

# 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) <sup>1</sup>H NMR, <sup>13</sup>C-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 -thio keto 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 sulphadiazine as antibacterial [8] sulphadoxin 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 antimicrobial 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 Veego electronic apparatus VMP-D (Veego Instrument Corporation, Mumbai, India) and are uncorrected. IR spectra (4000-400 cm<sup>-1</sup>) of synthesized compounds were recorded on a Perkin Elmer-Spectrum RX-FTIR 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. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on an Advance-II (Bruker) model using DMSO as a solvent and TMS as internal standard with <sup>1</sup>H resonant frequency of 400 MHz and <sup>13</sup>C resonant frequency of 100 MHz. The <sup>1</sup>H NMR and <sup>13</sup>C NMR chemical shifts were reported as parts per million (ppm) downfield from TMS

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(Me<sub>4</sub>Si). The splitting patterns are designated as follows; s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. All standard strains for screening of antibacterial and antifungal activities were procured from 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 barbituric acid (3)

To a solution of diethylmalonate **1** (20 g, 118.9 mmol), urea **2** 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 **1** (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<sub>2</sub>SO<sub>4</sub> 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**) and thiobarbituric acid (**3'**) (6.4 g, 44.39 mmol) in acetic anhydride (150 ml), few drops of H<sub>2</sub>SO<sub>4</sub> 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, 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].

### General synthetic procedure for the thiobarbituric acid based compounds 5 (a'-k')

To a well stirred solution of compound 5-acetyl thiobarbituric 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].

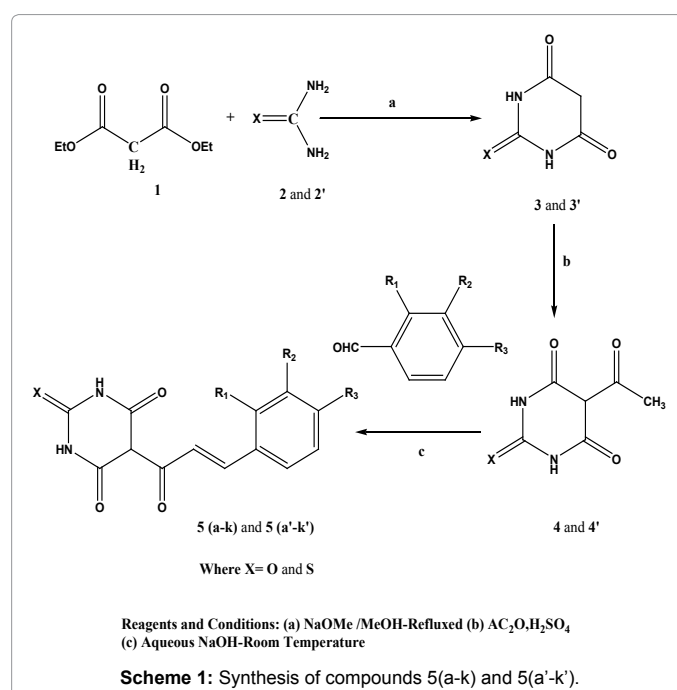
### Chemistry

The synthetic strategy adopted to obtain the target compounds were depicted in Scheme 1. Diethylmalonate on reaction with urea and thiourea at 65°C yielded barbituric acid and thiobarbituric acid. The key intermediate 5-acetyl barbituric acid and 5-acetyl thiobarbituric acid were prepared in an excellent yield by refluxing compound barbituric acid and thiobarbituric acid with acetic anhydride in the presence of H<sub>2</sub>SO<sub>4</sub> respectively. Compounds such as 5-acetyl barbituric acid and 5-acetyl thiobarbituric acid were treated with aromatic aldehydes (a-k) gave the corresponding chalcone derivatives **5 (a-k)** and **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 FT-IR spectra of compounds **5 (a-k)** and **5 (a'-k')** revealed a characteristic bands between 3010 cm<sup>-1</sup> and 3085 cm<sup>-1</sup> confirms the presence of (C=C) groups. Furthermore, in the FT-IR spectra the bands between 1682-1697 cm<sup>-1</sup> confirms the presence of (C=O) group. Moreover, a characteristic band appeared at 2526-2544 cm<sup>-1</sup> corresponded to the presence of (C=S) groups. The FT-IR spectra of compounds **5a** and **5d'** revealed peaks at 1512 cm<sup>-1</sup>



