Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(6):346-352
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, characterization and antimicrobial evaluation of isoxazole derivatives

Gollapalli Naga Raju*, P. V. Suresh, Rama Rao Nadendla and Katta Anusha

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India

---

ABSTRACT

Isoxazole derivatives possess antibacterial, antiviral, anti-fungal, anti-inflammatory insecticidal activities. Claisen-Schmidt Condensation method was adopted to get chalcones. Acetophenone on condensation with aldehyde in the presence of base produced chalcones. Chalcones were subjected for reaction with hydroxyl amine hydrochloric acid and potassium hydroxide to give isoxazoline derivative of chalcone. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Benzyl pencillin and Ketoconazole. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

Keywords: Isoxazole, Chalcone, Antibacterial activity, Antifungal activity.

INTRODUCTION

The dramatically rising prevalence of multidrug-resistant microbial infection in the past few decades has become a serious health care problem. In order to prevent this serious medical problem, the elaboration of the new types of the previously known drugs is a very actual task. In recent years, the synthesis of novel isoxazole derivatives remains a main focus of medicinal research. Isoxazole is a five membered heterocyclic compound.

Derivatives of Isoxazole have played a crucial role in the history of heterocyclic chemistry and been used extensively important pharmacophores and synthons in the field of organic chemistry. Owing to their versatile chemotherapeutic importance, a significant amount of research effort has been focused on these nuclei. Isoxazole derivatives exhibit various biological activities such as, Antibacterial [1,2], Anticonvulsant [3,4], Anticancer [5-7], Anthelmintics [8]. Antiinflammatory [9-11], Adenosine antagonist [12], Fungicidal [13-15], Herbicidal [16], Hypoglycemic [17], Muscle relaxant [18], Nematocidal [19,20], Insecticidal [21], Antiviral [22] and Antimicrobial [23].

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active Isoxazoles. Therefore, this work deals with the synthesis of the isoxazole derivatives from chalcones and screening their antimicrobial activities.

MATERIALS AND METHODS

Material and Methods: Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR and ^{13}C NMR was determined in $CDCl_3$ solution on a Bruker Ac 400 MHz spectrometer. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Preparation Resacetophenone: Freshly fused $ZnCl_2$(33g) was dissolved in glacial acetic acid (32ml) while heating and when all the $ZnCl_2$ is almost dissolved resorcinol (22 g) was added and heated to 140-150^{0}C for 15 min with stirring. After that this is left for 1 hr and then 1:1 HCl (100ml) was added to break the zinc chloride complex. Within 5 minutes precipitate obtained was crystallized from HCl 20% to give resacetophenone needles. R_f : 0.4 (Hexane: EtOAc, 8:2); M.P: 145^{0}C. Molecular formula: $C_8H_8O_3$. Elemental analysis: Found (%): C, 63.06: H, 5.23%; Required (%): C, 63.15; H, 5.26%. ^{1}H NMR ($CDCl_3$/TMS): δ2.65(s, 3H, $COCH_3$), δ6.35(s, 1H), δ6.5 (d, 1H), δ7.62 (d, 1H), δ11.55(s, OH). IR (ν_{max}): 3450cm^{-1}(-OH str.); 1685cm^{-1}(C=O str).

Nuclear prenylation of resacetophenone: A solution of isoprene (1.5 ml, 0.015 mol) in xylene (5ml) was added drop wise for 8 hr to a mixture of resacetophenone (1.41g, 0.0072 mol) and PPA (2ml) in xylene (3ml) with constant stirring at 30-35^{0}C stirring was continued for further 14hrs. The reaction mixture was taken in chloroform (100 ml) and the chloroform solution was washed with $NaHCO_3$ (5%, 3 × 60ml) water; dried over $MgSO_4$ and removed under reduced pressure to give gummy material which had three spots on TLC. This on separation using column chromatography over silica gel yielded three compounds 3,4-dihydro-5-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran; 3,4-dihydro-7-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran and 3, 4, 9, 10-tetrahydro-2, 2, 8, 8-tetramethyl-6-acetyl-2H, 8H-benzo [1, 2-b: 3,4b'] dipyran in the ration 2:3:1, on elution with hexane Ethylacetate.

