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Research Article

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Synthesis and evaluation of novel pyrrolidine chalcone derivatives with anticancer, anti-inflammatory and antibacterial activities

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ABSTRACT

In search of molecules that possess biological activity, synthesis of novel pyrrolidines chalcones (AP-JP) obtained by condensing different aldehydes with diazotized 4-amino acetophenone coupled to pyrrolidine and evaluate them for in vitro anticancer, anti-inflammatory and antibacterial activities was undertaken. The synthesized compounds were screened for anticancer activity against human breast cancer cell lines- MCF-7 and MDA-MB-468. In vitro methods for anti-inflammatory activity like inhibition of bovine albumin denaturation and heat induced hemolysismethod was used.Antibacterial and antitubercular activities were also performed.The structures of synthesized compounds were confirmed based on the IR, ^{1}H NMR and mass spectral data.Among the synthesized compounds, 3IP shown IC_{50} value of 25-30 µg against MCF-7 cell line, whereas 3FP shown IC_{50} value of 25 µg against MDA-MB-468 cell lines.Compound 3GP has moderate anti inflammatory activity in bovine denaturation and heat induced hemolytic method.Compounds 3BP, 3CP and 3DP shown MIC of 0.025µg/ml against Staphylococcus aureus, while compounds 3AP and 3IP shown MIC of 0.025µg/ml against E. faecalis. 3CP is sensitive to mycobacterium tuberculosis with a MIC of 6.25 µg/ml.

Keywords: Pyrrolidines, anticancer, antitubercular, antimicrobial, anti-inflammatory.

INTRODUCTION

Serious infections caused by microorganisms resistant to commonly used antimicrobials have become a major healthcare problem worldwide in the 21st century. This is responsible for the significant increase in morbidity and mortality, longer hospitalization and increased health care costs. Keeping in view the seriousness of this problem, the World Health Organization (WHO) has selected "Antimicrobial resistance: No action today, no cure tomorrow" as the theme for World Health Day 2011 as a preventive measure.[1]Pyrrolidine is found in the leaves of tobacco and carrot. The pyrrolidine ring structure is present in numerous natural alkaloids such as nicotine and hygrine. It is found in many pharmaceutical drugs such as levetiracetam, procyclidine and bepridil. It also forms the basis for theracetam compounds (e.g. piracetam, aniracetam). Chalcones are found to have varied biological activities such as antimicrobial, [2] anti-inflammatory, [3] anticancer etc.[4]

As part of the research work, we have synthesized chalcones containing pyrrolidine heterocycle and screened them for various biological activities such as anticancer, anti-inflammatory, antibacterial and antitubercular. Diazotisation

of 4-aminoacetophenone and coupling it with pyrrolidine, which was then made to condense with different aldehydes to obtain chalcone derivatives by Claisen-Schimdt reaction [5].In vitro anticancer activity was performed on MCF-7 and MDA-MB-468 cell lines. In vitro anti inflammatory activity was done by inhibition of bovine albumin denaturation method and heat induced hemolytic method. The compounds were also tested for antibacterial activity against *E.coli, Klebsiella, Staphylococcus aureus* and *E.faecalis* microorganisms. Antitubercular activity was done using *M.tuberculosis.*

EXPERIMENTAL SECTION

The melting point of the compounds was determined using open capillary melting point apparatus and were reported uncorrected. Ultraviolet, visible spectroscopic analysis has been carried out in UV-visible double beam spectrophotometer (LAB INDIA 3000+), IR spectra was recorded by a KBr pellet method using a Bruker FTIR ALPHA Transmission Mode spectrophotometer. The ^{1}H NMR spectra were recorded in $CDCl_3$ by NMR 300MHZ spectrometers using tetramethylsilane as an internal standard. All the chemicals and solvents used in this study were of analytical grade (S.D. FINE Chem. Limited, Mumbai). Reaction progress was checked by TLC in a solvent-vapor-saturated chamber on glass plates coated with Silica Gel GF_{254} followed by visualization under UV light (254 nm). The solvent system used for thin layer chromatography was n-Hexane: Ethyl acetate (6:4).

Procedure for the diazotization and coupling of amine group in 4-Amino Acetophenone with pyrrolidine1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}ethanone:[6] 2.7g of 4-amino acetophenone taken in a 100 ml beaker containing 12 ml of water and 6 ml of HCl and cooled to 5°C. In another beaker, 2.7g of $NaNO_2$ was taken, dissolved in 12ml of water cooled to 5°C. The contents of latter beaker were added slowly to former beaker with stirring and temperature was maintained below 5°C. The obtained diazonium salt was added in portions into a beaker containing 30 ml 10% NaOH, 5ml pyrrolidine and 20g of crushed ice. The formed precipitate is filtered, washed with cold water and dried.

