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Microwave Synthesis and Characterization of Antibacterial Activity Evaluation of 2-(4, 5-dihydro-5-(4-chlorophenyl)-1H-pyrazol-3-yl) phenol

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ABSTRACT: Chalcone derivatives were synthesized by reaction of some benzaldehyde derivatives with acetophenone, then the products obtained were allowed to react with urea, thiourea and hydroxylamine, to give the heterocyclic derivatives of oxazine, thiazine and isoxazole, respectively.In this study, a series of chalcones and substituted pyrazole compounds were synthesized according to green chemistry methods of conventional and microwave irradiation by using substituted acetophenone, substituted benzaldehyde, hydrazine hydrate and PEG-400. The synthesized compounds were characterized by UV-Visible, FT-IR and ^{1}H NMR spectral techniques. The purity of the synthesized compounds were monitored by TLC and tested for their antibacterial activity by Minimum Inhibitory Concentration (MIC) method against two different microorganisms *Staphylococcus auras* (MTCC3381), and *Escherichia coli* (MTCC739).

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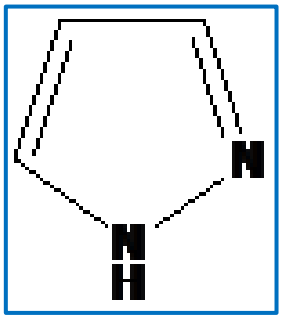
Keywords: Chalcones, Pyrazoles, antibacterial activity, PEG-600, MWI, Synthesis

Introduction

Heterocycles are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, antibiotics etc [1-5]. Identification of novel compounds which treat both infectious and inflammatory states more effectively and which lack side effects associated with current therapies remains a major challenge in biomedical research [6, 7]. In addition, from the pharmaco economic cost-effective stand-point and seeking for a better patient compliance, a dual anti-inflammatory, antimicrobial agent with minimum adverse effects and a high safety margin is highly desirable. This promoted us searching for agents that have a dual effect as anti -inflammatory, antimicrobial agents. Consequently, the chalcone backbone could be a versatile scaffold for drug design [8-10]. A survey of the literature revealed that some natural and synthetic chalcones showed significant ALR_2 inhibitory activities and this prompted us to investigate potential ARIs derived from chalcone-based compounds [11-15]. Thus, we focused on the compounds having a carboxylic acid moiety that was incorporated into the chalcone backbone and synthesized these compounds.



Experimental procedure

Melting point of the synthesised compounds were determined in open capillary tubes and were found uncorrected. UV spectra were also recorder using Alpha Bruker UV spectrophotometer. FTIR spectra (KBr pellets)were measured using Alpha Bruker FTIR instrument scanning with the entire region of 4000 - 400 cm^{-1} with typical resolution of 1.0cm^{-1}.The NMR spectra of the compounds have been recorded on Bruker AV400 spectrometer operating at 400 MHz for recording 1H spectra in DMSO solvent using TMS as internal standard Microwave reactions are carried out commercially available IFB domestic microwave oven having a maximum power output of 110W operating at 2450Hz. Purity of the compounds is checked by TLC plates (Merck) using hexane and ethyl acetate. Silicagel (column grade) was purchased from Merck. The solvents were purified as per the standard procedure reported elsewhere.

Synthesis of 1-(2-hydroxyphenyl)-3-(4-chlorophenyl) prop-2-en-1-one by PEG-600 as Recyclable Solvent (CH1)

Method – A: (Conventional Method):