3,4-dihydro-5-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran: M.P**:** 148-149^{0}C; Molecular formula: $C_{13}H_{16}O_3$; Elemental analysis: Found (%): C, 70.85; H, 7.3% Required (%): C, 70.90; H, 7.27%. ^{1}H NMR ($CDCl_3$/TMS): δ1.46 (s, 6H), δ1.91(t, 2H), δ2.72(t, 2H), δ6.35(d, 1H), δ7.4(d, 1H); δ2.5(s,3H,$COCH_3$); δ13.1(s,1H). IR (ν_{max}): 3350cm^{-1}(-OH str.); 1680cm^{-1}(C=O str).

3,4-dihydro-7-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran: M.P: 92-93^{0}C; Molecular formula: $C_{13}H_{16}O_3$; Elemental analysis: Found (%): C, 70.85; H, 7.3%. Required (%): C, 70.90; H, 7.27%^{1}H NMR ($CDCl_3$/TMS): δ1.3 (s, 6H), δ1.8(t, 2H), δ2.7(t, 2H), δ6.3(d, 1H),δ7.5(d,1H); δ2.5(s,3H,$COCH_3$); δ12.2(s,1H). IR (ν_{max}): 3350cm^{-1}(-OH str.); 1680cm^{-1}(C=O str).

3, 4, 9, 10-tetrahydro-2, 2, 8, 8-tetramethyl-6-acetyl-2H, 8H-benzo [1, 2-b: 3,4b'] dipyran: M.P: 107-108^{0}C; Molecular formula: $C_{18}H_{24}O_3$; Elemental analysis: Found (%): C, 74.85; H, 8.29%. Required (%): C, 75.0; H, 8.33%. ^{1}H NMR ($CDCl_3$/TMS): δ1.33 (s, 6H), δ1.36(s, 6H), δ1.6(s, 2H), δ1.7 (t, 2H), δ2.61 (t, 2H); δ2.71 (t, 2H); δ7.49(s, 1H); δ2.59(s, 3H, $COCH_3$). IR (ν_{max}): 1685.68cm^{-1}(C=O str).

Preparation of Chalcones: A mixture of 2,2-dimethyl-6-acetyl-7-hydroxy chroman (0.01mol), substituted benzaldehydes (0.01 mol) in ethanol (30 ml) and aqueous potassium hydroxide (15 g in 15 ml of water) was kept at room temperature for 24 hours. On acidification with 1:1 hydrochloric acid, yellow (or) orange red chalcone derivatives were obtained in 80-85% yield. It was filtered and crystallized from appropriate solvent and characterized using spectral data and elemental analysis.

2, 2-dimethyl-6-acetyl-7-hydroxy chroman was condensed with various benzaldehydes, veratraldehyde, 4-N, N-dimethylamino benzaldehyde, 4-chlorobenzaldehyde, 4-methoxy benzaldehyde and 4-cyano benzaldehyde to furnish

the respective chalcones viz.,

7-Hydroxy-6-(3,'4'-dimethoxy) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo(1,2b)pyran : Molecular formula: $C_{22}H_{24}O_5$; Elemental analysis: Found (%): C, 74.85; H, 8.29%. Required (%): C, 71.72 H, 6.57%

