Synthesis, Antimicrobial and Antioxidant Activity of Chalcone Derivatives Containing Thiobarbitone Nucleus

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Abstract
In this paper we reported the synthesis of novel series of 5-[1,3-bis (4-substituted phenyl) prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-diones (5a-k). The target compounds were synthesized by the Knoevenagel condensation of different chalcones (3a-k) with thiobarbituric acid using acetic acid as a catalyst in ethanol. These compounds were screened for their antimicrobial and antioxidant activities. From antimicrobial activity results it was found that compounds 5e, 5i and 5k displayed good antibacterial and antifungal activity against all tested strains. Further, the selected compounds were studied for docking using the enzyme, Glucosamine-6-phosphate synthase and the compounds 5a, 5e and 5k have emerged as an active antimicrobial agents with least binding energy (-4.52 and -4.41 kJ mol\(^{-1}\)). Compounds 5c and 5f showed promising free radical scavenging and Fe+2 ion chelating activity.

Keywords: Chalcone; Thiobarbituric acid; Knoevenagel condensation; Antimicrobial; Antioxidant; Molecular docking study

Introduction
The resistance of pathogenic microorganisms to accessible antibiotics, anxiolytics, sedatives, hypnotics and anti-convulsants is rapidly forming a foremost problem worldwide. On the other hand, prime and opportunistic fungal infections continue to rise rapidly because of the increased number of immune compromised patients [1]. In order to combat this new problem, novel, structurally diverse antibiotic compounds are very much essential [2]. The condensed thiobarbiturates possesses diverse pharmacological profile such as antimicrobial, selective cell adhesion inhibitors and DNA cleavage activities [3]. Additionally, recent literature survey has indicated that barbituric acid derivatives may also act as immune modulators [4,5]. Barbiturate and thiobarbiturate derivatives attracted considerable attention owing to their various biological effects such as inhibiting collagenase-3 (MMP-3) [6], recombinant cytome P450 enzymes [7], methionine aminopeptidase-1 (MetAP-1) [8], anti-inflammatory, analgesic [9], CYP19 inhibitor activity, molecular docking [10], cytoxicity properties [11] and broad spectrum pharmacological properties including hypotonic [12] and sedative [13]. Chalcones are known for their multiple anti-infective activities including antimalarial, antileishmanial, antitrypanosomal, antibacterial, anti-tubercular, antifungal and antiviral [14-17]. The chalcones are found to possess antioxidant activity; the impact is more acute in developing countries due to non-availability of desired medicines and emergence of widespread drug resistance. Glucosamine-6-phosphate synthase (GlcN-6-P) a key enzyme in cell wall biosynthesis catalyzes the first step in hexosamine biosynthesis, converting D-fructose 6-phosphate into D-glucosamine 6-phosphate using glutamine as the ammonia source [18-20]. GlcN-6-P is a precursor of uridine diphospho-N-acetyl glucosamine from which other molecules containing amino-sugar were derived. One of these products, N-acetyl glucosamine, is an important constituent of the peptidoglycan layer of bacterial cell walls and fungal cell wall. Accordingly, GlcN-6-P serves as a promising target for antibacterial and antifungal drug discovery. Earlier, our research group has synthesized different derivatives of benzofuran bearing barbitone and thiobarbitone moieties [21] and other biologically important heterocyclic compounds [22-27]. These results encouraged us to extend the scope of this methodology to build new systems for improving the activity of these scaffolds.

Experimental
Materials and methods
The thiobarbituric acid with 98% purity was purchased from Sigma Aldrich Company. Melting points were recorded on electro thermal melting point apparatus and are uncorrected. Column chromatography was performed using silica gel (230–400 mesh), silica gel GF254 plates from Merck were used for TLC and spots were identified under ultraviolet radiation. Ethyl acetate: pet ether was used in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer. 1HNM and 13CNMR spectra were recorded on Bruker 400 MHz spectrometer and chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. LCMS were obtained using C-18 column on Shimadzu, LCM5010A, Japan. In antimicrobial activity, the zone of inhibition and in antioxidant activity the IC\textsubscript{50} values are expressed as mean ± SD of three replicates.

