobial activity: The antimicrobial activity of the two synthesized compounds was determined by agar cup method according to CLSI standard protocols. A 36 hour old culture of *S.aureus*(ATCC 6538) &*E.coli*(ATCC 25922) was used for the antimicrobial testing. Varying concentrations of the two compounds in DMSO were prepared and checked for their zones of inhibition. DMSO, erythromycin disc (15 µg) and ciprofloxacin disc (5 µg) were used as controls.

III. RESULTS & DISCUSSION

Standard antibiotic discs served as the positive control and DMSO served as the negative control in the analysis. The zones of inhibition for the azine and DAD against controls are tabulated below. The range of concentrations tested was from 0.2 mg/ml to 5.0 mg/ml. However the highest zones of inhibition were obtained at a concentration of 2.5 mg/ml. DMSO did not inhibit the growth of either of the two microorganisms. Ciprofloxacin being a broad spectrum antibiotic inhibited the growth of both the Gram positive as well as the Gram negative bacteria.

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		Concentration (mg/ml) & Zone of inhibition (mm)							
Azine	*E.coli*	Conc.	0.4	0.6	0.8	1.0	1.5	2.0	2.5
		Zone	13	15	18	19	19	20	20
	S. aureus	Conc.	0.4	0.6	0.8	1.0	1.5	2.0	2.5
		Zone	10	12	15	18	22	23	24
DAD	*E.coli*	Conc.	0.4	0.6	0.8	1.0	1.5	2.0	2.5
		Zone	4	7	7	7	7	7	7
	S. aureus	Conc.	0.4	0.6	0.8	1.0	1.5	2.0	2.5
		Zone	0	0	12	12	14	15	15

	Erythromycin (10µg)	Ciprofloxacin (5 µg)	DMSO
E.coli	5	34	-
S.aureus	20	23	-

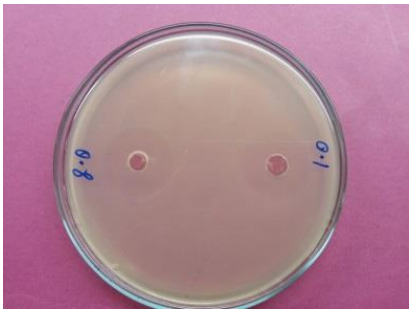

Fig. 3

Fig. 4

Fig.3: Zone of inhibition of 4-hydroxybenzalazine at concentrations 0.8 mg/ml and 1.0 mg/ml

Fig.4: Positive control of erythromycin disc and negative control of DMSO on on*E.coli*

The azine was found to be more active against both *S.aureus*and *E.coli* as compared to the DAD. The two structural isomers had a distinct difference in their antimicrobial activities, hence pointing towards a crucial role played by the azine framework in exhibition of biological activity. 1,4-DAD had moderate activity against the Gram positive *S.aureus* but had negligible activity against Gram negative *E.coli*.

IV. CONCLUSION

This work resonates the importance of azine functional group (>C=N-N=C<) in the antimicrobial compounds. It also highlights an important attribute of the azine – its broad spectrum nature of action. The compound, as against its structural isomer 1,4-DAD exhibited commensurable activities against both Gram positive as well as Gram negative bacteria. The 1,4-DAD exhibited low biological activity against Gram positive bacteria but did not show any appreciable activity against Gram negative bacteria indicating not only an overall lowered potency of this isomer but also its narrow spectrum of biological activity.

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