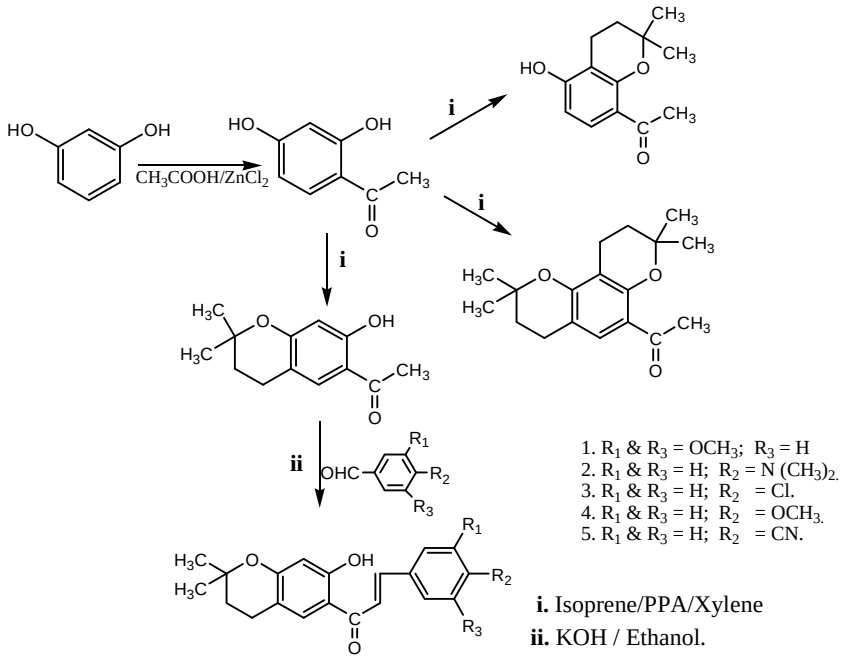
7-Hydroxy-6-(4'-N, N-dimethyl amino) cinnamoyl, 3, 4-dihydro-2,2-dimethyl-2H benzo(1,2b)pyran: Molecular formula: $C_{22}H_{25}O_3N$; Elemental analysis: Found (%): C, 75.08; H, 7.11; N, 3.85%. Required (%): C, 75.19 H, 7.17; N, 3.99%**.**

7-Hydroxy-6-(4'-chloro) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2Hbenzo (1,2b) pyran: Molecular formula: $C_{20}H_{19}O_3Cl$; Elemental analysis: Found (%): C, 70.01; H, 5.47; Cl, 10.28%. Required (%): C, 70.07; H, 5.59; Cl, 10.30%

7-Hydroxy-6-(4'-methoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2Hbenzo (1,2b) pyran: Molecular formula: $C_{21}H_{22}O_4$; Elemental analysis: Found (%): C, 74.47; H, 6.52%; Required (%): C, 74.54; H, 6.55%.

7-Hydroxy-6-(4'-cyano) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo(1,2b) pyran: Molecular formula: $C_{21}H_{19}O_3N$; Elemental analysis: Found (%): C, 75.59; H, 5.73% N, 4.15%; Required (%): C, 75.66; H, 5.74; N, 4.20% .

Figure 1: Preparation of Chalcones



Synthesis of 3-substituted phenyl-5-(2",2"-dimethyl, 7"-hydroxy chroman) isoxazoles (IC-1 TO IC-5): The above chalcones were condensed with hydroxylamine hydrochloride in presence of KOH/ethanol; after usual work up and purification by column chromatography the major compound was isolated and crystallized from methanol which furnished crystalline products. The elemental analysis for the above compounds were determined and found satisfactory. In IR spectra the absence of chalcone carbonyl was noticed and the characteristic ring stretching vibrations of isoxazoles between 1600-1300 $cm^{-1}$and 1300 - 1200 cm^{-1} was observed, confirming the formation of isoxazole ring.

The five new 3-ubstituted phenyl-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazoles thus synthesized were:
3-(3', 4'-dimethoxy-phenyl)-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (IC-1): 7-Hydroxy-6-(3,'4'-dimethoxy) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo (1,2b) pyran (324 mg, 1 mmol), hydroxylamine

hydrochloride (600 mg), KOH (500 mg) in ethanol was refluxed on a water bath for 4 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30ml). Where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from methanol as pale yellow needles in 60% yield.