General procedure for preparation of chalcones((2*E*)-3-phenyl-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl} prop-2-en-1-one) from 1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}ethanone-(3AP to 3JP): About 0.003 moles of 1-{4-[(E)-pyrrolidin-1-yldiazenyl]phenyl}ethanone was taken in a 100 ml round bottom flask containing 25ml of ethanol, also0.003 moles of aldehyde was added to the the round bottom flask and were stirred with the help of a magnetic stirrer to form a solution. Then 1 drop of 40% KOH solution was added and stirred for about 1hour. The precipitate obtained in the round bottom flask was filtered, washed with cold ethanol and dried.

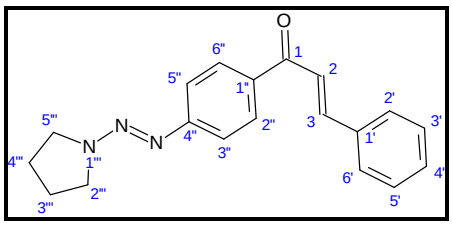


Fig 1: Common structure of the synthesized compounds

Anti-TB activity using Alamar Blue Dye[7]

The antimycobacterial activity of compounds was assessed against *M. tuberculosis* using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method. Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink. Standard values were Pyrazinamide- 3.125µg/ml and Streptomycin-6.25µg/ml. Standard Strain used was H37 RV of *Mycobacteria tuberculosis*.(Vaccine strain).

Antibacterial activity by serial dilution method[8]

9 dilutions of each drug have to be done with BHI for MIC. In the initial tube 20microliter of the drug was added into the 380microliter of BHI broth.For dilutions 200microliter of BHI broth was added into the next 9 tubes separately.Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of BHI broth. This was considered as 10^{-1} dilution. From 10^{-1} diluted tube 200microliter was transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-9} dilution for each drug. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of BHI (brain heart infusion) broth. To each serially diluted tube 200microliter of above culture suspension was added. The tubes were incubated for 24 hours and observed for turbidity.

Anticancer activity activity by the MTT Assay method[9]

MTT solution preparation (stock solution) is prepared by dissolving 5 mg in 1 ml of PBS. The cell line used for the study were MCF-7 and MDA-MB-468 (human procured from NCCS, Pune). The cell lines were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of the mixture of Gentamicin (10ug), Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in the presence of 5% CO_2 at 37ºC for 48-72 hours.

Cytotoxicity Assay: In vitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and add fresh MEM solution and treat with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. In your study, 10, 20, 25, 30 and 50ul of the stock solution (10mg / ml prepared in DMSO) were added to respective wells containing 100ul of the medium. So, the final concentrations were 10, 20, 25, 30 and 50ug / ml.After 48hrs incubation at 37ºC in a humidified atmosphere of 5% CO_2, stock solution of MTT was added to each well (20µl, 5mg per ml in sterile PBS) for further 4 hour incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue" were solubilised by adding DMSO (100µl) and optical density was measured at wavelength of 570nm by using LISA plus. The results represent the mean of five readings. The concentration at which the OD of treated cells was reduced by 50% with respect to the untreated control.

$$\text{Surviving cells } (\%) = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100$$

In Vitro Anti-Inflammatory Activity[10,11,12]

Inhibition of bovine albumin denaturation method

To 2ml of various concentrations of test or standard solutions, 2.8ml of normal saline (pH=7.4) and 0.2ml of 1% bovine albumin solution was added. Simultaneously blank samples were prepared for each concentration without the addition of 1% bovine albumin solution and equal volume of normal saline (pH7.4) was added to each blank sample. To 4.8ml of normal saline (pH 7.4), 0.2ml of 1% bovine albumin solution was added and used as control. The test/standard samples were incubated for 15 min at 70° C. Then the tubes were cooled under running tap water and then absorbance was recorded at 660nm. % inhibition of denaturation of bovine albumin was calculated using the formula

$$\% \, Inhibition = [\![(A - A1) \div A]\!] \times 100$$

Where A=absorbance of the control,
A1= absorbance of the test /standard.

