One Pot Synthesis, Characterization and Biological Evaluation of Bisnaphthylamine Substituted s-triazine Based Molecules

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ABSTRACT

The objective of the present investigation to synthesize trisubstituted traizine derivatives containing bisnapthylamine using a one pot synthesis method and evaluate the antibacterial activity of the compounds. The zone of inhibition was measured to assess the preliminary antibacterial activity of the trisubstituted derivatives and the broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds against S. aureus (gram positive) and E. coli (gram negative) bacteria. The products were soluble in water (BTb, BTc) and chloroform (BTd, BTe) and were obtained in 67-76% yield. The proton NMR spectra presented the chemical shifts presence of protons of aromatic ring (6.7-7.7 ppm), amine hydrogen (5.55 ppm) in all compounds. The chemical shifts of the aliphatic protons (2.5-3.7 ppm) were found in BT a and BTb. 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 BTc against both gram positive (88.24 µg/mL) and gram negative bacteria (55.37 µg/mL).

Keywords: Antibacterial, Solubility, Temperature, Rf value, UV Spectroscopy.

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INTRODUCTION

Heterocycles represents one of the most established and extensively studied structural classes of organic chemistry. This class includes some distinct elements, in addition to carbon atoms, as members of its ring, such as nitrogen, oxygen, and sulphur, among others. This class has made significant contributions to the literature over the last few decades due to their various synthetic methods and diverse biological applications. 1-8 Nitrogen-containing heterocyclic molecules, such as pyrrole, pyridine, pyrazole, imidazole, triazole, tetrazole, and others, have shown researchers interest over decades of organic synthesis history. [1]

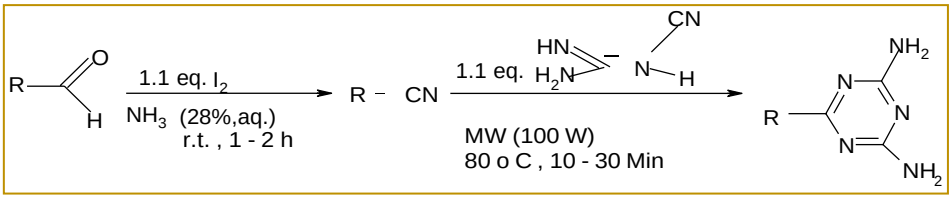
Synthetic organic chemists were particularly interested in monocyclic and bicyclic compounds with one or more heteroatoms. As a structural framework of natural and naturally derived compounds of biological interest, azoles make the maximum contribution among all these

subclasses. Azoles have aided in the biological and industrial growth of society, as well as in the study of living processes in order to increase quality of life. These compounds are found in many sectors of life science and technology. [2,3]

1,3,5-triazine isomer also called as s-triazine due to the symmetry of three nitrogen in the ring. This isomer is an oldest known organic compound. 1,3,5-Triazines represent a broadly used lead structure with remarkable applications in various fields.[4] s-Triazine derivatives are an important class of compounds showing many pharmacological activities (like antimicrobial activities).[5]

Different Methods of synthesis of Triazine [6-8]

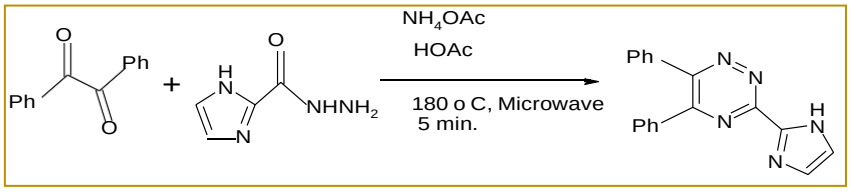
Synthesis of 1, 3, 5-triazines



A series of primary alcohols and aldehydes were treated with iodine in ammonia water under microwave irradiation to give the intermediate nitriles, which without isolation underwent [2 + 3] cycloadditions with dicyandiamide and sodium azide to afford the corresponding triazines and tetrazoles in high yields.

By the application of microwave technology

A general protocol has been developed for the rapid synthesis of diverse 3,5,6-trisubstituted 1,2,4-triazines in excellent yield and purity, including many previously unknown 3-heterocyclic-1,2,4-triazines.



