Synthesis, Characterization and Biological Evaluation of Chalcones and its derivatives for Antileishmanial activity

Abstract:-

Chalcones is a generic term given to compounds bearing the 1,3-diarylprop2-en-1-one**,** which can be functionlized in the propane chain by the presence of olefinic, keto and/or hydroxyl group. Chalcones belongs to the flavonoid family. Chemically chalcones consisted of open chain flavonoids in which the two aromatic rings are joined by a three carbon *a,ß*-unsaturated carbonyl system (Dhar, 1981). Microorganisms are a heterogeneous group of several distinct classes of living beings. They were classified under third kingdom, the Prostita. Based on differences in cellular organization and biochemistry, the kingdom prostita has been divided into two groups, Prokaryotes and Eukaryotes. Bacteria and blue green algae are prokaryotes while fungi, other algae, slime moulds and protozoa are eukaryotes. Antileishmenia drugs used or effective against leishmaniasis : destroying protozoa of the genus Leishmania antileishmanial activity Pentavalent antimony compounds have been the mainstay of antileishmanial therapy for half a century, but lipid formulations of amphotericin B represent a major advance for treating visceral leishmaniasis.

Key Words:- Synthesis, Chalcone, Hetrocyclic compound, Antileishmenia activity

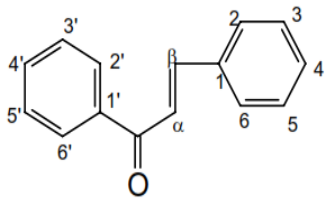
1.0 INTRODUCTION

Chalcones is a generic term given to compounds bearing the 1,3-diarylprop2-en-1-one**,** which can be functionlized in the propane chain by the presence of olefinic, keto and/or hydroxyl group. Chalcones belongs to the flavonoid family. Chemically chalcones consisted of open chain flavonoids in which the two aromatic rings are joined by a three carbon *a,ß*-unsaturated carbonyl system (Dhar, 1981). Pharmacological properties of chalcones are due to the presence of both a,ß-unsaturation and an aromatic ring. Chalcones considered as precursors of flavonoids and isoflavonoids are abundant in plants (Ni *et al.* 2004; Nowakowska, 2007; Dimmock *et al.* 1999). Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus Leishmania. It is transmitted through the bite of female phlebotomine sandflies and can range from mild self-healing cutaneous lesions to lethal visceral leishmaniasis. Lic A **(1)**, efficiently inhibited the proliferation of *Leishmania donovani* and *Leishmania major* promastigotes and amastigotes *in vitro* by inhibiting fumarate

reductase (Chen *et al.* 1993), a selective target present in the parasite mitochondria. The Lic C **(113)** inhibited the growth of the *L. major* parasite to the same extent as Lic A **(1)** (Nielson *et al.* 1995).

The oxygenated chalcones **114-117** and Lic A **(1)** inhibited the *in vitro* growth of *L. major* promastigotes (IC_{50} in the range of 4.0-10.5 µM) measured by 3H-thymidine incorporation and *L. donovani* amastigotes (IC_{50} in the range of 0.65-6.1 µM) in human monocyte-derived macrophages (MDM) and also inhibited the respiration of the parasite (Zhai *et al.* 1999).

General structure of chalcone:



Chalcones are one of the major classes of natural products which occur widely in nature particularly in colored flowers and wide spread distribution in fruits, vegetables, spices and tea. Various natural or synthetic chalcones have been found to posses diverse biological activites (Di Carlo *et al.* 1999).

All the chalcones give dark red coloration with concentrated sulphuric acid wilson test) and violet red coloration with alcoholic ferric chloride solution. Chalcones on heating with traces of iodine in dimethylsulphoxide (DMSO) for two hours give the corresponding flavones. Chalcones were converted into the corresponding flavonols by their oxidation using hydrogen peroxide in methanolic sodium hydroxide solution and these flavonols showed characteristic greenish yellow fluorescence in ethanolic solution as well as with concentrated sulphuric acid.

2.0 MATERIAL AND METHODS:

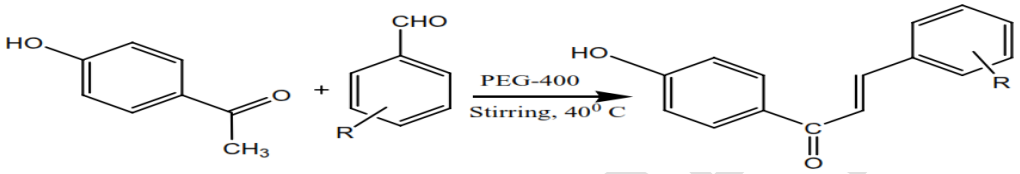
The following methods for synthesizing an Chalcones and chalcones derivatives. All the chemicals used were obtained from Sigma-Aldrich, Spectrochem and High Media.The melting point is determined by using an open capillary and are uncorrected .TLc were performed on silica sel G plates with observation in under UV or iodine chamber . IR spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometer in KBr disks and Elemental analysis were done on a Perkin-Elmer 2400C, H, N analyzer and values were found to be within the acceptable limits of the calculated values. The ^{1}H-NMR spectra of the synthesized compounds in $CDCl_3$/DMSO were recorded at 400 MHz by Bruker Advance II 400 NMR spectrometer. Chemical shift values are given in scale using tetramethylsilane (TMS) as an internal standard. Significant ^{1}H-NMR data are written in order: number of protons, multiplicity (b, broad; s, singlet; d, doublet; dd, double doublet; t, triplet;

m, multiplet), coupling constants in Hertz, assignment. The FAB mass spectra (at room temperature) were recorded on TOF MS ES^+ mass spectrometer.