Heat induced hemolytic method

To 1ml of various concentrations of test or standard solutions, 1ml of 1% RBC′s suspension was added. Simultaneously blank samples were prepared for each concentration without the addition of 1% RBC′s solution and equal amount of normal saline was added to each blank sample. An equal amount of 1% RBC′s solution and normal saline was added and was used as a control.

All these samples were taken into centrifuge tubes and incubated in a water bath at 56^0C for 30 min. The tubes were cooled under running tap water and then centrifuged at 2500 rpm for 15 min and absorbance of supernatant was taken at 560 nm. % inhibition was calculated using formula

$\%\ Inhibition = [\![(A - A1) \div A]\!] \times 100$

Where A=absorbance of the control, A1= absorbance of the test /standard.

IC_{50} Values:IC_{50} was calculated using Graphpad prism software.

Statistical analysis: All the data were expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Turkey′s test using a computer based fitness program (Graph pad prism 5)

RESULTS AND DISCUSSION

Chemistrty

The reaction sequences for the synthesis of compounds**(3AP-3JP)** is mentioned in the scheme I. 1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}ethanone synthesis was done by following the common procedure for the coupling reactions of diazonium salts. Various substituted aldehydes were made to react with 1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}ethanone to obtain chalcones**-(3AP-3JP)**, which is a Claisen Schmidt condensation reaction. All the newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectra.

(2*E*)-3-(4-chlorophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3AP)

Yellow crystals; yield: 60%; m.p.: 210-212°C; IR (KBr)V_{max} in cm^{-1}**:** Ar-H str =3073.72, Ali C-H str = 2875.35, =C-H str = 2971.94, N=N str =1394.72, C=O str =1656, C=C str =1604.67, , Ar-N-O str =1310.24, 1523.08; ^{1}H NMR ($CDCl_3$) in δ (ppm):δ 8.295 (d) 2 ArH H of 3′ & 5′ *J*= 8.7; δ 8.06 (d) 2 ArH H of 2′ & 6′ *J*= 8.7; δ 7.846–7.664(m) 4H (2ArH of 2″& 6″+ 2H of 2 & 3 *J*=15.6 trans isomer); 7.546 (d) 2 ArH of 3″ & 5″ ; δ 3.8 4H of 2‴ & 5‴; δ 2.1 4H of 3‴ & 4‴.LC-MS m/z=$[M+H]^+$ =351.4

(2*E*)-3-(3-nitrophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3BP)

Yellow crystals; yield: 55%; m.p.: 206-208°C; IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =3073.72, Aliphatic C-H str = 2875.35, =C-H str = 2971.94, N=N str =1394.72, C=O str =1656, C=C str =1604.67, , Ar-N-O str =1310.24, 1523.08; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.522 (s) 1 ArH of 2′ ; δ 8.265 (d) 1 ArH of 4′*J*= 8.1; δ 8.074 (d) 2 ArH 2″& 6″*J*= 8.7; δ 7.93 (d) 1 H ArH of 6′*J*=7.8; δ 7.860 (d) 1H, of CO-CH=C<u>H</u>*J=15.6* trans isomer); δ 7.675 (d) 1H, of CO-C<u>H</u>=CH *J*=15.6 , 7.63 (m, 1H, ArH 5′); 7.550 (d, 2 ArH of 3″& 5″); δ 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴). LC-MS m/z=$[M+H]^+$ =351.2

(2*E*)-3-(4-chlorophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3CP)

Cream color amorphous solid; yield: 50%; m.p.: 223-225°C;IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =2975, =C-H str = 2947.54, Aliphatic C-H str = 2870.02, N=N str =1398.14, C=O str =1654.70, C=C str =1603.5, Ar-C=C str =1603.5, C-Cl = 817.85 cm^{-1}; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.042 (d, 2H, ArH of 2″& 6″, *J*=8.4) ; δ 7.78 (d, 1H, of CO-CH=C<u>H</u>*J=15.6)*; δ 7.598 (m, 4ArH of Chlorobenzene); δ 7.527 (m, 2H, ArH of 3″& 5″);7.405 (d, 1H, of CO-C<u>H</u>=CH *J=15.6* ; δ 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^+$ =340.6

