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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization, solvatochromic properties, and antimicrobial evaluation of 5-acetyl-2-thioxo-dihydro-pyrimidine-4,6-dione-based chalcones

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Abstract A new series of chalcone analogs namely 5-(3-phenyl-acryloyl)-2-thioxo-dihydro-pyrimidine-4,6-dione have been synthesized from the key intermediate 5-acetyl-2-thioxo-dihydro-pyrimidine-4,6-dione **4′** with different aldehyde derivatives were performed to get the target compounds as thiobarbituric acid-based chalcones **5(a′–k′)** and they were obtained in excellent yields. The newly synthesized compounds were characterized by spectral analysis (FT-IR, ^{1}H NMR, ^{13}C NMR, and UV spectroscopy) and elemental analysis. The synthesized compounds were evaluated for their antimicrobial activity against five bacterial strains (*S. aureus*, *S. pyogenes*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*) and four fungal strains (*C. albicans*, *A. clavatus*, *T. rubrum*, and *Penicillium wild strain*). Among the screened compounds, **5e′** and **f′** showed comparable activity (minimum inhibitory concentration = 500 μg/mL) nearly to that of standard antibiotics griseofulvin.

Keywords Chalcones · Antibacterial activity · Antifungal activity

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Introduction

The medicinal importance of pyrimidine derivatives such as barbituric acid and thiobarbituric acid play vital role among various heterocyclic compounds due to their anti-neoplastic (Basavaraja *et al*., 2010; Holy *et al*., 2002), antiviral (Andres and Marhold, 1996), antibiotic (Reddick *et al*., 2001), and anti-inflammatory (Basavaraja *et al*., 2010) activity. The pyrimidine ring system is present in various natural compounds such as nucleic acids, vitamins, coenzymes, uric acid, purines, and some marine microorganisms (e.g., *sponge*) (Kashman *et al*., 1989). Many synthetic drugs (e.g., *barbituric and thiobarbituric acid*) derivatives and chemotherapeutic agents (e.g., *sulfadiazine)* (Fattah *et al*., 2010). The diverse biologic activity and coverage of a broad chemical space make barbituric and thiobarbituric acid derivatives excellent target compounds for organic and medicinal chemists. Owing to their ready availability and various functionalization possibilities, the parent barbituric and thiobarbituric acid are convenient starting materials for the preparation of different fused heterocycles and literature survey also ascribes that 5-substituted derivatives are pharmacologically active compounds (Nace and Danijel, 2011). Chalcones and their derivatives are attractive molecular scaffolds for the search of new biologically active molecules (Nowakowska, 2007). Chalcones or 1,3-diaryl-2-propen-1-ones are natural or synthetic compounds belonging to the flavonoids family (Lee *et al*., 2006). Chalcone analogs are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. Chalcones have been reported to possess many useful properties, including anti-inflammatory (Tomar *et al*., 2007), antimicrobial (Lopez *et al*., 2001), antifungal (Anto *et al*., 1995), antioxidant (Lui *et al*., 2003), antimalarial (Yi *et al*., 2000), antitumor (Hsu *et al*., 2006), and anticancer

(Siddiqui *et al*., 2011) activities. So, in light of the above facts of pyrimidine, barbituric acid, thiobarbituric acid, and chalcones, we have reported the synthesis and reaction of 5-acetyl-2-thioxo-dihydro-pyrimidine-4,6-dione **4′** with different aldehydes to form chalcones **5(a′–k′)**. All the novel derivatives were screened for their antimicrobial activity and their results are discussed.

Experimental

Chemicals and solvents were obtained from commercial sources and used as received throughout the investigation. Melting points were determined in open capillaries on a Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4,000–400 cm^{-1}) of the synthesized compounds were recorded on a Perkin Elmer—Spectrum RX-IFTIR spectrophotometer using KBr pellets. Thin layer chromatography was performed on object glass slides (2 × 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. ^{1}H- and ^{13}C NMR spectra were recorded on an Advance-II (Bruker) model using dimethyl sulfoxide (DMSO) as a solvent and TMS as internal standard with ^{1}H resonant frequency of 400 MHz and ^{13}C resonant frequency of 100 MHz. The ^{1}H- and ^{13}C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS (Me_4Si). The splitting patterns are designated as follows; s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. UV spectra were recorded on Maya pro 2000 (Ocean Optics, USA). All standard strains for screening of antibacterial and antifungal activities were procured from the Institute of Microbial Technology, Chandigarh. DMSO was used as diluents/vehicle to get desired concentration of drugs to test upon standard bacterial strains.

Synthesis of 2-thioxo-dihydro-pyrimidine-4,6-dione (**3′**)

To a solution of diethylmalonate **1′** (20 g, 118.9 mmol) and **2′** thiourea (7.5 g, 98.5 mmol) in methanol, anhydrous sodium methoxide was added and refluxed at 65 °C for 8 h. A white solid separates. Then, in the above reaction mixture, 125 mL of hot (50 °C) water was added and hydrochloric acid was used to make the solution acidic. After the completion of the reaction, the resulting clear solution was filtered and cooled in an ice bath overnight. The white product formed was filtered, washed with 50 mL of cold water, dried, and recrystallized from acetone to afford compound **3′** (10.2 g) as a white powder (Jacobson, 1937). Yield: 72 %, MW 144.15, mp 252–255 °C; ^{1}H NMR (DMSO-d_6): δ 2.98 (2H, s, –C<u>H</u>$_2$), 11.72 (1H, s, N<u>H</u>), 11.96 (1H, s, N<u>H</u>); ^{13}C NMR (DMSO-d_6): δ (C-2, 44.12, C-4, C-6, 166.23, C-2, 175.34); IR (KBr, cm^{-1}): 1252 (C=S), 1698 (C=O), 3339 (N–H); Anal. Calcd. for $C_4H_4N_2O_2S$: C, 33.33; H, 2.80; N, 19.43 %. Found: C, 33.27; H, 2.89; N, 19.48 %.

Synthesis of 5-acetyl-2-thioxo-dihydro-pyrimidine-4,6-dione (**4′**)

To a solution of 2-mercapto-pyrimidine-4,6-dione **3′** (6.4 g, 44.39 mmol) in acetic anhydride (150 mL), few drops of H_2SO_4 was added and refluxed for 1 h. The reaction in the beginning was a suspension; but, after about 10 min of reflux, it changes to brown color clear solution. The reaction mixture was concentrated into 1/2 of its original volume and cooled at about 10 °C. The solid product was formed, filtered, washed with hot water then acetone, dried, and recrystallized from acetone to give compound **4′** (7.4 g) yield, as a brown powder (Branko and Donna, 2001). Yield: 90 %, MW 174.19, mp 242–245 °C; ^{1}H NMR (DMSO-d_6): δ 2.60 (3H, s, –C<u>H</u>$_3$), 2.62 (1H, s, C<u>H</u> of pyrimidine ring), 11.01 (1H, s, N<u>H</u> of pyrimidine ring), 11.73 (1H, s, N<u>H</u> of pyrimidine ring); ^{13}C NMR (DMSO-d_6): δ (C-8, 27.52, C-5, 69.12, C-4, C-6, 168.12, C-2, 172.35, C-7, 178.69); IR (KBr, cm^{-1}): 1250.52 (C=S), 1700.34 (C=O), 3034.69 (C–CH_3), 3210.46 (CH of pyrimidine ring), 3285.78 (NH of pyrimidine ring); Anal. Calcd. for $C_6H_6N_2O_3S$: C, 38.70; H, 3.25; N, 15.05 %. Found: C, 38.77; H, 3.21; N, 15.11 %.