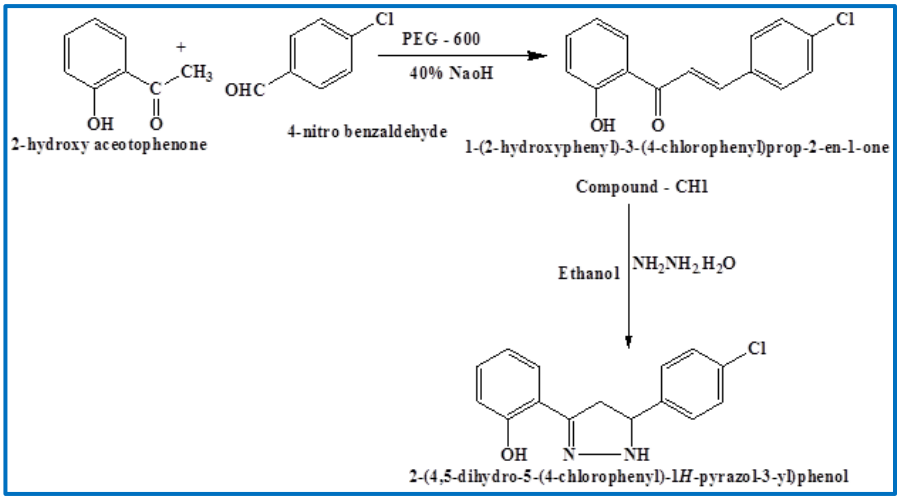
A mixture of compounds 4-chloroBenzaldehyde (0.01mol) and 2-hydroxyacetophenone (0.01mol) and NaOH (0.02 mol) was stirred in PEG-600 (20 mL) for 1 hour at 60°C. After completion of the reaction (monitored by TLC), the crude mixture was worked up in ice-cold water (100 mL). The product which separated out was filtered. The filtrate was evaporated to remove water leaving PEG behind. The same PEG was utilized to synthesize further chalcones (Yield – 85% &melting point: 104-108°C) (Table.1).

Method – B (Microwave irradiation Method):

A mixture of compounds 4-chlorobenzaldehyde (0.01mol) and 2-hydroxyacetophenone (0.01mol) and NaOH (0.02 mol) were grinded in to the mortar. Then it was mixed with 15mL of PEG – 600. The mixed compounds were taken in a 100mL beaker and it was irradiated in a microwave oven for the 3 minutes at 110 W operating at 2450Hz at 30 seconds of intervals. After completion of reaction as followed by TLC examination, chilled water was added to the reaction mixture and neutralized by an acid. The solid product was obtained, which was filtered, dried and crystallized from an ethanol. The filtrate was evaporated to dryness to remove water leaving behind PEG-600. (Yield – 85% & melting point: 106-110°C).

Synthesis of 2-(4, 5-dihydro-5-(4-Chlorophenyl)-1H-pyrazol-3-yl) phenol (CH2)

A mixture of chalcone (CH1) (0.01 mol) in 25ml of absolute alcohol and hydrazine hydrate (0.01 mol) was refluxed in oil bath at temperature 80-90°C for 6 hours. Then the reaction mixture poured in to ice. The product was isolated and crystallized from ethanol. (Yield – 78% & melting point: 106-110°C)



Synthetic scheme

Green chemistry approach

Chalcones are also key precursors in the synthesis of many biologically important heterocycles such as benzothiazepine, pyrazolines, 1, 4-diketones and flavones. Hence, the synthesis of chalcones has generated vast interest among organic as well as medicinal chemists. Reducing or eliminating the use of volatile organic solvents can minimize the generation of waste, which is a requirement of one of the principles of green chemistry. Recently, Poly ethylene glycol (PEG) has been found to be an interesting solvent system.

In continuation work on chalcones as precursors in the synthesis of various heterocycles, I have planned to synthesize a series of novel hetero chalcones by applying the principles of green chemistry, using PEG-600. PEG is an environmentally benign reaction solvent; it is non-toxic, inexpensive, potentially recyclable and water soluble, which facilitates its removal from the reaction product.

Table 1 Characterization and data of Compounds

Compound Code.	Compound General Name	Molecular formula	Molecular Weight	Melting point^{0}C	R_f Value	Percentage of Yield,%
CH1	Chalcone	$C_{15}H_{11}ClO_2$	258.7	104-108^{o}C	0.65	85%
CH2	Pyrazole	$C_{15}H_{13}ClN_2O$	272.73	106-110^{o}C	0.41	78%