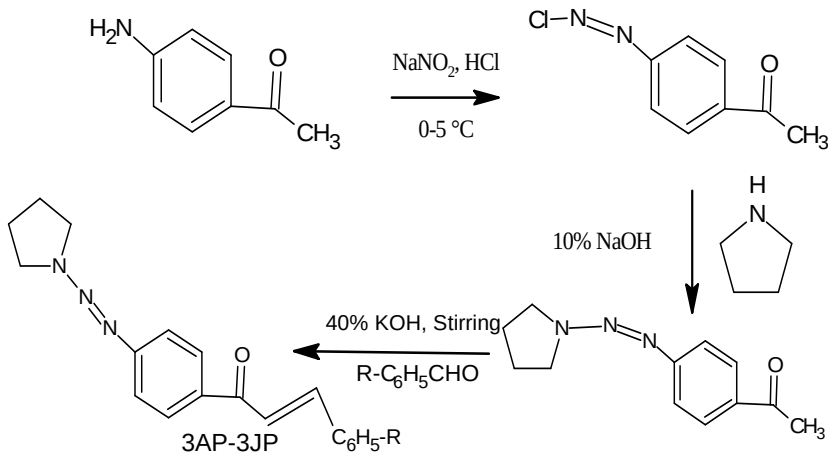
(2*E*)-3-(3-chlorophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one: (3DP)

Cream color amorphous solid; yield: 60%; m.p.: 122-124°C; IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =3065.05, =C-H str = 2976.23, Aliphatic C-H str = 2874.59, N=N str =1400.34, C=O str =1655.76, C=C str =1593.67, C-Cl = 784.51; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.05 (d, 2H, ArH of 2″& 6″, *J*=8.7) ; δ 7.763 (d,1H, CO-CH=C<u>H</u>); δ 7.645 (d, 2H, ArH of 3″& 5″*J=12.9)* ; δ 7.550 (m, 4H, 1H of CO-C<u>H</u>=CH & 3ArH of chlorobenzene); δ 7.368 (s, 1ArH 2′); δ 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^+$ =340.6

(2*E*)-3-(2-chlorophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3EP)

Yellow crystals; yield: 75%; m.p.: 178-180°C; IR (KBr)V_{max} in cm^{-1}**:**Aliphatic C-H str = 2874.06, N=N str =1400.40, C=O str =1649.49, C=C str =1593.39, Ar-H str =3067, Ar-C=C str =1464.40, =C-H str = 2966.13, C-Cl = 746.62; ^{1}H NMR ($CDCl_3$) in δ (ppm):δ 8.201 (d, 1H, CO-CH=C<u>H</u>); δ 8.046 (d, 2H, ArH 2″& 6″); δ 7.71 (d,1H, ArH 3′); δ 7.5 (m, 3H, 2 ArH of 3″& 5″& 1H of CO-C<u>H</u>=CH) ; δ 7.4 (m, 1H, ArH 6′); δ 7.327 (m, 2 ArH 4′& 5′); δ 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^+$ =340.6

Scheme



R=H, 2-Cl, 3-Cl, 4-Cl, 4-NO$_2$, 3-NO$_2$, 4-OCH$_3$, 2,3-(OCH$_3$)$_2$, 4-CH$_3$, 4-F

(2*E*)-3-(4-methoxyphenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3FP)
Brick red color crystals; yield: 80%; m.p.: 195-197°C; IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =3062.74 , =C-H str = 2976.96, Aliphatic C-H str = 2873.76 , N=N str =1405.57, C=O str =1649.49, C=C str =1596.72, Ar-C=C str =1511.66, C-Cl = 817.85 ; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.04 (d, 2H, ArH 2″& 6″*J*=8.4); δ 7.8 (d, 1H CO-CH=CH , *J*=15.6); δ 7.6 (d, 2H, ArH 3″& 5″*J*=8.7); δ 7.5 (m, 3H, 2ArH 2′& 6′, 1H of CO-CH=CH ,); δ 6.95 (d, 2H, ArH 3′& 5′); δ 3.8 (m, 7H, 4H of 2‴& 5‴, 3H of OCH_3); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^+$ =336.5

(2*E*)-3-(3,4-dimethoxyphenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3GP)
Brick red color crystals; yield: 65%; m.p.: 172-174°C; IR (KBr)V_{max} in cm^{-1}**:**Aliphatic C-H str = 2871.97, Ar-H str =3065.59, =C-H str = 2969.90, C=O str =1654.89, C=C str =1598.02; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.043 (d, 2H, ArH 2″& 6″*J*=8.4); δ 7.79 (d, 1H CO-CH=CH , *J*=15.6); δ 7.5 (m, 3H, 2ArH 3″& 5″, 1H of CO-CH=CH); δ 7.265 (m, 1H, ArH 5′); δ 7.17 (s, 1H, ArH 2′); δ 6.9 (d, 2H, ArH 6′); δ 4.0 (m, 8H, of which 2H pyrollidine& 6H $(OCH_3)_2$); δ 3.8 (s, 2H pyrollidine); δ 2.2 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^+$ =366.3