General method for the preparation of chalcones **5(a′–k′)**

To a well-stirred solution of compound (0.2 g, 1.148 mmol) **4′** in 40 % aqueous sodium hydroxide solution, equimolecular amount of the appropriate aldehydes (for e.g., benzaldehyde 0.121 g, 1.148 mmol) was added. The reaction mixture was stirred at room temperature for about 12 h. The confirmation of the reaction was carried out by TLC using chloroform–methanol and hexane–ethyl acetate (4:1 v/v) mixture. After the completion of the reaction, final compound was isolated from water at 6–7 pH. Further purification of isolated compound was done by recrystallization in methanol. Similarly, other compounds **5(b′–k′)** were synthesized (Dhorajiya *et al*., 2012).

Characterization data of synthesized compounds **5(a′–k′)**

*5-(3-Phenyl-acryloyl)-2-thioxo-dihydro-pyrimidine-4, 6-dione (**5a′**)*

Pale yellow powder, MW 274.30, Yield 77 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.53 (2H, s, J = 16.2,

trans-CH=CH), 3.82 (1H, s, –CH of pyrimidine ring at C-5), 6.49–7.39 (5H, m, Ar–H), 7.91 (1H, s, barbituric acid NH), 8.14 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 77 (C-5), 114.32 (C-9), 114.67 (C-8), 122.95 (C-13), 127.12 (C-12, C-14), 129.27 (C-11, C-15), 132 (C-10), 155 (C-7), 163.15 (C-4, C-6), 168.05 (C-2); IR (KBr, cm^{-1}): 1251.15 (C-SH), 1612.12 (C=C aromatic), 1696.23 (C=O), 3335.52 (N–H); λ_{max}: 237.84, 274. 59, 308.37; €: 4.93, 5.00, 5.05; Anal. Calcd. for $C_{13}H_{10}N_2O_3S$: C 56.92, H 3.67, N 10.21 (%). Found: C 56.89, H 3.71, N 10.19 (%).

*5-[3-(2-Hydroxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5b′**)*

Light brown, MW 290.29, Yield 58 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.51 (2H, s, J = 16.1, *trans*-CH=CH), 3.72 (1H, s, –CH of pyrimidine ring at C-5), 6.59–7.49 (4H, m, Ar–H), 7.89 (1H, s, barbituric acid NH), 8.19 (1H, s, barbituric acid NH), 9.29 (1H, s, o- Ar–OH); ^{13}C NMR (DMSO-d_6): δ 76 (C-5), 115.10 (C-9), 115.40 (C-8), 122.82 (C-13, C-14), 126.72 (C-12, C-15), 129.67 (C-11), 131.89 (C-10), 155.15 (C-7), 165.15 (C-4, C-6), 168.25 (C-2); IR (KBr, cm^{-1}): 1253.21 (C–SH), 1608.14 (C=C aromatic), 1693.36 (C=O), 3339.27 (N–H); λ_{max}: 237.84, 283.99, 308.37; €: 4.91, 4.99, 5.05; Anal. Calcd. for $C_{13}H_{10}N_2O_4S$: C 53.79, H 3.47, N 9.65 (%). Found (%): C 53.76, H 3.51, N 9.62 (%).

*5-[3-(4-Hydroxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5c′**)*

Dark brown, MW 290.29, Yield 82 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.54 (2H, s, J = 16.4, *trans*-CH=CH), 3.74 (1H, s, –CH of pyrimidine ring at C-5), 6.49–7.67 (2H, dd, Ar–H), 7.88 (1H, s, barbituric acid NH), 8.15 (1H, s, barbituric acid NH) 9.37 (1H, s, p- Ar–OH); ^{13}C NMR (DMSO-d_6): δ 78 (C-5), 113.10 (C-9), 113.38 (C-8), 121.39 (C-13), 126.56 (C-12, C-14), 129.60 (C-11, C-15), 133.29 (C-10), 157.55 (C-7), 163.15 (C-4, C-6), 168.05 (C-2); IR (KBr, cm^{-1}): 1257.33 (C–SH), 1614.17 (C=C aromatic), 1689.15 (C=O), 3328.54 (N–H); λ_{max}: 237.84, 277.41, 311.65; €: 4.91, 4.98, 5.03; Anal. Calcd. for $C_{13}H_{10}N_2O_4S$: C 53.79, H 3.47, N 9.65 (%). Found: C 53.74, H 3.50, N 9.61 (%).

*5-[3-(3-Methoxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5d′**)*

Yellow powder, MW 304.32, Yield 62 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.55 (2H, s, J = 16.6, *trans*-CH=CH), 3.69 (1H, s, –CH of pyrimidine ring at C-5), 3.76 (3H, s, *m*-OCH_3), 6.78–7.08 (2H, dd, Ar–H), 7.90 (1H, s, barbituric acid NH), 7.93 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 58.08 (C-18), 78.85 (C-5), 113.30 (C-9), 113.47 (C-8), 122.95 (C-11, C-15), 129.27 (C-12, C-14), 131.17 (C-10, C-13), 157 (C-7), 162.66(C-4, C-6), 167.05 (C-2); IR (KBr, cm^{-1}): 1260.73 (C–SH), 1603.57 (C=C aromatic), 1687.97 (C=O), 3343.39 (N–H); λ_{max}: 237.84, 272.24, 308.84; €: 4.89, 4.95, 5.00; Anal. Calcd. for $C_{14}H_{12}N_2O_4S$: C 55.25, H 3.97, N 9.21 (%). Found: C 55.28, H 3.93, N 9.23 (%).