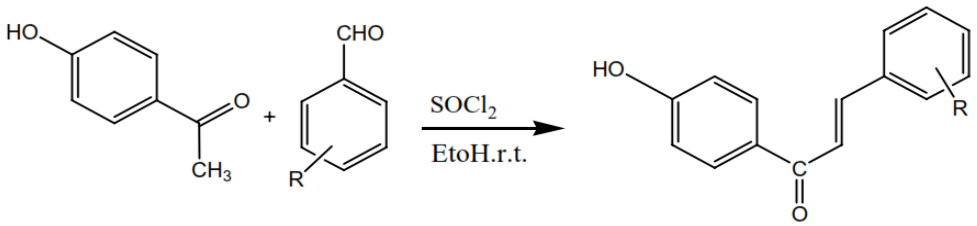
2.1 General methods of synthesis of chalcones

Chalcones are well known intermeadiates for synthesizing various heterocyclic compounds. They can be obtained by the acid or base catalyzed aldol condensation of acetophenones with benzaldehydes (Guida *et al.* 1997).

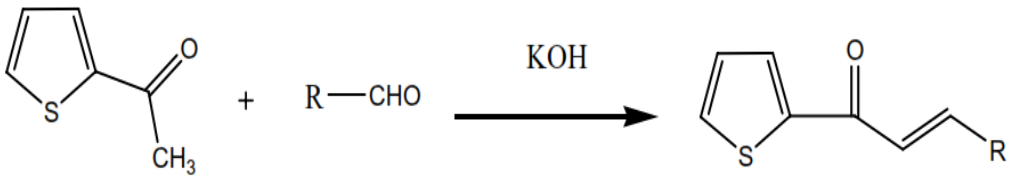
1) Claisen–Schmidt condensation between 4-hydroxy acetophenone and benzaldehyde was carried out in the presence of a base catalyst was stirred in PEG-400 as a recyclable solvent form 4' -hydroxy chalcones (Sreedhar *et al.* 2010).



2) Stirred mixture of 4-hydroxy acetophenone and various benzaldehyde in the presence of thionylchloride in absolute ethnol form substituted 4'-hydroxy chalcones (Eddarir, 2003).



A mixture of 2-acetyl thiophene substituted aldehydes was stirred in ethnol then an aqueous solution of KOH was added to form chalcones (Romanelli *et al.* 2011).



2.3 Synthesis of intermediates

2.3.1 Synthesis 3-N-(N'-p-chlorophenylurenyl)acetophenone

Synthesis of methyl ketone derivative was carried out by making *m*-amino acetophenone react with the *p*-chlorophenyl isocyanate. A mixture of the *m*-aminoacetophenone (2.7 g, 20 mmol) and *p*-chlorophenyl isocyanate (3 g, 20 mmol) was dissolved in dry acetone (100 mL). The mixture

was stirred for 6-7 hr at room temperature, filtered, and the crude compound urenylacetophenone was recrystallized using ethanol (Sonmez *et al.,* 2011).

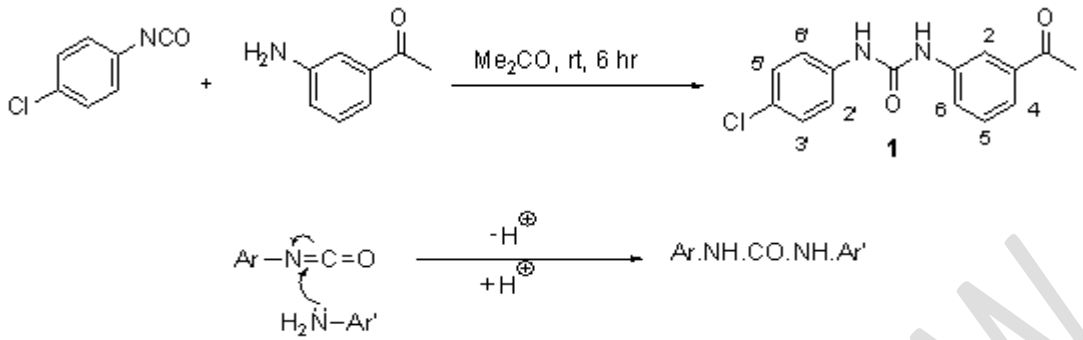


Figure 1 : Scheme for synthesis of *3-N-(N'-p-chlorophenylurenyl)acetophenone*

Yield 3.3 g, 58%, White solid; mp 272-274 ^{o}C; IR(KBr) ν_{max} /cm^{-1} 3372 (N-H), 3056 (ArC-H), 2962 2872 (C-H), 1711 ($COCH_3$), 1645 (C=O), 1614, 1534, 1461 (Ar C=C), 1515, 1290, 1185 (ArC-N), 1147 (Ar-Cl) 756, 687 (Ar); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 9.12 (br s, 1H, NH), 8.91 (br s, 1H, NH); 8.18 (1H, s, H-2), 7.78 (1H, d, *J* 5.9, H-6), 7.53 (3H, m, H-4, 2', 6'), 7.30 (1H, t, *J* 6.30, H-5), 7.21 (2H, d, *J* 6.65, H-3', 5'), 2.53 (s, 3H, 3-$COCH_3$).

