ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Effect of Novel Chalcone Derivatives on Clinical Isolates of Multidrug Resistant Bacterial Strains.

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ABSTRACT

We synthesized and studied the efficacy of hydroxy and corresponding tosyloxy chalcone derivatives to inhibit the growth of MDR strains of bacteria. The required compounds were synthesized using literature methods and characterized by spectroscopic techniques. Bacterial strains were isolated by clinical methods. Our studies show hydroxy chalcone derivative is effective in inhibiting the growth of MDR bacterial strains whereas tosyl derivative is inactive.
Keywords: Chalcone, hydroxy chalcones; tosyloxy chalcone; MDR strains.

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ISSN: 0975-8585

INTRODUCTION

Chalcone and their derivatives are found to possess various biological activities like antimicrobial, anti-inflammatory, anti-hyperglycemic, and antimalarial properties [1,2]. They are natural biocides and are reactive intermediates in the synthesis of heterocyclic compounds like pyrazoles and pyrimidine derivatives which exhibit different biological activities [3]. The presence of α, β-unsaturated keto function in chalcones is reported as responsible for their antimicrobial activity. A number of chalcones having hydroxy and alkoxy groups in different position have been reported in the literature to possess the activities like inhibition of chemical mediator's release, inhibition of tyrosinase and inhibition of aldoses reductase activities. There is a growing interest in the pharmacological potential of chalcones [2, 3]. Chalcone derivatives with differing substitutions are effective in inhibiting the antimicrobial growth [4-7]. Recent studies of chalcone derivatives are also extended to their application in the field of materials science [8-13].

Multidrug resistant organisms (MDROs) are microorganisms that are resistant to one or more classes of antimicrobial agents. These organisms deserve special concern in healthcare facilities as they are associated with increased length of patient stay, costs, and mortality. They can also be transmitted between patients and healthcare workers and can lead to the spread of antimicrobial resistance [14]. The likelihood of treatment failure and serious complications, particularly the development of antimicrobial resistance, is more common in complicated infections. Although a broad range of pathogens can cause complicated medical problems, *Escherichia coli* remain the most common. These organisms becoming increasingly resistant to the drugs that are normally being used. The treatment of bacterial infections remain a challenging therapeutic problem as there is an increase in emergence of infectious disease and there is an increase in the number of multidrug resistant pathogens which desire the screening and development of newer antimicrobial agents [15]. A recent review highlights the biological properties of chalcones [16]. Chalcone derivatives act as an active biological moiety and the ease of synthesis from variety of aldehydes and ketones make them the focus of study by several researchers. In most cases purifications of these compounds can easily be achieved by crystallizations avoiding tedious chromatographic techniques. These types of compounds are also chemically and thermally stable. In the present study we have tested the antibacterial activity of hydroxy and tosyloxy substituted chalcones against the clinically isolated multi-drug resistant bacterial strains and the results are reported.

MATERIALS AND METHODS

Chemistry:

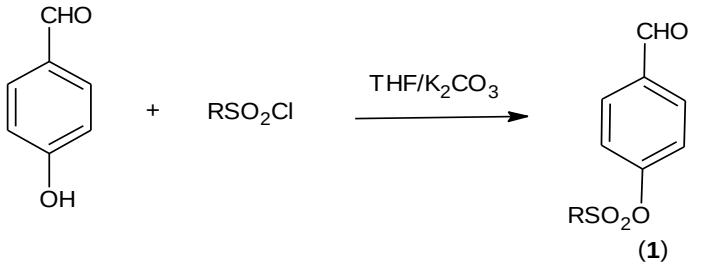
*Preparation of 4-tosyloxybenzaldehyde (**1**):*

A mixture of 4-hydroxybenzaldehyde (0.1mole) and p-toluenesulphonyl chloride (0.15mole) in tetrahydrofuran was added potassium carbonate (0.1mole) under stirring [17] (**Scheme 1**). The reaction mixture was heated at 70^0C for 6 hrs. After the reaction was complete, the solvent was removed by distillation. The residue was poured into ice-cold water, acidified and stirred well. The product was filtered, washed with water; dried and taken for next step without further purification.
Yield: 70%; m.p= 68-70^0C

