Research Journal of Chemical Sciences _____ ISSN 2231-606X
Vol. **4(2),** 56-59, February **(2014)** *Res. J. Chem. Sci.*

Synthesis and *in vitro* Antimicrobial Evaluation of 5'-Acetamido-2'-Hydroxy Chalcone derivatives

Amita S. Rao[1], Lalitha Simon[2*], K.K. Srinivasan[3], Sudheer Moorkoth[4], Zarah A. Haleem[2], Shivender S. Jadon[2], Ram M. Matsa[2] and Ravivarma Sagiraju[2]

[1]Department of Microbiology, Melaka Manipal Medical College (Manipal Campus), Manipal University, Manipal, Karnataka, INDIA
[2]Department of Chemistry, Kasturba Medical College International Center, Manipal University, Manipal, Karnataka, INDIA
[3]Department of Chemistry, Shri Madhwa Vadiraja Institute of Technology and Management, Bantakal, Udupi , Karnataka, INDIA
[4]Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, INDIA

Available online at: www.isca.in, www.isca.me
Received 8th January 2014, revised 23rd January 2014, accepted 10th February 2014

Abstract

A series of chalcones (3a-j) were synthesized by Claisen-Schmidt condensation of aromatic aldehydes with 5'-acetamido-2'-hydroxyacetophenone. Paracetamol was acetylated using acetic anhydride and sulphuric acid. The acetylated paracetamol underwent Fries rearrangement to form 5'-acetamido-2'-hydroxy acetophenone. The antibacterial and antifungal activity of the test compounds were evaluated using the agar well diffusion method. The test compounds displayed activity against Candida albicans. The fluorinated chalcone 3c exhibited maximum inhibition. However the test compounds failed to show antibacterial activity.

Keywords: Chalcone, antibacterial, antifungal, agar well diffusion method.

Introduction

Chalcones (*trans*-1,3-diaryl-2-propen-1-ones) are precursors of open chain flavonoids and isoflavonoids, which are abundant in edible plants. Chalcones have been reported to be very active compounds and have displayed a broad spectrum of pharmacological activities. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilized as antibacterial, antifungal, anti-inflammatory, antiviral, antioxidant, anti-allergic, antimalarial, and anticancer agents[1-6].

A number of chalcone derivatives have also been found to inhibit several important enzymes in cellular systems, including xanthine oxidase, aldose reductase, epoxide hydrolase, protein tyrosine Kinase, and quinone reductase[7]. The presence of α-β unsaturated ketone group in chalcones is responsible for their diverse activities.

Resistance to antimicrobial drugs has become an increasingly important global problem[8,9]. Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the lifespan of antifungal agents such as the azoles, the antiviral agents such as the non-nucleoside reverse transcriptase inhibitors[9], and various antibacterial agents including lactams and quinolones[10]. Broad empirical screening of chemical entities for antimicrobial activity represents a strategy for the development of novel drugs.

The aim of the present study was to evaluate the antimicrobial activity of synthesized 2'-Hydroxy chalcones against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans u*sing the agar well diffusion method. The scheme for synthesis is shown in figure 1.

