Medicinal chemistry

Venkatesh et al., Med chem (Los Angeles) 2016, 6:7
DOI: 10.4172/2161-0444.1000383

Research Article Open Access

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-convalsunts 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 cytochrome P450 enzymes [7], methionine aminopeptidase-1 (MetAP-1) [8], anti-inflammatory, analgesic [9], CYP19 inhibitory activity, molecular docking [10], cytotoxicity properties [11] and broad spectrum pharmacological properties including hypnotic [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 (1:2) is used as a mobile phase. The FTIR spectra were taken in KBr pellets (100 mg) using Shimadzu FT-IR spectrophotometer. ^{1}HNMR and ^{13}CNMR 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, LCMS 2010A, Japan. In antimicrobial activity, the zone of inhibition and in antioxidant activity the IC_{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-diaryl-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 $NaHCO_3$ 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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Received June 21, 2016; **Accepted** June 25, 2016; **Published** June 30, 2016

Citation: Venkatesh T, Bodke YD, Kenchappa R, Telkar S (2016) Synthesis, Antimicrobial and Antioxidant Activity of Chalcone Derivatives Containing Thiobarbitone Nucleus. Med chem (Los Angeles) 6: 440-448. doi:10.4172/2161-0444.1000383

(EtOH); m.p 268-270°C; IR (KBr, υ cm^{-1}): 3313 (NH), 1657, 1653 (C=O), 1350 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.95 (s, NH), 8.30-7.53 (m, Ar-H), 7.10 (d, CH), 5.72 (d, CH); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm): 178.1 (C=S), 166.6 (C=O), 146.8 (C=C), 133.5 (C-Cl), 131.2 (CH=CH), 126.4 (2CH); MS (LCMS): m/z 368.2[M^+] and 370.4[M^++2].

5-[3-(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}): 3318 (NH), 1651, 1650 (C=O), 1358 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.98 (s, NH), 8.30-7.28 (m, Ar-H), 7.12 (d, CH), 5.62 (d, CH), 2.40 (s, CH_3); ^{13}CNMR (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 (LCMS): m/z 348.3[M^+].

5-[3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5c): Brown yellow solid (EtOH); m.p. 288-290°C; IR (KBr, υ cm^{-1}): 3342 (NH), 1659, 1656 (C=O), 1319 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.76 (s, NH), 8.20-7.80 (m, Ar-H), 7.21 (d, CH), 5.64 (d, CH), 3.80 (s, OCH_3); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):177.6 (C=S), 167.8 (C=O), 145.8 (C=C), 131.3 (CH=CH), 126.2 (2CH); MS (LCMS): m/z 364.7[M^+].

5-[3-[4-(Dimethylamino)phenyl]-1-phenylprop-2-en-1-ylidene}-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5d): Brown solid (EtOH); m.p 280-282°C; IR (KBr, υ cm^{-1}): 3326 (NH), 1667, 1663 (C=O), 1342 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.56 (s, NH), 8.10-7.85 (m, Ar-H), 7.08 (d, CH), 5.61 (d, CH), 2.32 (s, $N(CH_3)_2$; ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):178.1 (C=S), 167.2 (C=O),146.8 (C=C),131.1 (CH=CH), 126.2 (2CH); MS (LCMS): m/z 377.8[M^+].

5-[3-(4-Chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5e): Light yellow solid (EtOH); m.p 279-281°C; IR (KBr, υ cm^{-1}): 3400 (NH), 1665, 1661 (C=O), 1313(C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.65 (s, NH), 8.13-7.51 (m, Ar-H), 7.18 (d, CH), 5.67 (d, CH), 3.81 (s, OCH_3); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):179.3 (C=S), 167.1 (C=O), 146.2 (C=C),133.1 (C-Cl), 131.2 (CH=CH), 126.4 (2CH); MS (LCMS): m/z 398.00[M^+] and 400.02[M^++2].