General procedure for the synthesis of benzofuran barbitone derivatives (5a-k)
The mixture of 1,3-diyaryl-2-propen-1-ones (3a-k) (0.01mol) and thiobarbituric acid (0.01mol) was taken in ethyl alcohol, catalytic amount of AcOH was added and the reaction mixture was refluxed for 7 h. After the completion of reaction, the reaction mass was cooled to room temperature, poured into crushed ice and neutralized with NaHCO\textsubscript{3} solution. The product separated out was filtered, dried and recrystallized using ethanol.

5-[3-(4-Chlorophenyl)-1-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5a): Light yellow solid

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5-[(4-Methylphenyl)-1-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5b): Reddish brown solid (EtOH); m.p 281-283°C; IR (KBr, υ cm\(^{-1}\)); δ ppm): 3318 (NH), 1651, 1650 (C=O), 1358 (C=S). 1\(^{13}\)H NMR (400 MHz, DMSO-\(d_6\), δ ppm): 8.06 (s, NH), 8.44-7.80 (m, Ar-H), 7.21 (d, CH), 5.61 (d, CH), 2.32 (s, CH\(_3\)). 1\(^{13}\)C NMR (400 MHz, DMSO-\(d_6\), δ ppm): 178.3 (C=S), 167.0 (C=O), 144.8 (C=C), 131.1 (CH=CH), 126.2 (2CH); MS (LC/MS): m/z 348.3[M\(^{+}\)].

5-[(4-Methoxyphenyl)-1-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5c): Brown solid (EtOH); m.p 285-287°C; IR (KBr, υ cm\(^{-1}\)); δ ppm): 3375 (NH), 1674, 1672 (C=O), 1367 (C=S). 1\(^{13}\)H NMR (400 MHz, DMSO-\(d_6\), δ ppm): 8.08 (s, NH), 8.01-7.61 (m, Ar-H), 7.14 (d, CH), 5.64 (d, CH), 3.88 (s, OCH\(_3\)). 1\(^{13}\)C NMR (400 MHz, DMSO-\(d_6\), δ ppm): 178.4 (C=S), 167.3 (C=O), 146.6 (C=C), 131.5 (CH=CH), 126.4 (2CH); LCMS: m/z 364.7[M\(^{+}\)].

5-[(4-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5d): Light brown solid (EtOH); m.p 293-295°C; IR (KBr, υ cm\(^{-1}\)); δ ppm): 3502 (NH), 1669, 1662 (C=O), 1388 (C=S). 1\(^{13}\)H NMR (400 MHz, DMSO-\(d_6\), δ ppm): 8.70 (s, NH), 8.32-7.51 (m, Ar-H), 7.24 (d, CH), 5.62 (d, CH), 2.42 (s, CH\(_3\)). 1\(^{13}\)C NMR (400 MHz, DMSO-\(d_6\), δ ppm): 179.6 (C=S), 167.3 (C=O), 146.7 (C=C), 131.2 (CH=CH), 126.4 (2CH); LCMS: m/z 422.3[M\(^{+}\)].

5-[(1,3-Bis-(4-chlorophenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5e): Yellow solid (EtOH); m.p 272-274°C; IR (KBr, υ cm\(^{-1}\)); δ ppm): 3332 (NH), 1676, 1651 (C=O), 1354 (C=S). 1\(^{13}\)H NMR (400 MHz, DMSO-\(d_6\), δ ppm): 8.75 (s, NH), 8.30-7.51 (m, Ar-H), 7.10 (d, CH), 5.72 (d, CH). 1\(^{13}\)C NMR (400 MHz, DMSO-\(d_6\), δ ppm): 178.1 (C=S), 167.0 (C=O), 145.8 (C=C), 133.5 (C=C), 131.2 (CH=CH), 126.4 (2CH); LCMS: m/z 402.2[M\(^{+}\)].