Antimicrobial Activity

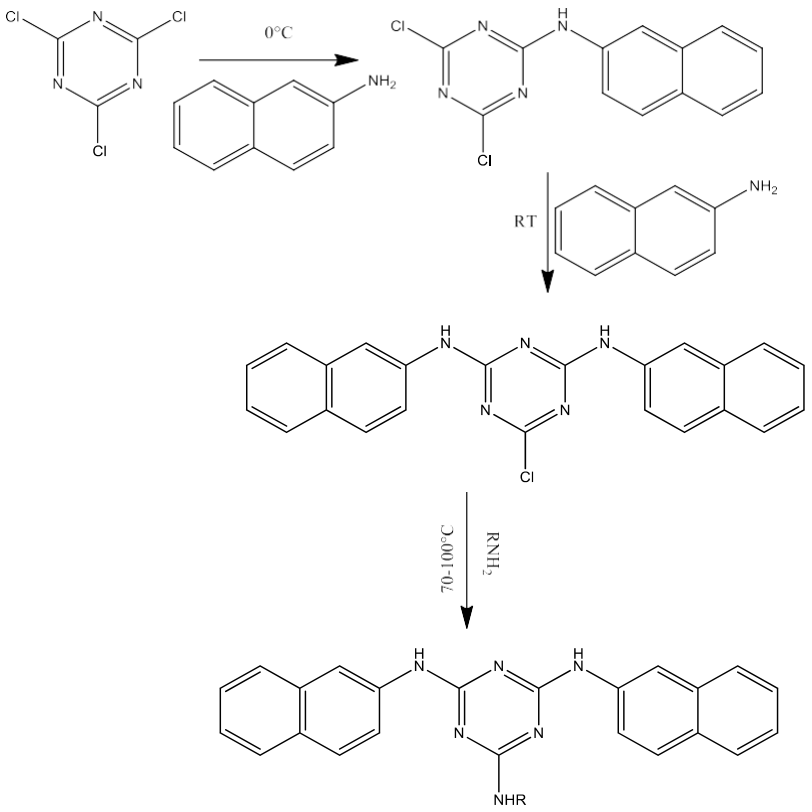
An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (Microbiostatic). [9]

MATERIAL AND METHODS

The objective of the present investigation to synthesize trisubstituted triazine derivatives using a one pot multicomponent reaction and evaluate the antibacterial activity of the synthesized compounds.

Synthesis Methodology

One pot synthesis, characterization and biological evaluation of bis naphtylamine substituted s-triazine based molecules. The synthetic pathway to prepare the desired trisubstituted triazine derivatives. The procedure was modified to suit the laboratory atmosphere and produce products in highest possible yields. A single pot reaction based 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. Throughout the reaction, no intermediate product was purified and separated from the reaction vessel. [10]