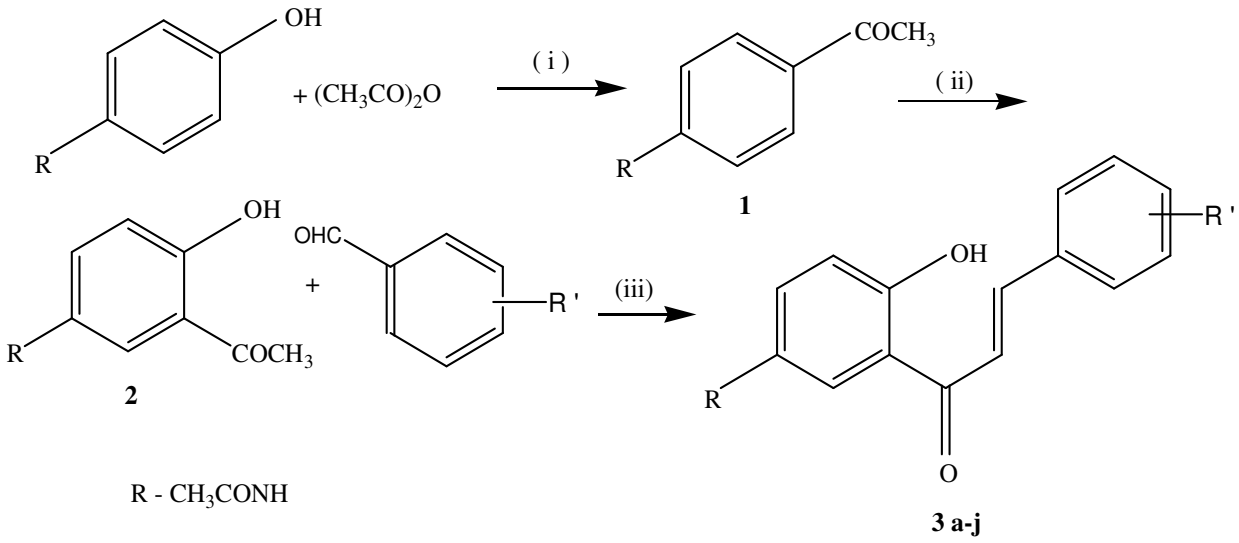
Material and Methods

Experimental: All purchased chemicals were of analytical grade and used without further purification. Melting points were determined by open capillary method and are uncorrected. Purity of synthesized compounds was checked by thin layer chromatography. R_f values were recorded by using pre-coated silica gel aluminium backed plates Kieselgel 60 F254 Merck (Germany).

Synthesis of N-(4-Acetylphenyl)acetamide (1): A mixture of paracetamol (0.1mol), acetic anhydride (0.01 mol) and concentrated sulphuric acid (0.001mol) was heated on a water bath at 50-60°C with occasional stirring for about 15min. After cooling the flask,100 ml water was added to it. The solid separated was filtered and crystallized from ethanol.

Synthesis of 5'-acetamido-2'-Hydroxyacetophenone (2): N-(4-Acetylphenyl) acetamide (0.1 mol) was added to anhydrous $AlCl_3$ (0.15mol) and nitromethane (0.4mol) and the mixture was refluxed at 130°C for 3hrs. To the cooled reaction mixture crushed ice was added slowly and the resulting solution was extracted with diethyl ether. The organic fraction was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure to get 5'-acetamido-2'-Hydroxyacetophenone.

Synthesis of substituted chalcone derivatives (3a-j): A mixture of 5'-acetamido-2'-Hydroxy acetophenone (0.01mol) and aryl aldehyde (0.01 mol) was dissolved in ethanol (30ml).To this, aqueous potassium hydroxide solution (0.03 mol) was added slowly and stirred for 24 h, at room temperature. After completion of the reaction, the reaction mixture was poured into crushed ice and acidified with HCl. The solid separated was filtered and crystallized from ethanol.



Reagents and conditions: (i) Con.H_2SO_4, reflux for 15 min, (ii) $AlCl_3$,130°C,3hrs (iii) NaOH/EtOH stir for 18-24 hrs

Figure-1
Scheme for the synthesis of compounds (3a-j)

Table-1
Physical data of compounds (3a-3j)

Compound	Mol Formula	R'	Yield (%)	MP (ºC)	Rf* value
3a	$C_{18}H_{17}O_5N$	4-OH,3-OCH_3	72	158-161	0.64
3b	$C_{17}H_{14}O_3NCl$	4-Cl	90	148	0.71
3c	$C_{17}H_{14}O_3NF$	4-F	88	160-162	0.69
3d	$C_{17}H_{15}O_5N$	Piperonaldehyde	93	188	0.60
3e	$C_{19}H_{19}O_4N$	4-OC_2H_5	88	158-160	0.66
3f	$C_{18}H_{17}O_4N$	3-OCH_3	89	187	0.58
3g	$C_{17}H_{15}O_3N$	H	90	163	0.64
3h	$C_{18}H_{17}O_5N$	3-OH,4-OCH_3	75	142	0.38
3i	$C_{19}H_{19}O_5N$	3,4-OCH_3	86	114	0.55
3j	$C_{17}H_{14}O_5N_2$	3-NO_2	79	138	0.60