*5-[3-(3,4-Dimethoxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5e′**)*

Dark yellow, MW 334.35, Yield 74 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.53 (2H, s, J = 16.3, *trans*-CH=CH), 3.78 (1H, s, –CH of pyrimidine ring at C-5), 3.85 (3H, s, *m*-OCH_3), 4.09 (3H, s, *p*-OCH_3), 6.75–7.18 (2H, dd, Ar–H), 7.76 (1H, s, barbituric acid NH), 7.95 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 58.08 (C-18, C-17) 75.35 (C-5), 114.56 (C-9), 114.87 (C-8), 122.95 (C-11, C-15), 129.27 (C-12, C-14), 131.17 (C-10, C-13), 158 (C-7), 162.66 (C-4, C-5), 167.05 (C-2); IR (KBr, cm^{-1}): 1261.69 (C–SH), 1607.12 (C=C aromatic), 1685.43 (C=O), 3337.64 (N–H); λ_{max}: 237.84, 275.06, 308.84; €: 4.85, 4.91, 4.96; Anal. Calcd. for $C_{15}H_{14}N_2O_5S$: C 53.88, H 4.22, N 8.38 (%). Found: C 53.85, H 4.26, N 8.36 (%).

*5-[3-(2-Chloro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5f′**)*

Brown powder, MW 308.74, Yield 84 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.51 (2H, s, J = 16.2, *trans*-CH=CH), 3.80 (1H, s, –CH of pyrimidine ring at C-5), 6.68–7.58 (2H, m, Ar–H), 7.74 (1H, s, barbituric acid NH), 7.97 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 74.53 (C-5), 113.26 (C-9), 113.59 (C-8), 122.92 (C-13, C-14), 126.51 (C-12, C-15), 129.39 (C-11), 131.78 (C-10) 157.23 (C-7), 162.66 (C-4, C-6), 168.62 (C-2); IR (KBr, cm^{-1}): 1254.71 (C–SH), 1615.42 (C=C aromatic), 1690.56 (C=O), 3334.28 (N–H); λ_{max}: 237.84, 276, 309.78; €: 4.88, 4.95, 5.00; Anal. Calcd. for $C_{13}H_9ClN_2O_3S$: C 50.57, H 2.94, N 9.07 (%). Found: C 50.54, H 2.97, N 9.02 (%).

*5-[3-(4-Chloro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5g′**)*

Dark brown powder, MW 308.74, Yield 86 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.53 (2H, s, J = 16.4, *trans*-CH=CH), 3.90 (1H, s, –CH of pyrimidine ring at C-5), 6.57–7.48 (2H, dd, Ar–H), 7.83 (1H, s, barbituric acid NH), 7.97 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 76.37 (C-5), 113.67 (C-9), 113.87 (C-8), 122.92 (C-13), 126.43 (C-14, C-15), 129.63 (C-11, C-12), 131.45 (C-10) 157.12 (C-7), 162.27 (C-4, C-6), 168.67 (C-2);

IR (KBr, cm^{-1}): 1604 (C=C aromatic), 1684 (C=O), 2527 (C=S), 3331 (N–H); λ_{max}: 238.31, 281.64, 308.37; €: 4.88, 4.96, 4.99; Anal. Calcd. for $C_{13}H_9ClN_2O_3S$: C 50.57, H 2.94, N 9.07 (%). Found: C 50.51, H 2.99, N 9.04 (%).

*5-[3-(3-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5h′**)*

Brown powder, MW 319.29, Yield 88 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.52 (2H, s, J = 16.7, *trans*-CH=CH), 3.65 (1H, s, –CH of pyrimidine ring at C-5), 6.78–7.08 (4H, m, Ar–H), 7.86 (1H, s, barbituric acid NH), 7.97 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 78.53 (C-7), 113.26 (C-9), 113.68 (C-8), 121.29 (C-13), 126.43 (C-12, C-14), 129.58 (C-11, C-15), 133.18 (C-10), 157.53 (C-7), 161.35 (C-4, C-6), 169.34 (C-2); IR (KBr, cm^{-1}): 1348 (C–NO_2), 1609 (C=C aromatic), 1693 (C=O), 2536 (C=S), 3329 (N–H); λ_{max}: 238.31, 275.06, 308. 37; €: 4.87, 4.93, 4.98; Anal. Calcd. for $C_{13}H_9N_3O_5S$: C 48.90, H 2.84, N 13.16 (%). Found: C 48.87, H 2.91, N 13.14 (%).

*5-[3-(4-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (**5i′**)*

Dark brown powder, MW 319.29, Yield 82 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.57 (2H, s, J = 16.7, *trans*-CH=CH), 3.70 (1H, s, –CH of pyrimidine ring at C-5), 6.68–7.28 (2H, dd, Ar–H), 7.79 (1H, s, barbituric acid NH), 8.03 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 78.53 (C-7), 113.26 (C-9), 113.68 (C-8), 121.29 (C-13), 126.43 (C-12, C-14), 129.58 (C-11, C-15), 133.18 (C-10), 157.53 (C-7), 161.35 (C-4, C-6), 169.34 (C-2); IR (KBr, cm^{-1}): 1353 (C–NO_2), 1613 (C=C aromatic), 1687 (C=O), 2535 (C=S), 3327 (N–H); λ_{max}: 238.26, 276, 306.5; €: 4.87, 4.93, 4.98; Anal. Calcd. for $C_{13}H_9N_3O_5S$: C 48.90, H 2.84, N 13.16 (%). Found: C 48.92, H 2.81, N 13.17 (%).

*Thioxo-5-(3-p-tolyl-acryloyl)-dihydro-pyrimidine-4,6-dione (**5j′**)*

Brown powder, MW 288.32, Yield 75 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.54 (2H, s, J = 16.3, *trans*-CH=CH), 3.69 (1H, s, –CH of pyrimidine ring at C-5), 6.48–7.38 (2H, dd, Ar–H), 7.81 (1H, s, barbituric acid NH), 7.08 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 77 (C-5), 113.45 (C-9), 113.76 (C-8), 121.78 (C-13), 126.34 (C-12, C-14), 129.87 (C-11, C-15), 132.22 (C-10), 158.25 (C-7), 166.15 (C-4, C-6), 169.26 (C-2); IR (KBr, cm^{-1}): 1607 (C=C aromatic), 1682 (C=O), 2544 (C=S), 3345 (N–H); λ_{max}: 238.31, 277.88, 306.5; €: 4.91, 4.98, 5.04; Anal. Calcd. for $C_{14}H_{12}N_2O_3S$: C 58.32, H 4.20, N 9.72 (%). Found: C 58.29, H 4.24, N 9.76 (%).

*5-(3-Naphthalen-2-yl-acryloyl)-2-thioxo-dihydro-pyrimidine-4,6-dione (**5k′**)*

Dark brown powder, MW 324.35, Yield 65 %; mp >250 °C; ^{1}H NMR (DMSO-d_6): δ 2.56 (2H, s, J = 16.3, *trans*-CH=CH), 3.83 (1H, s, –CH of pyrimidine ring at C-5), 6.75–7.14 (7H, m, Ar–H), 7.89 (1H, s, barbituric acid NH), 8.03 (1H, s, barbituric acid NH); ^{13}C NMR (DMSO-d_6): δ 76 (C-5), 114.52 (C-9), 114.77 (C-8), 122.95–132.67 (C-of naphthalene ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); IR (KBr, cm^{-1}): 1610 (C=C aromatic), 1697 (C=O), 2527 (C=S), 3338 (N–H); λ_{max}: 239.26, 279.76, 311.06; €: 4.86, 4.93, 4.98; Anal. Calcd. for $C_{17}H_{12}N_2O_3S$: C 62.95, H 3.73, N 8.64 (%). Found: C 62.92, H 3.69, N 8.67 (%).