and 1522  $\text{cm}^{-1}$ , which shows partial *H-Bonding* between H-atom of olefinic carbon atom and O-atom of pyrimidine ring. The  $^1\text{H}$  NMR data of chalcone derivatives showed signals between 6.48 - 7.67  $\delta$  ppm for aromatic protons corresponding to the phenyl ring. The  $^1\text{H}$  NMR data of compounds revealed signals between 7.08-11.73  $\delta$  ppm -NH of pyrimidine ring. From the  $^1\text{H}$  NMR of compounds 5-acetyl barbituric acid and 5-acetyl thiobarbituric acid and its chalcones proves that when chalcones forms -NH protons of the pyrimidine ring shifts towards the up field. Tautomeric study is important for other areas of chemistry. From  $^1\text{H}$  NMR signal at 12.34-17.36  $\delta$  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 tinctorial 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;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  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),  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  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,  $\text{cm}^{-1}$ ): 1136.11 (C-O), 1612.21 (C=C aromatic), 1696.32 (C=O), 3438.52 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4$ : C 60.47, H 3.09, N 10.84 (%). Found: C 60.45, H 3.05, N 10.87 (%).

**5-(3-Phenyl-acryloyl)-2-thioxo-dihydro-pyrimidine-4,6-dione (5a')**: Yellow Solid, M.W.274.30, Yield 77%; m.p. >250°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  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),  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  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); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1251.15 (C-SH), 1612.12 (C=C aromatic), 1696.23 (C=O), 3335.52 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_3\text{S}$ : 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]pyrimidine-2,4,6-trione (5b):** Brown Solid, M.W.274.22, Yield 81%; m.p. 293-296°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  2.58 (2H, s,  $J = 28$ , trans-CH=CH), 4.22 (1H, s, -CH of pyrimidine ring at C-5), 6.52-7.97 (4H, m, Ar-H), 10.61 (1H, s, barbituric acid NH), 10.85 (1H, s, barbituric acid NH) 6.52 (1H, s, o-Ar-OH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  77 (C-5), 116.56 (C-9), 117.16 (C-8), 123.28 (C-13, C-14), 127.29 (C-12, C-15), 130.71 (C-11), 132.91 (C-10) 156.23 (C-7), 167.08 (C-4, C-6), 169.52 (C-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1138.21 (C-O), 1614.24 (C=C aromatic), 1698.32 (C=O), 3437.23 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_5$ : C 56.94, H 3.68, N 10.22 (%). Found (%): 56.91, H 3.65, N 10.25 (%).

**5-[3-(2-Hydroxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione(5b')**: Light Brown Solid, M.W. 290.29, Yield 58%; m.p. >250°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  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);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  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); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1253.21 (C-SH), 1608.14 (C=C aromatic), 1693.36 (C=O), 3339.27 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4\text{S}$ : C 53.79, H 3.47, N 9.65 (%). Found (%): 53.76, H 3.51, N 9.62(%).

**5-[3-(4-Hydroxy-phenyl)-acryloyl]pyrimidine-2,4,6-trione (5c):** Yellow Solid, M.W. 274.22, Yield 79%; m.p. 206-210°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  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);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  79 (C-5), 114.08 (C-9), 115.81 (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-4, C-6), 167.35 (C-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1140.24 (C-O), 1616.07 (C=C aromatic), 1697.19 (C=O), 3437.45 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_5$ : C 56.94, H 3.68, N 10.22 (%). Found: 56.97, H 3.72, N 10.24 (%).

**5-[3-(4-Hydroxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5c')**: Dark Brown Solid, M.W. 290.29, Yield 82%; m.p. >250°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  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);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  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); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1257.33 (C-SH), 1614.17 (C=C aromatic), 1689.15 (C=O), 3328.54 (N-H); Anal. Calcd. For  $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_4\text{S}$ : C 53.79, H 3.47, N 9.65 (%). Found: 53.74, H 3.50, N 9.61(%).

**5-[3-(3-Methoxy-phenyl)-acryloyl]pyrimidine-2,4,6-trione (5d):** Yellow Solid, M.W.288.66, Yield 83%; m.p. 228-233°C;  $^1\text{H}$  NMR (DMSO- $d_6$ ):  $\delta$  2.62 (2H, s,  $J = 12$ , trans-CH=CH), 4.20 (1H, s, -CH of pyrimidine ring at C-5), 3.09 (3H, s, m-OCH<sub>3</sub>), 6.67-7.77 (2H, dd, Ar-H), 10.62 (1H, s, barbituric acid NH), 10.79 (1H, s, barbituric acid NH);  $^{13}\text{C}$  NMR (DMSO- $d_6$ ):  $\delta$  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-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1136.78 (C-O), 1613.24 (C=C aromatic), 1698.87 (C=O), 3439.33 (N-H); Anal. Calcd. For  $\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_5$ : C 58.33, H 4.20, N 9.72 (%). Found: C 58.31, H 4.23, N 9.75 (%).