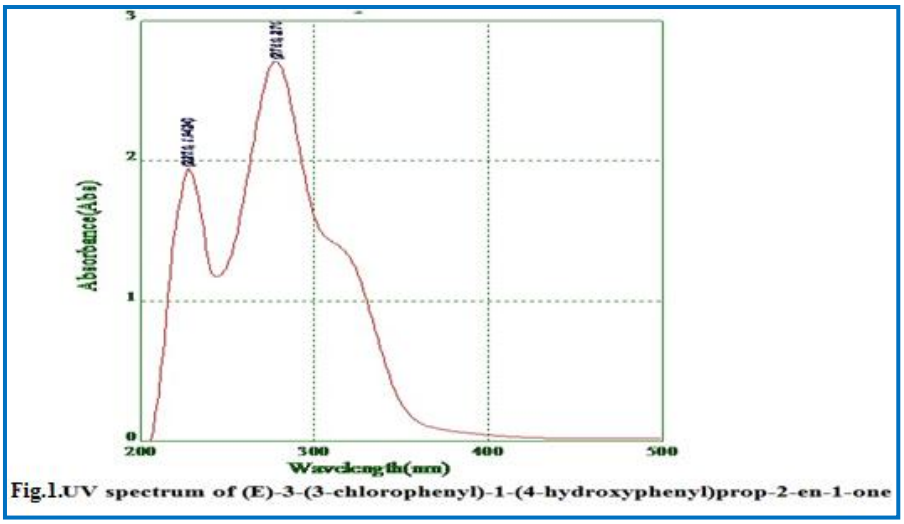


Fig.1.UV spectrum of (E)-3-(3-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

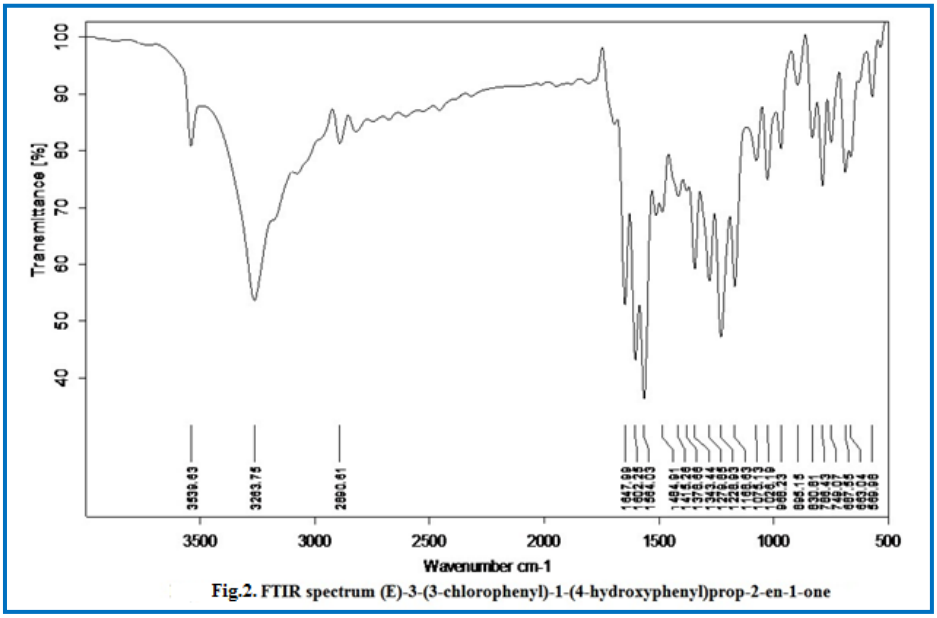


Fig.2. FTIR spectrum (E)-3-(3-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one

CH1: (R= Cl):m.p. **104-108°C**, yield: 85%, UV (in nm):226 (CH=CH), 366(C=O). IR (KBr in cm^{-1}):1633(Ar C=C str.), 3306(C-H str.), 1791(C=O str.), 1431(C-N str.),:R_f: 0.65: ^{1}H NMR(ppm)(CDCl3): 6.74- 7.01 (8H,m,Ar-H), 632- 6.47 (d,2H,CH_2=CH_2), 11.10(1H,s,Ar-OH), 3.9 (3H,S,CH_3).

CH2: (R= Cl):m.p. **106-110°C**, yield: 78%, UV (in nm):247 (CH=CH), 312(C=O). IR (KBr incm-1):1717(Ar C=C str.), 3152 (Ar-C-H str.), 1712 (C=N str.):R_f:0.41: 1H NMR(ppm)(CDCl3): 11.11(^{1}H,s,Ar-OH), 6.7 -7.4(4H,s,Ar-H), 4.83 (d,1H,CH=NH), 3.51- 3.63 (2H,t,CH_2).

