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Synthesis and screening of novel lipophilic diarylpropeones as prospective antitubercular, antibacterial and antifungal agents

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ABSTRACT

Infectious diseases including bacterial, fungal and tuberculosis are responsible for the suffering of humans worldwide. Based on this observation we predetermined to prepare and five novel lipophilic diarylpropenones (Chalcones) (3a-3e) against tubercular, bacterial and fungal strains. The compounds were prepared by base the catalyzed condensation of 2,4,6-trimethyl acetophenone with substituted aromatic aldehydes, purified by recrystallization and characterized by elemental analysis and IR, 1H NMR, and Mass spectroscopic techniques. Further, the compounds were biologically screened for their antitubercular, antibacterial, and antifungal actions. Results of these activities revealed that the compounds possess potential antifungal and antitubercular activities and poor antibacterial activity. The greater activities against tubercular and fungal strains may be due to the lipophilicity rendered by the three magic methyl groups and halogen atoms. The compounds showed no cytotoxicity against the normal human cell line L02. These compounds may act as new scaffolds for the design and development of new molecules against tubercular and fungal infections. Advanced studies need to be carried out in order to determine their potency *in vivo*.

Keywords: *2,4,6-trimethyl acetophenone; Diarylpropenones (Chalcones); Base-catalyzed condensation; Antibacterial, Antifungal; Antitubercular; Cytotoxicity*.

1. INTRODUCTION

Bacterial infections are the common problems causing serious health conditions to human race around the world [1]. According to a survey 20% of global burden of diseases have been recorded due to microorganisms. These organisms make human body as their hosts and cause exceptionable activities like diarrhoea, abdominal cramps, bloody cramps, vomiting (*Escherichia coli*) pneumonia (*Klebsiella pneumonia*e), Ocular infections, meningitis, musculo-skeletal infections (*Bacillus subtilis*). A number of infections including tuberculosis, gonorrhoea, blood poisoning and the infections originated from food are becoming hard to treat as the available antibiotics in the market are less useful. This is due to elevated levels of antibiotic resistance. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases Novel mechanisms of resistance are rising and disseminating globally, and lessening our ability to treat the regular infectious diseases [2-3]. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* and affecting one-third of the world's population. Antimicrobial agents of both synthetic and natural origin can provide the means to treat infections caused by microorganisms thus saving millions of lives. Diarylpropenones commonly called as chalcones has biological activities against microbial [4-12] and tubercular infections [13], oxidative stress 14], cancer, fungal infections, liver diseases, bronchitis [15-17], antimalarial [18] etc.,. Chalcone is chemically 1,3-diaryl-2-propen-1-one in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system, representing a class of flavonoids that occurs naturally in fruits and vegetables. The propenone linkage is partly responsible for the different bioactivities of diarylpropenones where as the substituents on the aryl rings influence the intensity and potency of these compoundsMedicinal chemists utilize three different structural manipulation strategies for making biologically useful chalcones including the modification of both the aryl rings, incorporation of novel aryl or heteroaryl rings, and clubbing the chalcone scaffold with biologically active skeletons through molecular hybridization approach. These three principles has resulted in the evolution of novel chalcone based agents for the treatment of infectious diseases [19-26]. Literature reveals chalcones have desirable antibacterial, antifungal and antitubercular activities and generally these compounds contain the aromatic rings substituted with electron withdrawing and releasing groups. The nature and position of these substituents have a great impact on the activity. In addition, lipophilicity and electronic effects of these substituents are two important governing factors for the activity of antimicrobial compounds. In the current study we predestined to synthesize, characterize and evaluate the influence of the position and nature of halogens and dimethylamino groups on the activities including the antibacterial, antitubercular and antifungal activity of five new lipophilic chalcones containing an unvarying trimethylphenyl ring. The lipophilic nature of these compounds is attributed because they contain three magical methyl groups on one of the aryl ring and electron withdrawing halogens and an electron releasing dimethylamino groups on the other ring. However, the compounds possess reasonable hydrophilicity as they contain the polar carbonyl group as a part of the propenone linkage.

