Design, Synthesis and Comparative Study of Anti-Microbial Activities on Barbituric Acid and Thiobarbituric Acid based Chalcone Derivatives Bearing the Pyrimidine Nucleus

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Abstract

A new comparative series of barbituric acid and thiobarbituric acid based chalcone derivatives bearing the pyrimidine nucleus were synthesized. The chemical structures of the resulting molecules were characterised by means of FT-IR (Fourier Transform Infrared) 1H NMR, 13C-NMR (Nuclear Magnetic Resonance) and HMBC (Heteronuclear Multiple-Bond Correlation) and Elemental Analysis. All synthesized compounds were subjected to in vitro antimicrobial screening against four bacterial strains i.e., one Gram Positive (Bacillus subtilis MTCC 441), two Gram Negative (E. coli MTCC 443, P. aeruginosa MTCC 1688), and one Fungal (C. albicans MTCC 227) Strains. The structure activity relationship is discussed on the basis of bioactivity results, various functional groups present and position of the functional group at various positions of the synthesized compounds. The comparative antimicrobial activity study of both of the series elucidated that shows the chalcone compounds containing -thioketo group are more potent than the -keto group.

Keywords: Antibacterial activity; Antifungal activity; Barbiturates; Chalcones; Structure activity relationship

Introduction

Development of new antimicrobial agents with novel structure and mode of action remains the primary goal of scientists for the solution of increasing bacterial resistance gained by microorganism to classical antimicrobial agents [1]. As resistance to antimicrobial drugs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent and less toxic antimicrobial agents [2]. The multiple pharmacological actions of unique synthetic compounds are a prerequisite for classifying a drug as highly efficacious, because these actions offer possibility of treating various diseases. Pyrimidine derivatives are synthesized by the reaction of 5-acetyl barbituric acid or 5-acetyl thiobarbituric acid and various aldehydes in alkaline condition at room temperature. These derivatives are considered to be important for drugs [3]. The presence of a pyrimidine base in thymine, cytosine and uracil, which are the essential binding blocks of nucleic acids, DNA and RNA is one possible reason for their activity. The literature survey indicated that compounds having pyrimidine nucleus possess broad range of biological activities, like 5-fluorouracil as anticancer, idoxuridine and trifluridine as antiviral [4-6] zidovudine and stavudine as anti-HIV [7] trimethoprim, sulphamethiazine and sulphasoxazole as antibacterial [8] sulphasoxazole as antimalarial and antibacterial [9]. Fungi are widely distributed in nature and frequently appear as pathogens in the animal and plant kingdoms. The pyrimidine ring system being present in various natural compounds such as nucleic acids, vitamins, coenzymes, uric acid, purines, some marine microorganisms [10]. The therapeutic importance of pyrimidine motif - derivatives such as barbituric acid and thiobarbituric acid play vital role among various heterocyclic compounds due to their anti-neoplastic [11,12], antiviral [13], antibiotic [14], and anti-inflammatory [11] activity. Many synthetic drugs of barbituric and thiobarbituric acid motif based derivatives and chemotherapeutic agents are well known [15]. Chalcones are the products of the condensation of a simple or substituted aromatic moiety with a simple or substituted acetophenone in presence of base. This group of compounds is widely used in anticancer research, as an antimicrobials or an antitubercular [16,17]. Chalcone analogues are very versatile as physiologically active compounds and substrates for the evaluation of various organic syntheses. So, in light of above facts of pyrimidine, barbituric acid, thiobarbituric acid and chalcones, we continue our earlier work on synthesis of 5-acetyl barbituric acid 4 and 5-acetyl thiobarbituric acid 4’ based chalcones 5 (a-k) and 5 (a’-k’).

All the analogs were screened for their antimicrobial activity and their comparative results are discussed with respect to one Gram Positive (Bacillus subtilis MTCC 441), two Gram Negative (E. coli MTCC 443, P. aeruginosa MTCC 1688), and one Fungal (C. albicans MTCC 227) Species and effects of functional groups and position of functional groups on various microbial strains.