5-[1-(4-Methoxyphenyl)-3-(4-methylphenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5f): Yellow solid (EtOH); m.p 274-276°C; IR (KBr, υ cm^{-1}): 3395 (NH), 1672, 1660 (C=O), 1365 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.70 (s, NH), 8.32-7.53 (m, Ar-H), 7.17 (d, CH), 5.68 (d, CH), 3.81 (s, OCH_3), 2.40 (s, CH_3); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):178.6 (C=S), 167.5 (C=O), 146.4 (C=C), 131.3 (CH=CH), 126.4 (2CH); LCMS: m/z 378.9[M^+].

5-[3-(4-Hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H,5H)-dione (5g): Brown solid (EtOH); m.p 281-283°C; IR (KBr, υ cm^{-1}): 3446 (OH), 3348 (NH), 1660, 1654 (C=O), 1336 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.61 (s, NH), 8.30-7.70 (m, Ar-H), 7.16 (d, CH), 6.25 (s, OH), 5.68 (d, CH), 3.86 (s, OCH_3); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm): 179.5 (C=S), 167.5 (C=O), 146.2 (C=C), 131.2 (CH=CH), 126.4 (2CH); LCMS: m/z 380.2[M^+].

5-[1-(4-Methoxyphenyl)-3-phenylprop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H, 5H)-dione (5h): White crystal (EtOH); m.p 285-287°C; IR (KBr, υ cm^{-1}): 3375 (NH), 1674, 1672 (C=O), 1367 (C=S); ^{1}HNMR (400 MHz, DMSO-d_6, δ ppm): 8.80 (s, NH), 8.01-7.61-(m, Ar-H), 7.14 (d, CH), 5.64 (d, CH), 3.88 (s, OCH_3); ^{13}CNMR (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-[3-(4-Methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-ylidene] pyrimidine-2,4,6(1H,3H,5H)-trione (5i): Orange solid (EtOH); m.p 296-298°C; IR (KBr, υ cm^{-1}): 3455 (NH), 1671, 1668 (C=O), 1374(C=S); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 8.75 (s, NH), 8.30-7.51 (m, Ar-H), 7.31 (d, CH), 5.75 (d, CH), 3.65 (s, OCH_3); ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):179.1 (C=S), 167.3 (C=O), 146.2 (C=C), 131.2 (CH=CH), 126.4 (2CH); LCMS: m/z 409.33[M^+].

5-[3-[4-(Dimethylamino)phenyl]-1-(4-nitro)prop-2-en-1-ylidene]-2-thioxodihydropyrimidine-4,6(1H, 5H)-dione (5j): Light black solid (EtOH); m.p 293-295°C; IR (KBr, υ cm^{-1}): 3502 (NH), 1669, 1662 (C=O), 1388 (C=S); ^{1}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, $N(CH_3)_2$; ^{13}CNMR (400 MHz, DMSO-d_6, δ ppm):179.6 (C=S), 167.3 (C=O), 146.2 (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 (5k): Yellow solid (EtOH); m.p 272-274°C; IR (KBr, υ cm^{-1}): 3323 (NH), 1676, 1651 (C=O), 1354 (C=S); ^{1}H NMR (400 MHz, DMSO-d6, δ ppm): 8.75 (s, NH), 8.30-7.51 (m, Ar-H), 7.10 (d, CH), 5.72 (d, CH); ^{13}CNMR (400 MHz, DMSO-d6, δ ppm): 178.1 (C=S),167.0 (C=O), 145.8 (C=C), 133.5 (C-Cl), 131.2 (CH=CH), 126.4 (2CH); LCMS: m/z 402.2 [M^+], 404.2 [M^++2] and 406.2 [M^++4].

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 12 amino acid residues as Ala602, Val399, Ala400, Gly301, Thr302, Ser303, Cys300, Gln348, Ser349, Thr352, Ser347 and Lys603 [30].