Antimicrobial assay

The synthesized compounds **5(a′–k′)** were examined for antimicrobial activity against several bacterial strains (*S. aureus MTCC 96*, *S. pyogenes MTCC 442*, *E. coli MTCC 443*, *K. pneumoniae MTCC 109*, and *P. aeruginosa MTCC 1688*) and four fungal strains (*C. albicans MTCC 227*, *A. clavatus MTCC 1323*, *T. rubrum MTCC 296*, and *Penicillium wild strain*) species by agar cup diffusion technique. Ciprofloxacin and griseofulvin were used as control drugs for antibacterial and antifungal activity, respectively. The lowest concentration (highest dilution) required to arrest the growth of bacteria and fungi was regarded as minimum inhibitory concentration (MIC), whereas minimum bactericidal concentration (MBC) was defined as the lowest drug concentration at which 99.9 % of the inoculums were killed. The susceptibility was assessed on the basis of diameter of zone of inhibition against bacterial and fungal strains. Inhibition zones were measured and compared with the controls.

Determination of zone of inhibition method

In vitro, antibacterial and antifungal activities were examined for all synthesized chalcone molecules. Antibacterial and antifungal activities of synthesized compounds against five pathogenic bacteria (two gram positive and three gram negative) and four pathogenic fungi were investigated by the agar disk diffusion method (Alzoreky and Nakahara, 2003; Bauer *et al*., 1966; Rios *et al*., 1988). Antimicrobial activity testing was carried out by agar cup method. Each synthesized compounds were dissolved in DMSO, sterilized by filtration using sintered glass filter, and stored at 4 °C. For the determination of zone of inhibition, pure gram-positive, gram-negative bacteria, and fungal strains were taken as a standard antibiotic for comparison of the results. All the newly synthesized

molecules were screened for their antibacterial activities against the (*S. aureus MTCC 96*, *S. pyogenes MTCC 442*, *E. coli MTCC 443*, *K. pneumoniae MTCC 109*, and *P. aeruginosa MTCC 1688*) and four fungal strains (*C. albicans MTCC 227*, *A. clavatus MTCC 1323*, *T. rubrum MTCC 296*, and *Penicillium wild strain*). The Sets of four dilutions (25, 50, 100, and 250 μg/mL) and standard drugs (25, 50, 100, and 250 μg/mL) were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37 °C for 3 h. Control experiments were carried out under similar condition using ciprofloxacin for antibacterial activity and griseofulvin for antifungal activity as standard drugs. All the plates were incubated at 37 °C for 18–24 h for bacteria and at 28 °C for 48–96 h for fungi. The zones of growth inhibition around the disks were measured after 18–24 h of in incubation at 37 °C for bacteria and 48–96 h for fungi at 28 °C. The sensitivity of the microorganism species to the synthesized compounds were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <10 mm were considered as not active against microorganisms. The growth inhibition zone measured ranged from 10 to 23 mm for all the sensitive bacteria, and ranged from 16 to 25 mm for fungal strains.

Chemistry

The synthetic strategy adopted to obtain the target compounds is depicted in Scheme 1. Diethylmalonate **1′** on reaction with thiourea **2′** at 65 °C yielded 2-thioxo-dihydro-pyrimidine-4,6-dione **3′**. The key intermediate 5-acetyl-2-thioxo-dihydro-pyrimidine-4,6-dione **4′** was prepared in an excellent yield by refluxing compound **4′** in acetic anhydride in the presence of H_2SO_4. Compound **4′** when treated with aromatic aldehydes (**a′–k′**) gave the corresponding chalcone analogs **5(a′–k′)**.

Results and discussion

Spectral characteristics and tautomerism

The structures of the synthesized compounds were confirmed by spectral data and elemental analysis and they were in full agreement with the proposed structures. The ^{1}H NMR data of the compounds revealed signals between 6.48 and 7.58 δ ppm for aromatic protons of substituted phenyl ring. The ^{1}H NMR data of compounds revealed signals between 7.74 and 8.19 δ ppm and –NH of pyrimidine ring. The ^{1}H NMR of compounds **4′** and **5d′** proves that when chalcones forms –NH protons of thee pyrimidine ring shifts toward the up field.

Tautomeric study is important for other areas of chemistry. ^{1}H NMR signal at 12.34 δ ppm indicates the presence of –OH group proton which confirms the formation of tautomeric mixture but it is in the minor amount. Tautomers not only have different colors but also have different tinctorial strengths and different properties. Chalcones **5(a′–k′)** can exist in different ten possible tautomeric forms, namely the **T1**, **T2**, **T3**, **T4**, **T5**, and **T6** as shown in Fig. 1.

^{13}C NMR spectra of compound **5d′** showed characteristic peak at 58.08, 78.85, 113.30, 113.47, 122.95, 129.27, 131.17, 157, 162.66, and 167.05 δ ppm. The FT-IR spectra of compounds **5(a′–k′)** revealed a characteristic bands between 3,010 and 3,085 cm^{-1} confirming the presence of (C=C) groups. Furthermore, in the IR spectra, the bands between 1,682 and 1,697 cm^{-1} confirm the presence of C=O group. Moreover, a characteristic band appeared at 2,526–2,544 cm^{-1} corresponding to the presence of C=S groups. The presence of $–NO_2$ group at aryl ring for compound **5h′** and **i′** was confirmed by IR spectrum which showed a characteristic band at 1,348 and 1,353 cm^{-1}, respectively.