2.3.2 Synthesis of 3'-N [(2'', 5''-dichlorophenyl) sulfonyl-amide] acetophenone

The intermediate compound 3'-N[(2'',5''-dichlorophenyl) sulfonyl-amide] acetophenone was synthesized adopting the procedure described by Leon *et al*. (2007) with some modifications (Figure 4.4).

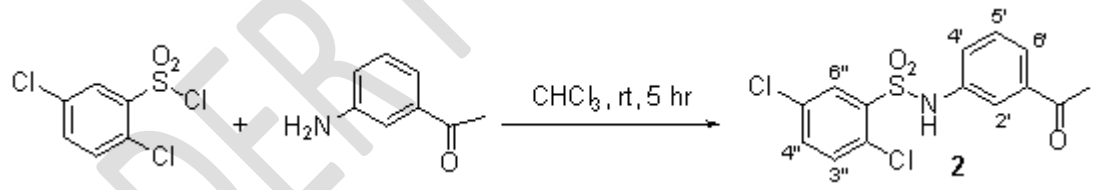


Figure 2 : Scheme for synthesis of 3'-N[(2'',5''-dichlorophenyl) sulfonyl-amide] acetophenone

A mixture of 3-aminoacetophenone (2.7 g, 20 mmol) and 2, 5-dichloro-benzene sulfonyl chloride (4.9 g, 20 mmol) in 5 mL of chloroform was stirred at room temperature (rt) for 3–6 hr. The resulting precipitate was washed with acetone, filtered, and the crude material obtained was recrystallized in acetonitrile to give pure compound 3'-N[(2'',5''-dichlorophenyl) sulfonyl-amide] acetophenone. Yield 3.6 g, 52%, Brown crystals; mp 230–232 °C; IR 3216 (N-H); 1667 (C=O); 1715 ($COCH_3$), 1337, 1270 (SO_2), 1142 (Ar-Cl), 3060 (Ar-H), 2967 (C-H), 1584, 1461, 1357, 1297, 1273, 1166, 993, 852, 819, 795, 720 (Ar); ^{1}H-NMR: δ_H 11.38 (s, 1H, NH), 7.94 (1H, s, H-6´´), 7.70 (1H, d, *J* 8.44, H-3´´), 7.25-7.44 (3H, m, H-2´, 5´, 6'), 7.71 (d, 1H, *J* 6.42, H-4´´), 6.94 (1H, d, *J* 8.91, H4'), 2.51 (s, 3H, CH_3CO).

2.3.3.General method of synthesis of chalcone derivatives (1a-1e)

Chalcones are synthesized by Claisen-Schmidt condensation (Furniss *et al.,* 1989; Kumar *et al.*, 2010) of aldehyde and ketone by base catalyzed or acid catalyzed followed by dehydration to yield chalcones (Figure 3).

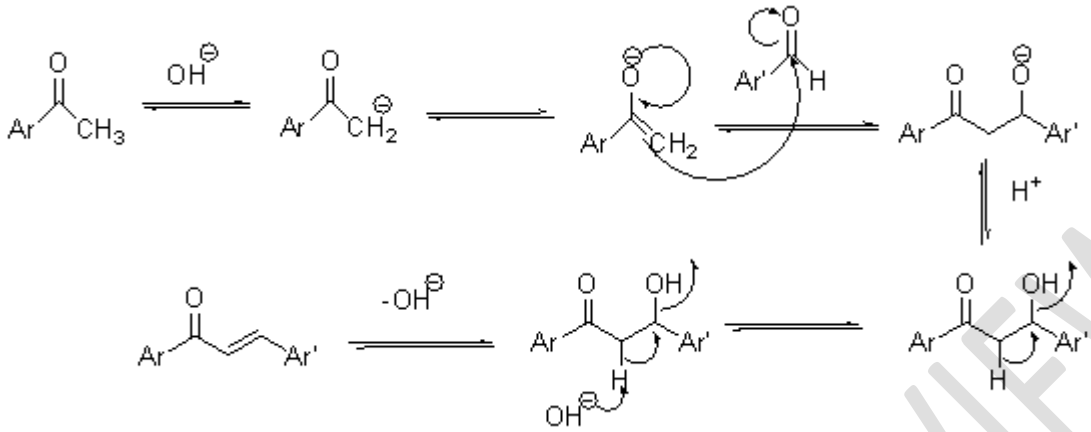


Figure 3 : Mechanism of reaction for synthesis of chalcone derivatives (1a-1e)

To a solution of substituted acetophenone (16 mmole) in 10 mL of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (60 mL) was added and stirred for 10 min. To this, appropriate aldehyde (16 mmole) was added and stirred at room temperature for 12-24 hr. The reaction mixture was cooled on an ice bath, neutralized with diluted HCl and the precipitate was washed three times with 50 mL distilled water to give the crude product. The product was recrystallized from methanol or ethanol/ water.

The purity of the product was checked by TLC using ethyl acetate and hexane (4:6) as mobile phase and iodine vapors as detecting agent.