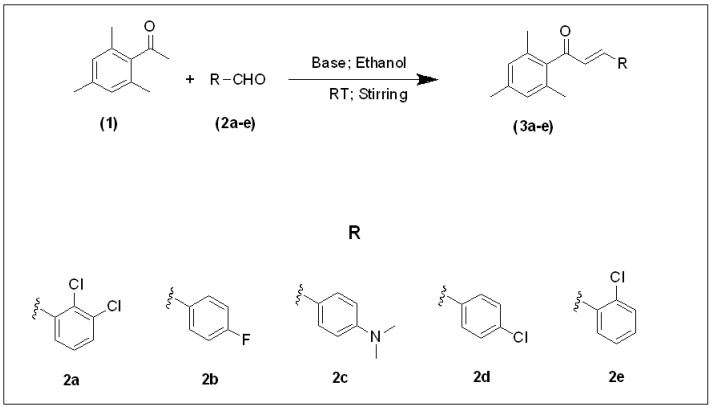
2. MATERIALS AND METHODS

Chemistry.

The organic solvents were purchased from S.D Fine Chem. Ltd, Mumbai, India which are of spectral grade and used as such without additional purification. The aldehydes i.e., 2,3-dichlorobenzaldehyde, 4-fluorobenzaldehyde, 2-chlorobenzaldehyde, 4-dimethylaminobenzaldehyde and 4-chlorobenzaldehyde and the ketone 2,4,6-trimethylacetophenone were purchased from Sigma Aldrich Chemical Co. (Melwaukee, Wisconsin, USA). Reactions were monitored on precoated TLC plate's (Merck grade) containing silica gel 60 F254 as the adsorbent. The compositions of the solvent systems employed for developing the TLC are indicated at the data of individual compound. The compounds on the plate were visualized by the non-destructive UV lamp method and the destructive procedure involving the spraying the plates with the universal spraying reagent 10% sulphuric acid in methanol. All the compounds were purified by recrystallization. Melting points of the compounds were expressed in ^{0}C after determining the same in open capillaries employing Boetius melting point apparatus and were uncorrected. Recording of the FT-IR spectra (values given in cm^{-1}) was done using Bruker alpha-T where as the the ^{1}H-NMR spectra on a Bruker 400 Avance NMR spectrophotometer. Tetramethylsilane (TMS) was employed as the standard for the NMR studies and the values are expressed in δ ppm. The peaks in the mass spectrum were recorded on an Agilent LC-MS spectrometer. Carlo Erba 1108 elemental analyzer was utilized to perform the elemental analysis and the experimental values of the results were within ± 0.4% of the calculated values.

General strategy for the synthesis of the target compounds.

We synthesized the diarylpropenones (chalcones) by the condensation of 2,4,5-trimethylacetophenone with different substituted aromatic aldehydes using inorganic bases (Scheme 1) [27]. The detailed procedure for the preparation and spectral and other properties of individual compound are given below (**Table 1**).



Scheme 1. Synthesis of diarylpropenoses (chalcones) of 2', 4',6'-trimethylacetophenone.

Synthesis of (*E*)-1-(2',4',6'-trimethylphenyl)-3-(2'',3''-dichlorophenyl)-prop-2-en-1-one (3a): A mixture of 2,4,6-trimethylacetophenone (1 mmol) and 2,3-dichloro benzaldehyde (1 mmol) was stirred in ethanol (7.5 ml) and then 4 mL of alcoholic KOH solution (40%) was added drop wise to it. The mixture was stirred for 24 hours and it was acidified with 1:1 HCl and H_2O which resulted in the formation of precipitate. The precipitate was then filtered under vacuum and the solid was washed with excess of water.Then the precipitate was dried and purified by recrystallization using ethanol as solvent. Appearance: Pale yellow powder; Melting point: 96 ^{o}C; R_f: 0.61 (15 % Ethyl acetate in n-Hexane); Molecular weight: 319.23; Molecular formula: $C_{18}H_{16}Cl_2O$; Yield: 90% (After recrystallization); Elemental analysis-Calculated for: $C_{18}H_{17}ClO$: C, 75.92; H, 6.02; Found : C, 76.48; H, 6.52; Solubility: Chloroform and Ethanol; FT-IR (KBr) (cm^{-1}) 1657.22 (C=O), 1609.09 (C=C of Ar), 1553.52 (CH=CH), 782.43, (C-Cl) 854.91 (C-Cl). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ-6.86 (d, 1H, *J* =16 Hz), 7.65 (d, 1H, *J* =16 Hz), 7.24-7.59 (m, 5H), 2.23 (s, 6H), 2.34 (s, 3H); **MS** (*m/z*, %): 320.23 (M+1, 99.10).

