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SUSTAINABLE SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND ANTIMICROBIAL PROFILING OF 1,3,5-TRIAZINE DERIVATIVES VIA ULTRASOUND APPROACH

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ABSTRACT

The present study describes the sustainable synthesis, structural characterization, and antimicrobial evaluation of trisubstituted 1,3,5-triazine derivatives using an ultrasound-assisted approach. A three-step synthetic pathway involving successive nucleophilic substitution of cyanuric chloride was employed to obtain five derivatives (TSa-e). The compounds were characterized by FTIR, 1H-NMR, and mass spectrometry, confirming the incorporation of functional groups and substitution patterns. Physicochemical properties including solubility, melting point, and retention factor were determined. Antibacterial activity was assessed against Escherichia coli (MTCC 40) and Staphylococcus aureus (MTCC 3160) using disc diffusion and broth dilution methods. Among the synthesized derivatives, TSc (N2,N4,N6-triphenyl-1,3,5-triazine-2,4,6-triamine) exhibited the most potent activity with IC50 values of 52.15 µg/mL (E. coli) and 76.13 µg/mL (S. aureus), suggesting enhanced lipophilicity improved antibacterial efficacy. Electron-withdrawing substituents (Cl, Br) reduced activity compared to unsubstituted or alkyl-substituted derivatives. The study highlights ultrasound-assisted synthesis as a green and efficient method for preparing bioactive triazine derivatives with promising antibacterial potential.

Key words: 1,3,5-Triazine; Ultrasound-assisted synthesis; Antimicrobial evaluation; Escherichia coli; Staphylococcus aureus

1 INTRODUCTION

Heterocyclic compounds, particularly triazine derivatives, have attracted significant attention in medicinal chemistry due to their diverse biological activities including antimicrobial, anticancer, and anti-inflammatory properties [1,2]. Among them, 1,3,5-triazines are versatile scaffolds that allow substitution at three reactive sites, enabling the design of molecules with tailored physicochemical and pharmacological profiles [3]. Traditional synthetic methods for triazines often involve harsh reaction conditions, prolonged reaction times, and the use of environmentally hazardous solvents [4]. In recent years, ultrasound-assisted synthesis has emerged as a sustainable alternative, offering advantages such as reduced reaction time, improved yields, and minimal use of organic solvents [5,6]. Sonochemistry promotes cavitation phenomena, which enhance mass transfer and accelerate chemical transformations under mild conditions, making it a green approach for heterocyclic synthesis [7].

The structural characterization of synthesized triazine derivatives is crucial for confirming substitution patterns and functional group incorporation. Techniques such as Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry provide valuable insights into the molecular framework [8].

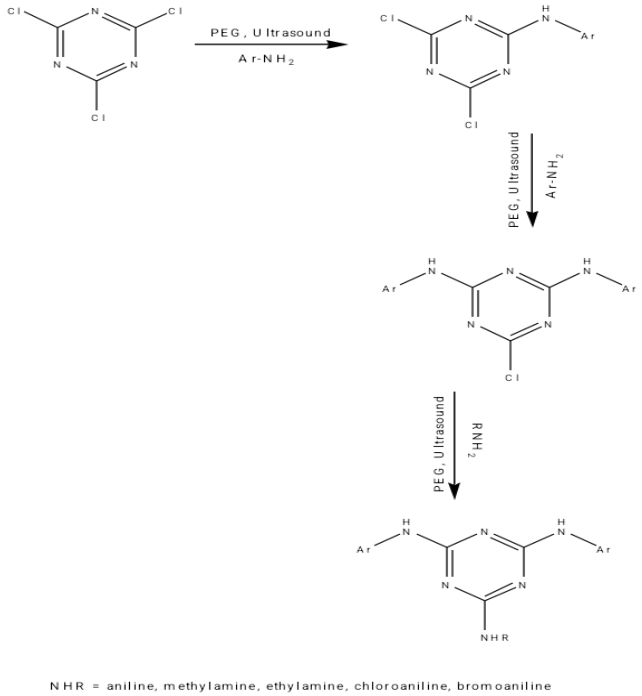
Furthermore, evaluation of antimicrobial activity against clinically relevant pathogens such as Escherichia coli and Staphylococcus aureus is essential to establish the therapeutic potential of these compounds[9]. In this study, we report the ultrasound-assisted synthesis of trisubstituted 1,3,5-triazine derivatives, their structural characterization, and antimicrobial evaluation. The work emphasizes eco-friendly methodology while exploring the relationship between structural modifications and antibacterial efficacy.