2.4 Synthesis of trisubstituted pyrazolines (2a-2e)

General method for synthesis of 1, 3, 5-trisubstituted pyrazolines (2a-2e)

1,3,5-trisubstituted pyrazolines (**2a-2e**) were synthesized according to the scheme depicted in Figure 4.6 (Ozdemir *et al.,* 2008). In this method, chalcone and nicotinic acid hydrazide were refluxed in *n*-butanol in order to synthesize the desired product (Kini and Gandhi, 2008). Factors such as the structure and position of the substituents have profoundly influenced the rate of the reaction. The generally accepted interpretation of this reaction, involves the initial formation of an aryl hydrazone with subsequent nucleophilic attack of nitrogen upon the carbon-carbon double bond at position. Hence the electropositive nature of carbon may control the overall rate of the reaction. The electropositive nature of carbon is controlled by the aromatic ring directly connected to it. Halogens being electron withdrawing in nature significantly increase the positive character of carbon lead to faster reaction while electron donating alkyl and alkoxy groups contributed for slower reaction.

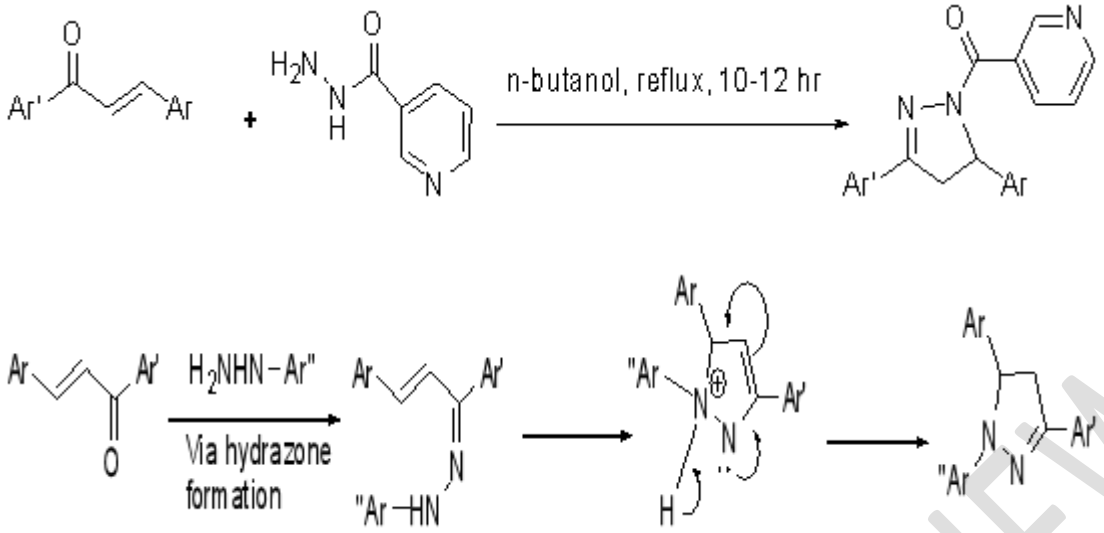
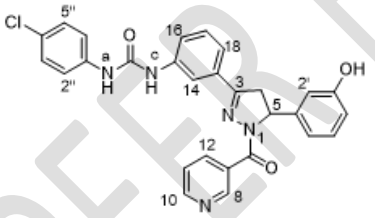


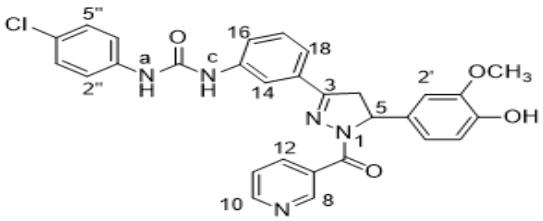
Figure 4 : Scheme and mechanism of reaction for synthesis of compounds (2a-2e)

a-(4"-chlorophenyl)-c-(3-(5"-(3'-hydroxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2a)



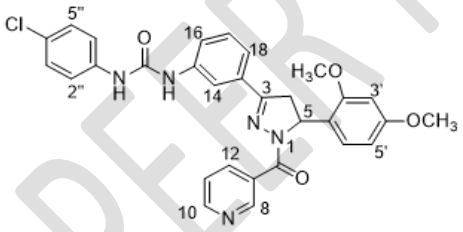
Synthesized by method from chalcone **1a** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 14 hrs reflux; Yield 67%, Pale yellow solid; mp 165-167°C; IR (KBr) ν_{max}/cm^{-1} 3414 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR, δ 10.02 (1H, s, 3'-OH), 9.15 (1H, br s, NH), 8.96 (1H, br s, NH), 9.02 (1H, s, 8-H), 8.73 (1H, d, *J* 3.7, 10-H), 8.25 (2H, t, *J* 6.50, H-12, 14), 7.80 (1H, d, *J* 6.70, H-16), 7.45-7.58 (6H, m, H-11, 17, 18, 3", 5", 6"), 7.10 (2H, dd, H-5', 6'), 6.85 (2H, dd, H-2', 4'), 5.95 (1H, dd, *J 12.1* and 6.8, H-5), 3.83 (1H, dd, *J 17.7* and 11.6, 4-H$_y$), 3.18 (1H, dd, *J 17.1* and 4.3, 4-H$_x$); FAB-MS *m/z*: 511.54 $[M+H]^+$; Analysis Calcd. (%) for $C_{28}H_{22}ClN_5O_3$: C, 65.69; H, 4.33; N, 13.68;Found: C, 65.38; H, 4.18; 13.85;

a-(4"-chlorophenyl)-c-(3-(5"-(4'-hydroxy,3'-methoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2b)