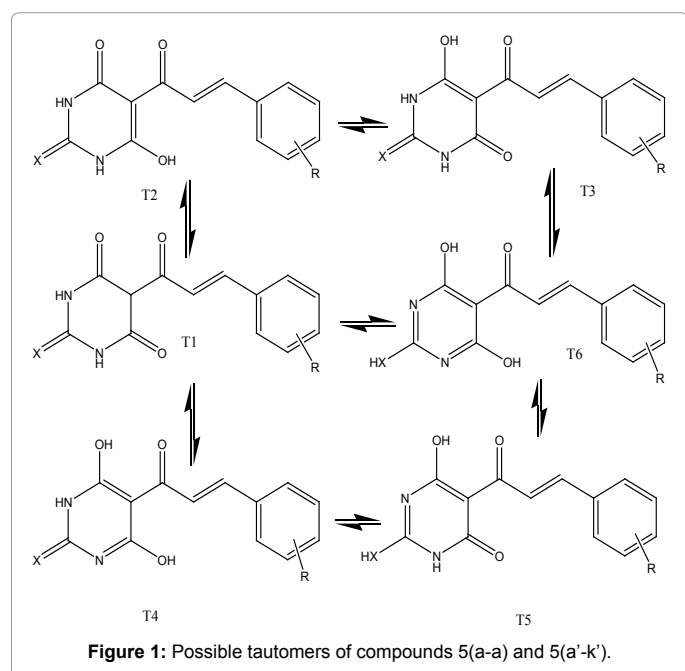


Figure 1: Possible tautomers of compounds **5(a-a')** and **5(a'-k')**.

**5-[3-(3-Methoxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5d')**: Yellow Solid, M.W.304.32. Yield 62%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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<sub>3</sub>), 6.78-7.08 (2H, dd, Ar-H), 7.90 (1H, s, barbituric acid NH), 7.93 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr, cm<sup>-1</sup>): 1260.73 (C-SH), 1603.57 (C=C aromatic), 1687.97 (C=O), 3343.39(N-H); Anal. Calcd. For C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>S: 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]pyrimidine-4,6-trione (5e)**: Yellow Solid, M.W. 318.28, Yield 83%; m.p. 191-196°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 2.57 (2H, s, *J* = 24, trans-CH=CH), 4.24 (1H, s, -CH of pyrimidine ring at C-5), 2.98 (3H, s, m-OCH<sub>3</sub>), 3.09 (3H, s, p-OCH<sub>3</sub>), 6.67-7.79 (2H, dd, Ar-H), 10.68 (1H, s, barbituric acid NH), 10.89 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 57.36 (C-18, C-17) 76.23 (C-5), 113.96 (C-9), 115.17 (C-8), 123.55 (C-11, C-15), 128.78 (C-12, C-14), 130.87 (C-10, C-13), 157.32 (C-7), 161.69 (C-4, C-5), 168.56 (C-2); FT-IR (KBr, cm<sup>-1</sup>): 1138.58 (C-O), 1615.17 (C=C aromatic), 1699.47 (C=O), 3436.56 (N-H); Anal. Calcd. C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>6</sub>: C 56.60, H 4.43, N 8.80 (%). Found: C 56.58, H 4.26, N 8.82 (%).

**5-[3-(3,4-Dimethoxy-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5e')**: Dark Yellow Solid, M.W. 334.35, Yield 74%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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<sub>3</sub>), 4.09 (3H, s, p-OCH<sub>3</sub>), 6.75-7.18 (2H, dd, Ar-H), 7.76 (1H, s, barbituric acid NH), 7.95 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr, cm<sup>-1</sup>): 1261.69 (C-SH), 1607.12 (C=C aromatic), 1685.43 (C=O), 3337.64 (N-H); Anal. Calcd. C<sub>15</sub>H<sub>14</sub>N<sub>2</sub>O<sub>5</sub>S: 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]pyrimidine-2,4,6-trione (5f)**: Yellow Solid, M.W. 292.67, Yield 69%; m.p. 237-242°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 2.59 (2H, s, *J* = 28, trans-CH=CH), 4.25 (1H, s, -CH of pyrimidine ring at C-5), 6.59-7.94 (2H, m, Ar-H), 10.62 (1H, s, barbituric acid NH), 10.81 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 75.31 (C-5), 114.67 (C-9), 115.37 (C-8), 123.28 (C-13, C-14), 127.19 (C-12, C-15), 128.89 (C-11), 130.84 (C-10) 156.32 (C-7), 161.68 (C-4, C-6), 167.28 (C-2); FT-IR (KBr, cm<sup>-1</sup>): 764.13 (C-Cl), 1137.69 (C-O), 1620.23 (C=C aromatic), 1701.51 (C=O), 3434.32 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>4</sub>: C 53.35, H 3.10, N 9.57 (%). Found: C 53.38, H 3.13, N 9.55 (%).