Antimicrobial activity

Antibacterial activity of the synthesized compounds was tested against five bacterial strains and three fungal strains using agar well diffusion method [28]. Dimethyl sulfoxide was used as solvent control. The bacterial culture was inoculated on nutrient agar and fungal culture was inoculated on potato dextrose agar media (20 ml). The test compounds were dissolved in DMSO to get a concentration of 12.79M and 100 µL of this sample was loaded into the wells of agar plates directly. Plates inoculated with the bacteria were incubated at 37°C for 24 h and the fungal culture was incubated at 25°C for 72 h. All determinations were done in triplicates. The Streptomycin (1.71M and 0.85M) and Griseofulvin (3.26M and 1.6M) were used as standard drugs for antibacterial and antifungal activities respectively.

The minimum inhibitory concentration (MIC) was performed by serial broth-dilution method [29] at different concentrations like 1, 10, 25, 50 and 100 µg/mL. After the incubation period, the minimum inhibition zone at which the microorganism growth was inhibited was measured in µg/mL.

In silico molecular docking studies

The compounds synthesized in the present investigation were subjected for molecular docking studies using Auto Dock (version 4.2) with Lamarckian genetic algorithm. The synthesized compounds having 2D structure were converted to energy minimized 3D structures and were further used for in silico protein-ligand docking. The synthesized compounds were used as ligand. The docking of receptor GlcN-6-P with newly synthesized compounds exhibited well established bonds with one or more amino acids in the receptor active pocket. The active pocket was considered to be the site where glucosamine-6-phosphate complexes with GlcN-6-P of 2VF5. The active pocket consisted of Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603 [30]. The Graphical User Interface program “AutoDock Tools” was used.
to prepare, run and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added to the receptor for the preparation of protein in docking simulation. Since ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were merged.

Antioxidant activity

Free radical scavenging activity by DPPH method: Free radical-scavenging capacities of synthesized compounds were determined according to the reported procedure [32]. The newly synthesized compounds at different concentrations (25-100 µg/mL) were added to each test tube and volume was made up to 4 ml using methanol. To this, 3 ml of 0.004% DPPH in methanol was added and the mixtures were incubated at room temperature under dark condition for 30 min. The absorbance was recorded at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Butylated hydroxy toluene (BHT), dissolved in distilled water was used as a reference. Control sample was prepared using the same volume without any compound and BHT, 95% methanol served as blank. Test was performed in triplicate and the results were averaged. Radical scavenging activity was calculated using the formula:

% of radical scavenging activity = \[(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}\] × 100

Where \(A_{\text{control}}\) is the absorbance of the control sample (DPPH solution without test sample) and \(A_{\text{test}}\) is the absorbance of the test sample (DPPH solution+test compound).

Iron chelating ability: The chelating effect was determined according to the literature method [33]. The test solution (2 ml) of different concentrations (25-100 µg/mL) in methanol was added to a solution of 2mM FeCl₂ (0.05 ml), the reaction was initiated by adding 5mM ferrozine (0.2 ml) and total volume was adjusted to 5 ml with methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used as a standard. The inhibition percentage of ferrozine-Fe⁺² complex formations was calculated using the formula:

Metal chelating effect (%) = \[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}\] × 100

Where \(A_{\text{control}}\) is the absorbance of control and \(A_{\text{sample}}\) is the absorbance of test compounds. Ascorbic acid is used as control. Test was performed in triplicate and the results were averaged.