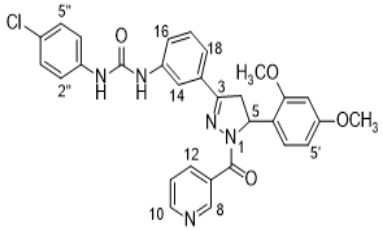
Synthesized by method from chalcone **1b** (1.29 g, 4 mmol) and nicotinic acid hydrazide (0.55 g, 4 mmol); Yield 0.97 g, 55%, Pale yellow powder; mp 135-137°C; IR(KBr) $_{max}$/cm^{-1} 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR, □ 10.05 (1H, s, 4'-OH), 9.10 (1H, br s, NH), 8.96 (1H, br s, NH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.16 (1H, d, *J* 7.2, 12-H), 7.68 (1H, d, *J* 7.6, H-11), 7.48-7.58 (5H, m, H-17, 18, 2", 5", 6"), 7.40 (1H, d, *J* 4.2, H-4''), 6.87-6.94 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5), 3.89 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.83 (3H, s, OCH$_3$-3'), 3.10 (1H, dd, *J 17.8* and 4.8, 4-H$_x$); FAB-MS *m/z*: 541.31 [M +H]$^+$; Analysis Calcd. (%) for $C_{29}H_{24}ClN_5O_4$: C, 64.27; H, 4.46; N, 12.92; Found: C, 64.36; H, 4.26; N, 12.71

a-(4"-chlorophenyl)-c-(3-(5-(2',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2c)



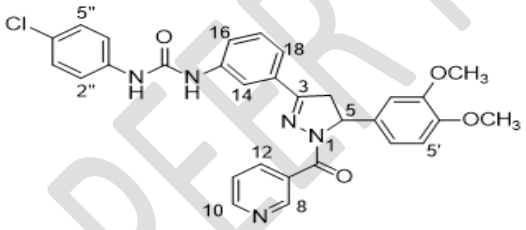
Synthesized by method above from chalcone **1c** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 69%, Light yellow solid; mp 156-159°C; IR (KBr) $_{max}$/cm^{-1} 3294 (N-H), 1668 (N-C=O), 1591 (Ar C=C), 1560 (C=N), 1262, 1096 (C–O), 1210 (C-N), 1102 (C–Cl), 3041, 2954 (C-H), 1501, 1467, 922, 815, 798 (Ar); ^{1}H-NMR, □ 9.12 (1H, br s, NH), 8.91 (1H, br s, NH), 9.02 (1H, s, 8-H), 8.70 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 8.18 (2H, dd, *J 12.3* H-12, 14), 7.85 (1H, d, *J* 6.70, H-16), 7.68-7.75 (3H, m, H-18, 2", 6"), 7.50-7.60 (4H, m, H-11, 17, 3",6"), 7.08 (1H, d, *J* 6.54, H-6'), 6.60 (2H, t, *J* 6.54, H-5', 3'), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.70 (6H, s, OCH$_3$-2',4'), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H$_x$); FAB-MS *m/z*: 556.31 [M +H]$^+$; Analysis Calcd. (%) for $C_{30}H_{26}ClN_5O_4$: C, 64.80; H, 4.71; N, 12.60; Found: C, 64.39; H, 4.17; N, 12.25

a-(4"-chlorophenyl)-c-(3-(5-(2',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2d)



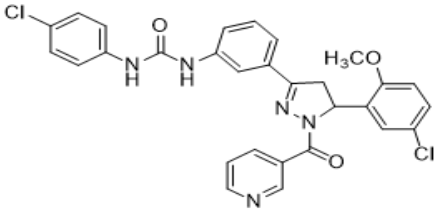
Synthesized by method above from chalcone **1d** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 69%, yellow solid; mp 151-153°C; IR (KBr) $_{max}$/cm^{-1} 3298 (N-H), 3045, 2953 (C-H), 1660 (N-C=O), 1599 (Ar C=C), 1561 (C=N), 1256, 1084 (C–O), 1227 (C-N), 1127 (C–Cl), 1501, 798 (Ar); ^{1}H-NMR, 9.10 (1H, br s, NH), 8.91 (1H, br s, NH), 9.08 (1H, s, 8-H), 8.76 (1H, d, *J* 3.9, 10-H), 8.20 (1H, d, *J* 7.2, 12-H), 8.18 (2H, dd, *J 12.3* H-12, 14), 7.80 (1H, d, *J* 6.70, H-16), 7.58-7.63 (3H, m, H-18, 2", 6"), 7.48-7.53 (4H, m, H-11, 17, 3",6"), 7.11 (1H, d, *J* 6.54, H-6'), 6.60 (2H, t, *J* 6.54, H-5', 3'), 5.91 (1H, dd, *J 12.3* and 6.2, H-5), 3.81 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.72 (6H, s, OCH$_3$-3',4'), 3.18 (1H, dd, *J 17.5* and 4.6, 4-H$_x$); FAB-MS *m/z*: 556.16 [M +H]$^+$; Analysis Calcd. (%) for $C_{30}H_{26}ClN_5O_4$: C, 64.80; H, 4.71; N, 12.60; Found: C, 64.72; H, 4.19; N, 12.23

a-(4"-chlorophenyl)-c-(3-(5-(3',4'-dimethoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2e)