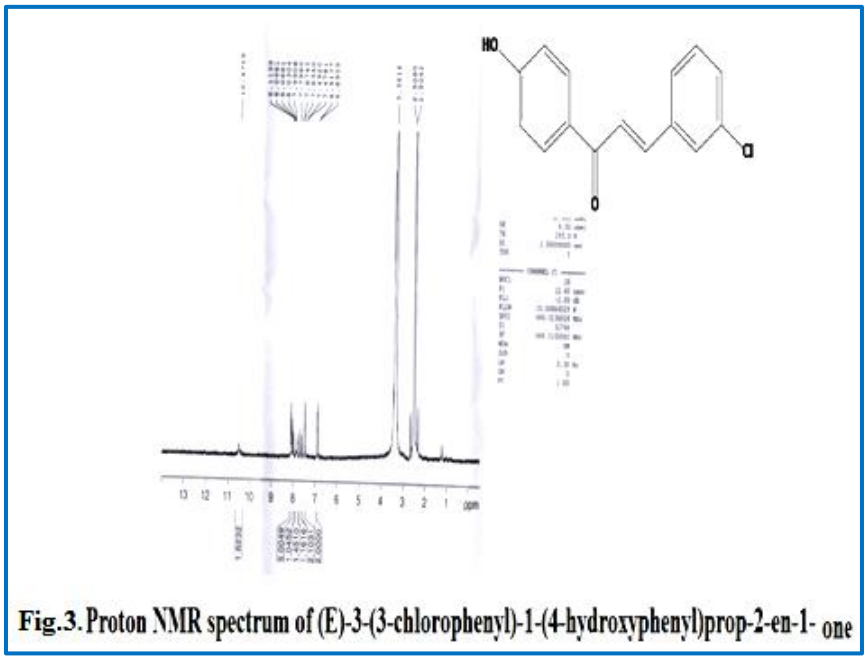


Fig.3. Proton NMR spectrum of (E)-3-(3-chlorophenyl)-1-(4-hydroxyphenyl)prop-2-en-1- one

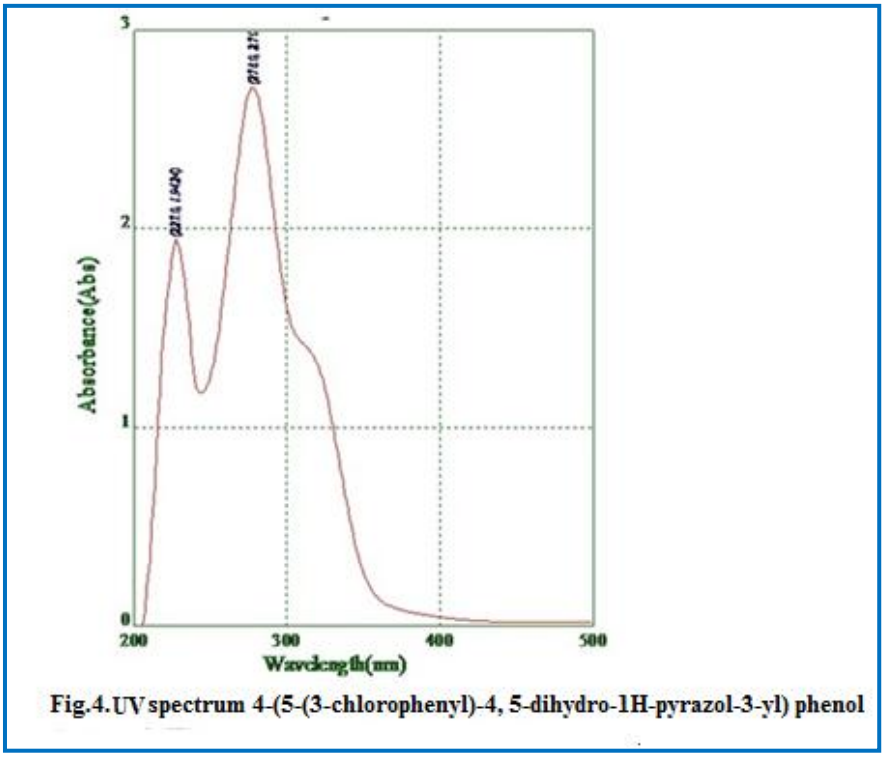


Fig.4. UV spectrum 4-(5-(3-chlorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl) phenol