Effects on UV spectra

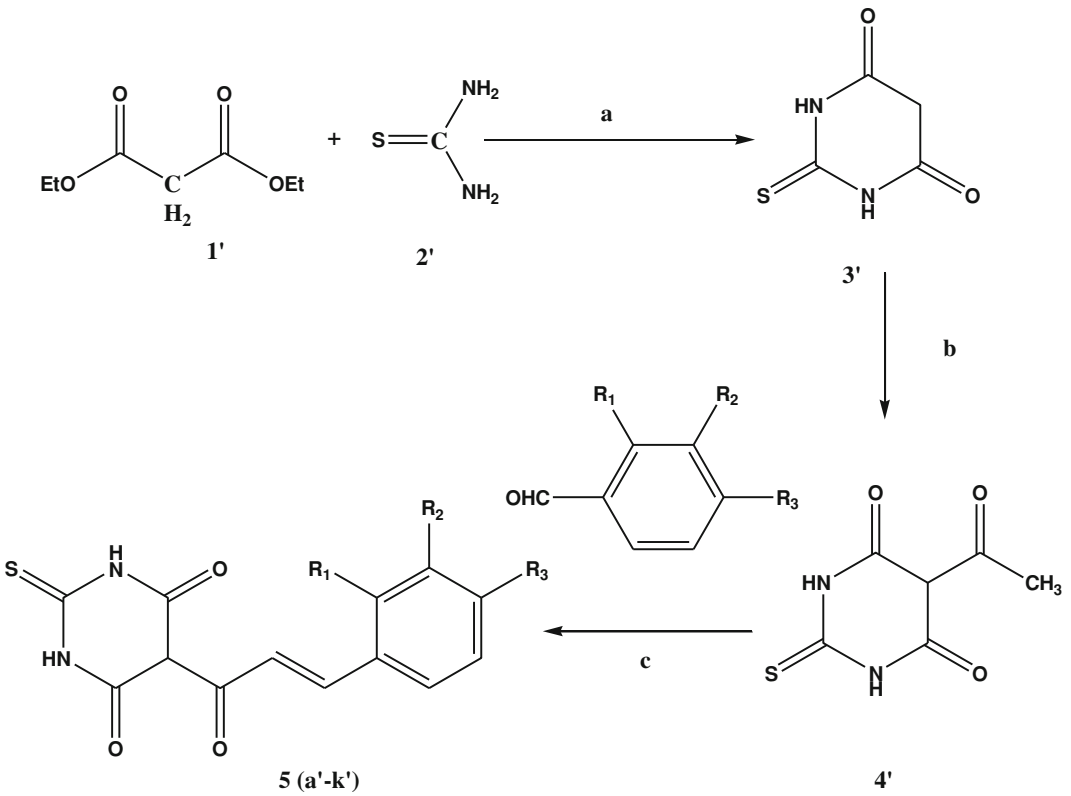
The UV absorption spectra of chalcones **5(a′–k′)** were recorded over the range of between 230 and 350 nm using DMSO as a solvent in concentrations (10^{-5}). The first absorption peak (λ_{max}) in UV spectra of compounds showed signals between 237.84 and 239.26 confirming the presence of olefinic HC=CH double bond with conjugated C=O group. The second and third absorption peak (λ_{max}) revealed signals between 274.59–283.99 and 306.5–317.37, respectively, confirming the presence of aromatic ring with different substitution group at different position (Silverstein and Webster, 1997). Also, it has been confirmed that in these types of chalcone molecules, π–π* occurs due to the presence of unsaturated bonds and this π–π* is responsible for biologic activities.

Structure–activity relationship for microbial study

The antimicrobial activity of the synthesized compounds with their MIC, MBC, minimum fungal concentration (MFC), and zone of inhibition values are summarized in Tables 1, 2, 3, and 4, respectively.

Antibacterial studies

A close investigation of the MIC values indicates that all the compounds exhibited a varied range of MIC (62.5–500 μg/mL) of antibacterial activity against all the



Scheme 1

Reagents and Conditions: **a**-NaOMe/ Methanol-Refluxed, **b**-AC2O, H2SO4-Heat, **c**-Aqueous NaOH Solution, Room Temperature

Compounds	R1, R2, R3 Groups		
	R1	R2	R3
5a'	-H	-H	-H
5b'	-OH	-H	-H
5c'	-H	-H	-OH
5d'	-H	-H	-OCH3
5e'	-H	-OCH3	-OCH3
5f'	-Cl	-H	-H
5g'	-H	-H	-Cl
5h'	-H	-NO2	-H
5i'	-H	-H	-NO2
5j'	-H	-H	-CH3
5k'	-Naphthyl Ring		

Scheme 1 Synthesis of compounds **5(a′–k′)**

tested bacterial strains. The MIC, MBC, and zone of inhibition in mm are given in Tables 1 and 2, respectively. The compounds **5a′** without any substituent at *ortho*, *meta*, and *para* position of the aryl ring attached as HC=CH of chalcone moiety showed MIC = 250 μg/mL against both gram-positive and gram-negative bacteria, except against *K. pneumoniae* where the MIC = 200 μg/mL. In the case of compound **5b′**, which was having hydroxyl substituent at *ortho* position of aryl ring at chalcone moiety, it exhibited significant activity in terms of MIC = 125 μg/mL against *S. pyogenes* at chalcone and *E. coli* same compound showed similar MIC = 250 μg/mL against *P. aeruginosa* and *K. pneumoniae*. Compound **5c′** having hydroxyl group at *para* position of the phenyl ring showed better MIC values against *S. pyogenes*, *S. aureus*, and *K. pneumoniae* compared to compound **5b′**. Chalcone **5d′** having electron donating methoxy group on *para* position of aryl ring showed good ability to inhibit all bacterial strain in terms of MIC in the range of 100–200 μg/mL. Compound **5d′** was also found to exhibit best MIC = 100 μg/mL and MBC = 125 μg/mL against *P. aeruginosa* along with compound **5k′** among all the synthesized derivatives.

