Microwave assisted synthesis of triazine substituted pyrimidinone derivatives for antibacterial activity

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Abstract

The objective of this investigation was to synthesize some novel triazine derivatives coupled to 3,4-dihydropyrimidine nucleus by using microwave method and assess their antimicrobial potential in culture medium. The synthesis was carried out in three steps involving beginning with the synthesis of dihydropyridimidine derivatives which were converted to corresponding carboxamides and finally cyclized to target triazine derivatives (6a-e). The compounds were obtained in yield of 78-84% and displayed varying solubility with all compounds soluble to some extent in chloroform. 6c and 6d were also soluble to some extent in water. In the IR spectrum, stretching bands of N-H (3500-3300 cm^{-1}), C=O (1818-1705 cm^{-1}), and C-C (1300-800 cm^{-1}), were observed in each compound. Bending vibration of N-H (1650-1580 cm^{-1}) and C-N (1250-1020 cm^{-1}) were also visible. The NMR spectra revealed protons of NH of pyrimidine as well as of primary amine. The protons of aromatic ring as well as aliphatic methyl group were also found in the compounds. The IC$_{50}$ values of the compounds was calculated and the lowest IC$_{50}$ was obtained for **6e** against both gram positive (56.73 µg/mL) and gram negative bacteria (53.55 µg/mL). The presence of electron withdrawing group on the compounds decreased the antibacterial potential **6b** (IC$_{50}$ 140.5 µg/mL (*E. coli*) and 127.76 µg/mL (*S. aureus*)) and **6c** (IC$_{50}$ 152.15 µg/mL (*E. coli*) and 134.20 µg/mL (*S. aureus*)).

Keywords: Dihydropyridine, microwave, antibacterial, triazine, Inhibitory concentration

Introduction

In the past years, health-care associated infections have become an important cause of morbidity and mortality, whilst the incidence of antibiotic-resistant bacteria has increased dramatically and become a serious threat. In fact, the management of bacterial infections is getting increasingly tough due to augmented prevalence of MDR pathogens, which represent a major challenge to antimicrobial therapy. Microbial resistance is now frequently confronted to common antibiotics being used in clinical settings, and there is an imperative demand for newer anti-infective agents to overcome emerging multi-drug resistance. Today, the need for novel antimicrobials has been greater than ever in the face of increasing resistance to the older ones and increasingly tough management of bacterial infections. In spite of urge for such agents, the scientific progression in terms of antimicrobial research and discovery of new antibacterial molecules has declined dramatically in the past few years. Despite the fact that there is no existing antibiotic to which resistance does not develop the majority of research on antibiotics advances are in terms of upgrading previously existing antibiotic classes, and upgrades for novel classes are poor. Triazine and pyrimidine-based compounds have been known to possess good anti-microbial properties [1-7]. The objective of this investigation was to synthesize some novel triazine derivatives coupled to 3,4-dihydropyrimidine nucleus by using microwave method and assess their antimicrobial potential in culture medium.

Material and Methods

All the material used for the present investigation have been procured from various sources through Science World, Bhopal and used as they were obtained. Melting point were uncorrected and analyzed by open capillary procedure. *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation.
The synthesis was done as per the synthetic pathway presented in Figure 1.