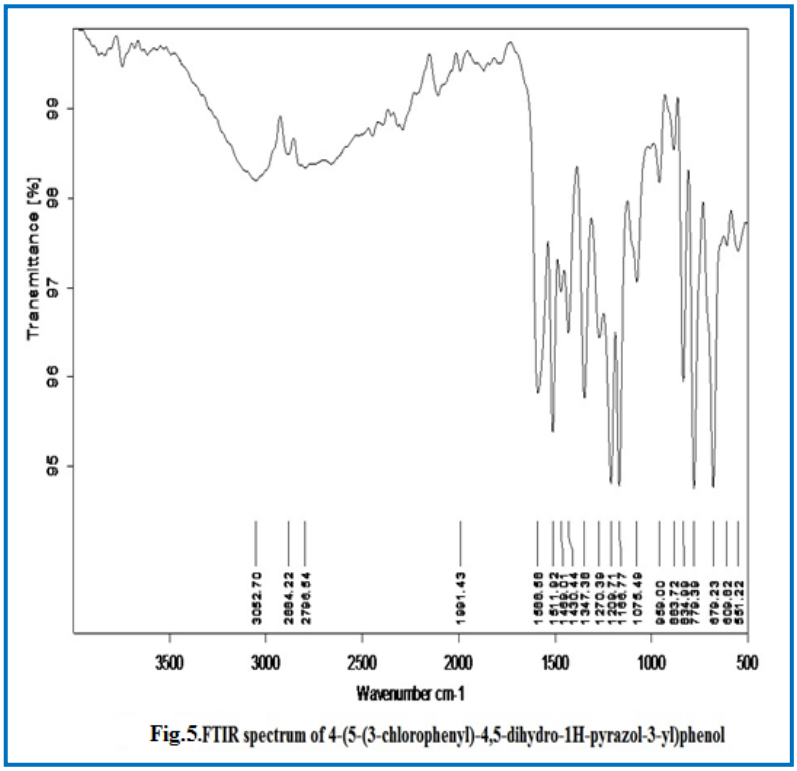


Fig.5.FTIR spectrum of 4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol

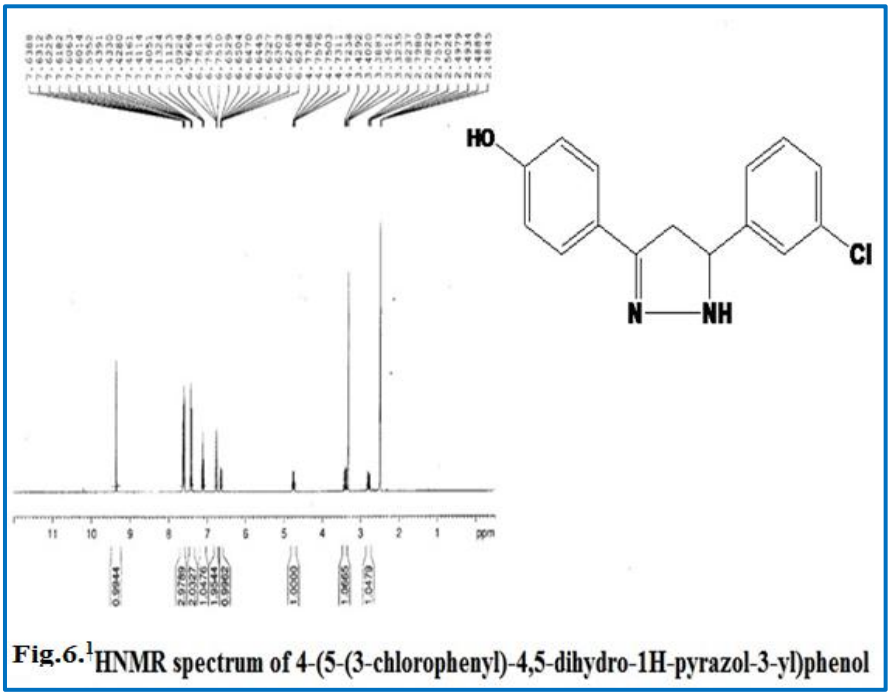


Fig.6.^{1}HNMR spectrum of 4-(5-(3-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenol

