Research Article

Synthesis of Chalconalides as Antimicrobial Agents

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ABSTRACT

In a wide search program towards new and efficient antimicrobial agents, substituted chalconanilides have been synthesized by condensing benzaldehyde derivatives with N-phenyl acetanilide derivatives in dilute ethanolic sodium hydroxide solution at room temperature according to Claisen – Schmidt condensation. The structures of these compounds have been investigated by IR spectral analysis. The antimicrobial activity of the synthesized compounds was evaluated by Filter Paper Disc diffusion Method. The compound A 4 & A1 has maximum activity at both concentration against gram positive bacteria & gram negative bacteria respectively.

Keywords: Chalcone, aldehyde, antibacterial.

INTRODUCTION

Chalcones are the aromatic ketones belonging to 1, 3-diaryl-2-propen-1-ones. Chalcones are also known as benzylideneacetophenones or phenyl styryl ketones. Naturally occurring chalcones belong to the flavanoids[1-3]. Chemically, they consist of open chain flavonoids in which the two aromatic rings are joined by a three carbon α,β unsaturated carbonyl system The presence of a reactive α,β unsaturated keto function in chalcones is found to be responsible for their antimicrobial activity[4]. Chalcones are one of the major classes of natural products with widespread distribution in fruits vegetables, spices, tea & soya based foodstuff and variety of trees and plants. Chalcones exist as either *E* or *Z* isomers. *E* isomer is the most stable form and consequently majority of chalcones are isolated as *E* isomer[5].

In recent years a variety of chalconilide have been reviewed for their cytotoxic, anticancer chemopreventive and mutagenic as well as antiviral, insecticidal and enzyme inhibitory properties[6]. A number of chalcones having hydroxy, alkoxy groups in different position have been reported to possess anti-bacterial[7], antiulcer[8], antifungal[9], antioxidant[10], vasodilatory[11], antimitotic[12], and inhibition of chemical mediators release, inhibition of leukotriene B_4[13], inhibition of tyrosinase and inhibition of aldose reductase[14] activities. Appreciation of these findings motivated us to synthesize chalconalides as a potential template for antimicrobial agents. It must be noted that this scaffold provides substitution pattern on benzylidenacetophenones nucleus.

EXPERIMENTAL

Chemistry

The melting points were recorded in open sulphuric acid or oil bath using thermometer and were uncorrected. IR spectra were recorded using Perkin-Elmer FTIR-RX_1 spectrophotometer. All the reagents and solvents used were of analytical grade and were used as supplied unless otherwise stated. Progress of the reactions was monitored using TLC, performed on aluminium plates precoated with silica gel-G, using chloroform: methanol (92:8) as the solvent systems and the spots were visualized by exposure to iodine vapors.

Synthesis of chalconalide

Chalconalide were synthesized by base catalyzed Claisen-Schmidt condensation reaction of appropriately substituted acetanilide and aldehydes by known literature method[15].

A mixture of benzaldehyde derivatives (0.01 mol) and acetanilide derivatives (0.01 mol) was dissolved in 10 ml rectified spirit in a 250 ml round-bottomed flask equipped with a magnetic stirrer. Then 10 ml NaOH solution (1g in 10ml H_2O) was added drop wise to the reaction mixture on vigorous stirring for 30 minutes when solution became turbid. The reaction temperature was maintained between 20-25˚ C using a cold water bath on the magnetic stirrer. After vigorous stirring for 4-5 hours the reaction

mixture was neutralized by 0.1-0.2N HCl whereby the precipitation occurred. On filtering off, the crude chalcones were dried in air and recrystallized by rectified spirit. The residue was purified on column chromatography (silica gel with 10% ethyl acetate in hexane) to afford pure chalcones (fig no. 1).

Antibacterial Activity

Antibacterial activity of all synthesized compounds were determined by disc diffusion method[16]. All human pathogenic bacteria viz *E.coli*, *Pseudomonas aeruginosa* were procured from SGRRITS, Dehradun. The stock cultures were obtained from stock culture bank of microbiology laboratory of SGRRITS, Dehradun and maintained on nutrient agar media slants at 4°C. In order to activate these cultures, subculture, were freshly prepared and incubated at 37°C for 18 hrs to 24 hrs before use. A volume of 0.1 ml of each such culture was used as inoculums in all tests. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Discs measuring 6.25 mm in diameter were punched from Whatman No.1 filter paper. Stock solutions of synthesized compounds diluted in dimethyl sulphoxide (1% DMSO) to give final concentration of 500µg/ml and 1000 µg/ml. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of amoxicillin (500 and 1000 µg/mL, respectively) in sterile distilled water, separately. The incubation was carried out at 37ºC for 24h. All the experiments were carried out in triplicate. Simultaneously, controls were maintained by employing 0.1 mL of dimethylsulfoxide which did not reveal any inhibition. Zones of inhibition produced by each compound was measured in mm.