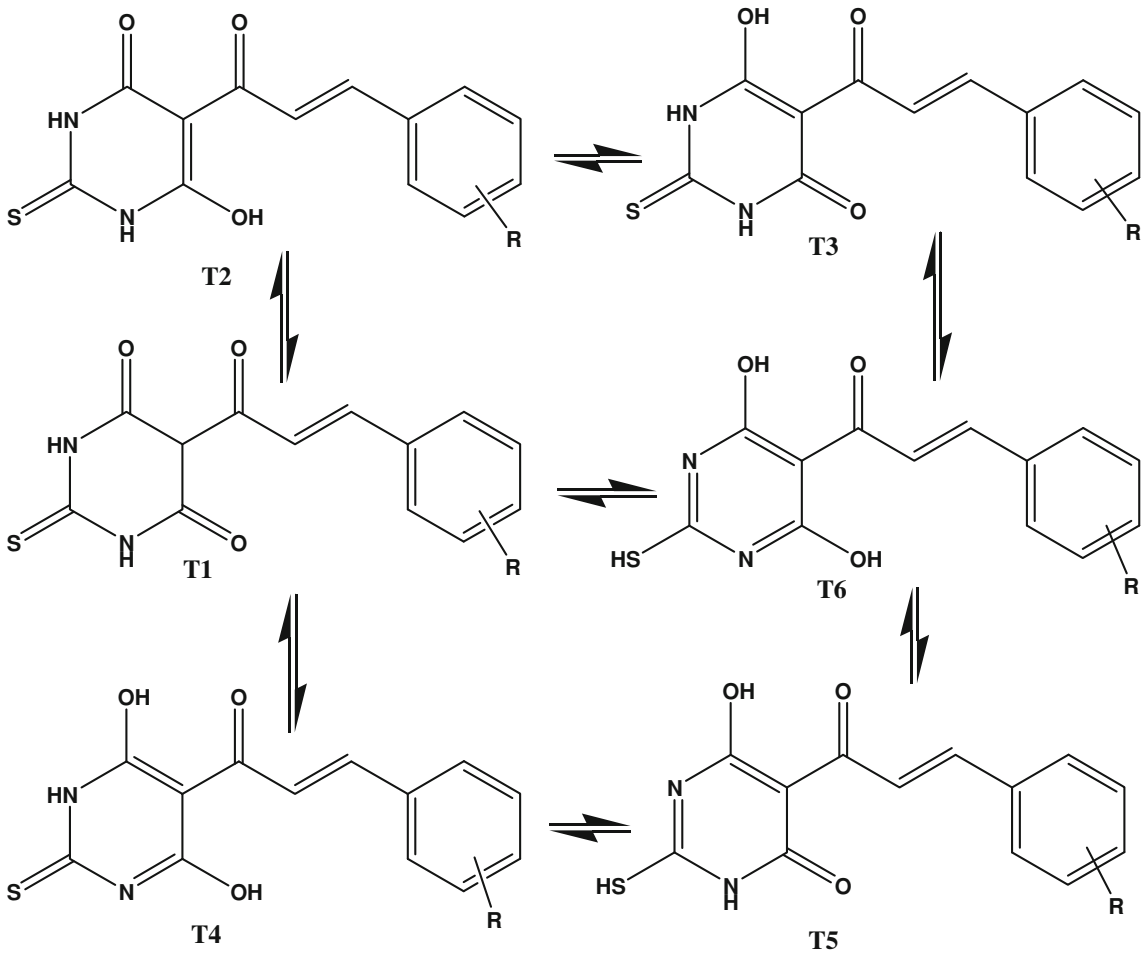


Fig. 1 Possible tautomers of compounds **5(a′–k′)**

Table 1 MIC (μg/mL) and MBC (μg/mL) results of the compounds **5(a′–k′)**

Compounds	Gram positive bacteria				Gram negative bacteria					
	S. pyogenes MTCC 442		*S. aureus MTCC 96*		*E. coli MTCC 443*		*P. aeruginosa MTCC 1688*		*K. pneumoniae MTCC 109*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
5a′	250	500	250	250	250	500	250	250	200	250
5b′	125	200	500	500	125	200	250	250	200	250
5c′	100	250	250	500	250	250	250	500	125	200
5d′	200	250	200	250	200	250	100	125	200	250
5e′	62.5	100	500	500	500	500	200	200	250	500
5f′	250	250	250	500	250	500	250	500	200	250
5g′	100	125	100	100	125	125	200	250	100	125
5h′	200	250	200	250	100	100	125	200	62.5	100
5i′	100	100	125	200	250	500	250	250	200	200
5j′	250	200	125	125	100	200	250	500	100	100
5k′	250	250	200	250	250	500	100	125	125	200
Ciprofloxacin	50		100		25		25		25	

MIC (μg/mL) minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of bacteria completely, *MBC* (μg/mL) minimum bactericidal concentration, that is, the lowest concentration of the compound for killing the bacteria completely

Table 2 Zone of inhibition (mm) of the compounds **5(a′–k′)**

Compounds	Diameter of zone of inhibition (mm)									
	Gram positive bacteria								Gram negative bacteria	
	S. pyogenes MTCC 442				*S. aureus MTCC 96*				*E. coli MTCC 443*	
	25	50	100	250	25	50	100	250	25	50
5a′	12± (0.1)	15± (0.2)	15± (0.2)	17± (0.1)	10± (0.3)	13± (0.2)	15 ± (0.2)	17± (0.2)	12± (0.3)	15± (0.1)
5b′	13± (0.2)	15± (0.1)	16± (0.1)	17± (0.3)	11± (0.1)	13± (0.1)	17± (0.3)	19± (0.2)	13± (0.2)	15± (0.1)
5c′	15± (0.1)	17± (0.2)	16± (0.1)	17± (0.2)	10± (0.3)	14± (0.2)	16± (0.2)	18± (0.2)	15± (0.2)	17± (0.1)
5d′	15± (0.1)	18± (0.1)	20± (0.2)	21± (0.3)	13± (0.3)	17± (0.1)	18± (0.1)	21± (0.2)	15± (0.3)	18± (0.2)
5e′	12± (0.1)	16± (0.2)	18± (0.1)	21± (0.3)	11± (0.4)	15± (0.2)	18± (0.1)	20± (0.1)	12± (0.4)	16± (0.2)
5f′	13± (0.2)	14± (0.1)	15± (0.1)	16± (0.1)	10± (0.3)	14± (0.2)	17± (0.1)	18± (0.1)	13± (0.1)	14± (0.1)
5g′	11± (0.3)	14± (0.1)	17± (0.1)	19± (0.2)	11± (0.1)	12± (0.1)	13± (0.1)	15± (0.3)	11± (0.3)	14± (0.1)
5h′	15± (0.1)	17± (0.3)	18± (0.2)	18± (0.2)	14± (0.2)	16± (0.1)	17± (0.1)	19± (0.4)	15± (0.2)	17± (0.2)
5i′	12± (0.1)	15± (0.2)	19± (0.3)	23± (0.2)	11± (0.1)	13± (0.1)	18± (0.4)	20± (0.2)	12± (0.3)	15± (0.3)
5j′	15± (0.1)	15± (0.1)	18± (0.2)	19± (0.2)	10± (0.1)	12± (0.1)	16± (0.3)	17± (0.2)	15± (0.1)	15± (0.1)
5k′	14± (0.2)	16± (0.2)	18± (0.2)	21± (0.2)	10± (0.1)	12± (0.1)	15± (0.2)	19± (0.3)	14± (0.1)	16± (0.2)

Compounds	Diameter of zone of inhibition (mm)									
	Gram negative bacteria									
	E. coli MTCC 443		*P. aeruginosa MTCC 1688*				*K. pneumoniae MTCC 109*			
	100	250	25	50	100	250	25	50	100	250
5a′	15± (0.1)	17± (0.2)	10± (0.4)	13± (0.2)	15± (0.1)	17± (0.3)	10± (0.1)	12± (0.2)	14± (0.2)	17± (0.3)
5b′	16± (0.1)	17± (0.1)	11± (0.1)	13± (0.2)	17± (0.4)	19± (0.2)	12± (0.1)	13± (0.1)	15± (0.2)	17± (0.2)
5c′	16± (0.2)	17± (0.1)	10± (0.1)	14± (0.2)	16± (0.2)	18± (0.2)	10± (0.1)	11± (0.1)	16± (0.3)	18± (0.2)
5d′	20± (0.1)	21± (0.1)	13± (0.4)	17± (0.2)	18± (0.1)	21± (0.3)	11± (0.1)	13± (0.2)	14± (0.1)	17± (0.2)
5e′	18± (0.2)	21± (0.1)	11± (0.1)	15± (0.4)	18± (0.3)	20± (0.2)	14± (0.1)	15± (0.1)	18± (0.3)	19± (0.1)
5f′	15± (0.1)	16± (0.1)	10± (0.2)	14± (0.3)	17± (0.2)	18± (0.1)	12± (0.1)	13± (0.1)	15± (0.2)	18± (0.4)
5g′	17± (0.2)	19± (0.2)	11± (0.1)	12± (0.1)	13± (0.1)	15± (0.2)	13± (0.1)	15± (0.2)	17± (0.2)	20± (0.1)
5h′	18± (0.1)	18± (0.1)	14± (0.2)	16± (0.1)	17± (0.1)	19± (0.2)	10± (0.3)	14± (0.1)	17± (0.2)	18± (0.1)