The crystal structure of GlcN-6-P synthase (PDB ID 2VF5) from the PDB (http://www.pdb.org/pdb/home/home.do) was selected and edited by removing the heteroatoms and adding C-terminal oxygen [31]. 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:

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

Where $A_{control}$ is the absorbance of the control sample (DPPH solution without test sample) and A_{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 $FeCl_2$ (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:

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

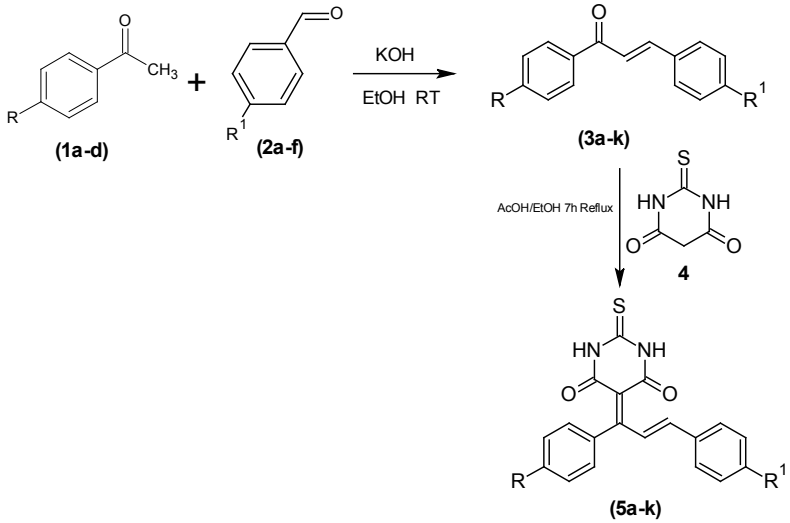
Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of test compounds. Ascorbic acid is used as control. Test was performed in triplicate and the results were averaged.

Reducing power assay: The reducing power of the test samples was evaluated by following the literature method [34]. Various concentrations of test compounds were mixed thoroughly with the mixture of 2.5 ml of 0.2mM phosphate buffer (pH7.4) and 2.5 ml of potassium ferricyanide. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution was collected and mixed with 2.5 ml distilled water and later with 0.5 ml of ferrous chloride (0.1% w/v). The absorbance was measured at 700 nm against a blank sample. Increase in the absorbance of the reaction mixture indicated higher reducing power of the test compounds.

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



Comp	R	R_1	Comp	R	R_1
5a	H	Cl	**5g**	OCH_3	OH
5b	H	CH_3	**5h**	OCH_3	H
5c	H	OCH_3	**5i**	NO_2	OCH_3
5d	H	$N(CH_3)_2$	**5j**	NO_2	$N(CH_3)_2$
5e	OCH_3	Cl	**5k**	Cl	Cl
5f	OCH_3	CH_3			

Scheme 1: Synthesis of chalcone derivatives containing thiobarbitone nucleus (5a-k).

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 ion which was formed in the former step can accept a proton from the methylene unit of thiobarbituric acid and generate a carbanion. The electron-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 δ 7.10 and 5.72 ppm due to two vinyl protons, the multiplet between δ 8.30-7.53 ppm correspond to aromatic protons and a singlet at δ 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 δ 178.1 ppm due to the C=S carbon and another signal at δ167 ppm correspond to C=O carbon of thiobarbituric acid ring. Another signal at δ 133.50 ppm attributed to C-Cl carbon and rest of carbon atoms displayed the signals at respective δ values pertaining to the structure. The mass spectrum showed molecular ion peak M^{+} at m/z at 368.20 which corresponds to molecular weight of the compound **5a** 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 tyhphi* (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.