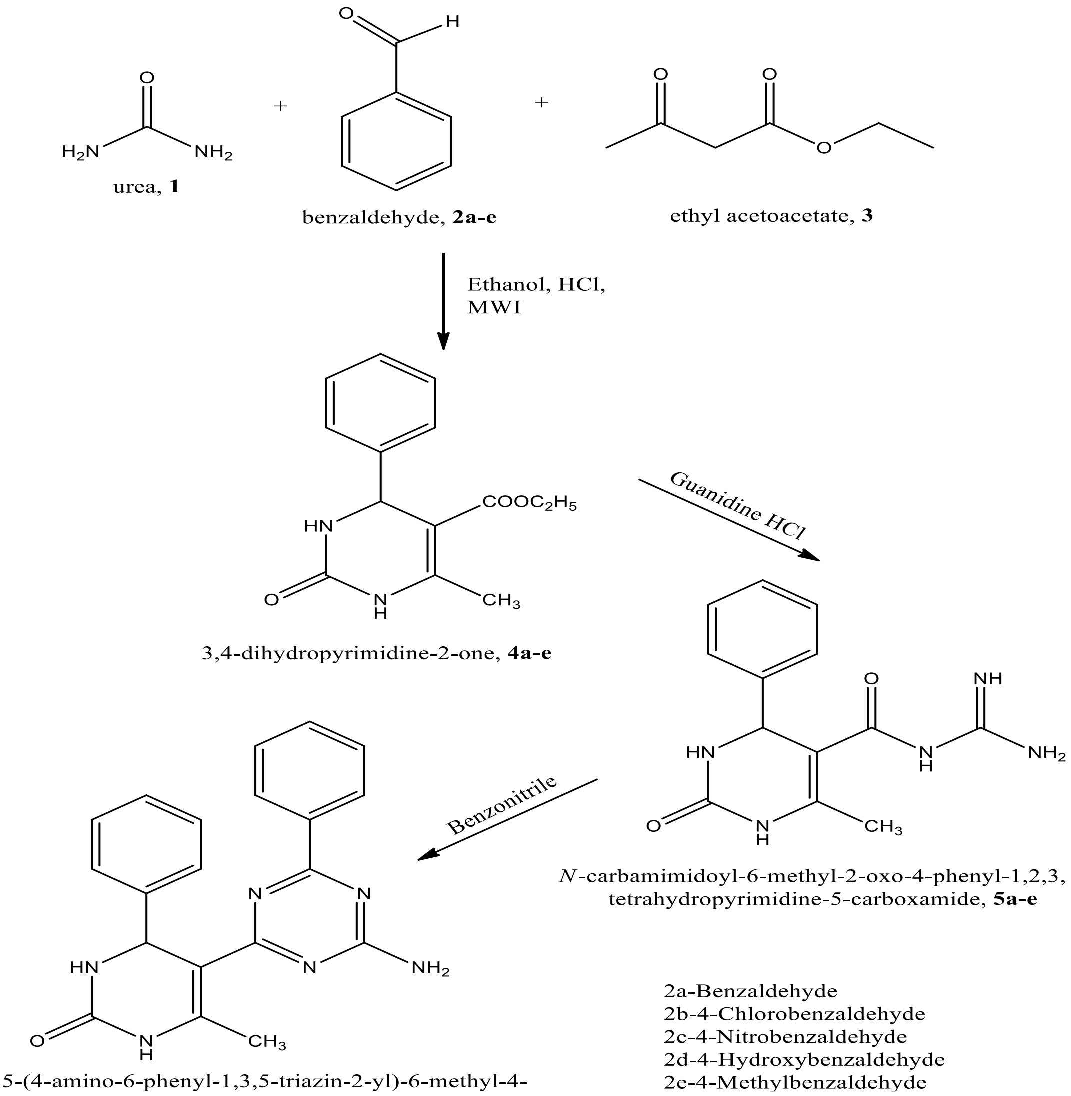


Figure 1 Synthetic Scheme

Synthesis of pyrimidine nucleus (4a-e)

0.5 moles of urea, 0.75 moles of ethylacetoacetate and 0.5 moles of substituted benzaldehyde were mixed in 25 mL of ethanol. Catalytic amount of concentrated hydrochloric acid (5 drops) was added to the mixture and the mixture was refluxed in microwave at 100 Watt power until the completion of the reaction (approximately 10 min). On cooling, a solid separated which was filtered and recrystallized using ethanol to give the product **4a-e.** Completion of the reaction was monitored by TLC (pale yellow) [8].

Synthesis of carboxamide derivatives (5a-e)

A solution of 4a-e (0.01 mol) in ethanol (5mL), guanidine hydrochloride (0.15 mol) was added. The resulting solution was irradiated under microwave for 3 min, reaction completion was monitored by TLC, the reaction mass was cooled to room temperature. The compound (5a-e) were recrystallized from ethanol to offer a solid product (pale yellow) [9].

Synthesis of triazine derivative (6a-e)

A solution of 5a-e (0.001 mol) in ethanol (10 mL), benzonitrile (0.015 mol) was added. The resulting solution was refluxed in microwave at 100 Watt power for 15 min and the reaction completion was monitored by TLC. The reaction mass was cooled to room temperature, evaporated ethanol completely and the compound (6a-e) was recrystalised in acetone [9].