Table 2 continued

Compounds	Diameter of zone of inhibition (mm)									
	Gram negative bacteria									
	E. coli MTCC 443		*P. aeruginosa MTCC 1688*				*K. pneumoniae MTCC 109*			
	100	250	25	50	100	250	25	50	100	250
5i′	19± (0.4)	23± (0.3)	11± (0.1)	13± (0.3)	18± (0.2)	20± (0.1)	11± (0.1)	13± (0.2)	15± (0.2)	16± (0.1)
5j′	18± (0.3)	19± (0.2)	10± (0.2)	12± (0.1)	16± (0.3)	17± (0.1)	12± (0.1)	14± (0.2)	15± (0.1)	17± (0.2)
5k′	18± (0.2)	21± (0.3)	10± (0.1)	12± (0.1)	15± (0.3)	19± (0.4)	14± (0.1)	15± (0.1)	18± (0.3)	20± (0.2)

Table 3 MIC (μg/mL) and MFC (μg/mL) results of compounds **5(a′–k′)**

Compounds	Fungal strains							
	C. albicans MTCC 227		A. clavatus MTCC 1323		T. rubrum MTCC 296		Penicillium wild strain	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
5a′	500	500	1000	1000	1000	1000	1000	>1000
5b′	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
5c′	>1000	>1000	500	500	>1000	>1000	>1000	>1000
5d′	>1000	>1000	500	1000	>1000	>1000	500	500
5e′	500	500	1000	1000	500	500	1000	1000
5f′	250	1000	>1000	>1000	500	500	>1000	>1000
5g′	500	500	500	500	>1000	>1000	>1000	>1000
5h′	1000	1000	1000	1000	1000	1000	1000	1000
5i′	1000	1000	1000	1000	1000	1000	500	500
5j′	1000	1000	>1000	>1000	>1000	>1000	>1000	>1000
5k′	>1000	>1000	>1000	>1000	1000	1000	1000	1000
Griseofulvin	100		100		500		100	

MIC (μg/mL) minimum inhibitory concentration, that is, the lowest concentration of the compound to inhibit the growth of fungus completely, *MFC* (μg/mL) minimum fungicidal concentration, that is, the lowest concentration of the compound for killing the fungus completely

On the other hand, **5e′** which was having two methoxy group one at *meta* and another at *para* position of phenyl ring showed strong inhibition in terms of MIC = 62.5 μg/mL against *S. pyogenes* and compound was found to be most potent against same bacteria among all the chalcone analogs. A very interesting observation made for compound **5d′** and **e′** was that except activity against *S. pyogenes*, **5d′** showed very good MIC and MBC values compare to compound **5d′** against *S. aureus*, *E. coli*, *P. aeruginosa,* and *K. pneumoniae*. In the case of compound **5f′** and **g′**, which were having electron-withdrawing chloro substituent respectively at the and position of phenyl ring attached at chalcone shows effective inhibitory effect with MIC range 100–250 μg/mL against all bacterial strains. Compound **5g′** exhibited the greatest activity against *S. aureus* in terms of MIC and MBC = 100 μg/mL among all the compounds. Same compound also showed similar MIC = 100 μg/mL and MBC = 125 μg/mL against *S. pyogenes* and *K. pneumoniae*. In comparison of compounds **5f′** and **g′** by means of activity data compound **5g** was found superior than compound **5f′**. Chalcones **5h′** and **i′** with $-NO_2$ group substituted at phenyl ring on *meta* and *para* position, respectively, exhibit MIC in the range of 62.5–250 μg/mL. Compound **5h′** was found to exhibit strong inhibition against gram-negative bacteria *E. coli* and *K. pneumoniae* in terms of MIC = 100 μg/mL and 62.5 μg/mL, respectively, with similar MBC = 100 μg/mL among all synthesized derivatives. $-NO_2$ group at *para* position containing compound **5i′** showed MBC = 100 μg/mL against *S. pyogenes* similar to most potent compound **5e′**, but **5e′** showed better MIC = 62.5 μg/mL than **5i′** having MIC = 100 μg/mL against *S. pyogenes* against all the gram positive bacterial strains. Compound **5i′** was found