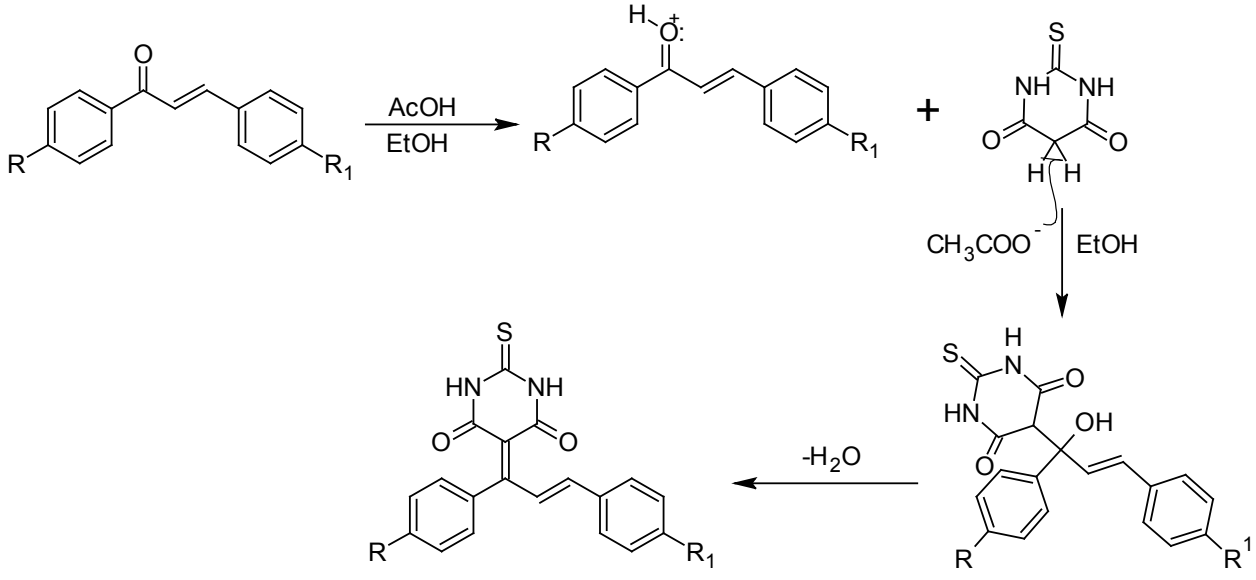


Figure 1: Proposed mechanism for formation of compounds (5a-k).

Compd	R	R_1	Yield (%)	Mol. Wt.
5a	H	Cl	79	368.83
5b	H	CH_3	90	348.41
5c	H	OCH_3	86	364.41
5d	H	$N(CH_3)_2$	83	377.45
5e	OCH_3	Cl	85	398.86
5f	OCH_3	CH_3	76	378.44
5g	OCH_3	OH	91	380.45
5h	OCH_3	H	75	364.41
5i	NO_2	OCH_3	83	409.41
5j	NO_2	$N(CH_3)_2$	79	422.45
5k	Cl	Cl	81	403.20

Table 1: Characterization data of synthesized compounds (5a-k).

Comp. No	Conc. in (mg/mL)	Zone of inhibition in mm (mean ± S.D.)				
		S. a ± S.D*	***B. s*** ± S.D*	***E. c*** ± S.D*	***S. t*** ± S.D*	***P. a*** ± S.D*
5a	0.5	9 ± 0.13	6 ± 0.17	6 ± 0.16	00	00
	1.0	20 ± 0.12	10 ± 0.15	9 ± 0.12	8 ± 0.14	9 ± 0.133
5b	0.5	8 ± 0.14	08 ± 0.1	7 ± 0.17	00	6 ± 0.17
	1.0	17 ± 0.18	10 ± 0.2	9 ± 0.16	8 ± 0.14	9 ± 0.18
5c	0.5	11 ± 0.19	12 ± 0.18	6 ± 0.12	6 ± 0.15	7 ± 0.17
	1.0	19 ± 0.15	21 ± 0.15	14 ± 0.16	12 ± 0.14	13 ± 0.13
5d	0.5	6 ± 0.14	6 ± 0.12	6 ± 0.18	6 ± 0.13	00
	1.0	13 ± 0.16	10 ± 0.14	8 ± 0.17	8 ± 0.14	9 ± 0.14
5e	0.5	7 ± 0.19	7 ± 0.17	7 ± 0.17	8 ± 0.17	7 ± 0.17
	1.0	15 ± 0.12	12 ± 0.16	14 ± 0.16	13 ± 0.16	13 ± 0.16
5f	0.5	6 ± 0.14	5 ± 0.18	8 ± 0.15	00	6 ± 0.17
	1.0	13 ± 0.16	11 ± 0.15	13 ± 0.18	6 ± 0.17	9 ± 0.12
5g	0.5	6 ± 0.14	7 ± 0.17	6 ± 0.17	6 ± 0.16	00
	1.0	10 ± 0.18	15 ± 0.18	10 ± 0.15	9 ± 0.17	6 ± 0.14
5h	0.5	7 ± 0.13	6 ± 0.12	6 ± 0.14	00	00
	1.0	14 ± 0.17	13 ± 0.17	9 ± 0.13	7 ± 0.19	00
5i	0.5	11 ± .015	10 ± 0.18	9 ± 0.17	7 ± 0.12	6 ± 0.12
	1.0	20 ± 0.15	18 ± 0.16	16 ± 0.15	11 ± 0.14	12 ± 0.16
5j	0.5	12 ± 0.18	10 ± 0.15	9 ± 0.16	9 ± 0.16	8 ± 0.15
	1.0	21 ± 0.16	18 ± 0.13	16 ± 0.16	13 ± 0.15	13 ± 0.17
5k	0.5	13 ± 0.2	11 ± 0.1	09 ± 0.2	11 ± 0.3	09 ± 0.2
	1.0	21 ± 0.3	18 ± 0.2	16 ± 0.1	14 ± 0.1	14 ± 0.3
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 tyhphi*; *P. a* **-** *Pseudomonas aeruginosa*

