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Design, Synthesis and Antimicrobial Evaluation of Some Novel α, β-Unsaturated Ketones

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ABSTRACT

A few α,β-unsaturated ketones DN1, DN2, DN3, DN4 and NN1, NN2 have been synthesized and characterized on the basis of elemental analysis, FT-IR, 1H-NMR, DART-MS spectral data. The synthesized compounds were screened for in vitro growth inhibiting activity against different bacterial strains viz, Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and against Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa* and fungi *Candida albicans* and *Aspergillus niger* were compared with the standard drugs Gentamycin for antibacterial and Ketoconazole for antifungal screening. Amongst all, compound DN3 showed highly significant ($p>0.001$) antibacterial activity against all the bacterial strains used in this study, when compared to standard. DN1, NN1 & NN2 showed moderate significant ($p>0.01$) inhibition against all bacterial strains when compared to standard. All the compounds were shown negligible or a mild activity against the fungal strains used in this study.

Key words: α,β-unsaturated ketones, Antimicrobial Evaluation, Agar Well Diffusion Method.

INTRODUCTION

A significant cause of death in the developing countries is infection. In particular, the rise of antibiotic resistance and the emergence of more recent pathogenic agents are to blame for this. Bacterial resistance to antimicrobial drugs has developed throughout time as a result of the evolution of bacteria and the large, unwise use of antibiotics in clinical practice. Antimicrobial resistance is acknowledged as a significant issue for treating microbiological infections Inactivation of antibiotics, target alteration, altered permeability, and "bypass" of metabolic pathway are a few examples of the biochemical resistance methods employed by bacteria. The identification of mutations that cause bacterial resistance to antibiotics (genetic analysis) and the phenotypic manifestations of this resistance in bacteria are useful

Clinicians will benefit from a better understandin g of the mechanisms underlying antibiotic resista nce when deciding how and when to use antibioti cs.

A flexible lead molecule for creating possible bioactive compounds is, *α,β* -unsaturated ketones. Numerous biological actions, including antitubercular, antioxidant, anti-inflammatory, anti-cancer, antiviral, antifilarial, and broad spectrum antibacterial, have been linked to the, *α,β* -unsaturated ketone derivatives. It is well known that α, β-unsaturated ketones are effective on antimicrobial studies especially in the case of antibacterials.

Keeping this context in mind an attempt has been made to synthesis and in-vitro antibacterial activity of some novel α, β unsaturated ketone derivatives. This possibly led to the development of compounds with probable antimicrobial activity especially in antibacterial study to overcome the strains those are resistant with earlier α, β-unsaturated ketone derivatives by developing structural modifications. [1-5]

MATERIALS AND METHODS

Experimental

Melting points were determined in open glass capillaries using an Electro thermal IA 9000 SERIES digital melting point apparatus (Electrothermal, UK) and are uncorrected. IR spectra (KBr wafers) were taken on Shimadzu FT-IR Spectrophotometer, Model No.8400S (Japan). 1H NMR spectra were recorded on Bruker 300 MHz NMR spectrometer (Switzerland) using CDCl3 as solvent. Mass spectra were documented on Joel SX-102(EI/CI/DART/MS) (USA). Microanalysis was done on a Vario EL III Elementor C, H, N analyzer (Germany). Iodine vapour was used as the developing agent and the solvent system used was benzene: ethanol (8:2). Respective aldehydes and ketones were procured from sigma-Aldrich, USA. All other chemicals used in the present studies were either of A.R or