*Solvent system Hexane: EtOAc 6:5

Table-2
Spectral data of a representative compound *3a*

UV λ max (nm)	IR data IR (KBr) ν cm^{-1}	Mass spectral data (ESI MS)	^{1}H NMR data δ values (DMSO, 400MHz)
240, 330	3244 (br., Ar-OH str), 1674 (C=O str. Side chain), 1626 (C=O str. Chalcone), 3342 (NH def), 3404 (NH str), 3036 (CH str Ar)	328 (M+1) Peak in the positive ionisation mode	2.38 (s, 3H, CH_3 of acetamido), 3.58 (s, 3H, CH_3 of methoxy), 6.77 (s, 1H, -C=CH), 10.07 (s, 1H, -NH), 9.78 (s, 1H, C-OH), 9.85 (s,1H,C-OH), 7.50-8.73 (m, 7H, Ar-H)

Antimicrobial Screening[12-15]: All the synthesized 2'-Hydroxy chalcone derivatives were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and clinical isolates *Klebsiella pneumonia. Candida albicans* was used for the antifungal activity study.

The antimicrobial activity was determined using the agar well diffusion method. The selected bacterial and fungal strains were revived by plating on nutrient agar medium and Sabouraud dextrose agar medium respectively. After overnight incubation at 37°C, isolated colonies were selected and identities of the organisms were confirmed. Isolated bacterial colonies were then transferred to sterile Muller-Hinton Broth (MHB) and Candida albicans was transferred to Sabouraud Dextrose Broth (SDB) and incubated overnight. The growth concentration was adjusted to 10^5 organisms/ml by using 0.5 McFarland's turbidity standard.

Determination of Antibacterial Activity: Petri dishes containing 20 ml of Muller-Hinton Agar (MHA) were used. The bacterial culture was spread over the surface of the MHA plate.4 mm diameter wells were punched into the agar and filled with 20µl solution of 2'-Hydroxy chalcones of various concentrations (1000 µg/ml, 500 µg /ml, 250 µg /ml and 50 µg/ml).The plates were then incubated at 37°C for 18 hrs. Tests were done in triplicates and the average was taken.

Determination of antifungal activity: The agar well diffusion method[11] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. Overnight cultures were grown at 37°C in Sabouraud's dextrose Broth (SDB) and adjusted to contain 10^5 organisms/ml. Petri dishes containing 20 ml of Sabouraud's dextrose agar (SDA) , were used. The fungal culture was spread over the surface of the SDA plate.4 mm diameter wells were punched into the agar and filled with 20µl of the solution of 2'-Hydroxy chalcones of various concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml and 50 µg/ml). The plates were then incubated at 37°C for 24 hrs. Tests were done in triplicates and the average was taken.

Results and Discussion

A series of chalcones (3a-3j) was prepared by reacting acetylated 5'-acetamido-2'-hydroxyacetophenone with various aryl aldehydes in the presence of alkali (figure-1 and table-1).Formation of the substituted chalcones were confirmed by various spectral techniques. All the chalcones were obtained in good yield. Physical data such as melting point and R_f values also were determined and are presented in table 1. The UV, IR, mass and 1HNMR spectral data of a representative compound is shown in table 2.

The effect of synthesized chalcones on bacterial and fungal strains are summarized in Table 3. The diameter of the clear zone of inhibition surrounding the well was measured in mm. The results indicate that 5'-Acetamido-2'-Hydroxy Chalcones displayed promising anti-fungal activity against *Candida albicans.* Among the chalcones, the fluorinated chalcone (*3c*) showed maximum activity against *Candida albicans* at all concentrations. However, the chalcones did not show any antibacterial activity.