Synthesized by method above from chalcone **1e** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 69%, Light yellow solid; mp 156-159°C; IR (KBr) $_{max}$/cm^{-1} 3293 (N-H), 1665 (N-C=O), 1595 (Ar C=C), 1563 (C=N), 1266, 1091 (C–O), 1216 (C-N), 1106 (C–Cl), 3045, 2953 (C-H), 1501, 798 (Ar); ^{1}H-NMR, 9.10 (1H, br s, NH), 8.91 (1H, br s, NH), 9.02 (1H, s, 8-H), 8.70 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 8.12 (2H, dd, *J 12.3* H-12, 14), 7.86 (1H, d, *J* 6.70, H-16), 7.61-7.67 (3H, m, H-18, 2", 6"), 7.53-7.57 (4H, m, H-11, 17, 3",6"), 7.08 (1H, d, *J* 6.54, H-6'), 6.60 (2H, t, *J* 6.54, H-5', 3'), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.70 (6H, s, OCH$_3$-3',4'), 3.15 (1H, dd, *J 17.5* and 4.6, 4-H$_x$); FAB-MS *m/z*: 556.72 [M +H]$^+$; Analysis Calcd. (%) for $C_{30}H_{26}ClN_5O_4$: C, 64.80; H, 4.71; N, 12.60; Found: C, 64.25; H, 4.52; N, 12.73

a-(4"-chlorophenyl)-c-(3-(5-(5'-chloro,2'-methoxyphenyl)-1-nicotinoyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)urea (2e)



Synthesized by above mentioned method from chalcone **1e** (1.36 g, 4 mmol) and nicotinic acid hydrazide (0.55 g, 4 mmol); Yield 0.77 g, 42%, Light-yellow powder; mp 142-145°C; IR (KBr) $\square_{max}$/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR, $\square$ 9.10 (1H, br s, NH), 8.90 (1H, br s, NH), 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.26 (1H, d, *J* 7.2, 12-H), 8.18 (2H, dd, *J 12.3* H-12, 14), 7.85 (1H, d, *J* 6.70, H-16), 7.68-7.75 (3H, m, H-18, 2", 6"), 7.50-7.60 (4H, m, H-11, 17, 3",6"), 7.40 (1H, s, H-6'), 7.20 (1H, d, H-3'), 6.80 (1H, d, H-4'), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 6.5, 4-H$_y$), 3.87 (3H, s, OCH$_3$-2'), 3.08 (1H, dd, *J* 17.5 and 8.1, 4-H$_x$); FAB-MS *m/z*: 559.52 [M +H]$^+$; Analysis Calcd. (%) for $C_{29}H_{23}Cl_2N_5O_3$: C, 62.15; H, 4.14; N, 12.50; Found: C, 62.83; H, 4.35; N, 12.16;

3.0 BIOLOGICAL EVALUATION FOR ANTILEISHMANIAL ACTIVITY

3.1 *In vitro screening Anti-promastigote activity*

The effect of compounds on the viability of Leishmania promastigotes was assessed by monitoring the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] metabolism (Sigma Chemical Co.) after a 96 h culture period in the presence of the respective compounds. Parasites in stationary culture stage were seeded at 1 × 106 /100 µL medium 199 per well in 96-well flat bottom microtitre plates (Cellstar). Further 100 µL of medium 199 per well with different concentrations of test compounds or drug standard, dissolved in DMSO (Gupta et al., 2005) were added in triplicate to achieve desired concentrations (12.5 – 200 µg mL–1). Parallel dilutions of DMSO alone did not affect the parasite growth. The plates were incubated at 25 °C for 92 h prior to MTT (20 µL per well of a 5 mg mL–1 PBS stock) addition and then for further 4–5 hours. MTT processing was stopped and formazan crystals solubilized by adding 50 µL per well acidified 20% SDS (Qualigens, India) and incubating overnight at 37 °C. The relative amount of formazan per well produced by viable cells was measured photometrically at 570 nm. Two independent experiments were performed for the determination of sensitivity of each compound. As a control, the activity of each compound was determined, and no substantial interaction was found.

3.2.Anti amastigote (semi - *in vivo*) activity

For assessing the activity of compounds against the amastigote stage of the parasite, mouse macrophage cell line (J-774A.1) infected with promastigotes in stationary culture stage were used. Cells were seeded in a 16 well chamber slides (Nunc) (5x10^4 cell/100µL/well) in RPMI1640 containing 10% foetal calf serum and the slides were incubated at 37°C in a CO_2 incubator. After 24h, the medium was replaced with fresh medium containing stationary– phase promastigotes (2.5x10^5 /100µL/well). Promastigotes invade the macrophage and are transformed into amastigotes. At 24hr of internalization of promastigotes, test material in appropriate concentrations (2.5-50µg/mL) in complete medium was added after replacing the previous medium and the plates were incubated at 37^{o}C in a CO_2 incubator for 72 hrs. After incubation, the drug containing medium was decanted

and cells are fixed with methanol and stained with 5% geimsa stain for 45 min and at least 100 infected macrophages per sample were counted under optical microscope. Efficacy was expressed as percent inhibition of amastigote multiplication using formula:

$$Percentage\ Inhibition\ (PI) = \frac{PT \times 100}{PC}$$

PI : Percent inhibition of amastigote multiplication.