Experimental Methods

Chemicals and solvents were obtained from commercial sources and used as received throughout the investigation. Melting points were determined in open capillaries on a Vego electronic apparatus VMP-D (Vego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4000-400 cm⁻¹) of synthesized compounds were recorded on a Perkin Elmer-Spectrum RX-1FTIR spectrophotometer using KBr pellets. Thin layer chromatography was performed on object glass slides (2 x 7.5 cm) coated with silica gel-G and spots were visualized under UV irradiation. 1H NMR and 13C NMR spectra were recorded on an Advance-II (Bruker) model using DMSO as a solvent and TMS as internal standard with 1H resonant frequency of 400 MHz and 13C resonant frequency of 100 MHz. The 1H NMR and 13C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS...
Synthesis of barbituric acid (3)

To a solution of diethylmalonate (20 g, 118.9 mmol), urea 2 (7.5 g, 125 mmol) in methanol, anhydrous sodium methoxide was added and refluxed at 65°C for 8 h. A white solid separates. Then in above reaction mixture 125 ml of hot (50°C) water was added and hydrochloric acid was used to make the solution acidic. After completion of the reaction, the resulting clear solution was filtered and cooled in an ice bath overnight. The white product formed and it was filtered, washed with 50 ml of cold water, dried and recrystallized from acetone to afford compound 3 as a white powder [18-20].

Synthesis of thiobarbituric acid (3')

To a solution of diethylmalonate (20 g, 118.9 mmol), thiourea 2' (7.5 g, 125 mmol) in methanol, anhydrous sodium methoxide was added and refluxed at 65°C for 8 h. A white solid separates. Then in above reaction mixture 125 ml of hot (50°C) water was added and hydrochloric acid was used to make the solution acidic. After completion of the reaction, the resulting clear solution was filtered and cooled in an ice bath overnight. The white product formed and it was filtered, washed with 50 ml of cold water, dried and recrystallized from acetone to afford compound 3' as a Light yellowish white powder [18-20].

Synthesis of 5-acetyl barbituric acid (4)

To a solution of barbituric acid (3) (6.4 g, 44.39 mmol) in acetic anhydride (150 ml), few drops of H$_2$SO$_4$ was added and refluxed for 1 h. The reaction in the beginning was a suspension but after about 10 min of refluxed, it changes to orange/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, and dried to give compound 4 as a yellow powder [21].

Synthesis of 5-acetyl thiobarbituric acid (4')

To a solution of barbituric acid (3) (6.4 g, 44.39 mmol) in acetic anhydride (150 ml), few drops of H$_2$SO$_4$ was added and refluxed for 1 h. The reaction in the beginning was a suspension but after about 10 min of refluxed, it changes to orange/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, and dried to give compound 4 and 4' as a brown powder [21].

General synthetic procedure for the barbituric acid based chalcone compounds 5 (a-k)

To a well stirred solution of compound 5-acetyl barbituric acid (4) in 40% aqueous sodium hydroxide solution, equimolecular amount of the appropriate aldehydes were 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 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 (a-k) were synthesized [22,23].

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 FT-IR spectra of compounds 5 (a-k) and 5 (a’-k) revealed a characteristic bands between 3010 cm$^{-1}$ and 3085 cm$^{-1}$ confirms the presence of (C=O) group. Moreover, a characteristic band appeared at 1630 cm$^{-1}$ and 1512 cm$^{-1}$ revealed peaks at 1512 cm$^{-1}$ & 1682-1697 cm$^{-1}$ confirms the presence of (C=O) group. Furthermore, in the FT-IR spectra the bands between 1682-1697 cm$^{-1}$ confirms the presence of (C=O) group. Moreover, a characteristic band appeared at 2526-2544 cm$^{-1}$ corresponded to the presence of (C=O) groups. The FT-IR spectra of compounds 5a and 5d revealed peaks at 1512 cm$^{-1}$.
and 1522 cm⁻¹, which shows partial $H$-Bonding between H-atom of olefinic carbon atom and O-atom of pyrimidine ring. The $^1H$ NMR data of chalcone derivatives showed signals between 6.48 - 7.67 δ ppm for aromatic protons corresponding to the phenyl ring. The $^1H$ NMR data of compounds 5-acylbarbituric acid and 5-acyl thiobarbituric acid and its chalcones proves that when chalcones –NH protons of thee pyrimidine ring shifts towards the up field. Tautomeric study is important for other areas of chemistry. From $^1H$ NMR signal at 12.34-17.36 δ ppm indicates the presence of –OH group proton which confirms the formation of tautomeric mixture but it is in the minor amount [24].