Results and Discussion

Chemistry

The reaction pathway used for the synthesis of target compounds (5a-k) has been shown in Scheme 1. The key intermediates, 1,3-diaryl-2-propen-1-ones (3a-k) were synthesized by the reaction of substituted

![Scheme 1: Synthesis of chalcone derivatives containing thiobarbitone nucleus (5a-k).](image-url)
acetophenones with different aromatic aldehydes according to the reported procedure [35]. The Knoevenagel condensation of 1,3-diaryl-2-propen-1-ones (3a-k) with thiobarbituric acid (4) furnished the target compound (5a-k). Initially, 1,3-diaryl-2-propen-1-one undergo protonation by acetic acid. The protonated form of the methanone then facilitates the addition reaction towards a nucleophile. The acetate-rich carbanion attacks on the electron deficient carbonyl carbon of 1,3-diaryl-2-propen-1-one to form an adduct which upon dehydration furnished the target compounds. The proposed mechanism is given in Figure 1.

The structures of synthesized compounds were confirmed by IR, NMR and Mass spectral data. The IR spectrum of compound 5a showed sharp absorption bands at 1657 cm\(^{-1}\) and 1653 cm\(^{-1}\) corresponding to carbonyl group (C=O). The absorption band in the region 3300-3313 cm\(^{-1}\) corresponds to (NH) stretching vibration and the band at 1350 cm\(^{-1}\) corresponds to (C=S) stretching vibration. The \(^1\)H NMR spectrum of compound 5a displayed two doublets at \(\delta\) 7.10 and 5.72 ppm due to two vinyl protons, the multiplet between \(\delta\) 8.30-7.53 ppm correspond to aromatic protons and a singlet at \(\delta\) 8.95 ppm is due to two NH protons. Further, \(^13\)CNMR spectrum of compound 5a confirmed the proposed structure by appearance of signal at \(\delta\) 178.1 ppm due to the C=S carbon and another signal at \(\delta\) 167 ppm corresponds to (C=S) stretching vibration. The \(\delta\) 133.50 ppm attributed to C-Cl carbon and rest of carbon atoms displayed the signals at respective \(\delta\) values pertaining to the structure. The mass spectrum showed molecular ion peak \(M^+\) at \(m/z\) 370.2 [M\(^{+2}\)] and isotopic peak at \(m/z\) 370.2 [M\(^{+2}\)]. The physical and analytical data of synthesized compounds (5a-k) have been given in Table 1.

**Biological activity**

*In vitro antibacterial and antifungal activity:* Though, we have many synthetic drugs in the market, the bacterial mutations are making them resistance. In view of this, the compounds synthesized in the present investigation (5a-k) were evaluated for their antimicrobial activity as primary screening at two different concentrations and the results have been displayed in Tables 2 and 3. The antimicrobial activity was carried out against five bacterial strains *Staphylococcus aureus (MTCC 3160)*, *Bacillus subtilis (MTCC 1134)*, *Escherichia coli (MTCC 1559)*, *Salmonella typhi* (MTCC 1160), *Pseudomonas aeruginosa (MTCC 1034)* and three fungi *Candida albicans (MTCC 1637)*, *Aspergillus niger (MTCC 4325)* and *Alternaria alternata (MTCC 3793)*. All these microorganisms were procured from IMTECH, Chandigarh, India.

The investigation of antimicrobial screening revealed that, test compounds showed varying degree of activity against all the tested microorganisms. Further, the compounds which showed good activity in primary screening were assessed by minimum inhibitory concentration (MIC) at different concentrations to quantify the antimicrobial potency of the compounds. The results of MIC values of antimicrobial activity have been given in Table 4.

From the structure–antimicrobial activity connection of the synthesized compounds, it revealed that, to assess the SAR studies, the effect of structural changes in the target compounds and the role of substituents in improving anti-microbial activities have been reported in the literature [36-41]. A close investigation of the MIC values indicates that all the compounds exhibited a varied degree of MIC (27.72 - 198.10 µg/mL) of antibacterial activity against the tested bacterial strains. The compounds 5a, 5e and 5k having Cl substituents on para position of phenyl ring were found to exhibit good antibacterial activity against *Staphylococcus aureus* and *bacillus subtilis* with MIC value 27.72-37.42 µg/mL. Compounds 5i and 5j showed very good activity against *Pseudomonas aeruginosa* with MIC value 28.11 µg/mL and 29.86 µg/mL respectively. Compound 5c is inactive against the *Staphylococcus aureus* and remaining compounds showed considerable activity against all tested strains.