Synthesis and characterization of (*E*)-1-(2',4',6'-trimethylphenyl)-3-(4''-fluorophenyl)- prop-2-en-1-one (3b): A mixture of 2,4,6-trimethylacetophenone (1 mmol) and 4-fluoro benzaldehyde (1 mmol) was stirred in 8 mL of ethanol for 24 hours by adding 5 mL of alcoholic NaOH solution (100%) as the catalyst. After completion of the reaction the mixture was precipitated by neutralizing with 1:1 HCl and H_2O. The precipitate was then filtered under vacuum and the solid was washed with excess of water, and then the precipitate was dried and purified by recrystallization using ethanol as solvent. Appearance: Cream colour powder; Melting point: 91 ^{o}C; R_f : 0.57 (15 % Ethyl acetate in Hexane); Molecular weight: 268.33; Molecular formula: $C_{18}H_{17}FO$; Yield: 30% (After recrystallization); Elemental analysis-Calculated for: $C_{18}H_{17}FO$: C, 80.57; H, 6.39; Found: C, 80.92; H, 7.06; Solubility: Chloroform and Ethanol; FT-IR (KBr) (cm^{-1}): 1635.92 (C=O), 1596.75 (C=C of Ar), 1508.59 (CH=CH), 979.45 (C-F); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ-6.87 (d, 1H, *J* =16 Hz), 7.17 (d, 1H, *J* =16 Hz), 6.91-7.54 (m, 6H), 2.21 (s, 6H), 2.34 (s, 3H); MS (*m/z*, %): 269.33 (M+1, 99.22).

Synthesis and characterization of (*E*)-1-(2',4',6'-trimethylphenyl)-3-(4''-dimethylaminophenyl)-prop-2-en-1-one (3c): A mixture of 2,4,6-trimethylacetophenone (1 mmol) and 4-dimethylamino benzaldehyde (1 mmol) was stirred in ethanol (7 ml) and then 4 mL of 50% alcoholic KOH solution was added to the above solution drop wise. The mixture was stirred for 24 hours and it was neutralized with 1:1 HCl and H_2O which resulted in the separation of the precipitate which was filtered under vacuum and the solid was washed with water, dried and recrystallized with ethanol. Appearance: Intense yellow colour; Melting point: 89 ^{o}C; R_f 0.38 (20 % Ethyl acetate in Hexane); Molecular weight: 293.40, Molecular formula $C_{20}H_{23}NO$; Yield: 80% (After recrystallization); Elemental analysis: Calculated for $C_{20}H_{23}NO$: C, 81.87; H, 7.90; N, 4.77; Found: C, 81.96; H, 8.01; N, 4.95. Solubility: Chloroform and Ethanol; FT-IR (KBr) (cm^{-1}): 1631.26 (C=O), 1590.40 (C=C of Ar), 1526.49 (CH=CH), 1434.28 (C-N); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ-6.77 (d, 1H, *J* =16 Hz), 7.12 (d, 1H, *J* =16 Hz), 6.66-7.77 (m, 6H), 2.21 (s, 6H), 2.34 (s, 3H), 3.04 (s, 6H); MS (*m/z*, %): 294.40 (M+1, 99.12).