Tautomers not only have different colors, but also have different tautomeric strengths and different properties. Chalcones 5 (a-k) and 5 (a-k’) can exist in different ten possible tautomeric forms, namely the T1, T2, T3, T4, T5 and T6 as shown in Figure 1.

Spectral Characterization data of synthesized compounds 5(a-k) and 5 (a-k’)

5-(3-Phenyl-acryloyl) pyrimidine-2,4,6-trione (5a): Yellow Solid, M.W.258.23, Yield 89%; m.p. 184-188°C; $^1H$ NMR (DMSO-d$_6$): δ 2.51 (2H, s, J = 23,8 trans-CH=CH), 4.16 (1H, s, -CH of pyrimidine ring at C-5), 7.40-7.93 (5H, m, Ar-H), 10.98 (1H, s, barbituric acid NH), 11.73 (1H, s, barbituric acid NH), $^13C$ NMR (DMSO-d$_6$): δ 78.55 (C-5), 116.12 (C-9), 116.73 (C-8), 129.13 (C-13), 130.70 (C-12, C-14), 129.13 (C-11, C-15), 132.38 (C-10, 167.29 (C-2, C-4, C-6, C-7); FT-IR (KBr, cm⁻¹): 1136.11 (C-O), 1612.21 (C=C aromatic), 1696.32 (C=O), 3438.52 (N-H); Anal. Calcld. For C$_{13}$H$_{10}$N$_2$O$_3$: C 56.92, H 3.50, N 9.65 (%). Found: C 56.91, H 3.65, N 10.25 (%).

5-(3-Phenyl-acryloyl) thio-dihydro-pyrimidine-4,6-dione (5b): Light Brown Solid, M.W. 250.29, Yield 58%; m.p. >250°C; $^1H$ 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), 6.57-7.49 (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); $^13C$ NMR (DMSO-d$_6$): δ 76.56 (C-6), 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=O, C-4), 168.25 (C=O); FT-IR (KBr, cm⁻¹): 1138.21 (C=O), 1614.24 (C=C aromatic), 1698.32 (C=O), 3437.23 (N-H); Anal. Calcld. For C$_{13}$H$_{10}$N$_2$O$_3$: C 56.94, H 6.88, N 10.22 (%). Found: C 56.91, H 3.65, N 10.25 (%).

5-(3-Phenyl-acryloyl) pyrimidine-2-thioxo-dihydro-pyrimidine-4,6-dione (5c): Yellow Solid, M.W. 274.22, Yield 79%; m.p. 206-210°C; $^1H$ NMR (DMSO-d$_6$): δ 2.61 (2H, s, J = 20, trans-CH=CH), 4.19 (1H, s, -CH of pyrimidine ring at C-5), 6.67-7.68 (2H, dd, Ar-H), 10.55 (1H, s, barbituric acid NH), 10.69 (1H, s, barbituric acid NH) 6.67 (1H, s, p- Ar- OH); $^13C$ NMR (DMSO-d$_6$): δ 79.5 (C-9), 115.08 (C-8), 120.92 (C-13), 125.61 (C-12, C-14), 128.66 (C-11, C-15), 132.17 (C-10), 158.52 (C-7), 164.67 (C-14, C-6), 167.35 (C-5); FT-IR (KBr, cm⁻¹): 1140.24 (C=O), 1616.07 (C=C aromatic), 1697.19 (C=O), 3437.45 (N-H); Anal. Calcld. For C$_{13}$H$_{10}$N$_2$O$_3$: C 56.94, H 6.68, N 10.22 (%). Found: C 56.97, H 3.72, N 10.24 (%).