M.P: 178^{0}C; Elemental analysis: Found (%): C, 69.01; H, 6.01; N, 3.45%; Required (%): C, 69.29; H, 6.04; N, 3.67%. ^{1}H NMR ($CDCl_3$/TMS): δ1.38(s, 6H, CH_3), δ1.7-1.9 (br t, 2H, 3"-CH_2), δ2.8 (t, 2H, 4" –CH_2), δ6.9 (s, 1H, C_4-H), δ7.55 (s, 1H, C"$_5$-H), δ6.35 (s, 1H, C"$_8$-H), δ6.8(s, 1H, C_2'-H), δ7.8(d, 1H, C_5'-H), δ7.8(d,1H, C'$_6$-H), δ7.6 (d, 1H, C_5'-H), δ3.9(s, OCH_3). IR (ν_{max}): 3435, 2974, 2927, 1615, 1524, 1387, 1277, 1196, 1040cm^{-1}.

3-(4'-N, N-dimethyl amino phenyl)-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (IC-2): 7-Hydroxy-6-(4'-N,N-dimethylamino)cinnamoyl,3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran (364 mg, 1mmol), hydroxylamine hydrochloride (600mg), KOH (500 mg) in ethanol was refluxed on a water bath for 5 hrs. then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was filtered and purified by column chromatography and crystallized from methanol as light brown colour needles in 62% yield.

M.P: 206^{0}C; Elemental analysis: Found (%): C, 72.51; H, 6.27; N, 7.43%; Required (%): C, 72.52; H, 6.59; N, 7.69%. ^{1}H NMR ($CDCl_3$/TMS): δ1.34(s, 3H, CH_3), δ1.38 (s, 3H, CH_3), δ1.78 (t, 2H, 3" –CH_2), δ2.6 (t, 2H, 4" –CH_2), δ7.52 (s, 1H, C"$_8$-H), δ7.55(s, 1H, C"$_5$-H), δ7.49(d, 2H, C_2'-H and C'$_6$-H), δ6.69(d, 2H, C'$_3$-H and C_5'-H), δ7.44(s,1H, C'$_4$-H), δ3.02 (N-Me_2). IR (ν_{max}): 3436, 3103, 2970, 2946, 1638, 1600, 1448, 1366, 1181, 1073, 980cm^{-1}.

3-(3', 4'-chloro-phenyl)-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (IC-3): 7-Hydroxy-6-(4'-chloro) cinnamoyl, 3,4-dihydro-2,2-dimethyl-2H benzo (1,2b) pyran (366 mg, 1 mmol), hydroxylamine hydrochloride (600 mg), KOH (500 mg) in ethanol was refluxed on a water bath for 5 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from methanol as pale yellow crystals in 56% yield.

M.P: 176^{0}C. Elemental analysis: Found (%): C, 67.41; H, 5.01; N, 3.83, Cl, 9.71%; Required (%): C, 67.60; H, 5.07; N, 3.94; Cl, 9.85%. ^{1}H NMR ($CDCl_3$/TMS): δ1.3(s, 3H, CH_3), δ1.4 (s, 3H, CH_3), δ1.8 (t, 2H, 3" –CH_2), δ2.7 (t, 2H, 4" –CH_2), δ6.35 (s, 1H, C"$_8$-H), δ7.1(s,1H, C"$_5$-H), δ7.32(s, 1H, C_4'-H) and δ 7.48(d,2H, C'$_2$-H, C'$_6$-H), δ7.37(d, 2H, C'$_3$-H and C_5'-H), IR (ν_{max}): 3247, 2975, 2620, 2582, 1342, 192, 1185, 1043, 745cm^{-1}.

3-(4'-methoxy-phenyl)-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (IC-4): 7-Hydroxy-6-(4'-methoxy)cinnamoyl,3,4-dihydro-2,2-dimethyl-2Hbenzo(1,2b)pyran (338 mg, 1mmol), hydroxylamine hydrochloride (600mg), KOH (500 mg) in ethanol was refluxed on a water bath for 4 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and purified by column chromatography and crystallized from methanol as pale yellow colour needles (64% yield).