**5-[3-(2-Chloro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5f')**: Brown Solid, M.W. 308.74, Yield 84%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr, cm<sup>-1</sup>): 1254.71 (C-SH), 1615.42 (C=C aromatic), 1690.56 (C=O), 3334.28 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>3</sub>S: 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]pyrimidine-2,4,6-trione (5g)**: Yellow Solid, M.W. 292.67, Yield 80%; m.p. 258-263°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 2.56 (2H, s, *J* = 28, trans-CH=CH), 4.23 (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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 77.72 (C-5), 112.78 (C-9), 114.07 (C-8), 123.02 (C-13), 127.30 (C-14, C-15), 128.86 (C-11, C-12), 131.52 (C-10) 156.02 (C-7), 163.07 (C-4, C-6), 167.57 (C-2); FT-IR (KBr, cm<sup>-1</sup>): 766.23 (C-Cl), 1139.45 (C-O), 1621.61 (C=C aromatic), 1700.22 (C=O), 3438 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>4</sub>: C 53.35, H 3.13, N 9.57 (%). Found (%): C 53.33, H 3.10, N 9.58 (%).

**5-[3-(4-Chloro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5g')**: Dark brown Powder, M.W. 308.74, Yield 86%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr, cm<sup>-1</sup>): 1604 (C=C aromatic), 1684 (C=O), 2527 (C=S), 3331(N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>3</sub>S: 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]pyrimidine-2,4,6-trione (5h)**: Yellow Solid, M.W. 303.23, Yield 78%; m.p. 203-208°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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<sup>-1</sup>): 1139.31 (C-NO<sub>2</sub>), 1351 (C-NO<sub>2</sub>), 1617 (C=C aromatic), 1700 (C=O), 3436 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>6</sub>: C 51.49, H 2.99, N 13.86 (%). Found: C 51.46, H 2.97, N 13.89 (%).

**5-[3-(3-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5h')**: Brown Solid, M.W. 319.29, Yield 88%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr, cm<sup>-1</sup>): 1348 (C-NO<sub>2</sub>), 1609 (C=C aromatic), 1693 (C=O), 2536 (C=S), 3329(N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>5</sub>S: 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]pyrimidine-2,4,6-trione (5i)**: Orange Solid, M.W. 303.23, Yield 71%; m.p. 209-213°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): δ 2.59 (2H, s, *J* = 8, trans-CH=CH), 4.27 (1H, s, -CH of pyrimidine ring at C-5), 6.76-8.14 (2H, dd, Ar-H), 10.66 (1H, s, barbituric acid NH), 10.75 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): δ 76.03 (C-7), 114.06 (C-9), 115.81 (C-8), 122.26 (C-13), 125.33 (C-12, C-14), 128.81 (C-11, C-15), 132.81 (C-10) 157.33 (C-7), 162.54 (C-4, C-6), 168.54 (C-2); FT-IR (KBr, cm<sup>-1</sup>): 1142.13 (C-O), 1355.12 (C-NO<sub>2</sub>), 1618 (C=C aromatic), 1698.56 (C=O), 3437 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>6</sub>: C 51.49, H 2.99, N 13.86 (%). Found: C 51.48, H 2.96, N 13.87 (%).

**5-[3-(4-Nitro-phenyl)-acryloyl]-2-thioxo-dihydro-pyrimidine-4,6-dione (5i')**: Dark brown Powder, M.W. 319.29, Yield 82%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>): 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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>): 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); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1353 (C-NO<sub>2</sub>), 1613 (C=C aromatic), 1687 (C=O), 2535 (C=S), 3327 (N-H); Anal. Calcd. C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>6</sub>: C 48.90, H 2.84, N 13.16 (%). Found: C 48.92, H 2.81, N 13.17 (%).

**5-(3-p-tolylacryloyl)pyrimidine-2,4,6(1H,3H,5H)-trione (5j):** Yellow solid, M.W. 272.25, Yield 87%; m.p. 182-187°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.61 (2H, s, *J* = 16, trans-CH=CH), 2.85 (s, 3H, -CH<sub>3</sub>), 4.22 (1H, s, -CH of pyrimidine ring at C-5), 6.75-8.14 (2H, dd, Ar-H), 10.66 (1H, s, barbituric acid NH), 10.72 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  77.37 (C-5), 112.58 (C-9), 113.16 (C-8), 122.08 (C-13), 127.40 (C-12, C-14), 128.17 (C-11, C-15), 133.02 (C-10), 157.56 (C-7), 167.52 (C-4, C-6), 168.64 (C-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1137 (C-O), 1614 (C=C aromatic), 1695 (C=O), 3443 (N-H); Anal. Calcd. C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>: C 61.74, H 4.44, N 10.29 (%). Found: C 61.74, H 4.46, N 10.31 (%).