2. MATERIAL AND METHODS

All the reagents and chemicals used were of synthetic or analytical grade and used without drying or purification unless otherwise mentioned.

2.1 Synthesis Methodology

The synthetic pathway to prepare the desired trisubstituted triazine derivatives was adapted from the previously reported methodology by Syafiq Bin Shahari et al33, Patel & Baldaniya36 and Sharma et al [10]. The procedure was modified to suit the laboratory atmosphere and produce products in highest possible yields. A three-step synthetic route (scheme 1) involving synthesis of mono substituted followed by di substituted and finally tri substituted compound was used to obtain the desired products. The product from each step was separated and purified by recrystallization.



Scheme 1. Synthetic pathway for trisubstituted triazines

2.1.1 General method for synthesis of monosubstituted cyanuric chloride

In a flat bottom stoppered flask sodium carbonate (0.005 mol), appropriate amine (0.001 mol) were added in methanol (10 mL). To the mixture was slowly added cyanuric chloride (0.001 mol). The contents were dissolved by shaking and subjected to sonication for 20 min. After completion of the reaction (monitored by TLC, n-hexane:ethylacetate, 9:1), the contents were allowed to cool in the refrigerator and the solid obtained was filtered and washed with water to obtain monosubstituted triazine derivative [11].

2.1.2 General method for synthesis of monosubstituted cyanuric chloride

The product obtained from step 1 (0.001 mol) was taken in a round bottom flask. To this was added 0.001 mol of the appropriate amine and methanol (10 mL). The contents were dissolved by shaking and subjected to sonication for 25 min at room 30°C. After completion of the reaction (monitored by TLC, n-hexane:ethylacetate, 9:1), the contents were allowed to cool in the refrigerator and the solid obtained was filtered and washed with water to obtain the di substituted triazine derivative [11].

2.1.3 General method for synthesis of monosubstituted cyanuric chloride

The product obtained from step 2 (0.001 mol) was taken in a round bottom flask. To this was added 0.001 mol of the appropriate amine and methanol (10 mL). The contents were dissolved by shaking and subjected to sonication for 50 min at room 40°C. After completion of the reaction (monitored by TLC, n-hexane:ethylacetate, 9:1), the contents were allowed to cool in the refrigerator and the solid obtained was filtered and washed with water to obtain the trisubstituted traizine derivative [11].

2.2. Chemical characterization of synthesized compounds

The various physical and chemical features of the synthesized compounds were studied using reported methods.

2.3 Yield, solubility and melting point determination

Weighing the resulting product using an electronic weighing balance allowed us to determine the practical yield of the dried product. The following formula was used to get the yield percent [12]. The solubility was determined to check whether the synthesized compounds were soluble in a variety of solvents, including water, methanol, chloroform, and dimethylsulfoxide [13]. The popular open capillary method was employed to find the melting points of the synthesized products [14].

2.4 Retention factor determination and structural characterization

Thin layer chromatography (TLC) was used to determine the retention factor (Rf) of the synthesised imine-benzothiazine derivatives using precoated TLC plates. The sample was identified on the lower end of the plate after being dissolved in methanol/ethanol. The solvent system was applied to the plate, and development time was permitted. When the solvent was ready to cover the top of the plate, the plate was removed from the solvent system. Following air drying, the plate was examined using a TLC cabinet and UV light to determine the distance that the solvent and samples had gone. The formula was used to compute the retention factor [15].

$$Rf = \frac{Distance\ travelled\ by\ sample}{Distance\ travelled\ by\ solvent}$$

Using infrared and nuclear magnetic resonance spectroscopy methods, the structural characteristics (functional groups incorporated into or present in the structure and the protons) were investigated [16].

3. ANTIBACTERIAL EVALUATION

3.1 Microorganisms used

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation. The lyophilized cultures were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria.

3.2 Preparation of test compounds

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

3.3 Screening Procedure (zone of inhibition)

A few drops of the bacterial suspension were injected onto the surface of pre-poured, 3 mm-thick nutritional agar plates. The disc diffusion method was used to screen for antibacterial activity [17]. Using a cork borer (10mm), wells were created in the agar plate at equal intervals, and 200 mL of the triazines (25, 50, 75, and 100 µg/mL) were poured into each one. The plates were kept at 37 ± 0.1°C for 24 hours to promote microbial development. Each plate's zone of inhibition was measured in millimetres.