M.P:185^{0}C. Elemental analysis: Found (%): C, 71.43; H, 5.82; N, 3.72%; Required (%): C, 71.79; H, 5.98; N, 3.98; %. ^{1}H NMR ($CDCl_3$/TMS): δ1.33(s, 3H, CH_3), δ1.4 (s, 3H, CH_3), δ1.8 (t, 2H, 3"–CH_2), δ2.8 (t, 2H, 4"–CH_2), δ6.3 (s,1H, C"$_8$-H), δ7.5 (s, 1H, C"$_5$-H), δ7.31(s, 1H, C_4'-H) and δ 7.7(d,2H, C'$_2$-H, C'$_6$-H), δ6.83(d, 2H, C'$_3$-H and C_5'-H), δ 3.9(s, OCH_3). IR (ν_{max}): 3299, 3210, 1605, 1518, 1465, 1440, 1255, 1030cm^{-1}.

3-(4'-cyano phenyl)-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole (IC-5): 7-Hydroxy-6-(3'-cyano) cinnamoyl, 3, 4-dihydro-2, 2-dimethyl-2H benzo (1,2b) pyran 25 (336 mg, 1 mmol), hydroxylamine hydrochloride (600 mg), KOH (500 mg) in ethanol was refluxed on a water bath for 5 hrs. Then the reaction mixture was neutralized with acetic acid and the whole contents were poured in ice-cold water (30 ml), where upon a light brown precipitate slowly separated out. The precipitate was collected and chromatographed over silica gel and crystallized from methanol as colourless crystals in 52% yield.

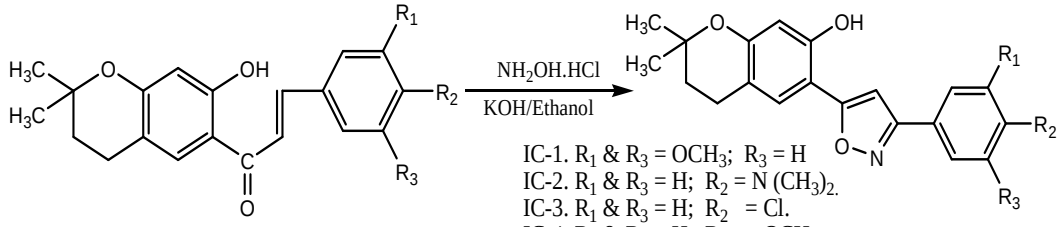
M.P: 146^{0}C. Elemental analysis: Found (%): C, 75.47; H, 5.31; N, 8.22%; Required (%): C, 75.90; H, 5.42; N, 8.43; %. ^{1}H NMR ($CDCl_3$/TMS): δ1.3(s, 3H, CH_3), δ1.45 (s, 3H, CH_3), δ1.85 (t, 2H, 3" –CH_2), δ2.8 (t, 2H, 4" –CH_2), δ6.3

(s, 1H, C"$_8$-H), δ7.6(s, 1H, C"$_5$-H), δ7.3(s, 1H,C$_4$'-H) and δ6.9(d,2H, C'$_2$-H, C'$_6$-H), δ7.7 (d, 2H, C'$_3$-H and C$_5$'-H), δ11.1(s, OH). IR (ν_{max}): 3263, 2973, 2931, 2360, 1608, 1587, 1456, 1338, 1250, 1153, 1082, 875cm^{-1}.

Tabel 1: Physical data of 3-substituted phenyl-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazoles (IC-1 TO IC-5)

Compd	M.F	M.W	M.P (^{0}C)	R_f	Yield (%)	R_t	Purity
1C-1	$C_{22}H_{23}NO_5$	381.42	178	0.4	60	4.323	95.79
IC-2	$C_{22}H_{24}N_2O_3$	364.44	206	0.6	62	3.931	95.62
IC-3	$C_{20}H_{18}NO_3Cl$	355.81	176	0.5	56	4.245	98.54
IC-4	$C_{21}H_{21}NO_4$	351.40	185	0.4	64	4.390	100
IC-5	$C_{21}H18N_2O_3$	346.38	146	0.7	52	3.914	100

Figure 2: Synthesis of 3-substituted phenyl-5-(2",2"-dimethyl, 7"-hydroxy chroman) isoxazoles (IC-1 TO IC-5)



IC-1. R_1 & R_3 = OCH_3; R_3 = H
IC-2. R_1 & R_3 = H; R_2 = N $(CH_3)_2$.
IC-3. R_1 & R_3 = H; R_2 = Cl.
IC-4. R_1 & R_3 = H; R_2 = OCH_3.
IC-5. R_1 & R_3 = H; R_2 = CN.