RESULTS AND DISCUSSION

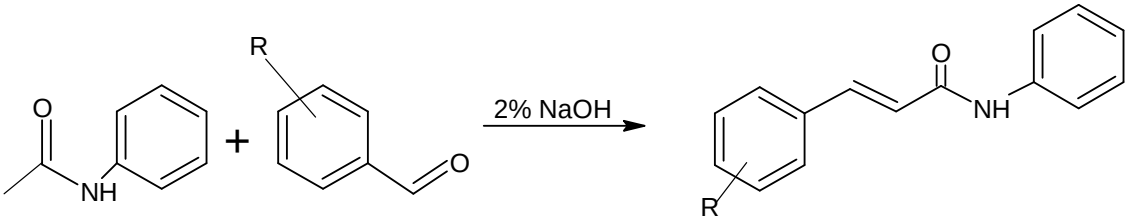
Synthesis and characterization of chalconalide derivatives

The synthesis was based on Claisen Schmidt reaction, which is condensation reaction of substituted benzaldehyde with substituted acetanilide in the presence of sodium hydroxide and ethanol. The products were characterized by comparison of physical data and IR spectral analysis. (Table 1 & 2).

IR spectrum of compounds revlealed the presence of conjugated keto carbonyl system, in the compounds A1-A5 the conjugated ketocarbonyl streching frequncies were observed between 1620-1693 cm it is well known that conjugation with c=c bond result in delocalization of π electron reduce the double bond of the c=o bond causing absorption at lower wave numbers. The absorption bonds for c=o of α, βord unsaturated corbonyl system in the compound A1-A5 were in good agreement with standard values repoted in the literature. The aromatic c=c stretching frequencies were observed in the 1602-1665 and 1450-1470 cm-1.

Antibacterial Activity

The synthesized compounds were evaluated for antibacterial activity by disc diffusion method. The results of preliminary antibacterial testing are shown in the table 3.



N-phenylacetamide Substituted benzaldehyde Substituted acetanilide chalcone

Fig. 1: Synthetic route to the title compounds

Table 1: The chemical profile of the synthesized compounds

S. No	Mol. Formula	Mol. Wt	Melting Point ^{0}C	% Yield
A1	$C_{15}H_{12}ClNO$	257	241-242	59.5%
A2	$C_{16}H_{15}NO_3$	269	290-293	61.12%
A3	$C_{15}H_{13}NO_2$	239	285-287	53.1%
A4	$C_{17}H_{15}N_2O$	266.3	268-269	63%
A5	$C_{17}H_{17}NO_2$	269.32	282-284	69%

Table 2: IR Spectral analysis of synthesized compounds

S. No.	IR(Nujol) Cm-1
A1	1621(C=O Stretch), 3508 (NH Stretch), 577 (C – C Stretch), 1668 (C=C Stretch)
A2	1683 (C =C Stretch), 1645 (C=O Stretch), 3649 (NH Stretch), 3296 (OH Stretch)
A3	1693 (C=C Stretch), 1612 (C=O) Stretch), 3644 (NH Stretch), 1259 (C-O-C Stretch),
A4	1696 cm^{-1} (C=C Stretch), 1306 cm^{-1} (C-N Stretch), (1644 cm^{-1} C=O Stretch), 3542 cm^{-1} (NH Stretch)
A5	1696 (C=C Stretch), 1623 (C=O Stretch), 3646 (NH Stretch), 3297 (O-H Stretch)

Table 3: Antibacterial activity of synthesized compound

Compounds	Zone of Inhibition (in mm)			
	E.coli		P.aergunosa	
	500 mg/ml	1000 mg/ml	500 mg/ml	1000 mg/ml
Compound A1	12	14	16	21
Compound A2	15	17	13	15
Compound A3	13	18	10	12
Compound A4	18	20	13	15
Compound A5	12	17	14	16
Amoxicillin	22	25	24	26

The results revealed that all synthesized compounds show varying degree of inhibition against gram positive and gram negative bacteria. The compound A4 has maximum activity against gram positive bacteria in both concentration and compound A1 has maximum activity at both concentrations against gram negative bacteria. The compound A3 showed least activity against gram negative bacteria.

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