3.4 Screening Procedure (Minimum inhibitory concentration)

We calculated the minimal inhibitory concentration of the synthesized compounds using the broth dilution method. The bacterial culture's ultimate inoculum size was kept at 10^5 CFU/mL. A set of tubes containing only the inoculated broth was used as the growth control, and one containing only the broth was used to ensure the sterility of the medium.

A set of six tubes was labelled as 1 to 6 and to each tube was added 3 mL of nutrient broth. To the first tube was added 300µL of 1mg/mL of the triazine sample. The contents were mixed by swirling between hands and 300µL of content from the 1st tube was transferred to 2nd tube. The process was repeated from 2nd to 3rd tube up to the 6th tube. From the 6th tube, 300µL of content was withdrawn and discarded.

To each of these tubes was added 200µL of the bacterial inoculum. 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 (ciprofloxacin) [17].

4. RESULTS

4.1 Chemical characterization of compounds

A total of ten compounds was planned to be synthesized but due to the non-availability of few chemicals, only five compounds were synthesized. The synthesized compounds were coded as **TS_{a-e}** and these compounds were tested for yield (%), solubility, retention factor (R_f) and melting point (°C).

All the synthesized triazine derivatives were assessed for their qualitative solubility in water, methanol, chloroform and DMSO. The solubility of compounds is important to select the solvent for preparation of test samples for pharmacological screening as well as selection of solvent for spectral studies. TS_a and TS_b were soluble in water, chloroform and DMSO whereas TS_{c-e} were soluble in chloroform and DMSO. All compounds were insoluble in methanol.

The melting temperature and R_f value of the synthesized compounds is presented in Table 1.

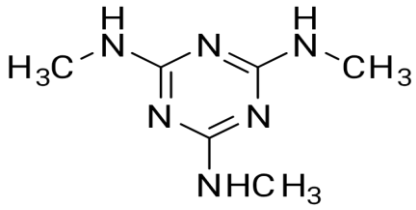
Table 1. Melting point and R_f value of TS_{a-e}

Compound Code	Melting Point (°C)	R_f Value (n-hexane: ethyl acetate (8:2)
TS_a	160-162°C	0.36
TS_b	164-166°C	0.69
TS_c	208-210°C	0.76
TS_d	204-206°C	0.58
TS_e	271-273°C	0.59

4.2 Structural study of the synthesized compounds

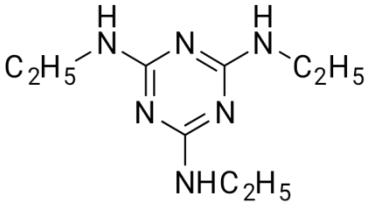
The structure of the synthesized compounds was confirmed by studying the 1H-NMR, and FTIR spectra of the molecules.

Structure elucidation of TS_a



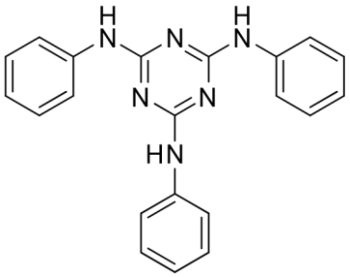
IUPAC - N^2,N^4,N^6-trimethyl-1,3,5-triazine-2,4,6-triamine; **Color** - Off-White; **^{1}HNMR** (δ, ppm): 6.23-6.26 (Protons of amine (N-H)), 3.04-3.05 (Aliphatic protons (CH_3)); **Mass** (m/e, calculated) – 168.20; FTIR (cm^{-1}): 3030.61 (Ar C-H stretch), 1603.33 (Ar C-C/Ar C=C stretch), 897.10 (C-N stretch)

Structure elucidation of TS_b



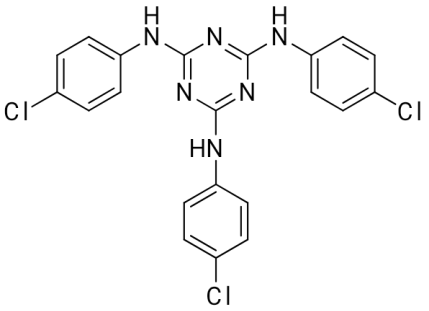
IUPAC - N^2,N^4,N^6-triethyl-1,3,5-triazine-2,4,6-triamine; **Color** - Off-White; **^{1}HNMR** (δ, ppm): 5.44-5.46 (Protons of amine (N-H)), 3.50-3.55 (Aliphatic protons (CH_2)), 1.28-1.31 (Aliphatic proton (CH_3)); **Mass** (m/e, calculated) – 210.28; FTIR (cm^{-1}): 3413.55 (N-H stretch), 3028.61, 3102.68 (Ar C-H stretch), 1586.89 (Ar C-C/Ar C=C stretch), 852.45, 897.35 (C-N stretch)