ANTIMICROBIAL STUDIES:

Preparation of media: 37 gms of nutrient agar medium was dissolved in 1000 ml of distilled water and the pH was adjusted to 7.0. Where as in case of antifungal activity studies, potato dextrose agar, 39 gm was dissolved in 1000 ml distilled water and the pH was adjusted to 5.6. Each 20ml portion of media was distributed to test tubes and these test tubes were plugged with non-adsorbent cotton and kept in autoclave (121.1^{0}C) for sterilization for an hour.

Plating of media: Sterilized media was heated in a water bath thoroughly. Molten media was poured on to the Petri dish (pre-sterilized in oven for 3 hours at 110^{0}C in order to avoid contamination). The plated Petri dishes were kept on plain surface to avoid non-uniform solidification of medium. Micro wells (6mm diameter) were made with bore-puncher at equidistance (four micro wells were made on a 4" assay-plate). All these operations were performed in "sterile room" which was equipped with a "laminar flow".

Antibacterial Activity: The synthesized compounds were tested for their antibacterial activity against namely *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 443), at concentrations of 5, 10, 20, 50, 100 and 200µg/ml. Nutrient agar medium was dissolved in water and pH was adjusted to 7.0. This was then disturbed in 20ml quantity in boiling tubes; they were then plugged tightly with non-absorbent cotton and sterilized in an autoclave. The bacterial culture (50µl) was then added aseptically to the agar medium maintained at 45^{0}C, mixed well and poured in to petriplates. Test solutions of different concentrations of compounds 26-30 were prepared in DMSO. After hardening, cups of 6mm diameter each were cut into agar and 50µl test solutions of varying concentrations (5, 10, 20, 50, 100 & 200µg/ml) were placed in these cups. The plates were incubated at 37^{0}C for 24 hours and the diameter of inhibition zone was measured in mm. Solvent DMSO alone was kept as control, which did not have any inhibition zone. The activity was compared with standard antibiotic Benzyl Penicillin and the antibacterial activities inhibition zones of the compounds are measured and presented in Table-2.

Antifungal activity: Antifungal activity of the synthesized compounds was tested against *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282) using agar well diffusion method**.** Potato dextrose agar was dissolved in water and pH was adjusted to 5.6. This was then distributed 20ml each in boiling tubes which were plugged tightly with non-absorbent cotton and sterilized. To this 50µl of fungal spore suspension was added and thoroughly mixed with 20 ml medium aseptically and poured in to petriplates. When agar solidified, cups of 6mm diameter were made on each of the seeded plates. These cups were filled with 50µl of test samples of concentrations of 5, 10, 20, 50, 100 & 200µg/ml the petriplates were incubated at 28^{0}C for 2 days. The inhibition zones produced by test compounds were compared with inhibition zones produced by pure ketoconazole used as standard. Inhibition zone of the compounds are presented in table 2.