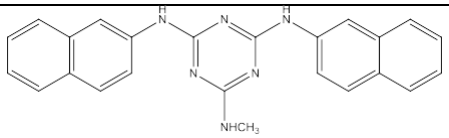
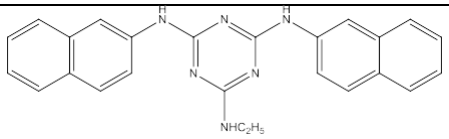
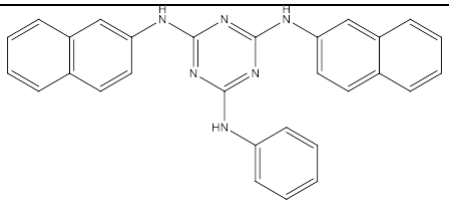
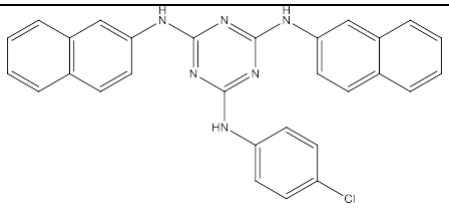
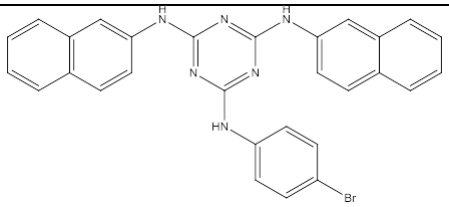
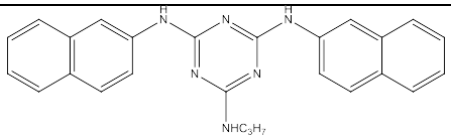
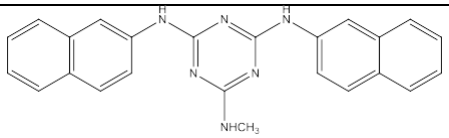
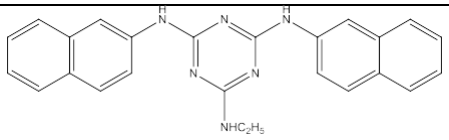
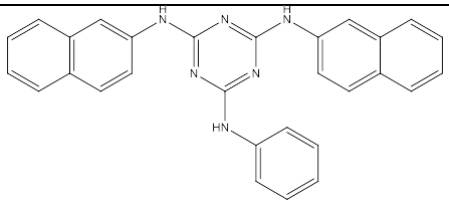
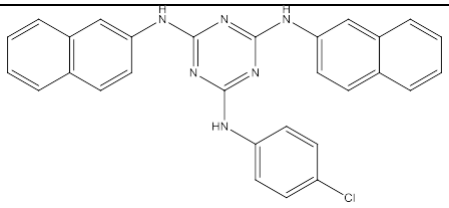
NHR = aniline, methylamine, ethylamine, chloroaniline, bromoaniline

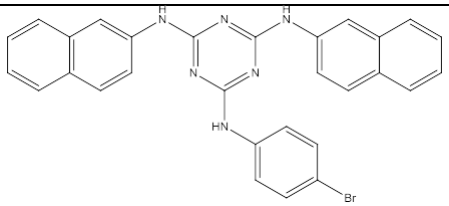
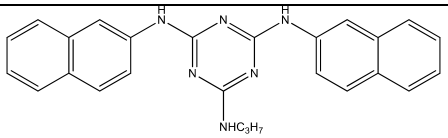
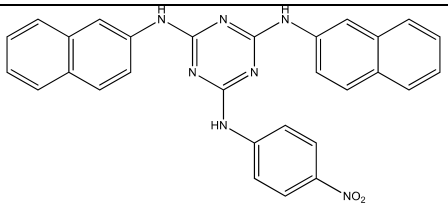
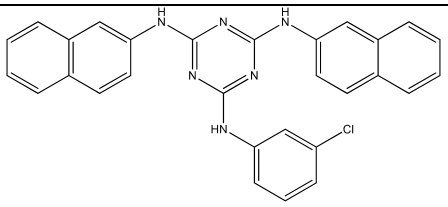
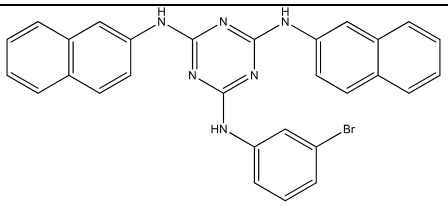
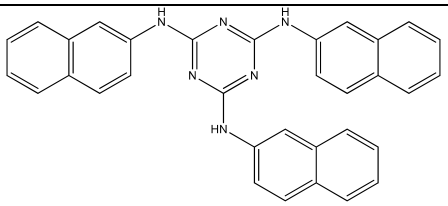
Scheme 1 Synthetic pathway for trisubstituted triazines

Ten novel bis-naphtylamine substituted triazine compounds were proposed for synthesis using the above scheme (Table 01)