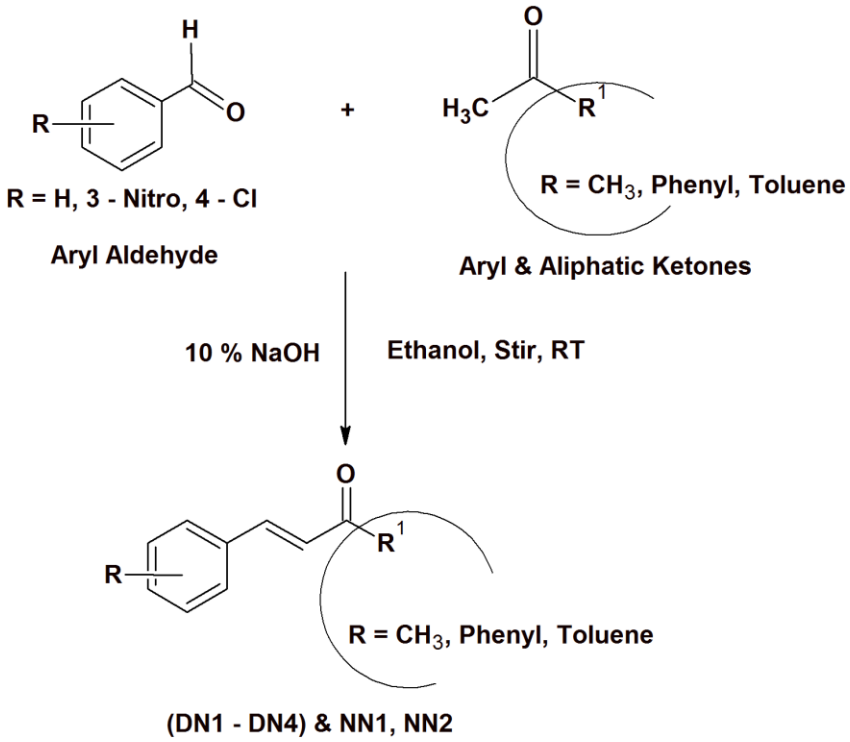
G.R quality. Anhydrous sodium sulphate was used as the drying agent. The purity of all compounds was established by a single spot on the TLC plates (Merck, Germany). Dimethyl formamide (Sigma), Ciprofloxacin; Ketoconazole (pure drug) was used. All the prototypes were dissolved in dimethyl sulfoxide for making the concentration of 100µg/ml.

Chemical Synthesis

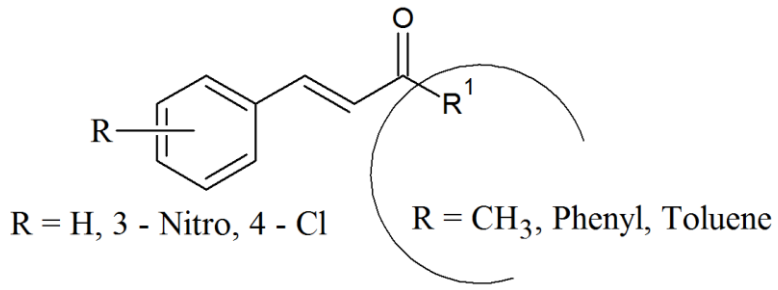
Synthesis of (1Z,4E)-1,5-diphenylpenta-1,4-dien-3-one [DN1]

Add 4 ml (0.039 M) of benzaldehyde and 2 ml (0.027 M) of acetone to a clean conical flask that has been filled with 25 ml of ethanol and 2 g (0.05 M)of 10 % alcoholic Sodium Hydroxide. Put the flask in a water bath and stir it constantly with a magnetic stirrer. Stir the mixture while maintaining a 30 °C temperature. After continuing to agitate for an additional hour, a crude yellow-colored product is produced. The product is then filtered, dried, and recrystallized from ethanol. To the reaction mixture, add dilute hydrochloric acid and then transfer it into a 250 mL separating funnel. To the mixture, apply 20ml of chloroform/ether and shake thoroughly. Shake the mixture completely, remove the organic layer and repeat the process twice. Cool the ice-water mixture. As a fine emulsion the compound separates initially and then forms yellow crystals. Under pressure, distill the residual portion and collect the fraction that boils at 150° C. Wash with cold water the yellow crystals, dry them and crystallize with ethanol.