PT : Average number of amastigotes/100 macrophage cells in treated groups

PC : Average number of amastigotes/100 macrophage cells in control groups

3.3. *In vivo evaluation:*

For *in vivo* evaluation of compounds, the method of Beveridge (1963) as modified by Bhatnagar *et al.* (1989) and Gupta *et al.* (2002) was employed. Male hamsters weighing 35–40 g each were infected with 1×10^7 amastigotes and the intensity of infection after 20 days was assessed by spleen biopsy. Animals with 2^+ infections (5–15 amastigotes per 100 cell nuclei) were selected for screening the compounds. The infected animals were randomized into several groups on the basis of their parasitic burdens. Usually four to six animals were used for each compound and the same numbers were kept as untreated controls. The drug treatment was given by intraperitoneal route / oral route for five consecutive days at 50 mg kg^{-1} dose level. To assess the effect of test compounds, spleen biopsies were performed on each animal after 7 days of last drug administration and amastigote counts were assessed by Giemsa staining. The percentage inhibition in amastigote multiplication was calculated using the following formula:

P.I. = 100 − ANAT × 100/ INAT × TIUC

P.I. = Percentage inhibition of amastigote multiplication.

ANAT = Actual no. of amastigotes in treated animal.

INAT = Initial no. of amastigotes in treated animals.

TIUC = Times increase of parasites in untreated control animals.

Selective Index: The selective Index (S.I.) were calculated using the following equation:

S.I. = IC_{50} (J774.A-1Cells) / IC_{50} *(Leishmania amastigotes*)

4. Data analysis

IC_{50} was calculated by Probit analysis (Finney, 1971). Compounds with more than 15 mg/ml IC_{50} were considered as inactive while compounds with IC_{50} between 15 and 5 mg/ml were considered as moderately active and less than 5 mg/ml are highly active compounds.

5.0 ANTILEISHMANIAL ACTIVITY

The antileishmanial activity of the pyrazoline derivatives **(2a-2e,)** against a clinically derived strain of *L.donovani* is shown in Table 1.

The *in vitro* efficacy of the synthesized compounds on promastigotes and amastigotes of *leishmania donovani* were assessed by a previously described method in chapter 4. The antileishmanial activity of the pyrazoline derivatives **(2a-2e)** against a clinically derived strain of *L.donovani* is shown in Table 1 while compounds having promastigote inhibition more than 80% were screened against amastigotes and their IC_{50} was calculated in as shown in Table 1.

Table 1 Antileishmanial *in vitro* activity against luciferase–promastigote system

S.No.	Compound	% Inhibition at 10 µg/ml promastigote
1	2a	90
2	2b	87.99
3	2c	94.93
4	2d	88.79
5	2e	90.92

NI: no inhibition.

Pentamidine shows 85–90% inhibition against promastigotes at 0.5 mg/ml.

SSG (sodium stibogluconate) shows 40–50% inhibition against promastigotes at 940 mg/ml.

The data in Table 2 suggests that pyrazoline derivatives represent interesting leads as antileishmanial agents. All the pyrazoline derivatives showed 80-100% inhibition against promastigotes.

Table 2: *in vitro* (against MQ amastigotes) and *in vivo* antileishmanial activity

S. no.	Compound no.	In vitro antiamastigote activity IC_{50} (µg/ml)	Cytotoxicity against J774A.1 cell lines CC_{50} (µg/ml)	S.I. (selectivity index) CC_{50}/IC_{50}
1	2a	11.83	20.81	
2	2b	6.93	17.92	
3	2c	10.83	NA	
4	2d	5.93	NA	
5	2e	13.84	27.92	
	Pentamidine	13.37	35.92	
	SSG	55.72	307.82	

6.0 RESULTS AND DISCUSSION

All the synthesized substituted chalcone and pyrazoline derivatives remitted in products with good yield. Purity of all the synthesized compounds was checked by their melting point as well as TLC. The structure of synthesized compounds has been established and confirmed by spectral and elemental data obtained viz, FT-IR, ^{1}HNMR and Mass. The synthesized compounds was screened for, Antileishmenia activity.

6.1 Antileishmanial Activity

The *in vitro* efficacy of the synthesized compounds on promastigotes and amastigotes of *leishmania donovani* were assessed by a previously described method in chapter 4. The antileishmanial activity of the pyrazoline derivatives **(2a-2e)** against a clinically derived strain of *L.donovani* is shown in Table 3 while compounds having promastigote inhibition more than 80% were screened against amastigotes and their IC_{50} was calculated in as shown in table.

Table 3: Antileishmanial *in vitro* activity against luciferase–promastigote system

S.No.	Compound	% Inhibition at 10 µg/ml promastigote
1	**2a**	90
2	**2b**	87.99
3	**2c**	94.93
4	**2d**	88.79
5	**2e**	90.92

NI: no inhibition.

Pentamidine shows 85–90% inhibition against promastigotes at 0.5 mg/ml.

SSG (sodium stibogluconate) shows 40–50% inhibition against promastigotes at 940 mg/ml.