(2*E*)-3-(4-fluorophenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3HP)
Brown amorphous solid; yield: 55%; m.p.: 205-207°C; IR (KBr)V_{max} in cm^{-1}**:** Aliphatic C-H str = 2877.01, N=N str =1403.24, C=O str =1657.62, C=C str =1596.72, Ar-H str =3053.76, Ar-C=C str=1510.94, =C-H str = 2985.76, C-F = 1336.67; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.043 (d, 2H, ArH of 2″& 6″, *J*=8.7) ; δ 7.802 (d, 1H, of CO-CH=CH *J=15.6)* ; δ 7.649 (m, 2ArH, 2′& 6′); δ 7.527 (m, 3H, 2ArH of 2″& 6″, 1H, of CO-CH=CH); 7.110 (m, 2H, ArH 3′& 5′); δ 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=M^+ =323.7

(2*E*)-3-(4-methylphenyl)-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3IP)
Yellow crystals; yield: 50%; m.p.: 212-214°C; IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =3029.21,=C-H str = 2966.92, Aliphatic C-H str = 2805.25, C=O str =1648.83 cm^{-1}, C=C str =1594.16 , Ar-C=C str =1511.15; ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.043 (d, 2H, ArH of 2″& 6″, *J*=8.7) ; δ 7.820 (d, 1H, of CO-CH=CH*J=15.6)* ; δ 7.520 (m, 5ArH, 2ArH 2′& 6′, 2ArH 3″& 5″, 1H, of CO-CH=CH); δ 7.236 (m, 2H, ArH of 3′& 5′);δ 3.8 (4H of 2‴& 5‴); δ 2.4 (s, 3H of CH_3); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=$[M+H]^-$ =317.6

(2*E*)-3-phenyl-1-{4-[(*E*)-pyrrolidin-1-yldiazenyl]phenyl}prop-2-en-1-one:(3JP)
Yellow crystals; yield: 72%; m.p.: 168-170°C; IR (KBr)V_{max} in cm^{-1}**:**Ar-H str =3028.33, =C-H str = 2968.18, Aliphatic C-H str = 2878.45, N=N str =1401.80, C=O str =1650.95, C=C str =1593.92, Ar-C=C str =1495.98; ^{1}H NMR: δ 8.053 (d, 2H, ArH of 2″& 6″, *J*=8.4) ; δ 7.842 (d, 1H, of CO-CH=CH*J=15.6)* ; 7.560 (m, 3H, 2ArH 3″& 5″; 1H, of CO-CH=CH); δ 7.412 (m, 1H, ArH of 4′);7.560 (m, 4H, ArH); 3.8 (4H of 2‴& 5‴); δ 2.1 (4H of 3‴& 4‴); LC-MS m/z=M^- =305.2

The IR spectrum, showed a strong absorption at 1656 cm^{-1} which is a characteristic band for the carbonyl group of the chalcones. Absorption around 1400 $cm^{-1}$was assigned to the N=N str. The other C=C, C-H, Ar-H stretching absorptions were noticed which were in accordance with the structure of the synthesized compounds. ^{1}H NMR has shown signal at δ 4 and 2 accounting for the 4 protons of the pyrrolidine nucleus. Signal for the olefinic and aromatic protons was present in between δ 8.5 and 6.9. The presence of olefinic group gives rise to geometrical isomers. The *J* value in all the compounds was found to be >14, which confirmed that, trans type of configuration is present at C=C. Thus, all the protons were accounted for the respective structures. Mass spectra were also in accordance with the proposed structures.