Chemical Characterization

All the synthesized compounds were characterized for melting point, solubility, yield and elucidation of the structure [10-13]. The structure elucidation was performed by spectroscopic analysis (NMR, Mass and IR).

Antibacterial Study

The synthesized compounds were dissolved in DMSO to obtain the solutions of 25, 50, 75 & 100 µg/mL. These solutions were used as the test samples.

The lyophilized cultures obtained from IMT, Chandigarh were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria. Agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labeled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening.

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [9]. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the Schiff's bases (25, 50, 75 & 100 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

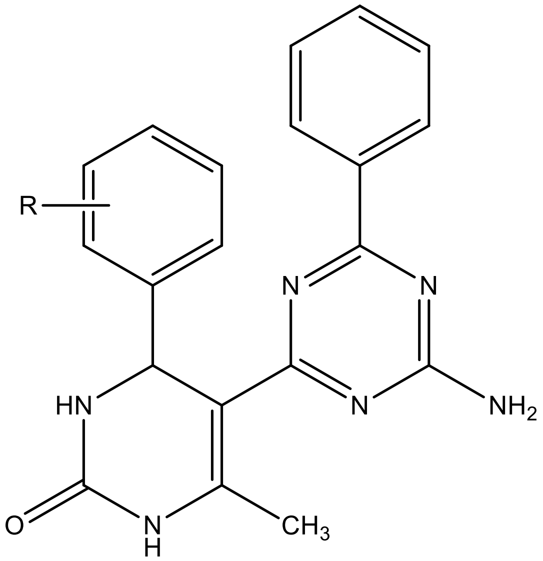
The broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds. The final inoculum size (of bacterial culture) was maintained to 10^5 CFU/mL. All the tubes were incubated at 37°C for 24-48 h to allow for growth of micro-organism. After incubation, the optical density of the content from each tube was observed at 600 nm using UV-Visible spectrophotometer. The concentration that led to half of the optical density (50%) of the growth control tube was observed for each sample and standard (norfloxacin) [14].

Results and Discussion

Results

Urea and ethylacetoacetate in a condensation reaction were reacted with different aromatic aldehydes to obtain five dihydropyrimidine compounds (4a-e). These compounds underwent substitution reactions with guanidine hydrochloride to produce carboxamide derivatives (5a-e). The compounds 5a-e on reaction with benzonitrile resulted in rearrangement and cyclization leading to the formation of target triazine derivatives (6a-e). The compounds were characterized for yield, melting point, and solubility (Table 1).

Table 1. Physicochemical features of 6a-e



Compound	**R**	**Color**	**Yield (%)**	**Melting Point (°C)**
6a	H	White	84	208-210
6b	Cl	White	81	184-186
6c	NO_2	White	87	198-200
6d	OH	White	78	203-206
6e	CH_3	White	83	211-213

The solubility of the synthesized compounds 6a-e was determined in water, methanol, chloroform and DMSO. The compounds displayed varying solubility with all compounds soluble to some extent in chloroform. 6c and 6d were also soluble to some extent in water.

The structure of the compounds was determined using proton NMR in $CDCl_3$ solvent, IR and Mass study.

Compound 6a: 5-(4-amino-6-phenyl-1,3,5-triazin-2-yl)-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one; **FTIR (cm-1):** N-H (3401.2), C=O (1705.3), C-N (1217.0)**; 1H NMR (δ, ppm):** 10.35, 7.08 (NH, pyrimidine), 6.52 (NH_2), 5.70,7.36 (CH, pyrimidine), 7.39-8.39 (CH, aromatic); **Mass (m/e):** 344.1

Compound 6b: 5-(4-amino-6-phenyl-1,3,5-triazin-2-yl)-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one; **FTIR (cm-1):** N-H (3587.6), C=O (1705.3), C-N (1224.4)**; 1H NMR (δ, ppm):** 10.35, 7.08 (NH, pyrimidine), 6.52 (NH_2), 5.82,7.36 (CH, pyrimidine), 7.29-8.39 (CH, aromatic); **Mass (m/e):** 378.3