Compound	Conc. µg/ml	Zone of inhibition in mm					
		Antibacterial Activity				Antifungal activity	
		B. subtilis	*S.aureus*	*E.coli*	*P.aeruginosa*	*C.albicans*	*A.niger*
IC-1	5 µg/ml	10	9	NA	NA	NA	NA
	10 µg/ml	11	10	NA	10	10	11
	20 µg/ml	12	10	9	9	11	12
	50 µg/ml	13	11	10	11	11	11
	100 µg/ml	14	12	10	9	12	11
	200 µg/ml	15	12	11	11	13	13
IC-2	5 µg/ml	NA	9	NA	9	NA	NA
	10 µg/ml	NA	10	NA	10	NA	10
	20 µg/ml	9	10	NA	10	9	11
	50 µg/ml	10	11	11	11	10	12
	100 µg/ml	10	11	12	11	11	12
	200 µg/ml	11	12	12	12	12	13
IC-3	5 µg/ml	NA	NA	NA	NA	NA	NA
	10 µg/ml	10	NA	NA	NA	10	9
	20 µg/ml	11	10	NA	9	10	10
	50 µg/ml	12	11	10	10	11	11
	100 µg/ml	12	11	12	10	12	12
	200 µg/ml	13	12	13	11	12	13
IC-4	5 µg/ml	10	9	NA	NA	NA	NA
	10 µg/ml	11	10	NA	10	NA	9
	20 µg/ml	11	10	10	10	9	10
	50 µg/ml	12	11	10	11	10	10
	100 µg/ml	13	11	11	11	10	11
	200 µg/ml	13	12	11	12	11	12
1C-5	5 µg/ml	NA	NA	NA	NA	NA	NA
	10 µg/ml	NA	NA	NA	NA	NA	NA
	20 µg/ml	NA	NA	9	10	NA	NA
	50 µg/ml	10	NA	10	10	NA	NA
	100 µg/ml	12	10	10	11	NA	NA
	200 µg/ml	13	11	11	11	10	10
Benzyl pencillin	200 µg/ml	25	22	25	25	--	--
Ketocon-azole	200 µg/ml	--	--	--	--	22	22

RESULTS AND DISCUSSION

Freshly fused $ZnCl_2$ was dissolved in glacial acetic acid then all the $ZnCl_2$ was dissolved resorcinol and heated to 140-150^{0}C for 15 min with stirring gives resacetophenone. A solution of isoprene in xylene was added drop wise to a mixture of resacetophenone and PPA in xylene. The reaction mixture was taken in chloroform and the chloroform solution was washed with $NaHCO_3$ water; dried over $MgSO_4$ gives three compounds like 3,4-dihydro-5-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran; 3,4-dihydro-7-hydroxy-2,2-dimethyl-6-acetyl-2H-1-benzopyran and 3, 4, 9, 10-tetrahydro-2, 2, 8, 8-tetramethyl-6-acetyl-2H, 8H-benzo [1, 2-b: 3,4b'] dipyran. A mixture of 2,2-dimethyl-6-acetyl-7-hydroxy chroman, substituted benzaldehydes in ethanol and aqueous potassium hydroxide was kept at room temperature for 24 hours, on acidification, chalcone derivatives were obtained. The chalcones were condensed with hydroxylamine hydrochloride in presence of KOH/ethanol; the new 3-substituted phenyl-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazoles are formed.

The results show that all the five compounds showed antibacterial activity against all the four organisms. The minimum inhibition concentration (MIC) was 5µg/ml against *B.subtilis* for compounds IC-1 and IC-4 where as it was 10µg/ml for compounds IC-3, 20µg/ml for compound IC-2 and 50µg/ml in case of compound IC-5. MIC was 5µg/ml against *S.aureus* for compounds IC-2, IC-4 and IC-5; 20µg/ml for compounds IC-3 and in case of compound IC-5 MIC was 100µg/ml. In case of gram-negative bacteria, *E.coli* MIC was 20µg/ml for compound IC-1, IC-4 and IC-5; 50µg/ml for IC-2 & IC-3; the minimum inhibition concentration (MIC) was 5µg/ml against *P.aeruginosa* for compound IC-2; 10µg/ml for IC-1 & IC-4 and 20µg/ml for compound IC-3 & IC-5. On the whole it was observed that compound IC-1 and IC-4 both having methoxy substituents in the phenyl ring showed more activity in all the organisms than that of other compounds.