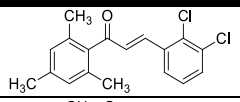
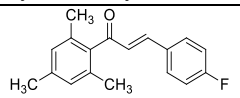
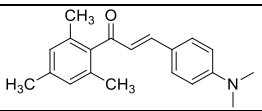
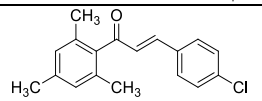
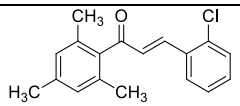
Synthesis and characterization of (*E*)-1-(2',4',6'-trimethylphenyl)-3-(4''-chlorophenyl)-prop-2-en-1-one (3d): 1 mmol of 2,4,6-trimethylacetophenone and 4-chlorobenzaldehyde (1 mmol) was dissolved in 7.5 ml of ethanol and then 4 mL of 100% alcoholic NaOH solution was added dropwise to the above mixture and stirred for 24 hours. When the reaction of completed, the mixture was added with 1:1 HCl and H_2O to neutralize the base. This resulted in the formation of a precipitate of the compound 3d. Then the precipitate was filtered under vacuum and the solid was washed with water and purified by recrystallization using ethanol as the solvent. Appearance: Pale Yellow; Melting point: 90 ^oC; R_f:0.64 (20 % Ethyl acetate in Hexane); Molecular weight: 284.78; Molecular formula: $C_{18}H_{17}ClO$; Yield: 92% (After recrystallization); Elemental analysis: Calculated for $C_{18}H_{17}ClO$: C, 75.92; H, 6.02; Found: C, 76.48; H, 6.52; Solubility: Chloroform and Ethanol; FT-IR (KBr) (cm^{-1}): 1642.48 (C=O), 1588.60 (C=C of Ar), 1489.12 (CH=CH), 836.09 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ-6.90 (d, 1H, *J* =16 Hz), 7.16 (d, 1H, *J* =16 Hz), 6.93-7.46 (m, 6H), 2.12 (s, 6H), 2.34 (s, 3H); MS (*m/z*, %): 285.78 (M+1, 99.01).

Synthesis and characterization of (*E*)-1-(2',4',6'-trimethylphenyl)-3-(2''-chlorophenyl)-prop-2-en-1-one (3e): A mixture of 2,4,6-trimethylacetophenone (1 mmol) and 2-chlorobenzaldehyde (1 mmol) was dissolved in ethanol (7.5 ml) and then 5 mL of alcoholic NaOH solution (100%, 7.5 ml) was added drop wise and magnetically stirred for 24 hours. 1:1 HCl and H_2O was used for neutralizing the reaction mixture to derive the precipitate of the compound. The precipitate was then filtered under vacuum and the solid was washed with water, purified by recrystallization using ethanol as solvent. Appearance: Yellowish-green; Melting point: 83 ^oC; R_f : 0.59 (20 % Ethyl acetate in Hexane); Molecular weight: 284.78; Molecular formula: $C_{18}H_{17}ClO$; Yield: 94% (After recrystallization); Elemental analysis- Calculated for $C_{18}H_{17}ClO$: C, 75.92; H, 6.02; Found: C, 76.48; H, 6.52; Solubility Chloroform and Ethanol; FT-IR (KBr) (cm^{-1}): 1644.35 (C=O), 1588.68 (C=C of Ar), 1554.01 (CH=CH), 854.28 (C-Cl); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ-6.89 (d, 1H, *J* =16 Hz), 7.40 (d, 1H, *J* =16 Hz), 7.28- 7.70 (m, 6H), 2.23 (s, 6H), 2.34 (s, 3H); MS (*m/z*, %): 285.78 (M+1, 99.01).

Biological protocols.

Anti-tubercular protocol. The initial antitubercular screening of the target compounds was done using *Mycobacterium tuberculosis* H_{37}Rv strain. Broth dilution assay was utilized to determine the Minimum Inhibitory Concentration (MIC) of each compound [28-29]. MIC is defined as the lowest concentration of drug or a compound, which inhibits ≤ 99% of bacteria present at the start of the assay. A frozen culture in Middle brook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was melted and diluted in broth to 10^5 cfu mL^{-1} (colony forming unit/mL) dilutions. Each one of the test compounds was dissolved separately in DMSO and subsequently diluted in broth two times at the required concentration. The ultimate concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 mL of standardized culture and then incubated at 37 °C for 21 days. The growth in the U-tubes was matched with visibility in opposition to a positive control (without drug), negative control (without drug and inoculum) and with standard pyrazinamide.

Table 1. Catalysts, structures and the yield of different diarylpropenones.

Entry	Catalyst	Structure	% Yield (After Recrystallization)
3a	40% alcoholic KOH		90%
3b	100% alcoholic NaOH		30
3c	50% alcoholic KOH		80
3d	100% alcoholic NaOH		80
3e	100% alcoholic NaOH		94

Antibacterial and antifungal protocols (Antimicrobial activity).