**dihydro-2-thioxo-5-(3-p-tolylacryloyl)pyrimidine-4,6(1H,5H)-dione (5j<sup>1</sup>):** Brown powder, M.W. 288.32, Yield 75%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  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), 7.08 (1H, s, barbituric acid NH), 7.81 (1H, s, barbituric acid NH); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  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); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1607 (C=C aromatic), 1682 (C=O), 2544 (C=S), 3345 (N-H); Anal. Calcd. C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S: 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)pyrimidine-2,4,6-trione (5k):** Yellow Solid, M.W. 308.29, Yield 93%; m.p. 187-193°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  77.39 (C-5), 113.58 (C-9), 113.37 (C-8), 121.05-134.52 (C-of Naphthalen ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1141 (C-O), 1619.32 (C=C aromatic), 1698 12 (C=O), 3439 (N-H); Anal. Calcd. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>: C 66.23, H 3.92, 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<sup>1</sup>):** Dark brown Powder, M.W. 324.35, Yield 65%; m.p. >250°C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  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); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  76 (C-5), 114.52 (C-9), 114.77 (C-8), 122.95-132.67 (C-of Naphthalen ring), 156.13 (C-7), 164.73 (C-4, C-6), 169.54 (C-2); FT-IR (KBr,  $\text{cm}^{-1}$ ): 1610 (C=C aromatic), 1697 (C=O), 2527 (C=S), 3338 (N-H); Anal. Calcd. C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S: 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<sup>1</sup>-k<sup>1</sup>) 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) species using paper disc diffusion technique. The Mullere Hinton agar media were sterilized (autoclaved at 120°C for 30 min), poured at uniform depth of 5 mm and allowed to solidify. The microbial suspension (10<sup>5</sup> CFU/mL) (0.5 McFarland Nephelometry Standards) was streaked over the surface of media using a sterile cotton swab to ensure even

growth of the organisms. The tested compounds were dissolved in dimethylsulfoxide to give solutions of 3.12-100 mg/mL. Sterile filter paper discs measuring 6.25 mm in diameter (Whatman No. 1 filter paper), previously soaked in a known concentration of the respective test compound in dimethylsulfoxide were placed on the solidified nutrient agar medium that had been inoculated with the respective microorganism and the plates were incubated for 24 h at (37 ± 1)°C. A control disc impregnated with an equivalent amount of dimethyl sulfoxide without any sample was also used and did not produce any inhibition. Ciprofloxacin (100 mg/ disc) were used as control drugs for antibacterial, Flucanazole and Griseofulvin for antifungal activity. MIC of the compound was determined by agar streak dilution method. A stock solution of the synthesized compound (100 mg/mL) in dimethylsulfoxide was prepared and graded quantities of the test compounds were incorporated in a specified quantity of molten sterile agar, i.e., nutrient agar for evaluation of antibacterial and sabouraud dextrose agar for antifungal activity, respectively. The medium containing the test compound was poured into a Petri dish at a depth of 4-5 mm and allowed to solidify under aseptic conditions. A suspension of the respective microorganism of approximately 10<sup>5</sup> CFU/mL was prepared and applied to plates with serially diluted compounds with concentrations in the range of 3.12-100 mg/mL in dimethylsulfoxide and incubated at (37 ± 1)°C for 24 h (bacteria) or 48 h (fungi). The lowest concentration of the substance that prevents the development of visible growth is considered to be the MIC value.

**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 four pathogenic bacteria (one Gram positive and two Gram negative) and one pathogenic fungi were investigated by the Agar disk diffusion method [25-27]. Antimicrobial activity testing was carried out by using Agar cup method. Each synthesized compounds were dissolved in dimethyl sulfoxide (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 and fungal strains were taken as a standard antibiotic for comparison of the results. All new synthesized molecules were screened for their antibacterial activities against the (*Bacillus subtilis* MTCC 441, *E. coli* MTCC 443, *P. aeruginosa* MTCC 1688) and one fungal strains (*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<sup>8</sup> 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 (Flucanazole 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, respectively. 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).

**Structure activity relationship antibacterial study:** A close investigation of the MIC values that all compounds exhibited a varied range of MIC (20-600 µg/mL) of antibacterial activity against three bacterial strains *Bacillus subtilis* MTCC 441, *P. aeruginosa* MTCC 1688 and *E. coli* MTCC 443.