Table 2: Antibacterial 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 3: Antifungal activity data 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	28.00	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	26.67	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 tyhphi*; *P. a* - *Pseudomonas aeruginosa*; *C. a* - *Candida albicans*; *A. n* - *Aspergillus niger*; *A. a* - *Alternaria alternata*

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

***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 347 and thiobarbitone oxygen 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 2.143Å). In *in vitro* studies too,

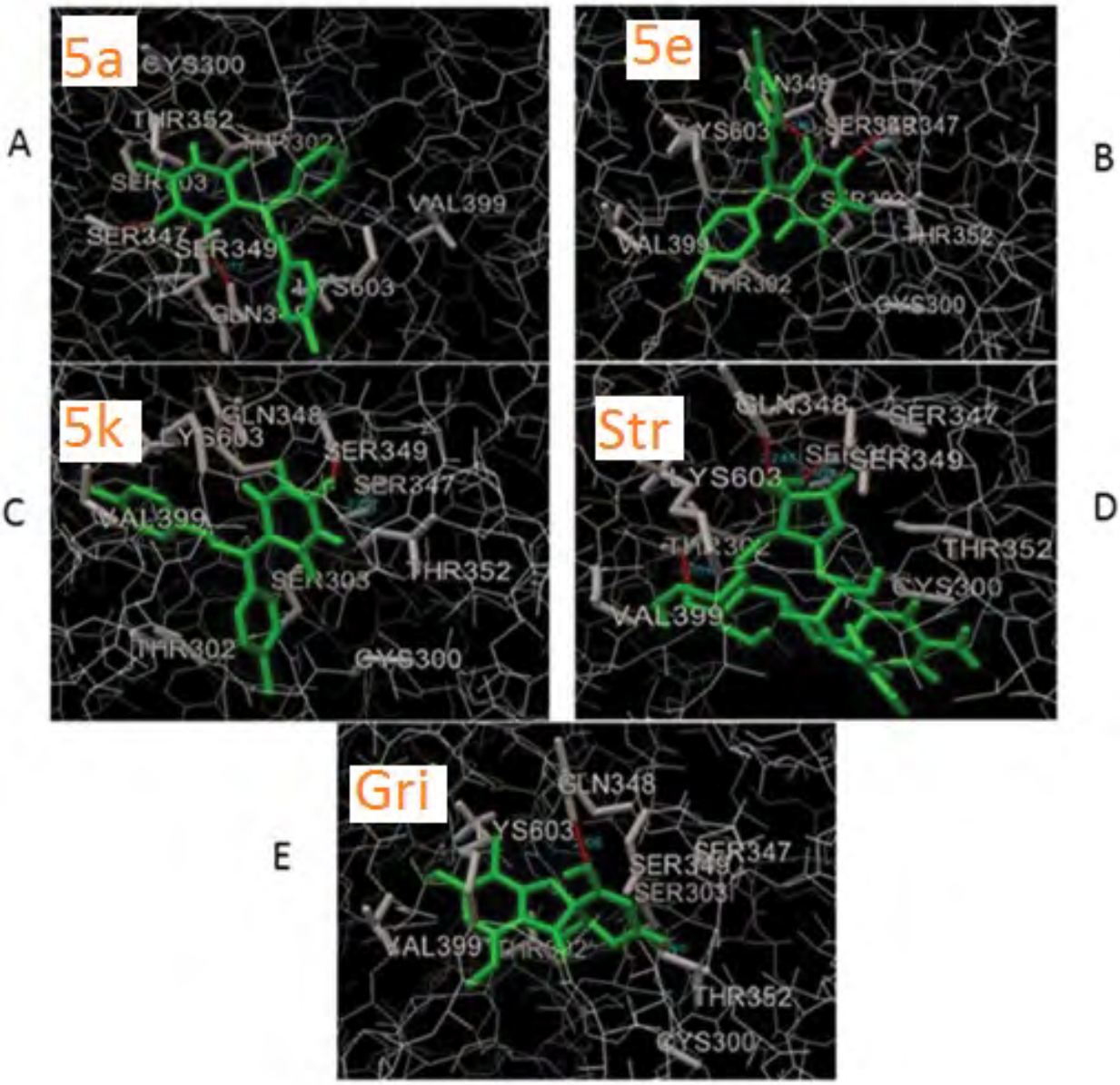


Figure 2: Interaction of ligand molecules 5a, 5e, 5k, Str and Gri with GlcN-6-P. A: interaction of 5a with GlcN-6-P; B: interaction of 5e with GlcN-6-P; C: interaction of 5k with GlcN-6-P; D: Interaction of Streptomycin (Str) with GlcN-6-P; E: interaction of Griseofulvin (Gri) with GlcN-6-P.

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 mm. This deep blue color generally fades when antioxidant is present in the solution. All compounds have exhibited varied free radial 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

Comp. No	Binding Energy (kJ mol^{-1})	Inhibition Constant (μM)	RMSd	Ligand efficiency	No of hydrogen bonds	Bonding residues	Bond length (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 2.143
5e	-4.41	589.5	0.0	-0.16	2	2VF5: GLN348: HE22: Ligands / 4e:: : H 2VF5:SER349: HG: Ligands/ 4c:: : O	2.246 2.163
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 2VF5:THR352:OG1: Ligands/ Streptomycin:: :H	1.922 1.894