Similarly the other derivatives of α, β-unsaturated ketones [**DN1-DN4 & NN1, NN2**] were synthesized by utilizing two phases with various aryl aldehydes (**DN1-DN4)** and aliphatic ketones (**NN1, NN2)**. [6-8]



Scheme: Synthesis OF α, β-Unsaturated Ketones [**DN1-DN4 & NN1, NN2**]



(DN1 - DN4) & NN1, NN2

DN1

DN2

DN3

DN4

NN1

NN2

COMPOUND	**R**	**MF**	**MR**	**MW**	**MP** (°C)	**Rf Value**	**DENSITY** (g/cm^3)
DN1	H	$C_{17}H_{14}O$	77.63	212.8	110	0.87	1.097
DN2	H	$C_{12}H_{12}O_2$	55.94	173.9	58	0.73	1.081
DN3	H	$C_{15}H_{12}O$	67.10	189.8	55	0.59	1.157
DN4	H	$C_{16}H_{14}O$	71.93	206.0	93.2	0.73	1.078
NN1	NO_2	$C_{10}H9_NO_3$	53.35	55.8	188	0.89	1.226
NN2	Cl	$C_{10}H_9ClO$	51.69	155.9	58	0.63	1.157

Table 1. Physico-chemical data of the synthesised compounds [DN1-DN4 & NN1, NN2]

[a] µg/disc,

[b] Gentamycin (10µg/disc); Ketoconazole (10 µg/disc) were used as positive reference standards antibiotic discs.

Prototype Compounds	Zone of Inhibition (mm)											
	Bacterial strains								Fungal strains			
	Staphylococcus Aureus		*Bacillus subtilis*		*Escherichia Coli*		*Pseudomonas Aeruginosa*		*Candida albicans*		*Aspergillus niger*	
	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]	50[a]	100[a]
DN1	17	30	16	33	16	32	16	33	1	2	0	1
DN2	21	33	18	30	20	33	15	33	0	2	0	1
DN3	16	23	15	25	15	23	15	25	0	0	0	0
DN4	09	12	08	21	08	17	07	20	0	0	0	0
NN1	20	26	19	31	18	31	19	31	1	2	0	1
NN2	11	18	10	20	09	20	11	23	0	1	0	0
Gentamycin[b]	**32**		**34**		**38**		**32**		-		-	
Ketoconazole[b]	-		-		-		-		**23**		**24**	

Table 2. Evaluation of *in vitro* antibacterial activity of α, β-unsaturated ketones

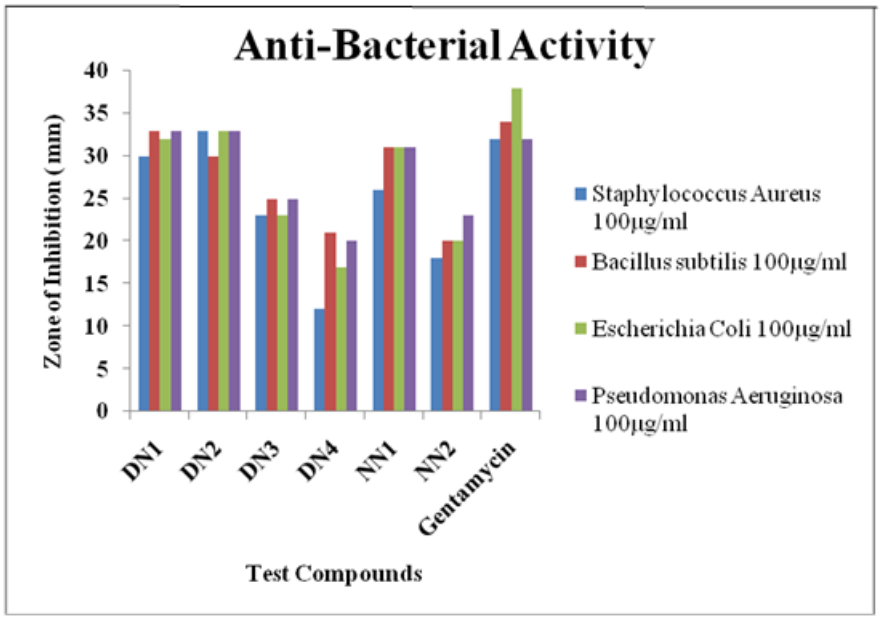


Fig.1: Effect of prototypes (100 µg/ml) and Gentamicin (10 µg/ml) on *in Vitro antibacterial* activity.