Antimicrobial activity was done using agar disc diffusion method [30] against three gram positive bacteria, four gram negative and two fungal strains such as *Bacillus subtilis(Bs)* (NCIM 2698), *Micrococcus variens (Mv)* (NCIM 2913), *Staphylococcus aureus (Sa)* (NCIM 2079), *Escherichia coli (Ec)* (NCIM 2344), *Klebsiella pneumonia (Kp)* (NCIM 2957), *Pseudomonas florescence (Pf)* (NCIM 2639), *Serratia marcescen (Sm)* (NCIM 2919), *Aspergillus niger (An)* (NCIM 548) *and Aspergillus flavus (Af)* (NCIM 536) in comparison with antibacterial and antifungal standard Gentamicin and Fluconazole respectively. Subcultures of the above bacterial and fungal strains were made using nutrient broth and Sabouraud dextrose media respectively. 24 hr subcultures of the organisms were used as inoculum. The bacteria were inoculated onto nutrient agar while, fungi on potato dextrose agar. Compound solutions of a concentration of 10, 20, 30, 40, 50, 75 and 100 µg/ml were prepared with DMSO. Discs soaked in dilutions of synthesised compounds were placed on inoculated media in the petridishes and incubated for 24-48 hours. The diameter of the inhibitory zone produced was measured in millimetres.

Cytotoxicity protocol. The compounds were screened for their cytotoxic activity in order to determine their safety. Mosmann's MTT assay was performed on all the five compounds using L02 (human normal cell line). This assay is based on the conversion of the soluble MTT (0.5 mg mL^{-1}, 100 µL), to a reduced formazan (blue-purple) product, by the mitochondrial reductase enzyme inside the living cells (Mosmann T *et al*., 1983). The cells employed in the cytotoxicity assay were cultivated in RPMI 1640 medium added with 10% fetal calf serum, penicillin, and streptomycin at 37 °C and humidified at 5% CO_2. For a short time cells were placed on 96-well plates at 100 µL total volume with a density of 1–2.5 × 10^4 cells per mL and were made to adhere for 24 h prior to treatment with tested drugs in DMSO solution (10^{-5}, 10^{-6}, 10^{-7} mol L^{-1} final concentration). Triplicate wells were treated

with media and agents. The viability of the cells was examined after 96 h of continuous drug exposure with a tetrazolium compound. The supernatant medium was removed, and 150 µL of DMSO solution was added to each well. The plates were smoothly agitated by means of mechanical plate mixer until the colour reaction was homogeneous and the OD570 was determined using micro plate reader. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that decreased the absorbance of the untreated wells by 50% of the vehicle in the MTT assay. Assays were performed in triplicate on 03 autonomous experiments. The results had good reproducibility between replicate wells with standard errors below 10%.

3. RESULTS

Chemistry.

The target compounds were prepared by the condensation of 2,4,6-trimethylacetophenone with differently substituted aldehydes in the presence of a alcoholic solutions of either sodium hydroxide or potassium hydroxide. Initially, the compounds were tried to synthesize in the presence of mineral acids like HCl and H_2SO_4 but were not preceded further due to formation of many impurities along with the intended compound. Hence we tried the reactions with inorganic bases and successfully synthesized the compounds with good yields (80-94%). However, among the five compounds, **3b** containing fluoro substituent was isolated in poor yield (30%). These results indicate that aromatic aldehydes with chlorine substituent can be synthesized in high yields with the base catalyzed reactions when condensed with the ketone-2,4,6-trimethylacetophenone. The R_f values of the compounds when detected on a normal phase precoated silica gel (weakly acidic) containing TLC plate by using ethylacetate and hexane as the solvent system was 0.61, 0.57, 0.38, 0.64 and 0.59 for **3a**, **3b**, **3c**, **3d** and **3e** respectively. The R_f value of **3c** was low among the others due to its basic dimethylamino group that have more affinity towards the weakly acidic silica gel than the mobile phase. The FT-IR spectrum was recorded by scanning the compound loaded KBr discs and found the characteristic absorption bands for the functional groups of diarylpropenones including the carbonyl (1640-1660 cm^{-1}), alkenyl (1480-1560 cm^{-1}) and aromatic double bond (1580-1600 cm^{-1}).