Structure elucidation of TS_c



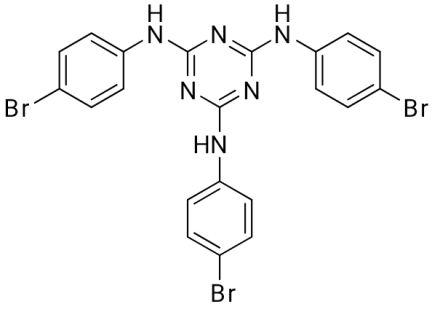
IUPAC - N^2,N^4,N^6-triphenyl-1,3,5-triazine-2,4,6-triamine; **Color** - Off-White; **^{1}HNMR** (δ, ppm): 8.42 (Protons of amine (N-H)), 6.84-7.77 (Aromatic protons (C-H)); **Mass** (m/e, calculated) – 354.41; FTIR (cm^{-1}): 3413.55 (N-H stretch), 3026.05 (Ar C-H stretch), 1589.72 (Ar C-C/Ar C=C stretch), 817.65 (C-N stretch)

Structure elucidation of TS_d



IUPAC - N^2,N^4,N^6-tris(4-chlorophenyl)-1,3,5-triazine-2,4,6-triamine; **Color** - Pinkish-White; **^{1}HNMR** (δ, ppm): 8.49 (Protons of amine (N-H)), 7.21-7.23 (Aromatic protons (C-H)); **Mass** (m/e, calculated) – 457.74; FTIR (cm^{-1}): 3365.77 (N-H stretch), 3024.82, 3103.07 (Ar C-H stretch), 1578.14 (Ar C-C/Ar C=C stretch), 815.82 (C-N stretch), 1082.39 (C-Cl stretch)

Structure elucidation of TS_e



IUPAC - N^2,N^4,N^6-tris(4-bromophenyl)-1,3,5-triazine-2,4,6-triamine; **Color** - Pinkish-White; **^{1}HNMR** (δ, ppm): 8.52 (Protons of amine (N-H)), 7.41-7.43 and 8.69-8.71 (Aromatic protons (C-

H)); **Mass** (m/e, calculated) – 591.10; FTIR (cm^{-1}): 3342.99 (N-H stretch), 3093.00 (Ar C-H stretch), 1587.97 (Ar C-C/Ar C=C stretch), 897.14, 809.11 (C-N stretch), 597.97 (C-Br stretch)

5. ANTIBACTERIAL ACTION - ZONE OF INHIBITION

The zone of inhibition was measured to assess the preliminary antibacterial activity of the triazine derivatives (trisubstituted). Four concentrations of the triazines were tested for antibacterial action. Ciprofloxacin 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. aureus				*E. coli*			
	25 µg	50µg	75 µg	100µg	25 µg	50 µg	750µg	100µg
TS_a	-	-	14	16	-	-	11	12
TS_b	-	-	13	14	-	-	14	17
TS_c	-	-	15	**20**	-	-	17	**22**
TS_d	-	-	13	15	-	-	14	15
TS_e	-	-	12	13	-	-	11	13
Ciprofloxacin	**24**	-	-	-	**22**	-	-	-

5.1 Minimum Inhibitory Concentration (MIC)

The MIC value of the test compounds was determined using the broth dilution method by measuring the optical density of the broth solution incubated with diluted drug samples. The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Figure 1, 2).