*Preparation of 4-tosyloxy substituted chalcone (**2**):*

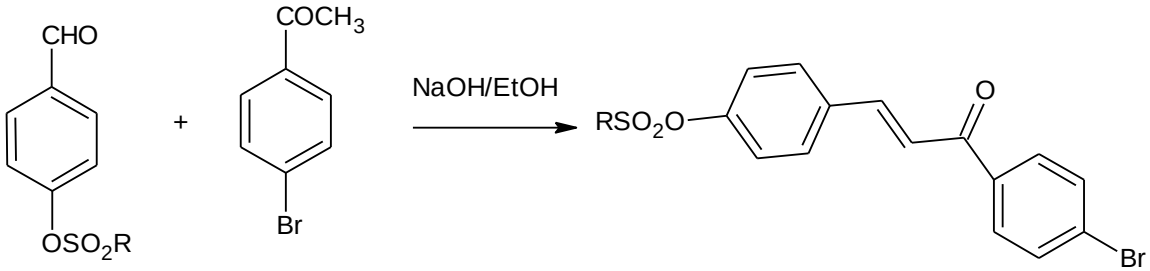
To a mixture of 4-tosyloxybenzaldehyde (0.1mole) and p-bromoacetophenone (0.1mole) in ethanol was added sodium hydroxide (0.1mole) under stirring (**Scheme 2**). The reaction mixture was kept at room temperature for 6 hrs [18]. After reaction was complete, the contents were poured into ice cold water and kept overnight under refrigeration. Resulting solid product was filtered, washed with water and dried. The crude product was purified by recrystallization from ethanol.
Yield: 57% ; m.p= 114-118^0C

ISSN: 0975-8585



(**1**)

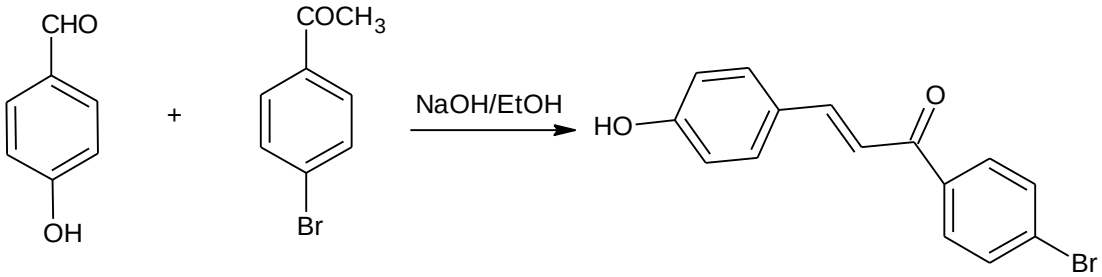
Scheme 1



(**2**)

R=4-$CH_3C_6H_4$-

Scheme 2



(**3**)

Scheme 3

*Synthesis of 4-bromo substituted chalcone (**3**)*:

To a mixture of 4-hydroxybenzaldehyde (0.1mole) and p-bromoacetophenone (0.1 mole) was added a solution of sodium hydroxide (0.3mole) under stirring (**Scheme 3**). The reaction mixture was kept at room temperature for 8hrs. The clear solution so obtained was neutralized by using 4N HCl and allowed to stand overnight [9, 10]. Resulting solid product was filtered, washed and dried. The compound was purified by recrystallization from ethanol.

Antimicrobial activity:

Isolation & identification:

Bacteria were isolated from clinical specimen over a period of two months during August and September 2013 from Kasturba medical college, Manipal. The bacteria were isolated from urine, sputum and pus samples.

The isolates were identified upto genus and species level by doing gram stain, motility testing and conventional biochemical tests using standard microbiological techniques. The isolates were identified as *Escherichia coli (5strains), Klebsiella pneumonia (11strains), Acinetobacter baumanni (8strains) and Enterobacter cloacae* (2strains). These strains were showed resistance to penicillins, first and second generation cephalosporins, Aminoglycosides, Fluroquinolones group of antibiotics.