**Effects of chalcones of barbituric acid and thiobarbituric acid on *Bacillus subtilis* MTCC-441:** The MIC in  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  and 62.5  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  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 for the chalcones compounds such 5d and 5d' which have electron donating Methoxy group at *para* position of the aryl ring at chalcone motif showed MIC 20  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  respectively in this case the chalcones of barbituric acid showed more MIC than that of thiobarbituric acid owing to more electron releasing nature of ketonic than the thioxo group. On other hand compounds such 5e and 5e'

which have two electron donating Methoxy group at *meta* and *para* position of the aryl ring at chalcone motif showed MIC 600  $\mu\text{g/mL}$  and 250  $\mu\text{g/mL}$  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 withdrawing chloro substituent at *ortho* position of the aryl ring at chalcone motif showed MIC >600  $\mu\text{g/mL}$  and 62.5  $\mu\text{g/mL}$  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 withdrawing chloro substituent at *para* position of the aryl ring at chalcone motif showed MIC >600  $\mu\text{g/mL}$  and 250  $\mu\text{g/mL}$  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 withdrawing nature of chloro group at *para* position intensify in the MIC. In case of compounds such 5h and 5h' which have strong electron withdrawing nitro substituent at *meta* position of the aryl ring at chalcone motif showed MIC >600  $\mu\text{g/mL}$  and 200  $\mu\text{g/mL}$  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 withdrawing nature 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 withdrawing nitro substituent at *para* position of the aryl ring at chalcone motif showed MIC 60  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  and 250  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  and 200  $\mu\text{g/mL}$  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).

Compound Code	For compound X=O	For compound X=S	R <sub>1</sub> , R <sub>2</sub> and R <sub>3</sub> are different functional groups		
			R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
5 (a-a')	5 (a)	5 (a')	-H	-H	-H
5 (b-b')	5 (b)	5 (b')	-H	-OH	-H
5 (c-c')	5 (c)	5 (c')	-H	-H	-OH
5 (d-d')	5 (d)	5 (d')	-H	-H	-OCH <sub>3</sub>
5 (e-e')	5 (e)	5 (e')	-H	-OCH <sub>3</sub>	-OCH <sub>3</sub>
5 (f-f')	5 (f)	5 (f')	-H	-Cl	-H
5 (g-g')	5 (g)	5 (g')	-H	-H	-Cl
5 (h-h')	5 (h)	5 (h')	-H	-NO <sub>2</sub>	-H
5 (i-i')	5 (i)	5 (i')	-H	-H	-NO <sub>2</sub>
5 (j-j')	5 (j)	5 (j')	-H	-H	-CH <sub>3</sub>
5 (k-k')	5 (k)	5 (k')	Naphthalene Ring		

Table 1: List of compounds with their different functional groups.

Compounds code	<i>Bacillus subtilis</i> MTCC-441									
	Barbituric acid				MIC $\mu\text{g/mL}$	Thiobarbituric acid				
	Zone of inhibition					Zone of inhibition				
	25	50	100	250		25	50	100	250	MIC $\mu\text{g/mL}$
5 (a-a')	10	10	10	10	600	10	12	14	18	100
5 (b-b')	10	10	11	12	100	11	13	17	19	62.5
5 (c-c')	10	10	10	10	600	10	13	15	18	100
5 (d-d')	10	10	13	15	20	13	16	19	21	100
5 (e-e')	10	10	10	10	600	11	14	17	19	250
5 (f-f')	10	10	10	10	>600	10	13	16	19	62.5
5 (g-g')	10	10	10	10	>600	11	13	16	18	250
5 (h-h')	10	10	10	10	>600	12	15	17	18	200
5 (i-i')	10	10	12	12	60	11	13	17	19	100
5 (j-j')	10	10	11	12	100	10	13	15	17	250
5 (k-k')	10	10	10	10	>600	10	12	15	18	200
Standard Drugs	Ciprofloxacin				05	Ciprofloxacin				05

Table 2: Comparative analysis data MIC and Zone of inhibition values of barbituric acid and thiobarbituric acid based chalcones with respect to *Bacillus subtilis* MTCC-441.