5-(3-Phenyl-acryloyl) pyrimidine-2-thioxo-dihydro-pyrimidine-4,6-dione (5d): Dark Brown Solid, M.W. 290.29, Yield 82%; m.p. >250°C; $^1H$ 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); $^13C$ NMR (DMSO-d$_6$): δ 78.56 (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=O); FT-IR (KBr, cm⁻¹): 1253.33 (C=O), 1614.17 (C=C aromatic), 1689.15 (C=O), 3328.54 (N-H); Anal. Calcld. For C$_{13}$H$_{10}$N$_2$O$_3$: C 53.79, H 3.47, N 9.65 (%). Found: C 53.74, H 3.50, N 9.61 (%).

5-(3-Methoxy-phenyl)-acryloylpyrimidine-2,4,6-trione (5e): Yellow Solid, M.W.288.66, Yield 83%; m.p. 228-233°C; $^1H$ NMR (DMSO-d$_6$): δ 2.62 (2H, s, J = 12, trans-CH=CH), 4.20 (1H, s, -CH of pyrimidine ring at C-5), 3.09 (1H, s, m-OCH$_3$), 6.67-7.77 (2H, dd, Ar-H), 10.62 (1H, s, barbituric acid NH), 10.79 (1H, s, barbituric acid NH); $^13C$ NMR (DMSO-d$_6$): δ 57.87 (C-18), 77.54 (C-5), 114.63 (C-9), 115.78 (C-8), 123.58 (C-11, C-15), 128.12 (C-12, C-14), 130.13 (C-10, C-13), 156.84 (C-7), 163.43 (C-4, C-6), 168.32 (C=O); FT-IR (KBr, cm⁻¹): 1136.78 (C=O), 1631.24 (C=C aromatic), 1698.87 (C=O), 3439.33 (N-H); Anal. Calcld. For C$_{15}$H$_{10}$N$_2$O$_3$: C 58.33, H 4.20, N 9.72 (%). Found: C 58.31, H 4.23, N 9.75 (%).
5-[(3-Methoxy-phenyl)-acryloyl]-2-thioxo-dihydro-
pyrimidine-4,6-dione (5d): Yellow Solid, M.W. 304.32. Yield 62%.
m.p. >250°C; 1H NMR (DMSO-d6): 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-
-OCH3), 6.78-7.08 (2H, dd, Ar-H), 7.90 (1H, s, barbituric acid NH),
7.93 (1H, s, barbituric acid NH); 13C NMR (DMSO-
d6): δ 58.08 (C-8), 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); FT-IR (KBr cm⁻¹): 1261.69 (C-O), 1620.23 (C=C aromatic), 1701.51 (C=O), 3343.28 (N-H);

5-[(3-Chloro-phenyl)-acryloyl]-2-thioxo-dihydro-
pyrimidine-4,6-dione (5g): Dark brown Powder, M.W. 308.74. Yield 86%; m.p. >250°C; 1H NMR (DMSO-d6): 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); 13C NMR (DMSO-
d6): δ 76.37 (C-5), 113.67 (C-9), 113.87 (C-8), 122.92 (C-12, C-14), 126.43 (C-13, C-15), 126.93 (C-11, C-12), 131.45 (C-10) 157.12 (C-7), 162.27 (C-4, C-6), 168.67 (C-2); FT-IR (KBr cm⁻¹): 1604 (C=C aromatic), 1684 (C=O), 2527 (C=N); 3331 (N-H); Anal. Calcld.
C13H15CN2O5S; C 50.57, H 2.94, N 9.07 (%). Found (%): C 50.51, H 2.99, N 9.04 (%).