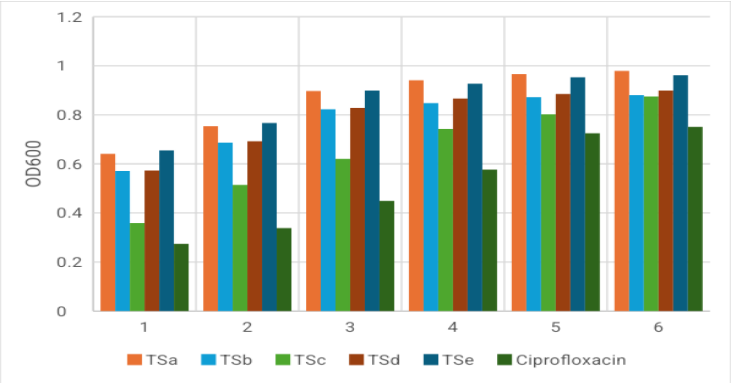


Figure 1. Optical density of TS_{a-e} against *E. coli*

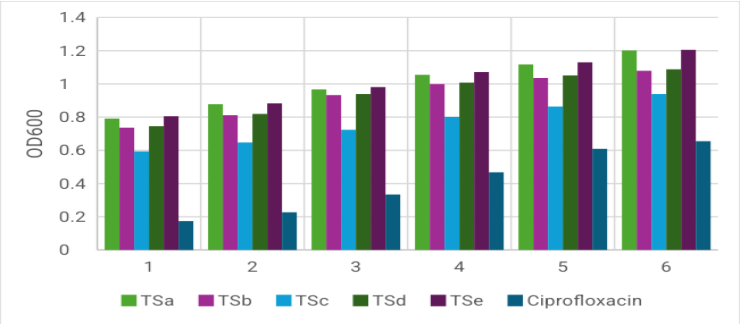


Figure 2. Optical density of TS_{a-e} against *S. aureus*

The MIC (IC_{50}) was calculated from the optical density data and the values are presented in Table 3.

Table 3. Calculated IC_{50} values of the test samples

Test sample	IC_{50} (µg/mL)	
	E. coli	*S. aureus*
TS_a	133.92	149.56
TS_b	111.58	132.68
TS_c	52.15	76.13
TS_d	111.61	135.88
TS_e	139.06	154.88
Ciprofloxacin	1.16	0.01

6. DISCUSSION

6.1 Chemistry

Ultrasonic irradiation was used to create the trisubstituted triazine. The three cyanuric chloride chloro groups undergo successive nucleophilic substitution in the process at different temperatures. The first attack is the formation of the compound. The second nucleophilic assault happens at room temperature as a result of the compound's other chloro groups being less reactive. The creation of the disubstituted triazine derivative further lowers the reactivity of the chloro group and the third nucleophile reacts at a higher temperature (40°C) leading to the formation of the trisubstituted derivative. The time of reaction changed with increasing replacements on the ring. The products were soluble in water (TSa, TSb) and DMSO and chloroform and were obtained in 71-76% yield. The structural identification of the compounds was done by FTIR spectroscopy to identify the functional groups present and 1H-NMR spectroscopy to identify the protons in the molecules.

All the compounds exhibited the stretching vibrations for aromatic C-H (3080-3030 cm-1), aromatic C-C/C=C (1625-1575 cm-1), N-H (3500-3100 cm-1) and C-N (900-800 cm-1), in the FTIR spectrum. The stretching vibrations corresponding to C-Cl (1080-1020 cm-1) and C-Br (600-500 cm-1) were also found in the compounds containing these groups. The proton NMR spectra presented the chemical shift presence of protons of aromatic ring (6.7-7.7 ppm), amine hydrogen in all compounds. The chemical shifts of the aliphatic protons (1.28-3.77 ppm) was found in TSa and TSb.

6.2 Antibacterial action

The antibacterial action against both gram positive and gram negative bacteria 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 IC50 values of the compounds were calculated and the lowest IC50 was obtained for TSc against both gram positive (76.13 µg/mL) and gram-negative bacteria (52.15 µg/mL). This suggests that increasing the lipophilicity of the molecule helped in improving its antibacterial action. The presence of electron withdrawing group on the compounds decreased the antibacterial potential TSd (IC50 111.61 µg/mL (E. coli) and 135.88 µg/mL (S. aureus)) and TSe (IC50 139.06 µg/mL (E. coli) and 154.88 µg/mL (S. aureus)).

7. CONCLUSIONS

Ultrasound-assisted synthesis provided an eco-friendly and efficient route for preparing trisubstituted 1,3,5-triazine derivatives with good yields and reproducibility. Structural characterization confirmed successful substitution, while antibacterial screening demonstrated moderate to significant activity against both gram-positive and gram-negative bacteria. The triphenyl-substituted derivative (TSc) showed the highest inhibitory effect, indicating that increased lipophilicity enhances antimicrobial potency. Conversely, derivatives containing electron-withdrawing substituents exhibited reduced activity. These findings suggest that structural modifications of triazine scaffolds can modulate antibacterial efficacy, and ultrasound-mediated synthesis offers a sustainable platform for developing new heterocyclic antimicrobial agents.

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