**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 chalcone motif of thioxo 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,

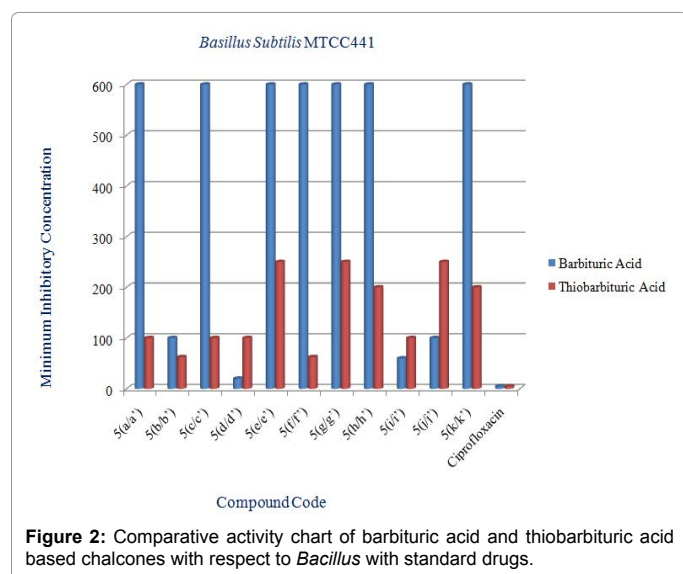


Figure 2: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to *Bacillus* with standard drugs.

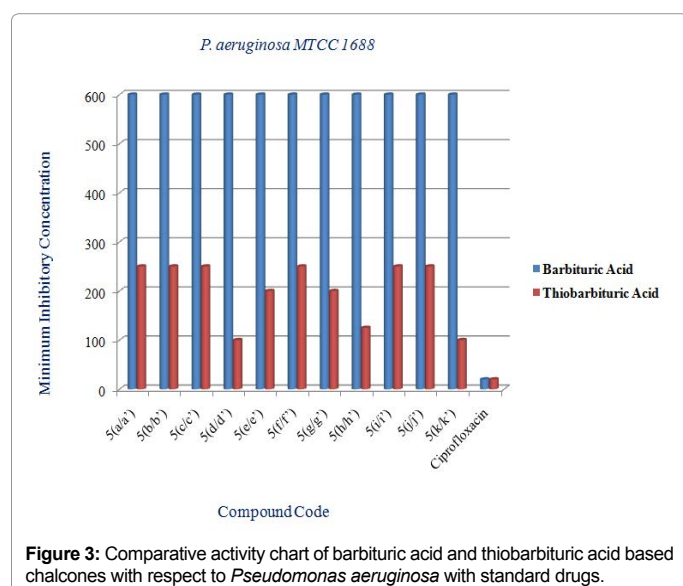


Figure 3: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to *Pseudomonas aeruginosa* with standard drugs.

5i and 5j compounds respectively. chalcones of the barbituric acid are more potent than the chalcones of thiobarbituric acid viceversa (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, where as 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 where as 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. albicans* 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.

Compounds code	<i>P. aeruginosa</i> MTCC-1688									
	Barbituric acid					Thiobarbituric acid				
	Zone of inhibition				MIC µg/mL	Zone of inhibition				MIC µg/mL
	25	50	100	250		25	50	100	250	
5 (a-a')	10	10	10	10	>600	10	13	15	17	250
5 (b-b')	10	10	10	10	600	11	13	17	19	250
5 (c-c')	10	10	10	10	600	10	14	16	18	250
5 (d-d')	10	10	10	10	>600	13	17	18	21	100
5 (e-e')	10	10	10	10	>600	11	15	18	20	200
5 (f-f')	10	10	10	10	>600	10	14	17	18	250
5 (g-g')	10	10	10	10	>600	11	12	13	15	200
5 (h-h')	10	10	10	10	600	14	16	17	19	125
5 (i-i')	10	10	10	10	600	11	13	18	20	250
5 (j-j')	10	10	10	10	>600	10	12	16	17	250
5 (k-k')	10	10	10	10	>600	10	12	15	19	100
Standard Drugs	Ciprofloxacin				20	Ciprofloxacin				20

Table 3: Comparative activity analysis data MIC and Zone of inhibition values of barbituric acid and thiobarbituric acid based chalcones with respect to *Pseudomonas aeruginosa*.

Compounds code	<i>E. coli</i> MTCC -443									
	Barbituric acid					Thiobarbituric acid				
	Zone of inhibition				MIC µg/mL	Zone of inhibition				MIC µg/mL
	25	50	100	250		25	50	100	250	
5 (a-a')	10	10	10	11	300	12	15	15	17	250
5 (b-b')	10	10	10	11	600	13	15	16	17	125
5 (c-c')	10	10	10	11	600	15	17	16	17	250
5 (d-d')	11	17	20	23	20	15	18	20	21	200
5 (e-e')	10	10	10	11	300	12	16	18	21	500
5 (f-f')	10	10	10	11	300	13	14	15	16	250
5 (g-g')	10	10	10	12	300	11	14	17	19	125
5 (h-h')	10	10	10	11	300	15	17	18	18	100
5 (i-i')	12	13	18	21	20	12	15	19	23	250
5 (j-j')	11	11	14	21	20	15	15	18	19	100
5 (k-k')	10	10	10	11	300	14	16	18	21	250
Standard Drugs	Ciprofloxacin				10	Ciprofloxacin				10

Table 4: Comparative activity analysis data MIC and Zone of inhibition values of barbituric acid and thiobarbituric acid based chalcones with respect to *Escherichia coli*.