Table 4 Zone of inhibition (mm) of the compounds **5(a′–k′)**

Compounds	Diameter of zone of inhibition (mm)							
	C. albicans MTCC 227				*A. clavatus MTCC 1323*			
	25	50	100	250	25	50	100	250
5a′	16± (0.2)	18± (0.2)	22± (0.3)	22± (0.1)	18± (0.1)	19± (0.1)	21± (0.2)	24± (0.2)
5b′	17± (0.2)	19± (0.1)	22± (0.3)	24± (0.2)	18± (0.1)	19± (0.1)	20± (0.2)	22± (0.1)
5c′	16± (0.3)	20± (0.1)	23± (0.2)	23± (0.2)	21± (0.1)	21± (0.1)	22± (0.2)	24± (0.1)
5d′	18± (0.1)	21± (0.3)	24± (0.1)	24± (0.1)	20± (0.1)	21± (0.1)	23± (0.2)	24± (0.1)
5e′	20± (0.3)	24± (0.1)	25± (0.1)	25± (0.1)	18± (0.2)	20± (0.2)	21± (0.1)	23± (0.2)
5f′	21± (0.1)	22± (0.2)	22± (0.2)	23± (0.1)	18± (0.1)	19± (0.2)	21± (0.1)	22± (0.1)
5g′	20± (0.1)	21± (0.1)	22± (0.1)	23± (0.1)	18± (0.2)	20± (0.3)	22± (0.1)	22± (0.1)
5h′	21± (0.2)	22± (0.1)	23± (0.1)	24± (0.2)	20± (0.2)	22± (0.1)	22± (0.1)	24± (0.2)
5i′	20± (0.1)	21± (0.4)	25± (0.2)	25± (0.2)	20± (0.1)	21± (0.1)	22± (0.2)	24± (0.1)
5j′	19± (0.1)	20± (0.1)	21± (0.1)	24± (0.3)	21± (0.2)	22± (0.1)	22± (0.1)	25± (0.3)
5k′	22± (0.1)	22± (0.1)	24± (0.2)	25± (0.1)	18± (0.2)	20± (0.4)	25± (0.1)	25± (0.1)
Compounds	Diameter of zone of inhibition (mm)							
	T. rubrum MTCC 296				*Penicillium wild strain*			
	25	50	100	250	25	50	100	250
5a′	20± (0.3)	24± (0.1)	24± (0.1)	25± (0.2)	19± (0.2)	21± (0.1)	22± (0.1)	24± (0.2)
5b′	18± (0.2)	21± (0.1)	21± (0.1)	22± (0.2)	18± (0.1)	19± (0.1)	22± (0.3)	23± (0.1)
5c′	22± (0.1)	22± (0.1)	24± (0.2)	24± (0.2)	18± (0.1)	19± (0.3)	22± (0.2)	24± (0.1)
5d′	19± (0.2)	20± (0.1)	21± (0.1)	22± (0.2)	21± (0.1)	21± (0.1)	23± (0.2)	25± (0.3)
5e′	21± (0.1)	22± (0.1)	23± (0.2)	24± (0.1)	19± (0.1)	20± (0.2)	22± (0.3)	24± (0.4)
5f′	19± (0.1)	20± (0.2)	19± (0.1)	21± (0.3)	18± (0.1)	18± (0.1)	21± (0.3)	22± (0.2)
5g′	18± (0.2)	20± (0.1)	21± (0.1)	22± (0.2)	19± (0.1)	22± (0.2)	23± (0.1)	25± (0.4)
5h′	18± (0.3)	21± (0.1)	22± (0.1)	27± (0.4)	18± (0.1)	20± (0.2)	21± (0.1)	22± (0.1)
5i′	20± (0.2)	22± (0.1)	23± (0.1)	25± (0.2)	21± (0.1)	21± (0.1)	23± (0.2)	25± (0.2)
5j′	19± (0.1)	20± (0.2)	22± (0.1)	25± (0.3)	18± (0.1)	18± (0.1)	19± (0.1)	22± (0.3)
5k′	18± (0.2)	20± (0.1)	22± (0.2)	23± (0.1)	18± (0.1)	18± (0.1)	21± (0.4)	23± (0.2)

to exhibit better activity (MIC and MBC) compare to **5h′** having superior activity than **5i′** against all gram-negative bacterial strains. Antibacterial activity of electron donating $-CH_3$ substituent on phenyl ring containing compound **5j′** showed very good MIC = 100 μg/mL against *E. coli* along with compound **5h′**, but compared to **5h′** showed better MBC = 100 μg/mL compared to MBC = 200 μg/mL of compound **5j′**. Compound **5j′** also showed similar MBC = 100 μg/mL along with the most active compound **5h′** against *K. pneumoniae*. Compound **5k′** containing naphthalene ring was found to be equipotent in terms of MIC = 100 μg/mL and MBC = 125 μg/mL along with compound **5d′**. *K. pneumoniae* was effectively inhibited with good MIC = 125 μg/mL by compound **5k′**. In most of the cases, the MBC of compounds were found to be one to two folds than that of the corresponding MIC results.

Antifungal studies

Antifungal activity was also done by Kirby–Bauer disk diffusion method. For assaying antifungal activity *C. albicans MTCC 227*, *A. clavatus MTCC 1323*, *T. rubrum MTCC 296,* and *Penicillium wild strain* were recultured in DMSO by agar diffusion method. The MIC, MFC, and zone of inhibition in mm are given in Tables 3 and 4, respectively. A close investigation of the MIC values indicates that all the compounds exhibited a varied range of MIC (250–>1,000 μg/mL) of antifungal activity against all the tested fungal strains. The antifungal screening data of the compounds **5(a′–k′)** showed good to moderate activity. Compound **5a′** and **k′** without any substituent at any position of the aryl ring attached as CH=CH of chalcone moiety showed MIC and MFC (1,000–>1,000 μg/mL) against all the strains; but, in the case of **5a′,** the presence of small phenyl ring compared to naphthyl ring of **5k′** showed good MIC and MFC = 500 μg/mL in the case of *C. albicans* as the fungal strain. In compound **5b′,** the presence of electron-releasing hydroxyl substituent at *ortho* position showed moderate activity against all the strains. While in the case of chalcone **5c′** containing same group, but at *para* position, showed good MIC and MFC values 500 μg/mL for *A. clavatus* as a fungal strain. Chalcone molecule **5d′** containing electron-releasing methoxy group at *para* position showed good MIC 500 = μg/mL for *A. clavatus* and MIC and MFC = 500 μg/mL for *Penicillium wild strains* as fungal strains. Compound **5e′** possesses same methoxy substituent at *meta* and *para* position showed good and comparable MIC and MFC = 500 μg/mL for *C. albicans* and *T. rubrum* as the fungal strains, respectively. Chalcone derivatives **5f′** having electron-releasing chlorine substituent at *ortho* position showed

MIC = 250μg/mL and comparable MIC and MFC = 500 μg/mL for *C. albicans* and *T. rubrum* as the fungal strains respectively but out of **5e′** and **f′** compound **5f′** is more superior. Chalcone molecule **5g′** containing same chlorine group at *para* position showed good MIC and MFC values 500 μg/mL in the case of *C. albicans* and *A. clavatus* as the fungal strains. Compounds **5h′** and **i′** containing electron-withdrawing nitro group at *meta* and *para* position, respectively, showed moderate MIC and MFC values, but compound **5i′** showed good MIC and MFC = 500 μg/mL in the case of *Penicillium wild strains* as the fungal strain. Chalcone molecule **5j′** possesses electron-releasing methyl group at *para* position showing moderate antifungal activity. The MFC of compounds were found to be one or two-folds higher than the corresponding MIC results. The antifungal activity of each compound was compared with griseofulvin as standard drugs. Inhibition zones were measured and compared with the controls. The fungal zones of inhibition values are given in Table 4.

Conclusion

From the antimicrobial study, we ascertained that some of the newly synthesized compound containing electron-withdrawing group like chloro and nitro as substituent on phenyl ring at chalcone **5g′** and **i′** showed significant activity against both the gram-positive as well as gram-negative bacteria. On the other hand, compound **5j′** having electron-donating group methyl as substituent on phenyl ring also showed good activity against both bacterial strains (gram positive and gram negative). Compounds **5e′** and **f′** were found to exhibit a comparable (MIC) against *T. rubrum* as a fungal strain with reference to griseofulvin as standard drugs. The antimicrobial study of the synthesized compounds permitted us to state that the variation of antimicrobial activity may be associated with the nature of tested bacterial and fungal strains and the substituent on phenyl ring at chalcone moiety. The results unfold the way for investigation of new potential lead compounds for investigating antimicrobial activity. The present investigation revealed that chalcone compounds **5(a′–k′)** can be potential lead for development of new antibacterial and antifungal agents. In future, 5-acetyl-2-thioxo-dihydropyrimidine-4,6-dione-based chalcones will use for the further development of new biologic entity.

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