The 1H NMR spectrum of all the compounds was performed by dissolving the compounds in deuterated chloroform ($CDCl_3$) and seen two diagnostic doublets corresponding to 1α-H and 1*β*-H around the chemical shift values 6.7-6.9 (α-H) and 7.10-7.65 (*β*-H) with the coupling constant (*J*) values 16 Hz between the individual doublets. Such large *J* values indicate that the geometry of the compounds at the alkenyl portion of the propenone bridge is *trans* but not *cis*. The spectrum also showed three peaks corresponding to the three methyl groups around 2-3 ppm as well as the peaks characteristic of aromatic protons (Ar-H) in the region of 6-8 ppm. The mass spectrum of the compounds were recorded in positive mode and shown the spectrum shown characteristic [M+1] peaks. The elemental analysis used to determine the elemental composition of the compounds has shown the results which were in agreement with those of the intended values.

Biological screening

Antitubercular activity. The compounds exhibited excellent antitubercular potency against *M. tuberculosis* $H_{37}Rv$ strain (Table 2). Halogenated compounds shown antitubercular potency greater than the standard pyrazinamide whereas the compound **3c** (MIC = 25 µg/mL) containing electron releasing 4”-dimethylamino group was less potent that the standard (MIC = 3.12 µg/mL). Among the halogenated derivatives, **3b** and **3d** containing 4”-fluoro and 4”-chloro substituents were the most potent of the five compounds with a similar MIC value of 0.8 µg/mL. This indicates that halogen atoms at 4”-position are essential for the activity. The compound **3e** having 2”-chloro substituent was potent with a MIC 1.6 µg/mL whereas **3a** containing 2”,3”-dichloro groups has a MIC similar to pyrazinamide and the activity was reduced (MIC= 3.2 µg/mL) indicating that the chloro group at position-3” is not a good contributor for antitubercular activity. Structure activity relationships (SAR) indicate that the presence of fluorine and chlorine substituents at positions-2” and 4” enhances the antitubercular potency. The electronic effect of compounds containing other substituents need to be studied further in order to frame a more detailed SAR.

Table 2. Results of the antitubercular activity of diarylpropenones (3a-3e).

S.No	Compound	MIC values (µg/mL) of *M. tuberculosis* $H_{37}Rv$
1	**3a**	3.12
2	**3b**	0.8
3	**3c**	25
4	**3d**	0.8
5	**3e**	1.6
6	**Pyrazinamide**	3.12

Antibacterial and Antifungal activities. The results of these two activities are displayed in Table 3

Antibacterial activity. The chalcones tested for their antibacterial activity have shown considerable activity against the tested organisms but is less than the standard Gentamicin. The compounds were much active against gram negative organisms compared to gram positive organisms. However, compound **3e** exhibited no activity against one gram positive organism *Staphylococcus aureus* and two of the gram negative organisms *Micrococcus variens* and *Klebsiella pneumoniae* respectively. The activities of the compounds were nearly equal to the standard only at a concentration, 100 µg/mL. Amongst the five compounds, the compound **3b** containing 4”-fluoro substituent exhibited a zone of inhibition of 17 mm against *Micrococcus variens* and 14 mm against *Serratia marcescen* and *Pseudomonas florescence* respectively at 100 µg/mL. The zone of inhibition of compound **3c** containing 4”-dimethylamino group was 15 mm against *Klebsiella pneumoniae* whereas **3d** bearing 4”-chloro substituent also shown an inhibition of 15 mm but against *Escherichia coli*. The structure activity relationship (SAR) based on the above results suggests

that the presence of electron withdrawing halogens (-F, -Cl) substituent at 4"-position is essential for the activity compared to electron releasing dimethyl amino group. Halogens at positions-2" and 3" are poor contributors to the antibacterial activity.

Antifungal activity. The antifungal activity of the compounds was performed against *Aspergillus niger* and *Aspergillus flavus* with fluconazole (1 mg/mL) as standard. The target compounds were tested at low concentrations ranging from10-100 µg/mL. All the compounds displayed antifungal activity greater than the standard, Fluconazole. However, the compound **3d** was inactive against *Aspergillus flavus*. Compound **3e** containing 2"-chloro substitution was the most active in the entire series with a zone of inhibition 10 mm at 10 µg/mL and 19 mm at 100 µg/mL. **3d** containing 4"-chloro group was next in potency. This inclination indicates that the presence of chlorine substitution on ring-B of chalcones has an influence on antifungal potency. But, **3a** with two chlorine atoms at 2",3"-positions were less potent representing that chlorine atom at 2" and 4" positions have greater antifungal activity. The compound **3c** containing 4"-dimethylamino group shown a zone of inhibition of 10 mm against both the organisms at the concentration 30 µg/mL and the activity was same even after increasing the concentration up to 100 µg/mL. The structure activity relationship (SAR) based on the above data represent that presence of chlorine atom at 2" and 4"-positions enhances the activity.