Compound 6c: 5-(4-amino-6-phenyl-1,3,5-triazin-2-yl)-6-methyl-4-(4-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one; **FTIR (cm-1):** N-H (3390.0), C=O (1710.7), C-N (1218.85)**; 1H NMR (δ, ppm):** 10.35, 7.09 (NH, pyrimidine), 6.52 (NH_2), 5.83,7.36 (CH, pyrimidine), 7.52-8.39 (CH, aromatic); **Mass (m/e):** 389.2

Compound 6d: 5-(4-amino-6-phenyl-1,3,5-triazin-2-yl)-4-(4-hydroxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-one; **FTIR (cm-1):** N-H (3401.2), C=O (1710.3), C-N (1142.4)**; 1H NMR (δ, ppm):** 10.35, 7.09 (NH, pyrimidine), 6.52 (NH_2), 5.81,7.36 (CH, pyrimidine), 7.10-8.39 (CH, aromatic); **Mass (m/e):** 360.7

Compound 6e: 5-(4-amino-6-phenyl-1,3,5-triazin-2-yl)-6-methyl-4-(p-tolyl)-3,4-dihydropyrimidin-2(1H)-one; **FTIR (cm-1):** N-H (3384.4), C=O (1705.2), C-N (1174.1)**; 1H NMR (δ, ppm):** 10.35, 7.09 (NH, pyrimidine), 6.52 (NH_2), 5.83,7.36 (CH, pyrimidine), 7.18-8.39 (CH, aromatic), 2.33 (CH_3, methyl); **Mass (m/e):** 360.7

Anti-bacterial activity

The zone of inhibition was measured to assess the preliminary antibacterial activity of the triazine derivatives. Four concentrations of the conjugates were tested for antibacterial action. Norfloxacin was used as the standard drug for antibacterial action (Table 2).

Table 2. Zone of inhibition exhibited by compounds

Compound Code	**Zone of Inhibition (mm)***							
	S. auerus				***E.coli***			
	25µg	50µg	100µg	150µg	25µg	50µg	100µg	150µg
6a	-	-	16	18	-	-	12	13
6b	-	-	15	16	-	-	16	19
6c	-	-	17	18	-	-	16	18
6d	-	-	**18**	**20**	-	-	**17**	**19**
6e	-	-	**19**	**20**	-	-	**18**	**20**
Norfloxacin	**24**	**-**	**-**	**-**	**25**	**-**	**-**	**-**

The MIC value of the test compounds was determined using broth dilution method by measuring the optical density of the broth solution incubated with diluted drug samples (Figure 2,3). The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Table 2).