ANTI-TB RESULTS

Table-1: MIC of synthesized compounds against *Mycobacterium tuberculosis*

Sl. No.	Samples	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	3AP	S	S	S	R	R	R	R	R
2	3BP	S	S	S	S	R	R	R	R
3	3CP	S	S	S	S	S	R	R	R
4	3DP	S	S	S	R	R	R	R	R
5	3EP	S	S	S	R	R	R	R	R
6	3FP	S	S	S	R	R	R	R	R
7	3GP	S	S	S	R	R	R	R	R
8	3HP	S	S	S	R	R	R	R	R
9	3IP	S	S	S	R	R	R	R	R
10	3JP	S	S	S	R	R	R	R	R

NOTE: *S - Sensitive, R – Resistant, Strain used:* ***M.tuberculosis*** *(H37 RV strain) : ATCC No- 27294. standard drug MIC values for the Anti-Tb test - Pyrazinamide- 3.125µg/ml, Streptomycin- 6.25µg/ml, Ciprofloxacin-3.125µg/ml.*

Antibacterial activity results

Table-2: MIC of synthesized compounds against *S.aureus*

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml	0.1 µg/ml	0.05 µg/ml	0.025 µg/ml	0.0125 µg/ml
S.aureus															
1	3AP	S	S	S	S	S	S	S	S	S	S	S	R	R	R
2	3BP	S	S	S	S	S	S	S	S	S	S	S	S	S	R
3	3CP	S	S	S	S	S	S	S	S	S	S	S	S	S	R
4	3DP	S	S	S	S	S	S	S	S	S	S	S	S	S	R
5	3EP	S	S	S	S	S	S	S	S	S	S	S	S	R	R
6	3FP	S	S	S	S	S	S	S	S	S	R	R	R	R	R
7	3GP	S	S	S	S	S	S	S	S	S	R	R	R	R	R
8	3HP	S	S	S	S	S	S	S	S	S	R	R	R	R	R
9	3IP	S	S	S	S	S	S	S	S	S	S	S	R	R	R
10	3JP	S	S	S	S	S	S	S	S	S	R	R	R	R	R

NOTE:*S– Sensitive, R – Resistant, standard values of MIC for anti-bacterial, Ciprofloxacin (10µg): S.aureus- 2µg/ml.*

Table-3: MIC of synthesized compounds against *E.faecalis*

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml	0.1 µg/ml	0.05 µg/ml	0.025 µg/ml	0.0125 µg/ml
	E.faecalis														
1	3AP	S	S	S	S	S	S	S	S	S	S	S	S	S	R
2	3BP	S	S	S	S	S	S	S	S	S	R	R	R	R	R
3	3CP	S	S	S	S	S	S	S	S	S	S	S	S	R	R
4	3DP	S	S	S	S	S	S	S	R	R	R	R	R	R	R
5	3EP	S	S	S	S	S	R	R	R	R	R	R	R	R	R
6	3FP	S	S	S	S	S	S	S	S	S	S	S	S	R	R
7	3GP	S	S	S	S	S	S	S	S	S	S	S	R	R	R
8	3HP	S	S	R	R	R	R	R	R	R	R	R	R	R	R
9	3IP	S	S	S	S	S	S	S	S	S	S	S	S	S	R
10	3JP	S	S	S	S	S	S	S	S	S	R	R	R	R	R

NOTE:*S – Sensitive, R – Resistant, standard values for MIC for anti-bacterial, Ciprofloxacin (10µg): E.faecalis- 2µg/ml*

Table-4: MIC of synthesized compounds against *E.coli*

Sl. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
	E.coli										
1	3AP	S	S	S	R	R	R	R	R	R	R
2	3BP	S	R	R	R	R	R	R	R	R	R
3	3CP	S	R	R	R	R	R	R	R	R	R
4	3DP	S	R	R	R	R	R	R	R	R	R
5	3EP	S	R	R	R	R	R	R	R	R	R
6	3FP	S	S	S	S	R	R	R	R	R	R
7	3GP	S	R	R	R	R	R	R	R	R	R
8	3HP	R	R	R	R	R	R	R	R	R	R
9	3IP	R	R	R	R	R	R	R	R	R	R
10	3JP	S	R	R	R	R	R	R	R	R	R

NOTE: *S – Sensitive, R – Resistant, standard values for MIC for anti-bacterial, Ciprofloxacin (10µg): E.coli - 2µg/ml*

Table-5: MIC of synthesized compounds against *Klebsiella.pneumoniae*

Sl.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
Klebsiella											
1	3AP	S	S	R	R	R	R	R	R	R	R
2	3BP	S	R	R	R	R	R	R	R	R	R
3	3CP	R	R	R	R	R	R	R	R	R	R
4	3DP	S	R	R	R	R	R	R	R	R	R
5	3EP	S	R	R	R	R	R	R	R	R	R
6	3FP	S	R	R	R	R	R	R	R	R	R
7	3GP	S	R	R	R	R	R	R	R	R	R
8	3HP	R	R	R	R	R	R	R	R	R	R
9	3IP	R	R	R	R	R	R	R	R	R	R
10	3JP	S	R	R	R	R	R	R	R	R	R