The MIC of antifungal activity of title compounds indicated that, the compound 5e was found to exhibit good activity against all the tested fungal strains with MIC value 23.50-28.00 µg/mL. Compounds 5i and 5j showed moderate activity against the tested fungal microorganisms *Aspergillus niger* and *Alternaria alternata* with MIC value 22.66-36.49 µg/mL.
Table 1: Characterization data of synthesized compounds (5a-k).

| Compd | R | R_i | Yield (%) | Mol. Wt. |
|-------|---|-----|-----------|----------|
| 5a    | H | Cl  | 79        | 368.83   |
| 5b    | H | CH₃ | 90        | 348.41   |
| 5c    | H | OCH₃| 86        | 364.41   |
| 5d    | H | N(CH₃)₂ | 83   | 377.45   |
| 5e    | OCH₃| Cl  | 85        | 398.86   |
| 5f    | OCH₃| CH₃ | 76        | 378.44   |
| 5g    | OCH₃| OH  | 91        | 380.45   |
| 5h    | OCH₃| H   | 75        | 364.41   |
| 5i    | NO₂| OCH₃| 83        | 409.41   |
| 5j    | NO₂| N(CH₃)₂| 79    | 422.45   |
| 5k    | Cl | Cl  | 81        | 403.20   |

Table 2: Antibacterial activity data of synthesized compounds (5a-k).

| Comp. No | Conc. in (mg/mL) | Zone of inhibition in mm (mean ± S.D.) |
|----------|-----------------|---------------------------------------|
| 5a       | 0.5             | S. a ± S.D.' 6 ± 0.17  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5b       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5c       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5d       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5e       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5f       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5g       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5h       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5i       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5j       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 5k       | 0.5             | S. a ± S.D.' 6 ± 0.12  B. s ± S.D.' 10 ± 0.15  E. c ± S.D.' 7 ± 0.17  S. t ± S.D.' 6 ± 0.17  P. a ± S.D.' 00 |
|          | 1.0             | S. a ± S.D.' 13 ± 0.16  B. s ± S.D.' 18 ± 0.14  E. c ± S.D.' 14 ± 0.16  S. t ± S.D.' 13 ± 0.16  P. a ± S.D.' 00 |
| 10% DMSO | -               | -                        | -           |
| Streptomycin | 24 ± 0.16 | 21 ± 0.12 | 18 ± 0.13 | 16 ± 0.17 | 15 ± 0.18 |

Each value is the mean of three replicate determinations ± standard deviation; S. a - Staphylococcus aureus; B. s - Bacillus subtilis; E. c - Escherichia coli; S. t - Salmonella typhph; P. a - Pseudomonas aeruginosa.
**Table 3:** Antifungal activity data of synthesized compounds (5a-k).