In case of antifungal activity studies, MIC was 10µg/ml for compounds IC-1 and IC-3; 20µg/ml for compounds IC-

2 & IC-4, whereas it was 200µg/ml for compound IC-5 against *C.albicans*. The minimum inhibition concentration (MIC) was 10µg/ml against *A.niger* for compounds IC-1, IC-2, IC-3 and IC-4, whereas it was 200µg/ml for compound IC-5 against *A.niger*.

CONCLUSION

In conclusion, the synthesis of various 3-substituted phenyl-5-(2",2"-dimethyl, 7"-hydroxy chroman) isoxazole derivatives were achieved by condensing the mixture of chalcones with hydroxylamine hydrochloride in presence of KOH/ethanol. All the synthesized 3-substituted phenyl-5-(2", 2"-dimethyl, 7"-hydroxy chroman) isoxazole derivatives evaluated for their antimicrobial activities. Results revealed that the compounds exhibiting the antimicrobial activity. On the whole it was observed that compound IC-1 and IC-4 both having methoxy substituents in the phenyl ring showed more activity in all the organisms than that of other compounds. The study would be a fruitful matrix for the development of isoxazole derivatives for further bio-evaluation.

Acknowledgement
The authors are grateful to Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur for providing facilities to perform this research work.

REFERENCES

[1] B. E. Kumara Swamy et al, *Der Pharma Chemica*, **2011**, 3 (6):224-234.
[2] Dodia et al, *The International Journal of Science and Technoledge* 2.4 (**2014**): 62-66.
[3] Ashok Kumar et al, *Int.J. ChemTech Res*.**2010**, 2(2).
[4] S. V. Manju et al, *International Bulletin of Drug Research*, **2013,** 1(1): 100-106.
[5] Yong JP, Lu CZ, Wu X et al, *Anticancer Agents Med Chem*. **2014**; 15(1):131-6. 2014; 15(1):131-6.
[6] Mostafa M. Ghorab et al, *Acta Pharmaceutica*, **2013**, Volume 64, Issue 4, Pages 419–431.
[7] Canzhong Lu et al, *Med. Chem. Commun.***, 2014**,5, 968-972.
[8] P.Mondal et al, *J Young Pharm,* v.4(1); Jan-Mar **2012**
[9] B.V. Kendre, et al., *Arabian Journal of Chemistry* (**2015**), http://dx.doi.org/10.1016/j.arabjc.2015.01.007.
[10] EL-Hawash SA et al, *Med Chem.* **2014** May; 10(3):318-38.
[11] S.S Panda et al, *Indian J Pharm Sci*. **2009** Nov-Dec; 71(6): 684–68.
[12] Choi K et al, *Chem Biol*. **2005** Apr; 12(4):469-75.
[13] Vicentini CB et al, *Pharm Biol*. **2011** May;49(5):545-52.
[14] Hyung Jin Kim, Kwang-Jin Hwang & Jae Hyun Lee, *Bioscience, Biotechnology, and Biochemistry*, **1994**, 58:6,1191-1192.
[15] K. A. Parmar et al, *Der Chemica Sinica*, **2011**, 2 (1):100-110.
[16] Yuhan Zhou et al, *Journal of Heterocyclic Chemistry*, Volume 47, Issue 6, pages 1310–1316, November **2010**.
[17] Kumar A et al, *Bioorg Med Chem*. **2009** Jul 15;17(14):5285-92
[18] Tochiro Tatee et al, *Chemical & Pharmaceutical Bulletin*; **2012**, vol.34; no.4; page.1643-1655.
[19] A. Srinivas et al, *Eur J Med Chem*. **2010** Jun;45(6):2353-8.
[20] Singh Rajvir et al, *Pesticide Research Journal*, year 2006, volume: 18, Issue: 2, 128-128.
[21] Qingmin Wang et al, *J. Agric. Food Chem.*, **2011**, 59 (9), pp 4851–4859
[22] Artemenko et al, *Future Med. Chem*.(**2011**) 3(1), 15–27.
[23] SA El-Hawash et al, *Med Chem*. **2014** May; 10(3):318-38.