NOTE: S – Sensitive, R – Resistant, standard values for MIC for anti-bacterial, Ciprofloxacin (10µg): standard values, K.Pneumoniae- 1µg/ml

Table-6:IC_{50} (µg) of synthesized compounds againstMCF-7 and MDA-MB-468

Sl. No.	Samples	IC_{50} (µg)	
		MCF-7	MDA-MB-468
1	3AP	-	50
2	3BP	-	50
3	3CP	30	30
4	3DP	50	30
5	3EP	-	-
6	3FP	-	-
7	3GP	30	-
8	3HP	50	-
9	3IP	25-30	-
10	3JP	50	25-30

Note: IC_{50} – is half maximal inhibitory concentration - it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50}

Antiinfalmmatory activity

Bovine albumin denaturation method

Table-7:Bovine albumin denaturation by the synthesized compounds

Conc. (µg/ml)	% Inhibition ± SEM*										
	Diclofenac sodium	**3AP**	**3BP**	**3CP**	**3DP**	**3EP**	**3FP**	**3GP**	**3HP**	**3IP**	**3JP**
20	75.3± 0.364	43.2± 0.536	30.2± 0.543	43.1± 0.249	43.9± 0.657	45.7± 0.442	29.3± 0.223	64.9± 0.424	41.7± 0.331	33.3± 0.289	53.2± 0.346
40	81.4± 0.234	34.0± 0.24	48.0± 0.542	46.1± 0.231	45.0± 0.632	48.6± 0.753	33.2± 0.521	68.3± 0.423	46.8± 0.234	36.2± 0.246	56.0± 0.236
60	86.0± 0.321	30.9± 0.24	40.9± 0.213	33.4± 0.271	27.8± 0.355	36.9± 0.205	26.5± 0.636	50.1± 0.334	28.7± 0.125	29.5± 0.26	37.2± 0.535
80	94.0± 0.423	41.0± 0.602	48.0± 0.102	50.9± 0.894	40.8± 0.624	49.7± 0.275	28.7± 0.491	64.30± 0.984	43.9± 0.721	20.7± 0.046	30.8± 0.648
100	96.4± 0.624	56.5± 0.856	50.5± 0.475	57.8± 0.129	45.4± 0.694	60.3± 0.117	30.7± 0.402	69.5± 0.264	45.5± 0.024	40.7± 0.452	35.4± 0.224
120	98.0± 0.245	42.5± 0.346	48.5± 0.626	56.3± 0.772	39.5± 0.58	36.5± 0.054	42.5± 0.342	60.6± 0.284	42.9± 0.492	36.5± 0.132	31.5± 0.85
IC_{50}(µg/ml)	7.873	90.2	92.8	56.2	119	66.2	240	19.9	110	35	75

NOTE: All the values are average of three readings, Mean ±SEM, SEM = Standard Error Mean, IC_{50}= Half maximal inhibitory concentration.

Table-8:Heat induced hemolysisby the synthesized compounds

Conc. (µg/ml)	% Inhibition ± SEM*										
	Diclofenac sodium	**3AP**	**3BP**	**3CP**	**3DP**	**3EP**	**3FP**	**3GP**	**3HP**	**3IP**	**3JP**
20	74.8± 0.282	33.7± 0.608	47.7± 0.122	37.0± 0.484	38.9± 0.229	42.8± 0.664	42.9± 0.145	42.3± 0.366	36.8± 0.398	32.5± 0.804	37.8± 0.286
40	78.2± 0.644	46.7± 0.238	42.84± 0.304	48.8± 0.405	39.2± 0.668	39.2± 0.945	48.2± 0.467	44.2± 0.205	38.2± 0.663	32.6± 0.244	32.5± 0.338
60	89.0± 0.482	48.8± 0.308	48.60± 0.25	48.2± 0.205	44.4± 0.508	49.0± 0.468	54.8± 0.62	62.6± 0.926	44.6± 0.608	33.4± 0.088	394± 0.286
80	91.2± 0.514	56.2± 0.362	55.6± 0.16	58.8± 0.064	48.5± 0.004	58.2± 0.094	52.6± 0.674	63.0± 0.62	52.8± 0.565	48.2± 0.145	49.8± 0.565
100	93.2± 0.321	52.3± 0.61	52.8± 0.864	58.7± 0.206	54.7± 0.467	49.1± 0.929	45.3± 0.676	58.2± 0.737	54.6± 0.91	52.6± 0.26	49.6± 0.688
120	94.4± 0.821	58.4± 0.626	5.82± 0.412	59.8± 0.825	57.4± 0.636	58.8± 0.024	58.4± 0.684	54.0± 0.636	46.7± 0.764	54.0± 0.802	48.6± 0.494
IC_{50}(µg/ml)	8.624	58.4	68.2	76.7	143	66.8	72	46.67	116.8	176.8	156.5