| Comp. No | Conc. in (mg/mL) | Zone of inhibition in mm (mean ± S.D.) |
|----------|------------------|---------------------------------------|
|          |                  | C. a ± S.D' | A. n ± S.D' | A. a ± S.D' |
| 5a       | 0.5              | 6 ± 0.13   | 6 ± 0.15   | 00         |
|          | 1.0              | 10 ± 0.14  | 12 ± 0.13  | 6 ± 0.18   |
| 5b       | 0.5              | 7 ± 0.12   | 8 ± 0.14   | 6 ± 0.17   |
|          | 1.0              | 10 ± 0.17  | 11 ± 0.12  | 8 ± 0.12   |
| 5c       | 0.5              | 8 ± 0.15   | 7 ± 0.17   | 7 ± 0.18   |
|          | 1.0              | 10 ± 0.15  | 11 ± 0.16  | 12 ± 0.15  |
| 5d       | 0.5              | 00         | 00         | 6 ± 0.17   |
|          | 1.0              | 8 ± 0.18   | 00         | 00         |
| 5e       | 0.5              | 7 ± 0.16   | 8 ± 0.12   | 6 ± 0.17   |
|          | 1.0              | 12 ± 0.17  | 16 ± 0.15  | 10 ± 0.13  |
| 5f       | 0.5              | 00         | 6 ± 0.18   | 6 ± 0.17   |
|          | 1.0              | 8 ± 0.12   | 8 ± 0.12   | 9 ± 0.15   |
| 5g       | 0.5              | 00         | 6 ± 0.18   | 6 ± 0.17   |
|          | 1.0              | 8 ± 0.12   | 8 ± 0.12   | 9 ± 0.15   |
| 5h       | 0.5              | 00         | 00         | 00         |
|          | 1.0              | 9 ± 0.15   | 00         | 00         |
| 5i       | 0.5              | 6 ± 0.13   | 7 ± 0.14   | 7 ± 0.19   |
|          | 1.0              | 11 ± 0.15  | 15 ± 0.15  | 12 ± 0.15  |
| 5j       | 0.5              | 6 ± 0.12   | 7 ± 0.14   | 6 ± 0.15   |
|          | 1.0              | 11 ± 0.16  | 10 ± 0.14  | 11 ± 0.17  |
| 5k       | 0.5              | 7 ± 0.18   | 8 ± 0.15   | 7 ± 0.17   |
|          | 1.0              | 13 ± 0.15  | 17 ± 0.15  | 13 ± 0.16  |
| 10% DMSO | --               | --         | --         | --         |
| Griseofulvin | 14 ± 0.16 | 19 ± 0.13 | 16 ± 0.15  |

Each value is the mean of three replicate determinations ± standard deviation; C. a - Candida albicans; A. n - Aspergillus niger; A. a – Alternaria alternata

**Table 4:** Minimum inhibitory concentration (MIC) of synthesized compounds (5a-k).

| Comp. No | Minimum inhibitory concentration (MIC µg/mL) |
|----------|---------------------------------------------|
|          | S. a | B. s | E. c | S. t | P. a | C. a | A. n | A. a |
| 5a       | 32.14| 31.08| --   | 31.11| 27.01| --   | 64.49| 42.61|
| 5c       | --   | 198.10| 182.98| 179.18| 159.34| 68.17| --   | 48.25|
| 5e       | 28.76| 27.72| 29.84| 32.54| 30.05| 26.00| 30.09| 23.50|
| 5i       | 31.02| 32.53| 31.16| 30.26| 29.86| 35.51| 35.51| 25.50|
| 5j       | 32.34| 32.68| 33.78| 43.21| 28.11| --   | 36.49| 22.66|
| 5k       | 35.59| 35.08| 37.42| 31.30| 34.31| 41.10| 42.22| 23.96|
| Streptomycin | 24.95| 24.95| 26.57| 26.24| 26.24| --   | --   | --   |
| Griseofulvin | --   | --   | --   | --   | 24.84| 24.84| 20.42|

S. a - Staphylococcus aureus; B. s - Bacillus subtilis; E. c - Escherichia coli; S. t - Salmonella typhi; P. a - Pseudomonas aeruginosa; C. a - Candida albicans; A. n - Aspergillus niger; A. a - Alternaria alternata

**In silico molecular docking studies:** Glucosamine-6-phosphate synthase (L-glutamine: D-fructose-6-phosphate amino transferase) catalyze the first step in hexamine biosynthesis, converting D-fructose-6-phosphate (Fru-6-P) into D-glucosamine 6-phosphate (GlcN-6-P) using glutamine as the ammonia source. The amino sugars are the significant building blocks of polysaccharides found in the cell wall of most human pathogenic microorganisms. Therefore not surprising that a number of GlcN-6-P synthase inhibitors of natural or synthetic origin display bactericidal or fungicidal properties [42]. In correlation to in vitro antimicrobial activity, it thought worthwhile to carryout in silico studies of target molecules 5a, 5e and 5k to predict the binding affinity and orientation at the active site of the receptor.