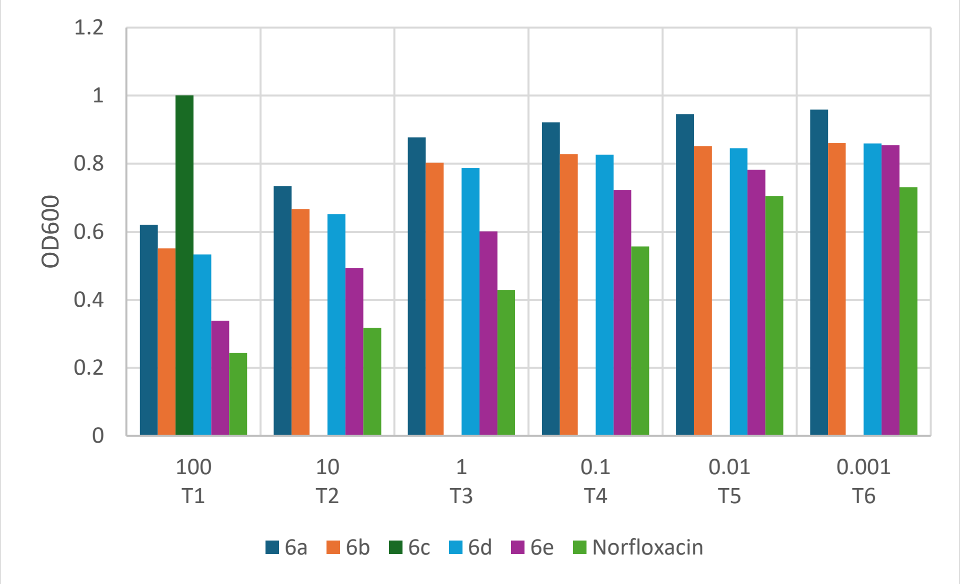


Figure 2 Plot of optical density for *E. coli*

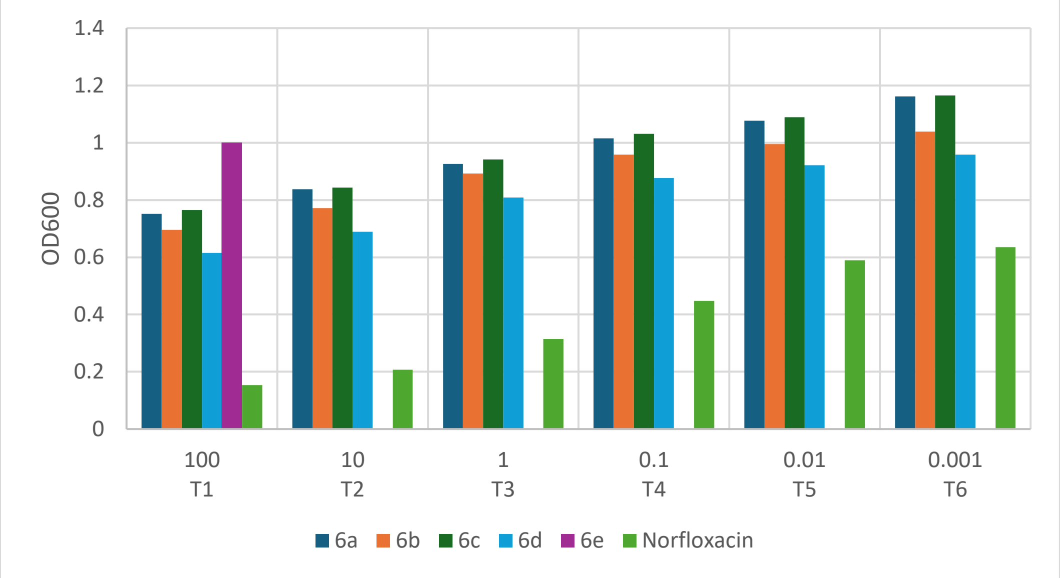


Figure 3 Plot of optical density for *S. aureus*

Table 2 Calculated IC_{50} values of the test samples

Test sample	**IC50 (µg/mL)**	
	E. coli	***S. aureus***
6a	150.24	159.57
6_b	127.76	140.5
6_c	134.20	152.15
6_d	107.43	132.35
6_e	56.73	53.55
Norfloxacin	0.99	0.02

Discussion

In the IR spectrum, stretching bands of N-H (3500-3300 cm^{-1}), C=O (1818-1705 cm^{-1}), and C-C (1300-800 cm^{-1}), were observed in each compound. Bending vibration of N-H (1650-1580 cm^{-1}) and C-N (1250-1020 cm^{-1}) were also visible. The NMR spectra revealed protons of NH of pyrimidine as well as of primary amine. The protons of aromatic ring as well as aliphatic methyl group were also found in the compounds. The antibacterial action against both gram positive and gram negative bacterial was exhibited by the compounds. The compounds were found to be possessing better inhibitory action against the gram negative bacteria in comparison to gram positive bacteria. The IC_{50} values of the compounds was calculated and the lowest IC_{50} was obtained for **6e** against both gram positive (56.73 µg/mL) and gram negative bacteria (53.55 µg/mL). The presence of electron withdrawing group on the compounds decreased the antibacterial potential **6b** (IC_{50} 140.5 µg/mL (*E. coli*) and 127.76 µg/mL (*S. aureus*)) and **6c** (IC_{50} 152.15 µg/mL (*E. coli*) and 134.20 µg/mL (*S. aureus*)).