5-[(3-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-
pyrimidine-4,6-dione (5h): Brown Solid, M.W. 319.29. Yield 78%; m.p. 203-208°C; 1H NMR (DMSO-d6): δ 2.57 (2H, s, J = 40, trans-CH=CH), 4.24 (1H, s, -CH of pyrimidine ring at C-5), 6.92-8.07 (4H, m, Ar-H), 10.68 (1H, s, barbituric acid NH), 10.75 (1H, s, barbituric acid NH); 13C NMR (DMSO-
d6): δ 77.32 (C-7), 114.24 (C-9), 114.83 (C-8), 122.09 (C-13), 125.37 (C-12, C-14), 129.53 (C-11, C-15), 132.82 (C-10) 156.32 (C-7), 162.05 (C-4, C-6), 168.41 (C-2); FT-IR (KBr cm⁻¹): 1139.31 (C=O), 1351 (C-NO2), 1617 (C=O aromatic), 1700 (C=O); 3436 (N-H); Anal. Calcld. 
C13H13N2O5S; C 51.49, H 2.99, N 13.86 (%). Found: C 51.46, H 2.97, N 13.89 (%).

5-[(3-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-
pyrimidine-4,6-dione (5i): Orange Solid, M.W. 303.23. Yield 71%; m.p. 209-213°C; 1H NMR (DMSO-d6): δ 2.59 (2H, s, J = 167, trans-CH=CH), 3.65 (1H, s, -CH of pyrimidine ring at C-5), 6.78-7.08 (4H, m, Ar-H), 8.76 (1H, s, barbituric acid NH), 7.97 (1H, s, barbituric acid NH); 13C NMR (DMSO-
d6): 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), 131.18 (C-10) 157.53 (C-7), 161.35 (C-4, C-6), 169.34 (C-2); FT-IR (KBr cm⁻¹): 1348 (C=O), 1609 (C=C aromatic), 1693 (C=O), 2536 (C=N); 3329 (N-H); Anal. Calcld. 
C13H11N2O5S; C 48.90, H 2.84, N 13.16 (%). Found: C 48.87, H 2.91, N 13.14 (%).

5-[(3-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-
pyrimidine-4,6-dione (5j): Dark brown Powder, M.W. 319.29, Yield 88%; m.p. >250°C; 1H NMR (DMSO-d6): δ 2.56 (2H, s, J = 16, trans-
-CH=CH), 3.69 (1H, s, -CH of pyrimidine ring at C-5), 6.71-7.24 (2H, dd, Ar-H), 10.65 (1H, s, barbituric acid NH), 10.77 (1H, s, barbituric acid NH); 13C NMR (DMSO-
d6): δ 77.72 (C-5), 112.78 (C-9), 114.07 (C-8), 123.02 (C-13), 127.30 (C-14), 128.86 (C-11, C-12), 131.52 (C-10) 156.02 (C-7), 163.07 (C-4, C-6), 167.37 (C-2); FT-IR (KBr cm⁻¹): 766.23 (C=Cl), 1139.45 (C-O), 1621.61 (C=C aromatic), 1700.22 (C=O), 3438 (N-H); Anal. Calcld. 
C13H12ClN2O4S; C 53.35, H 3.13, N 9.57 (%). Found: C 53.33, H 3.10, N 9.58 (%).
(5)-3-(p-tolylacryloyl)pyrimidine-2,4,6(1H,3H,5H)-trione (5J): Dark brown Powder, M.W. 288.32, Yield 75%; m.p. >250°C; 'H NMR (DMSO-_d6): δ 2.54 (2H, s, J = 16, trans-CH=CH), 4.24 (1H, s, CH of pyrimidine ring at C-5), 6.68-7.71 (7H, m, Ar-H), 7.08 (1H, s, barbituric acid NH), 7.89 (1H, s, barbituric acid NH), 8.03 (1H, s, barbituric acid NH); 13C NMR (DMSO-d6): δ 77.39 (C-5), 113.58 (C-9), 113.37 (C-8), 121.05-134.52 (C-of Naphthalene ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); FT-IR (KBr, cm^-1): 3345(N-H); Anal. Calcd. C_{16}H_{13}N_{3}O_{6}: C 48.90, H 3.95, N 9.11 (%). Found: C 48.92, H 3.95, N 9.09 (%).