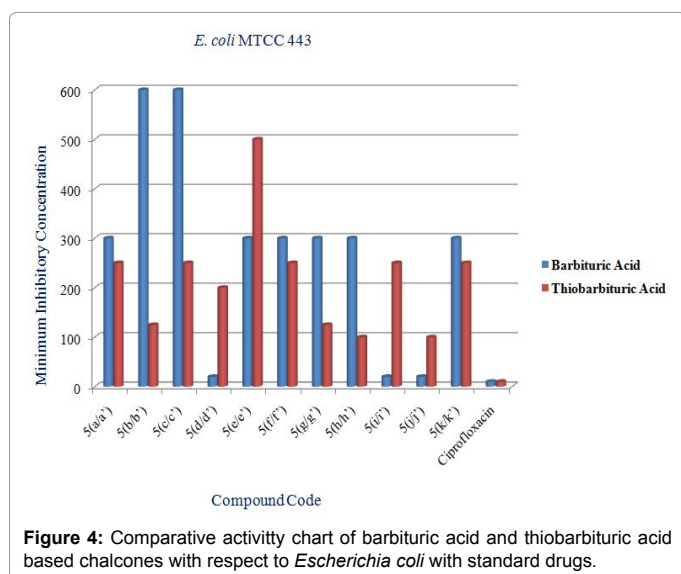


Figure 4: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to *Escherichia coli* with standard drugs.

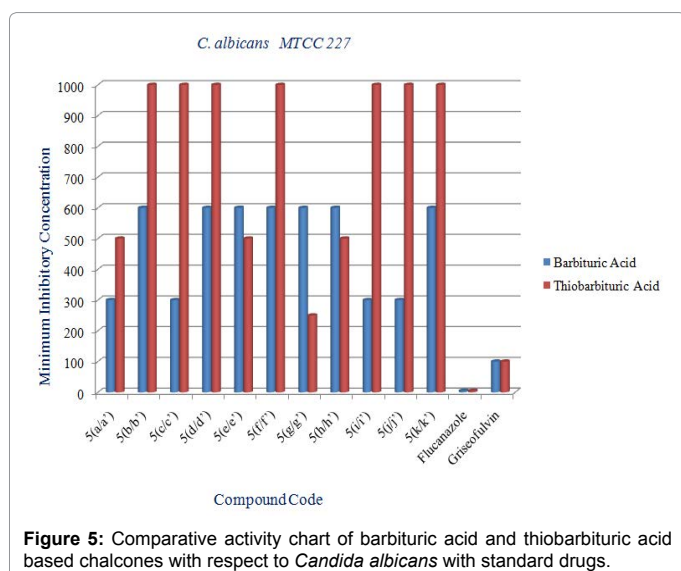


Figure 5: Comparative activity chart of barbituric acid and thiobarbituric acid based chalcones with respect to *Candida albicans* with standard drugs.

From the comparative antimicrobial activity analysis of both of the series elucidated that it has been proved that the chalcone compounds containing -thioxo 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.

Compounds code	<i>C. albicans</i> MTCC-227									
	Barbituric acid					Thiobarbituric acid				
	Zone of inhibition				MIC $\mu\text{g}/\text{mL}$	Zone of inhibition				MIC $\mu\text{g}/\text{mL}$
	25	50	100	250	$\mu\text{g}/\text{mL}$	25	50	100	250	$\mu\text{g}/\text{mL}$
5 (a-a')	10	10	10	11	300	16	18	22	22	500
5 (b-b')	10	10	10	10	600	17	19	22	24	1000
5 (c-c')	10	10	10	10	300	16	20	23	23	>1000
5 (d-d')	10	10	10	10	600	18	21	24	24	>1000
5 (e-e')	10	10	10	10	600	20	24	25	25	500
5 (f-f')	10	10	10	10	600	21	22	22	23	1000
5 (g-g')	10	10	10	10	600	20	21	22	23	250
5 (h-h')	10	10	10	10	600	21	22	23	24	500
5 (i-i')	10	10	10	10	300	20	21	25	25	1000
5 (j-j')	10	10	10	10	300	19	20	21	24	1000
5 (k-k')	10	10	10	10	>600	22	22	24	25	>1000
Standard Drugs	Flucanazole				05	Flucanazole				05
	Griseofulvin				100	Griseofulvin				100

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

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