Automated docking was used to assess the orientation of inhibitors bound in the active pockets of GlcN-6-P synthase. The molecular docking of molecules 5a, 5e and 5k with GlcN-6-P synthase revealed that all tested compounds have shown the bonding with one or the other amino acids in the active pockets as shown in Figure 2.

Among the three molecules 5a, 5e and 5k, the docking of GlcN-6-P synthase with compounds 5a and 5e were found with least binding energy (-4.41 kJ mol⁻¹). Compound 5a establishes two hydrogen bonds between thiobarbitone NH with ser 349 amino acids in the active site of the target protein with minimum bond length (2.003 and 2.143 Å). Compound 5e establishes two hydrogen bonds between NH with gln 348 and thiobarbitone oxygen with ser 349 amino acids in the active site of the target protein with minimum bond length (2.246 and 2.143 Å). In in vitro studies too,
compounds 5a and 5e have emerged as an active antimicrobial agent against the tested organisms. Molecular docking results of synthesized compounds have been given in Table 5.

**Free radical scavenging activity by DPPH method:** All the synthesized compounds were screened for their free radical scavenging activity by DPPH method. The freshly prepared solution exhibits a deep blue color with the absorption maximum at 517 nm. This deep blue color generally fades when antioxidant is present in the solution. All compounds have exhibited varied free radical scavenging capacity by comparison with the standard Butylated hydroxy toluene (BHT). The variation exhibited in DPPH scavenging activity could be attributed to the effect of different substituents. The compound substituted with phenolic hydroxy group has the high potential for scavenging radicals [43]. Among the tested compounds, compounds 5c and 5f displayed potent DPPH free radical scavenging activity with least IC$_{50}$ value (55.58-58.68 µg/mL). Compounds 5b and 5i displayed good activity with the IC$_{50}$ value 61.88 and 65.78 µg/mL respectively.

**Iron chelating ability:** The iron chelating study measures the ability of antioxidants to compete with Ferrozine in chelating ferrous ion [44]. The Fe$^{2+}$ chelating capacities varied significantly among different compounds. From the activity results it revealed that, among the tested compounds, compounds 5b, 5f and 5i showed very good chelating ability with IC$_{50}$ value 62.81-69.06 µg/mL. Further, it was observed that the compound 5c substituted with methoxy group at C-4 of aromatic

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**Figure 2:** Interaction of ligand molecules 5a, 5e, 5k, Str and Gri with GlcN-6-P. A: interaction of 5a with GlcN-6-P; B: interaction of 5e with GlcN-6-P; C: interaction of 5k with GlcN-6-P; D: Interaction of Streptomycin (Str) with GlcN-6-P; E: interaction of Griseofulvin (Gri) with GlcN-6-P.
| Comp. No | Binding Energy (kJ mol⁻¹) | Inhibition Constant (µM) | RMSd | Ligand efficiency | No of hydrogen bonds | Bonding residues | Bond Strength (Å) |
|---------|--------------------------|-------------------------|------|-------------------|---------------------|------------------|------------------|
| 5a      | -4.41                    | 583.09                  | 0.0  | -0.18             | 2                   | 2VF5: GLN348: HE22 : Ligands / 4a:: : H 2VF5: SER347: HG1: Ligands/ 4a:: : O | 2.003            |
| 5e      | -4.41                    | 589.5                   | 0.0  | -0.16             | 2                   | 2VF5: GLN348: HE22 : Ligands / 4a:: : H 2VF5:SER349: HG: Ligands/ 4c:: : O | 2.246            |
| 5k      | -4.52                    | 487.83                  | 0.0  | -0.17             | 1                   | 2VF5:SER349: HG: Ligands/ 4e:: : O | 2.007            |

| Streptomycin | -6.72 | -181.49 | 0.0  | -0.17 | 2 | 2VF5:SER349: HG: Ligands/ Streptomycin:: : O | 1.922 |
| Griseofulvin  | -4.84 | -282.81 | 0.0  | -0.12 | 2 | 2VF5:GLN348: HE22: Ligands/ Griseofulvin:: : O | 2.106 |