5-(3-Naphthalen-2-yl-acryloyl)-2-thioxo-dihydro-pyrimidine-4,6-dione (5k): Brown powder, M.W. 308.29, Yield 93%; m.p. 187-193°C; 'H NMR (DMSO-d6): δ 2.59 (2H, s, J = 32, trans-CH=CH), 4.24 (1H, s, CH of pyrimidine ring at C-5), 6.68-7.71 (7H, m, Ar-H), 10.62 (1H, s, barbituric acid NH), 10.78 (1H, s, barbituric acid NH); 13C NMR (DMSO-d6): δ 77.39 (C-5), 113.58 (C-9), 113.37 (C-8), 121.05-134.52 (C-of Naphthalene ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); FT-IR (KBr, cm^-1): 1141 (C-O), 1619.32 (C=O-acetic), 1698 12 (C=O), 3439 (N-H); Anal. Calcd. C_{16}H_{13}N_{3}O_{6}: C 66.23, H 3.95, N 9.09 (%). Found: C 66.25, H 3.95, N 9.11 (%).

5-(3-Naphthalen-2-yl-acryloyl)-2-thioxo-dihydro-pyrimidine-4,6-dione (5k): Dark brown Powder, M.W. 324.35, Yield 65%; m.p. >250°C; 'H NMR (DMSO-d6): 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); 13C NMR (DMSO-d6): δ 76 (C-5), 114.52 (C-9), 114.77 (C-8), 121.95-132.67 (C-of Naphthalene ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); FT-IR (KBr, cm^-1): 1141 (C-O), 1610.19 (C=O-acetic), 1697 (C=O), 3439 (N-H); Anal. Calcd. C_{16}H_{13}N_{3}O_{6}: C 62.95, H 3.73, N 8.64 (%). Found: C 62.92, H 3.69, N 8.67 (%).

Biological assay

In vitro antimicrobial activity evaluation: The synthesized barbituric acid and thiobarbituric acid derivatives 5 (a-a) and 5 (a-k) were examined for antimicrobial activity against several bacteria (Bacillus subtilis MTCC 441, P. aeruginosa MTCC 1688, E. coli MTCC 443) and fungi (C. albicans MTCC 227). 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 c.f.u.) and allowed to stay at 37°C for 3 h. Control experiments were carried out under similar condition by using Ciprofloxacin (and Fluconazole and Griseofulvin) standard drugs for antibacterial and antifungal activity respectively. All of the plates were incubated at 37°C for 18 to 24 h for bacteria and at 28°C for 48 to 96 h for fungi. The zones of growth inhibition around the disks were measured after 18 to 24 h of incubation at 37°C for bacteria and 48 to 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 10-23 mm for all the sensitive bacteria, and ranged from 10-25 mm for fungal strains (Table 1).
Effects of chalcones of barbituric acid and thiobarbituric acid on Bacillus subtilis MTCC-441: The MIC in µg/mL and Zone of inhibition in mm for B. subtilis are given in Table 2. For compounds such 5a and 5a’ without any substituent at ortho, meta and para position of the aryl ring attached as HC=HC of chalcone motif showed MIC 600 µg/mL and 100 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC (Minimum Inhibition Concentration) than that of chalcones barbituric acid. In the case of compounds such 5b and 5b’ which have hydroxyl group at ortho position of the aryl ring at chalcone motif showed MIC 100 µg/mL and 62.5 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due to electron releasing nature of thioxo than the ketone. The compounds such 5c and 5c’ which have hydroxyl group at para position of the aryl ring at chalcone motif showed MIC 600 µg/mL and 100 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone. A very interesting case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid due to electron releasing nature of thioxo than the ketone. For compounds such 5g and 5g’ which have electron donating chloro substituent at ortho position of the aryl ring at chalcone motif showed MIC >600 µg/mL and 250 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone and electron donating chloro substituent at para position intensity in the MIC. In case of compounds such 5i and 5i’ which have strong electron donating nitro substituent at meta position of the aryl ring at chalcone motif showed MIC >600 µg/mL and 200 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone and strong electron donating chloro substituent of nitro group at meta position intensify in MIC in case of Bacillus subtilis MTCC441 as the bacterial strain. For the compounds such 5i and 5i’ which have strong electron donating nitro substituent at para position of the aryl ring at chalcone motif showed MIC 60 µg/mL and 100 µg/mL respectively in this case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid due electron withdrawing nature of Nitro group at para position and less electron withdrawing nature of ketone group than thioxo group. From compounds 5h-5h’ having one electron withdrawing group at meta position where as 5i-5i’ having two electron withdrawing group at meta and para position, the presence of two electron withdrawing increases in MIC values than one in at meta position. In the case of compounds such 5j and 5j’ which have strong electron donating methyl substituent at para position of the aryl ring at chalcone motif showed MIC 100 µg/mL and 250 µg/mL respectively in this case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid due electron releasing nature of methyl group at para position. In compounds such 5k-5k’ naphthalene ring similar to 5a-5a’ where as phenyl ring attached as HC=HC of chalcone motif showed MIC >600 µg/mL and 200 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more potency towards MIC than that of the barbituric acid. Thus from MIC values it has proved that the substituent at para position is responsible for Bacillus subtilis as the bacterial strains in case of barbituric acid based chalcones while rest of the compounds having substituent at ortho and meta position in this case chalcones of thiobarbituric acid were more potent than the chalcones of barbituric acid (Table 2; Figure 2).