Screening for Antimicrobial activity

The antimicrobial activity of all the newly synthesized compounds [**DN1-DN4 & NN1, NN2**] was determined by well plate or agar diffusion method. The medium used were double strength nutrient broth (Hi-Media) for antibacterial activity and double strength malt yeast extract (HiMedia) for antifungal activity. The *in-vitro* antimicrobial activity was carried out against 24h old cultures of bacterial and 72h old cultures of fungal strain. The different strains of bacteria and fungi were used *viz*., *Bacillus subtilis* (NCIM 2193), Gram positive bacteria's, *Escherichia coli* (NCIM 2809). Gram negative bacteria's, and fungi strains *Candida albicans* (NCIM 3471), *Aspergillus niger* (NCIM 1056) were used. The compounds were tested at the concentrations of 10 and 50 µg/ml and solutions were prepared by dissolving in dimethylsulfoxide (DMSO). The petridishes used for antibacterial screening were incubated at 37±1ºC for 24 h, while those used for antifungal activity were incubated at 28 ºC for 48-72h. The results were compared to Gentamycin (10 µg/ml) and Ketoconazole (10 µg/ml) for antibacterial and antifungal activity respectively by measuring zone of inhibition in mm. The antibacterial screening results were presented in Table 2. [9-12]

RESULTS AND DISCUSSION

The results of antimicrobial activity indicated that all the compounds **DN1, DN2, DN3, DN4 and NN1, NN2** were found to be significant active against all bacterial strains used in this in vitro bioassay at the concentration range of 100 µg/ml whereas in case of anti fungal activity all the compounds exhibit a moderate activity at 50 µg/ml. The test compounds **DN1, NN1, DN2, NN1** exhibited a significant ($p>0.001$) activity against *Bacillus subtilis* and *Escherichia Coli*, the prototype compounds shown a significant activity against the both the gram positive and gram negative strains but mild or less activity against the same strains at 10 µg/ml concentration. In fact, these compounds exhibited comparatively equipotent activity at 100 µg/ml concentration with that of standard, Gentamycin at 10µg/ml. On the other hand all the synthesized compounds showed comparatively very less activity against the fungi strains *Candida albicans and Aspergillus niger*. Which has clearly shown that the title compounds α, β-unsaturated ketones (Fig.1) having a good antibacterial activity against the all the bacterial strains what we have been used in this research. Rather the test compounds DN3 and DN4 showed comparatively lesser activity than other compounds.

It was noticed that **DN4** were found to possess comparatively less activity than other derivatives due to the presence of methyl moiety in its second position. Introduction of meta nitro and para chloro at third and fourth position of the α,β-unsaturated ketones has significantly improved potency.

CONCLUSION

In the present attempt, all the newly synthesized few *α, β*-unsaturated ketones **DN1, DN2, DN3, DN4 and NN1, NN2** are related to chalcones. Therefore the probable mechanism of antibacterial activity is due to the presence of the reactive α, β-unsaturated system. Their flexibility to change their structure by incorporating different types of substituent groups into the aromatic ring can potentially achieve a higher potency, lower toxicity, and a wider spectrum of antibacterial activity.α, β-unsaturated ketones derivatives showed potential antibacterial activities against different pathogenic strains, including antibiotic-resistant bacteria. α, β-unsaturated ketones have shown strong antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, and against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa.* [13-16]

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