Table 4:*In vitro* (against MQ amastigotes) and *in vivo* antileishmanial activity

S. no.	Comd no.	*In vitro* antiamastigote activity IC_{50} (µg/ml)	Cytotoxicity *against* J774A.1 cell lines CC_{50} (µg/ml)
1	**2a**	11.83	20.81
2	**2b**	16.93	17.92

3	**2c**	10.83	NA
4	**2d**	17.93	NA
5	**2e**	13.84	27.92
	Pentamidine	13.37	35.92
	SSG	55.72	307.82

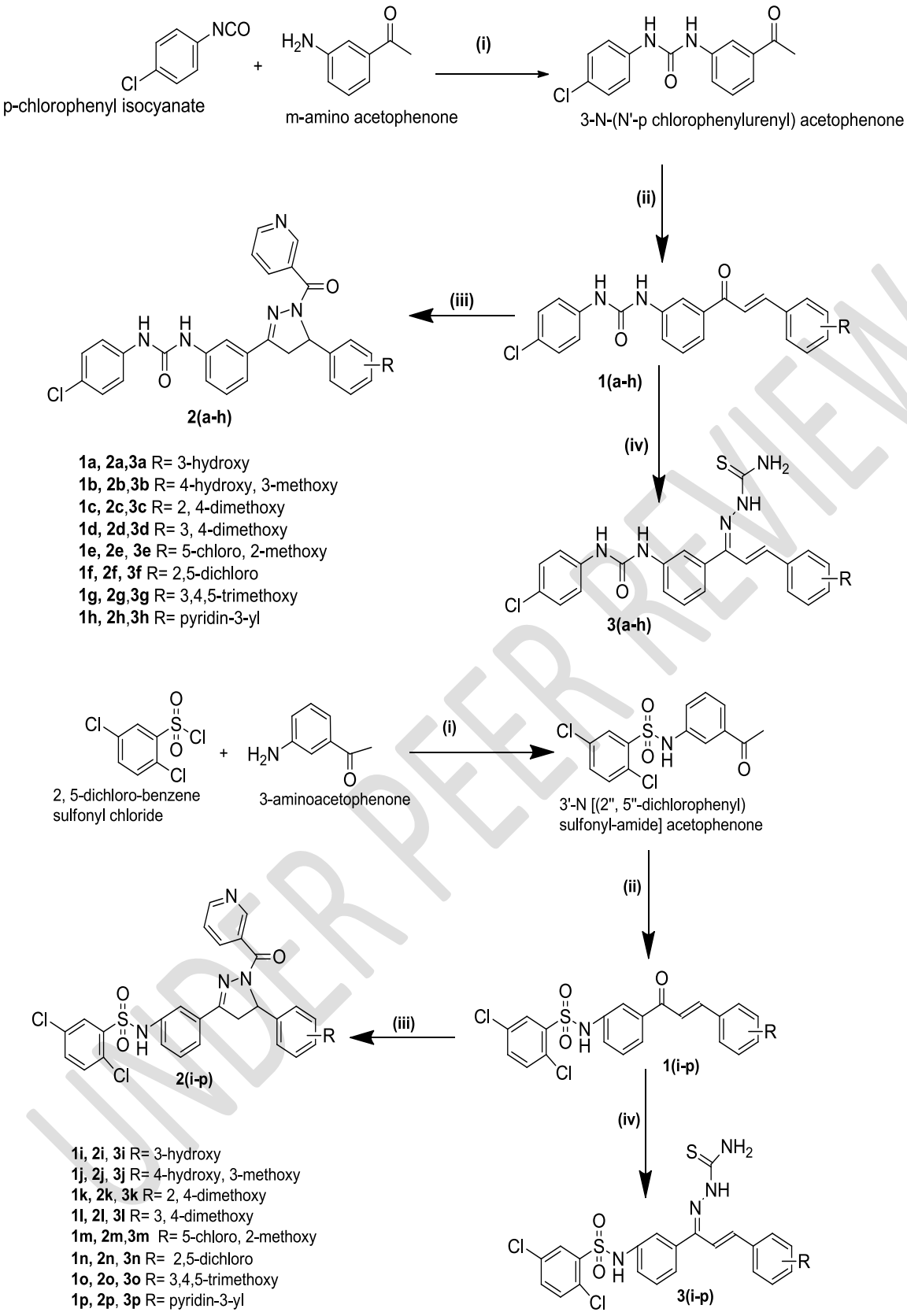
The data in Table suggests that pyrazoline derivatives represent interesting leads as antileishmanial agents. All the pyrazoline derivatives showed 80-100% inhibition against promastigotes.

7.0 CONCLUSION

With increasing resistance to available antileishmenial drugs, intensive drug discovery efforts aimed at developing new antimicrobial drugs or modifying existing agents are ongoing.

In this context, chalcones and Pyrazoline are promising candidates, as these individually possess multifarious pharmacological profiles including antimicrobial activities with different mode of action. The substitution on these two pharmacophores into novel scaffolds and evaluation of their biological activities have not yet been reported.

The strategy to synthesis of designed compounds **2a-2e** has been shown in Fig.



The synthesis of the designed compounds 1a-1e, 2a-2e,

Different substitutions on new synthesized substituted Chalcones and pyrazolines compounds (1a- 1e, 2a-2e,)

S.No	Comp. No.		R_2	R_3	R_4	R_5
1	**2a**	**3a**	-	OCH_3	-	-
2	**2b**	**3b**	-	OCH_3	OH	-
3	**2c**	**3c**	OCH_3	-	OCH_3	-
4	**2d**	**3d**	-	OCH_3	OCH_3	-
5	**2e**	**3e**	OCH_3	-	-	Cl

In conclusion, novel pyrazoline derivatives **(2a-2e)** were synthesized and their antileishmanial activity against *Leishmania donovani* was evaluated. Compound **2e** showed better activity in comparison to Pentamidine and Sodium Stibogluconate. As a consequence of the above results and considerations, these molecules can serve as promising prototypes for the development of potent antileishmanial agents.

These observations indicated that these chalcone and 2-pyrazoline derivatives constitute attractive chemical scaffold for the establishment of new chemical entities with antimicrobial, anti-inflammatory and analgesic activities.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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