Effects of Chalcones of barbituric acid and Thiobarbituric acid on P. aeruginosa MTCC 1688: From the MIC values of chalcones of thiobarbituric acid showed better potency towards P. aeruginosa as bacterial strain than that of the barbituric acid. In this case the thioketo group is intensifying the interaction with P. aeruginosa than the ketonic group. It proves that there is increase in interaction of P. aeruginosa and chalone motif of thixo group (Table 3; Figure 3).

Effects of chalcones of barbituric acid and thiobarbituric acid on E. coli MTCC 443: From MIC values it has proved that the substituents such as -Methoxy, -Nitro and -Methyl group at para position for 5d, which have two electron donating Methoxy group at meta and para position of the aryl ring at chalcone motif showed MIC 600 µg/mL and 250 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone and methoxy group at meta position reduces in the MIC. In the case of compounds such 5f and 5f’ which have electron with drawing chloro substituent at ortho position of the aryl ring at chalcone motif showed MIC >600 µg/mL and 62.5 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone. For compounds such 5g and 5g’ which have electron donating chloro substituent at ortho position of the aryl ring at chalcone motif showed MIC >600 µg/mL and 250 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone and electron donating chloro substituent of nitro group at para position intensity in the MIC. In case of compounds such 5i and 5i’ which have strong electron donating nitro substituent at meta position of the aryl ring at chalcone motif showed MIC >600 µg/mL and 200 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more MIC than that of barbituric acid due electron releasing nature of thioxo than the ketone and strong electron donating chloro substituent of nitro group at meta position intensify in MIC in case of Bacillus subtilis MTCC441 as the bacterial strain. For the compounds such 5i and 5i’ which have strong electron donating nitro substituent at para position of the aryl ring at chalcone motif showed MIC 60 µg/mL and 100 µg/mL respectively in this case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid due electron withdrawing nature of Nitro group at para position and less electron withdrawing nature of ketone group than thioxo group. From compounds 5h-5h’ having one electron withdrawing group at meta position where as 5i-5i’ having two electron withdrawing group at meta and para position, the presence of two electron withdrawing increases in MIC values than one in at meta position. In the case of compounds such 5j and 5j’ which have strong electron donating methyl substituent at para position of the aryl ring at chalcone motif showed MIC 100 µg/mL and 250 µg/mL respectively in this case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid due electron releasing nature of methyl group at para position. In compounds such 5k-5k’ naphthalene ring similar to 5a-5a’ where as phenyl ring attached as HC=HC of chalcone motif showed MIC >600 µg/mL and 200 µg/mL respectively in this case the chalcones of thiobarbituric acid showed more potency towards MIC than that of the barbituric acid. Thus from MIC values it has proved that the substituent at para position is responsible for Bacillus subtilis as the bacterial strains in case of barbituric acid based chalcones while rest of the compounds having substituent at ortho and meta position in this case chalcones of thiobarbituric acid were more potent than the chalone motif of thixo group (Table 2; Figure 2).