Table 01: Structure of proposed imine-linked benzothiazine derivatives

Code	Structure	IUPAC Name

BT_a		N^2-methyl-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_b		N^2-ethyl-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_c		N^2,N^4-di(naphthalen-2-yl)-N^6-phenyl-1,3,5-triazine-2,4,6-triamine
BT_d		N^2-(4-chlorophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_e		N^2-(4-bromophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_f		N^2,N^4-di(naphthalen-2-yl)-N^6-propyl-1,3,5-triazine-2,4,6-triamine
Code	**Structure**	**IUPAC Name**
BT_a		N^2-methyl-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_b		N^2-ethyl-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_c		N^2,N^4-di(naphthalen-2-yl)-N^6-phenyl-1,3,5-triazine-2,4,6-triamine
BT_d		N^2-(4-chlorophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

BT_e		N^2-(4-bromophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_f		N^2,N^4-di(naphthalen-2-yl)-N^6-propyl-1,3,5-triazine-2,4,6-triamine
BT_g		N^2,N^4-di(naphthalen-2-yl)-N^6-(4-nitrophenyl)-1,3,5-triazine-2,4,6-triamine
BT_h		N^2-(3-chlorophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_i		N_2-(3-bromophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine
BT_j		N_2,N^4,N^6-tri(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

General method for synthesis

In a flat bottom stoppered flask sodium carbonate (0.005 mol), naphthylamine (0.002 mol) and appropriate amine (0.001 mol) were added in methanol (10 mL). To the mixture was slowly added cyanuric chloride (0.001 mol) and flask was stirred at 0°C for 1h on a magnetic stirrer. The flask was brought to room temperature by removing from the ice bath and the stirring was continued for 3h at room temperature. The flask was then attached with a reflux condenser and the contents were refluxed at 70-80°C for 12h to obtain the solid product. [11]

Chemical characterization of synthesized compounds

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

Yield determination [12]

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.

$$\% \, yield = \frac{Practical \; yield \; (g)}{Theoretical \; yield \; (g)} x100$$

Solubility determination [13]

It was determined whether the synthesized compounds were soluble in a variety of solvents, including water, methanol, chloroform, and dimethylsulfoxide. In a test tube, a few crystals of the product were placed, and 1 mL of the solvent was added. After shaking the test tube for a while, it was checked to see if there were any undissolved particles still present.

Melting point determination [14]

The popular open capillary method was employed to find the melting points of the synthesized products. By heating on a Bunsen burner, a glass capillary tube was sealed from one end, and the dried solid product was poured in through the capillary's other end. The capillary was now inserted into the melting point equipment' heating head, and a thermometer was fastened to it. The temperature was gradually raised, and the thermometer was used to record the temperature at which the product could be seen to be melting.

Retention factor determination [15]

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.

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

UV Spectral study [16,17]

The synthesized compounds were dissolved in appropriate solvent to prepared a 1mg/mL solution. The solution was further diluted with the same solvent to obtain a solution of 30µg/mL. This solution was placed in the sample cell and was scanned over range of 200-400 nm using a UV-Visible spectrophotometer and the absorption maxima was recorded.

Study of structural features of the synthesized compounds

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.

Fourier-transformed infrared spectroscopic analysis (FTIR) [18]

On the infrared spectrophotometer's analytical window was placed the dried sample, and it scanned between 400 and 4000 cm^{-1} wavenumbers. We measured and assessed the transmittance brought on by the vibrations of functional groups.

Proton nuclear magnetic resonance study (^{1}H-NMR)

The sample was dissolved in the proper solvent before being placed in the spectrophotometer's sample container. The sample was scanned over various magnetic fields using the field sweep method. To investigate the proton in the sample, the chemical shift that was obtained was noted and examined.

Antibacterial evaluation

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.

Nutrient Broth preparation

Ready to use broth powder was used for preparing the nutrient broth (Microgen).

Composition of the nutrient broth powder (pH 6.8 ±0.2)

Ingredients	**Amount (g/L)**
Beef Extract	**1.5**
Peptone	**5.0**
Yeast dried	**1.5**
Sodium chloride	**5.0**

13 g of the nutrient broth powder was dissolved in 1000 mL of distilled water with the aid of a slight heat and sterilized using autoclave at 121°C and 15 lbs pressure for 15 min.

Revival of lyophilized cultures

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.