Antimicrobial susceptibility test:

The MDR bacterial isolates were subcultured on nutrient agar and maintained. The disk diffusion assay was performed according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 8th edition). The isolates were inoculated in peptone water and incubated at 37°C for 4 hours. The inoculum was adjusted to 0.5 McFarland standards ($1.5x10^8$cfu/ml).Sterile swabs were used in the process. The bacteria were seeded on Mulleur Hinton Agar plates performing lawn culture. Wells were punched in the agar plate using a sterile borer. 50 µl of each 3% stock solution the compound was dispensed in the wells. Colistin disc of disc potency 10µg was used as control.

Determination of minimum inhibitory concentration:

1%, 1.5%, 2%, 2.5% dilution suspensions of the compound **2** and **3** were prepared by dissolving 0.1gram, 0.15 gram, 0.2gram and 0.25gram of the compound in 10ml of distilled water respectively. This represents 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml respectively. Minimum inhibitory concentration was determined by performing serial dilutions. A control tube was kept which had only growth medium and inoculated organism, with no compound in it. The tubes were incubated at 37°C for 24 hours. Subculture was made from each tube including the control on nutrient agar and incubated at 37°C for 24 hours. The results of studies for compound (**3**) were presented in **Table 1**. The compound (**2**) didn't show significant activity at the tested concentration and hence the data not shown in the Table 1.

Table1: Results of minimum inhibitory concentration studies

Test organism	2.5%	2%	1.5%	1%
Escherichia coli	S	R	R	R
Klebsiella pneumonia	S	R	R	R
Acinetobacter baumanni	S	S	S	R
Enterobacter cloacae	S	R	R	R

S=Sensitive; R=Resistant

DISCUSSION

The formation of compound **1**, **2** and **3** are confirmed from their FTIR, proton NMR and LCMS data. The FTIR spectrum of compound **1** showed C=O showed strong absorption at 1704 cm^{-1}. Further the peak for OH group present in starting hydroxy benzaldehyde disappeared confirming the formation of tosyloxy benzaldehyde. The proton NMR spectrum showed a peak at δ 9.7 integrating for one proton dues to the presence of -CHO group. Other peaks are observed in the respective region of NMR spectrum. The LCMS of the compound showed protonated molecular ion as the base peak at *m/z* 277 clearly indicating the formation of compound (**1**). The reaction of compound (**1**) with p-bromoacetophenone under alkaline condition resulted in the formation of product (**2**). Interestingly, tosyloxy group was intact under the reaction conditions employed. The FTIR spectrum of the compound showed a characteristic absorption at 1681 cm^{-1} due to the formation of chalcone moiety having the extended conjugation in the molecule when compared to simple aldehyde. Further proton NMR and LCMS data are in agreement with the proposed structure. The FTIR spectrum of compound (**3**) showed characteristic peak at 1689 cm^{-1} due to C=O group of chalcone moiety whereas hydroxyl group observed as broad peak at 3263 cm^{-1}. The proton NMR spectrum of the compound showed a signal at δ 9.86 due the presence of OH group, whereas other peaks are in agreement with molecular structure of the compound (**3**). The NMR data also proved the *E*-configuration of CH=CH protons as evidenced by high coupling constant observed.

The antimicrobial testing showed that the compound (**3**) is moderately active against the MDR strains of all tested organisms at 2.5% dilution level. It is interesting to note that *Acinetobacter baumanni* exhibited activity upto 1.5% dilution level. The tosyloxy derivative of chalcone (**2**) didn't show antimicrobial activity against the tested MDR strain indicating the need of free hydroxyl group for exhibiting biological activity. Further work needed to assess the structure property relationships of related chalcone derivatives.

ISSN: 0975-8585

ACKNOWLEDGMENT

We are thankful to Central Instruments Facility, Manipal Institute of Technology, Manipal University for recording ^{1}H NMR and Dr Vasudeva PK of Sequent Scientific, Mangalore for recording LCMS.

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