Griseofulvin	-4.84	-282.81	0.0	-0.12	2	2VF5:GLN348: HE22: Ligands/ Griseofulvin:: : O 2VF5:THR352:OG1: Ligands/ Griseofulvin :: :H	2.106 2.346

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

Test Compd	DPPH assay (IC 50 μg/mL)	Fe $^{2+}$ 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	128.87 ± 0.12	176.05 ± 0.27
5k	87.41 ± 0.24	79.89 ± 0.21	81.24 ± 0.21
Std^{a,b,c}	48.63 ± 0.18	47.17 ± 0.13	52.3 ± 0.12

Each value is expressed as mean ± SD of three replicates; Std^{a} BHT used as standard for DPPH radical scavenging activity; Std^{b} EDTA is used as a standard for Fe^{2+} ion chelating activity; Std^{c} BHA used as standard for total reductive capability

Table 6: IC_{50} value of DPPH radical scavenging, Ferrous ion chelating and total reductive capability activity of test compounds (5a-k).

ring displayed excellent activity with minimum IC_{50} value 58.41 μg/mL. The other compounds showed moderate to good activity.

Total reductive capability: The reduction of Fe^{3+} to Fe^{2+} 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 Fe^{+3} to Fe^{+2} by donating electron(s). The amount of Fe^{+2} complexes was then monitored by measuring 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 IC_{50} value 62.18-63.13 μg/mL. IC_{50} 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.

Acknowledgements

The authors are thankful to the Chairman, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta, Karnataka, India for providing the laboratory facilities and IISc, Bangalore, Karnataka for providing spectral data.

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