NOTE: All the values are the average of three readings, Mean ±SEM, SEM = Standard Error Mean, IC_{50}= Half maximal inhibitory concentration.

Most of the compounds shown moderate to good antimicrobial activity. The α,β-unsaturared part of chalcone along with pyrrolidine seems to be essential for the antimicrobial activity. But substitution on the phenyl ring decides the extent of potency of the compounds. The The electron releasing group at para position of the phenyl ring . i.e, Cl substituent(3CP) has moderate antitubercular activity, while the meta substituent 3BP with electron withdrawing

group i.e, NO_2 is found to be active but not as a potent as 3CP with regard to antitubercular activity. The Cl substituted compounds were more potent than the standard drug ciprofloxacin against *S.aureus*. Para nitro and para methyl derivatives (3AP and 3IP) were also more potent against *E.faecalis*. Except for the para methoxy derivative (3FP), all the synthesised compounds were resistant against E.coli. No significant activity was found against *Klebsiella.*

Compound with substituents like chloro, methyl at para position and compound with 3,4-dimethoxy substituents(3CP, 3IP, 3GP) were cytotoxic against MCF-7 cell lines at IC_{50}30µg. Para chloro, meta chloro and the unsubstituted derivatives(3CP, 3DP,3JP) were cytotoxic against MDA-MB-468 cell lines at IC_{50}30µg. 3,4-dimethoxy substituents(3GP) shown mild anti-inflammatory activity.

CONCLUSION

Compound 3CP active against M.tuberculosis and gram positive microorganisms and thus can be called as broad spectrum antimicrobial agent. It is also has mild anticancer and anti-inflammatory activity. Thus molecule 3CP seems to have a wide variety of biological activities and can be considered for further research activities.

REFERENCES

[1] Jaswant Rai; Gurpreet Kaur Randhawa; Mandeep Kaur, *Int J Appl Basic Med Res*,**2013**, Jan-Jun, 3(1), 3–10.
[2] Y Rajendra Prasad;P Praveen Kumar; PA Ravi Kumar; Srinivasa rao, *E-Journal of Chemistry* 5(1), **2008**, 144-148.
[3] M Kouskoura; LD Hadjipavlou; M Giakoumakou, *Medicinal Chemistry*, **2008**, 4(6), 586-596
[4]SY Mohammad; AS Anees; , AA Mohamed; *J. Chinese Chemical Society*, **2007**, 54, 81-86
[5]BS Furniss, AJ Hannford, PWG Smith, AR Tatchell, Vogel's Textbook of Practical Organic Chemistry, Pearson Education India, 01-Sep-**1989**, 1032.
[6]BS Furniss, AJ Hannford, PWG Smith, AR Tatchell, Vogel's Textbook of Practical Organic Chemistry, Pearson Education India, 01-Sep-**1989**, 927.
[7] CS Maria Lourenco, Marcus VN deSouza; Alessandra C Pinheiro; L Marcelle de;Fer reira; B Rasnisb; Goncalves; Thais Cristina M Nogneira; Monica A Peralta, ARKIVOC **2007** (xv), 181-191.
[8] Schwalve; Moore; Goodwin, Antimicrobial susceptibility testing protocols. Crc Press **2007.**
[9]Alan Dolly; J. Brayan Griffiths, Cell And Tissue Culture For Medical Research . Jhon Willey and Son′s , June **2000**
[10]RS Joshi;PG Mandhane;SD Diwakar;SK Dabhade; CH Gill.. *Bioorg Med Chemlett* **2010**; 20, 3721-5.
[11]S Chandra; C Priyanka; D Protapaditya;S Bhattacharya, *Asian Pacific J of Tropical Biomed*, , **2012**,3, 178-180.
[12]PJ Jainey; IK Bhat;*J Young Pharmacists***2012**, 4,82-7.