Table 5: Molecular docking results of synthesized compounds (5a-k) with Glucosamine-6-Phosphate Synthase.

| Test Compd | DPPH assay (IC 50 µg/mL) | Fe²⁺ ion chelating (IC 50 µg/mL) | Total reductive capability (IC 50 µg/mL) |
|------------|--------------------------|----------------------------------|----------------------------------------|
| 5a         | 85.03 ± 0.21             | 88.62 ± 0.14                     | 91.25 ± 0.12                           |
| 5b         | 61.88 ± 0.12             | 67.2 ± 0.18                      | 64.43 ± 0.14                           |
| 5c         | 55.58 ± 0.31             | 58.41 ± 0.21                     | 63.13 ± 0.25                           |
| 5d         | 168.91 ± 0.43            | 176.05 ± 0.41                    | 204.91 ± 0.38                          |
| 5e         | 75.76 ± 0.15             | 81.69 ± 0.15                     | 92.59 ± 0.31                           |
| 5f         | 58.68 ± 0.18             | 62.81 ± 0.18                     | 72.25 ± 0.12                           |
| 5g         | 94.41 ± 0.24             | 96.89 ± 0.21                     | 91.24 ± 0.21                           |
| 5h         | 134.41 ± 0.18            | 121.36 ± 0.13                    | 181.16 ± 0.18                          |
| 5i         | 65.78 ± 0.21             | 69.06 ± 0.21                     | 62.18 ± 0.12                           |
| 5j         | 121.36 ± 0.13            | 128.67 ± 0.12                    | 176.05 ± 0.27                          |
| 5k         | 87.41 ± 0.24             | 79.89 ± 0.21                     | 81.24 ± 0.21                           |
| Std⁵a,b,c  | 48.63 ± 0.18             | 47.17 ± 0.13                     | 52.3 ± 0.12                            |

Each value is expressed as mean ± SD of three replicates; Std⁵a BHT used as standard for DPPH radical scavenging activity; Std⁵b EDTA is used as a standard for Fe²⁺ ion chelating activity; Std⁵c BHA used as standard for total reductive capability

Table 6: IC₅₀ value of DPPH radical scavenging, Ferrous ion chelating and total reductive capability activity of test compounds (5a-k).

ring displayed excellent activity with minimum IC₅₀ value 58.41 µg/mL. The other compounds showed moderate to good activity.

**Total reductive capability:** The reduction of Fe³⁺ to Fe²⁺ is often used as an indicator for electron donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidant in the synthesized compounds would result in the reduction of Fe³⁺ to Fe²⁺ by donating electron(s). The amount of Fe³⁺ complexes was then monitored by observing the formation of Perl’s Prussian blue at 700 nm. Absorbance at 700 nm indicates an increase in reducing ability [45]. It was found that the reducing power of all the synthesized compounds increased with the increase in their concentrations. The best reducing power was presented by the compounds 5c and 5i with IC₅₀ value 62.18-63.13 µg/mL. IC₅₀ values of DPPH radical scavenging and ferrous ion chelating activity of test compounds is given in Table 6.

**Conclusion**

We synthesized novel series of chalcone derivatives containing thiobarbiturate moiety and screened for antimicrobial and antioxidant activity. From the antimicrobial study results it revealed that, compounds 5a, 5e and 5k were most effective against all the tested pathogens compared with the other tested compounds. In case of antioxidant screening, compounds containing hydroxyl groups showed very good DPPH radical scavenging activity.

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