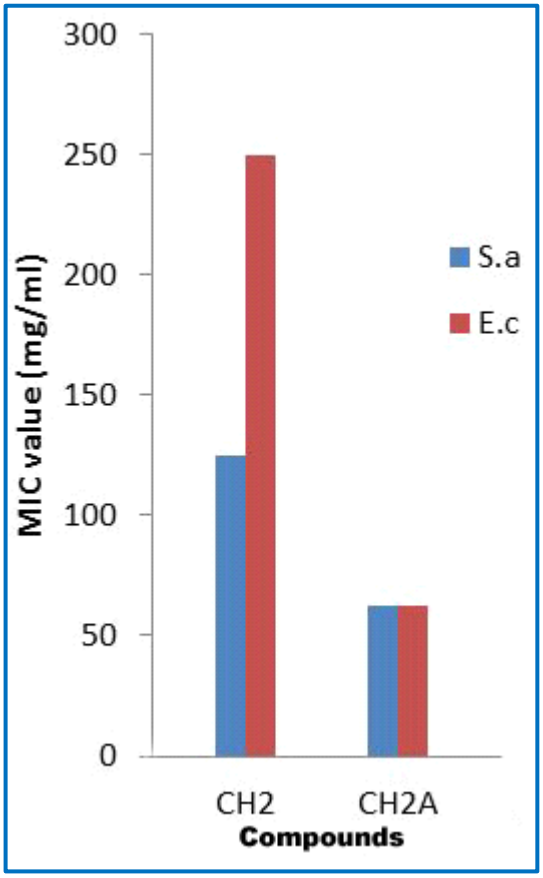
Antimicrobial activity

The micro dilution susceptibility test in Miller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) were used for the determination of antibacterial and antifungal activity. Test organisms: Escherichia coli (E. coli) ATCC 25922 and Salmonella typhimurium (S.typhimurium) ATCC 3311 as Gram-negative bacteria, Staphylococcus aureus (S. aureus) ATCC 19433 and Bacillussubtilis (B. subtilis) ATCC 1042 as Gram-positive bacteria and Candidaalbicans (C. albicans) as a yeast fungus. Ampicillin trihydrate and clotrimazole were used as standard antibacterial and antifungalagents, respectively. Solutions of the test compounds, ampicillin trihydrate and clotrimazole were prepared in DMSO at

concentration of 1600 mg/mL. From this stock different dilutions of the compounds (800, 400, down to 6.25 mg/mL) were prepared. The microorganism suspensions at 106 colony forming unit/mL/1c concentration were inoculated to the corresponding wells. Plates were incubated at 368C for 24 to 48 h. The incubation chamber was kept sufficiently humid. At the end of the incubation period, the minimal inhibitory concentrations (MIC) were determined. Controls with DMSO and uninoculated media were also maintained.

Compounds	**Minimum Inhibiting Concentration (g/ml)**	
	***Staphylococcus aureus* (S.a)**	***Escherichia coli* (E.c)**
CH1	120.00	250.00
CH2	62.5	62.5

Bar Graph



Results and Discussions

The Claisen-Schmidt condensation is an important C-C bond formation for the synthesis of 1, 3-diaryl-2-propen-1-ones (chalcones). The aim of my present study was to develop an efficient protocol using PEG-400 as a recyclable reaction solvent to obtain 1, 3-diaryl-2-propen-1-ones with excellent yields in a short span of time without formation of any side product and the same time the solvent can be reused again and again by adopting the recycling process.

The yields of the synthesized compounds were found to be excellent. The structure of the synthesized compounds was identified by IR (Fig.2 and 5) and UV-Visible (Fig.1 and 4) spectroscopy and confirmed by ^{1}HNMR spectroscopy (Fig.3 and 6).

Conclusion

All synthesised compounds were characterized by UV-Visible, FT-IR and 1HNMR spectroscopy techniques and were found to be in agreement with the chemical structures expected. Antibacterial activity of eight synthesised compounds tested against Staphylococcus aureus and Escherichia coli. From the antimicrobial evaluation of all the newly synthesized compounds it was concluded that out of two compounds CH1, CH2 showed excellent antibacterial activities against both *Staphylococcus aureus* and *Escherichia coli* with lowest value of 13.5(g/mL) and 31.25 ((g/mL) respectively. The activity data obtained during the study will be certainly useful to go for further research for drug designing and synthesizing new chalcone and pyrazole derivatives. Obviously, the comparative evaluation of active compounds will require further studies; the data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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