Cytotoxicity studies. The compounds **3a-3e** were screened for the *invitro* cytotoxic action by MTT assay against L02 (human normal cell line). The IC_{50} values of all the compounds were greater than 50 µg/mL (Table 4). These results indicate that the compounds were safer and non-toxic to normal human cells.

Table 3. Antibacterial and antifungal activities of target compounds.

Entry	Concentration (µg/mL)	Gram +ve bacteria			Gram –ve bacteria				Fungi	
		Bs	***Sa***	***Mv***	***Kp***	***Ec***	***Sm***	***Pf***	***An***	***Af***
3a	10	9	9	8	8	8	8	8	6	7
	20	10	9	9	8	10	9	10	8	8
	30	10	10	9	8	10	9	10	9	9
	40	10	10	9	9	11	10	10	9	10
	50	10	10	10	10	12	10	11	9	10
	75	11	11	10	11	12	11	11	10	11
	100	13	11	11	11	12	12	13	10	12
3b	10	9	8	6	7	9	10	7	-	-
	20	10	8	7	9	9	10	9	6	6
	30	12	9	9	11	10	10	10	8	7
	40	12	9	10	11	10	12	10	8	8
	50	12	10	11	12	10	13	11	9	8
	75	13	10	16	13	12	13	13	9	9
	100	13	11	17	14	12	14	14	10	9
3c	10	8	9	10	10	9	8	7	8	-
	20	9	10	10	10	9	9	8	9	-
	30	9	10	10	11	10	10	8	10	10
	40	9	10	10	11	10	10	8	10	10
	50	9	11	11	12	10	10	8	10	10
	75	10	12	12	12	10	11	9	10	10
	100	10	12	13	15	12	12	9	10	10
3d	10	8	8	9	8	9	8	10	8	-
	20	9	8	9	10	10	9	11	9	-
	30	9	9	9	10	10	9	11	9	-
	40	10	9	9	12	12	10	11	10	-
	50	10	9	10	12	13	10	11	10	-
	75	10	10	11	13	13	10	12	11	-
	100	11	10	13	14	15	10	12	11	-
3e	10	6	-	7	-	6	8	-	10	7
	20	8	-	7	-	7	9	-	11	7
	30	8	-	7	-	8	10	-	12	9
	40	8	-	7	-	8	10	-	12	9
	50	8	-	8	-	8	10	-	13	9
	75	9	-	8	-	9	12	-	13	10
	100	9	-	9	-	12	13	-	19	10
	Gentamycin	15	13	17	17	15	16	14		
	Fluconazole								10	10

Table 4. Cytotoxicity of compounds (**3a-3e**) against human normal cells (IC_{50} ±SD, µM)[a,b].

S.No	Compounds	Human normal cells (L02)
1	**3a**	>50
2	**3b**	>50
3	**3c**	>50
4	**3d**	>50
5	**3e**	>50

[a]Mean value ±SD (standard deviation from three experiments).

[b]Boldface: $IC_{50} \leq$ the control, (IC_{50} , µg mL^{-1})

4. CONCLUSIONS

In the present study we report the successful synthesis, characterization and biological activities of 2,4,6-trimethylacetophenone based diarylpropenones. These compounds can be easily prepared in high yields by base catalysed condensation reaction. They were purified by re-crystallization and characterized by physicochemical analysis data. The compounds exhibited excellent antitubercular and antifungal activities and poor antibacterial activity. The compounds containing halogen atoms on ring-B has been greatly responsible for the outstanding antitubercular and antifungal activities. Since aromatic rings with electron withdrawing groups contributed favorably to the activity other heterocyclic aldehydes containing electron withdrawing groups can be tried in the synthesis of chalcones in order to have still better activity. All the five compounds are safer as exemplified by their cytotoxicity results. Further studies need to be carried out in order to determine their potency *in vivo*.

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