Effects of Chalcones of barbituric acid and Thiobarbituric acid on P. aeruginosa MTCC 1688: From the MIC values of chalcones of thiobarbituric acid showed better potency towards P. aeruginosa as bacterial strain than that of the barbituric acid. In this case the thioketo group is intensifying the interaction with P. aeruginosa than the ketonic group. It proves that there is increase in interaction of P. aeruginosa and chalone motif of thixo group (Table 3; Figure 3).

Effects of chalcones of barbituric acid and thiobarbituric acid on E. coli MTCC 443: From MIC values it has proved that the substituents such as -Methoxy, -Nitro and -Methyl group at para position for 5d,
5i and 5j compounds respectively. Chalcones of the barbituric acid are more potent than the chalcones of thiobarbituric acid vice versa (Table 4; Figure 4).

Effects of chalcones of barbituric acid and thiobarbituric acid on C. albicans MTCC 227 as the fungal strain: The MIC and Zone of inhibition for C. albicans are given in Table 5. From the MIC values it has been proved that the chalcone motif of thiobarbituric acid possessing various functional group at -Meta-Para, -Ortho and -Meta positions in 5e’, 5g’ and 5h’ molecules shows better activity than the rest of molecules, whereas the chalcones molecules of barbituric acid shows better activity independent of substituent and its positions (Figure 5 and Supplementary figures).

**Conclusion**

In Summary, from the antimicrobial study we conclude that, for gram positive bacterial strain B. subtilis Ortho and Para position of the chalcones of barbituric acid is more responsible whereas in rest of the compounds thioxo group plays crucial role for the activity. For gram negative bacterial strains such as P. aeruginosa, thioxo group is more responsible for activity than the oxo group whereas in E. coli, the position of functional group at para position is more responsible independent of nature of functional group. For fungal strain i.e., C. albican nature of functional group and position of functional group both factors are responsible for activity. The results unfold the way for investigation of new potential lead compounds for investigating antimicrobial activity. The present investigation showed that chalcone compounds of barbituric acid and thiobarbituric acid can be potential lead for development of new antibacterial and antifungal agents. On the basis of comparative analysis chalcones compound containing -thioxo group is more potent than the -oxo group. In future, pyrimidine based chalcones will use for the further development of new biological entity with reference to nature of functional group and its position.
From the comparative antimicrobial activity analysis of both of the series elucidated that it has been proved that the chalcone compounds containing -oxo group are more potent towards bacterial as well as fungal strains than the -oxo group.

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Author Contributions

Bhaveshkumar D Dhorajiya conducted all the experimental section. Bhaveshkumar D Dhorajiya also wrote the manuscript which was revised by Bharatkumar Z Dholakiya. Both authors gave the approval to this final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References


Table 5: Comparative analysis data MIC and Zone of inhibition values of barbituric acid and thiobarbituric acid based chalcones with respect to Candida albicans.

### Table 5: Comparative analysis data MIC and Zone of inhibition values of barbituric acid and thiobarbituric acid based chalcones with respect to Candida albicans.

<table>
<thead>
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<th>Compounds code</th>
<th>C. albicans</th>
<th>MTCC-227</th>
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<tbody>
<tr>
<td></td>
<td>Barbituric acid</td>
<td>MIC µg/mL</td>
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<tr>
<td>5 (a-a’)</td>
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<td>25</td>
</tr>
<tr>
<td>5 (b-b’)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5 (c-c’)</td>
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<tr>
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<tr>
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Table 5: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to Eschschencia col with standard drugs.

![Figure 4: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to Eschschencia col with standard drugs.](image1)

![Figure 5: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to Candida albicans with standard drugs.](image2)