Table-3
Antimicrobial activity screening data

Compound Code	Zone of Inhibition of Different Concentrations of Compounds for Different Organisms in mm				
	Antifungal Activity				Antibacterial Activity
	Candida albicans				***Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia***
Concentration	**1000 µg /ml**	**500µg /ml**	**250 µg /ml**	**50 µg /ml**	All four concentrations of compounds 3a-j did not show activity against the bacterial organisms: *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia*
3a	8.0	8.0	5.0	00	
3b	13.0	11.0	9.0	00	
3c	17.0	15.0	15.0	12.0	
3d	7.0	5.0	00	00	
3e	14.0	13.0	11.0	9.0	
3f	14.0	10.0	8.0	7.0	
3g	10.0	9.0	6.0	6.0	
3h	12.0	10.0	7.0	6.0	
3i	13.0	8.0	8.0	7.0	
3j	9.0	7.0	00	00	
DMSO	00	00	00	00	

Conclusion

The 5'-Acetamido-2'-Hydroxy chalcones displayed promising anti-fungal activity against *Candida albicans*. The chalcone containing fluorine (*3c*) showed maximum activity at all concentrations. In the antibacterial screening, none of the chalcones showed activity against the bacterial strains used. It can be concluded that 5'-Acetamido-2'-Hydroxy chalcones may be considered as an antifungal agent and can be used as a source of antimycotic substance for possible treatment of fungal infections.

Acknowledgment

Authors thank Manipal University and KMC International Center for providing the necessary laboratory facilities.

References

1. Achanta G., Modzelewska A., Feng L., Khan S.R, Huang P., A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome, *Molecular Pharmacology*, **70**, 426-433 **(2006)**

2. Romagnoli R., Baraldi P.G., Carrion M.D., Cara C.L., Cruz-Lopez O., Preti D., Design, synthesis and biological evaluation of thiophene analogues of chalcones, *Bioorganic and Medicinal Chemistry*, **16**, 5367-5376 (**2008**)

3. Prasad R.Y., Kumar R.P., Synthesis and antimicrobial activity of some new Chalcones of 2-acetyl pyridine, *Eur. J. Chem.,* **5,** 236-241(**2006**)

4. Jevwon S., Liv CT., Sao LT., Weng JR. and Ko HH., Synthetic Chalcones as potential anti-inflammatory and cancer chemopreventive agents, *Eur. J. Chem.,* **40**, 103-112 **(2005)**

5. Shivakumar P.M., Babu G.S.M., Mukesh D., QSAR studies on Chalcones and flavonoids as antitubercular agents using genetic function approximation (GFA) method, *Chemical and Pharmaceutical Bulletin*, **55**, 44-49 **(2005)**

6. Sivakumar P.M., Prabhakar P.K., Doble, Synthesis, antioxidant evaluation and quantitative structure activity relationship studies of chalcones, *Medicinal Chemistry Research*, **19**, 1-17 **(2010)**

7. Nowakowska Z., A review of anti-infective and anti-inflammatory chalcones, *European Journal of Medicinal Chemistry.*, **42**,125-137 **(2007)**

8. Cunha B.A., Antibiotic resistance, *Drugs Today.,* **34(8),** 691-698 **(1992)**

9. Cohen M.L., Epidemiology of drug resistance: Implication for a post-antimicrobial era, *Science,* **257**, 1050-1055 **(1992)**

10. De Clercq E., New developments in anti-HIV chemotherapy, *Farmaco,* **56**, 3–12 **(2001)**

11. Poole K., Overcoming antimicrobial resistance by targeting resistance mechanisms, *J Pharm Pharmacol*, **53**, 283–94 **(2001)**

12. Perez C. and Anesini J., Screening of plants used in Argentine folk medicine for antimicrobial activity, *Ethnopharmacol.*, **44**, 41-46 **(1993)**

13. Nayak A., Nayak R.N., Soumya B.G., Bhat K. and Kudlakar M., Evaluation of antibacterial and anti candidial efficacy of aqueous and alchoholic extract of neem [Azadirachta indica] - An in vitro study, *Int J Res Ayurveda Pharm.*, **2,** 230-235 **(2011)**

14. Essawi T., Srour M., Screening of some Palestinian medicinal plants for antibacterial activity, *J Ethnopharmacol.*, **70**, 343-349 **(2000)**

15. Muntha K.R., Sashi K.G., Melissa R.J., Shabana I.K., Daneel F., Antioxidant, Antimalarial and Antimicrobial activities of Tannin-rich Fractions, Ellagitannins and Phenolic acids from Punica granatum L. *Planta Med.*, **73**(**5**), 461-467 **(2007)**