Conclusion

In the present study, triazine-dihydropyrimidine compounds were synthesized using urea and ethylacetoacetate in a condensation reaction were reacted with different aromatic aldehydes followed by substitution reactions with guanidine hydrochloride to produce carboxamide derivatives. The carboxamides on reaction with benzonitrile resulted in rearrangement and cyclization leading to the formation of target triazine derivatives (6a-e). The compounds were found to be of good purity and yield. Two compounds exhibited good anti-bacterial potential against *E. coli* and *S. aureus*. Compounds **6e** was found to be the most potent of all the compounds. This compound might be used to create newer similar compounds with the aid of computer aided drug design techniques like pharmacophore modeling or docking which might lead to generation of a new lead molecule for anti-bacterial activity.

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References

1. Noureen S, Ali S, Zia MA, Afzal M, Ayub AR, El-Naggar M. Synthesis, combined theoretical and spectral characterization of some new 1,3,5 triazine compounds, and their in vitro biological analysis. Reaction Chemistry and Engineering. 2023; 8: 465-481
2. Aldalbahi A, AlOtaibi BS, Thamer BM, El-Faham A. Synthesis of New S-Triazine Bishydrazino and Bishydrazido-Based Polymers and Their Application in Flame-Retardant Polypropylene Composites. *Polymers*. 2022; 14: 784. Doi: 10.3390/polym14040784

3. Park S, Ahn Y, Kim Y, Roh EJ, Lee Y, Han C, Yoo HM, Yu J. Design, Synthesis and Biological Evaluation of 1,3,5-Triazine Derivatives Targeting hA1 and hA3 Adenosine Receptor. *Molecules*. 2022; 27: 4016. Doi: 10.3390/molecules27134016
4. Xue X, Luo J, Kong L, Zhao J, Zhang Y, Du H, Chen S, Xie Y. The synthesis of triazine–thiophene–thiophene conjugated porous polymers and their composites with carbon as anode materials in lithium-ion batteries. *RSC Advances*. 2021; 11: 10688. Doi: 10.1039/d0ra10862f
5. Wippert N, Nieger M, Herlan C, Jung N, Bräse S. Synthesis of new pyrazolo[1,2,3]triazines by cyclative cleavage of pyrazolyltriazenes. *Beilstein Journal of Organic Chemistry*. 2021; 17: 2773–2780. Doi: 10.3762/bjoc.17.187
6. Chalermnon M, Cherdchom S, Sereemaspun A, Rojanathanes R, Khotavivattana T. Biguanide-Based Synthesis of 1,3,5-Triazine Derivatives with Anticancer Activity and 1,3,5-Triazine Incorporated Calcium Citrate Nanoparticles. *Molecules*. 2021; 26: 1028. Doi: 10.3390/ molecules26041028
7. Pan X, Song C, Zhou W, Cui D-M, Zhang C. Synthesis of imidazo[1,2-a][1,3,5]triazines by NBS-mediated coupling of 2-amino-1,3,5-triazines with 1,3-dicarbonyl compounds. *New Journal of Chemistry*. 2020. Doi: 10.1039/c9nj05794c
8. Mishra R, Mishra BJ, Moorthy NSHN. Synthesis and antimicrobial evaluation of some novel 3,4-dihydropyrimidine-2-one derivatives. Trends in Applied Sciences Research. 2008; 3(2): 203-208
9. Sandhya G, Sravanthi GG. Microwave assisted synthesis of 1,3,5-triazine containing [1,8]naphthyridine derivatives. Asian Journal of Research in Chemistry. 2018; 11(1): 109-110.
10. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR (Editors). In: Vogel's Textbook of Practical Organic Chemistry. 5th edition, Longman, Singapore, 1989, pp. 26-33
11. Ault, A.; Determination of physical properties. In techniques and experiments for organic chemistry; University science books: Susalito. 1998, 138-240.
12. Ault, A. Separate of substance publication of substances. In techniques 1 experiments for organic chemistry. University science books, chemistry. University science books. 1998, 44-37.
13. Furniss BS, Hannaford AJ, Smith PWG, Tatchell AR (Editors). In: Vogel's Textbook of Practical Organic Chemistry. 5th edition, Longman, Singapore, 1989, pp. 197-233
14. Mishra R, Jain S. Investigation of antimicrobial potential of some thiazolyl chalcone derivatives. PharmacologyOnline. 2013; 1: 190-193.