Nutrient agar preparation- Composition of the nutrient agar powder (pH 6.8 ±0.2)

Ingredients	**Amount (g/L)**
Beef Extract	**3.0**
Peptone	**5.0**
Agar	**15.0**

23 g of the nutrient agar powder was dissolved in 1000 mL of distilled water with the aid of a slight heat and sterilized using autoclave at 121°C and 15 lbs pressure for 15 min. The sterilized medium was added to sterilized petridishes that had been appropriately marked and labelled in order to make agar plates.

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.

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. [19] 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.

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 (norfloxacin).[20]

RESULTS AND DISCUSSION

Chemical characterization of compounds A total of five compounds were planned to be synthesized. The synthesized compounds were coded as BTa-e and these compounds were tested yield (%), solubility, retention factor (Rf) and melting point (°C). A list of the amines used for synthesizing the trisubstituted traizine derivative, the posisble structure of the synthesized compound, yield and molecular formula are presented in Table 02.

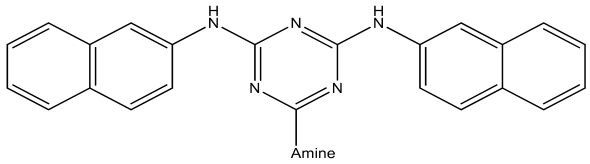
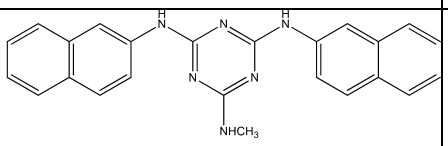
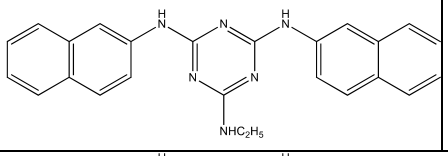
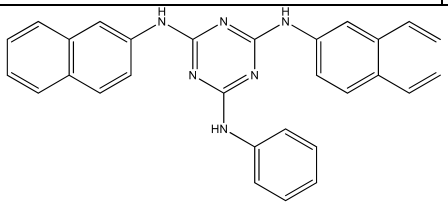
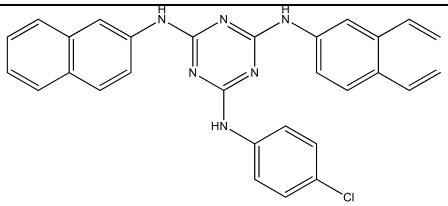
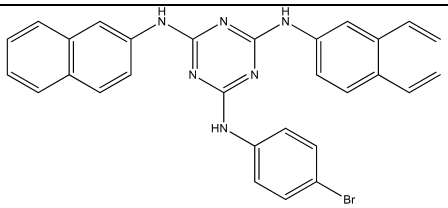


Table 02: Synthesized derivatives and their yield

Compound code	Amine used	Structure	Molecular Formula	% Yield
BT_a	Methyl amine		$C_{24}H_{20}N_6$	**72**
BT_b	Ethyla mine		$C_{25}H_{22}N_6$	**76**
BT_c	Aniline		$C_{29}H_{22}N_6$	**67**
BT_d	4-Chloroa niline		$C_{29}H_{22}ClN_6$	**71**
BT_e	4-Bromoa niline		$C_{29}H_{22}BrN_6$	**73**

All the synthesized triazine derivatives were assessed for their qualitative solubility in water, methanol, chloroform and DMSO. (Table 03).

Table 03: Solubility of trisubstituted triazine derivatives

Compound Code	Water	Methanol	Chloroform	DMSO

SBI_a	Insoluble	Insoluble	Insoluble	Insoluble
SBI_b	Soluble	Insoluble	Insoluble	Insoluble
SBI_c	Soluble	Insoluble	Insoluble	Insoluble
SBI_d	Insoluble	Insoluble	Soluble	Insoluble
SBI_e	Insoluble	Insoluble	Soluble	Insoluble

The melting temperature of the compounds was recorded using melting point apparatus equipped with thermometer (Table 04).

Table 04: Melting point of prepared trisubstituted triazines

Compound Code	Melting Point (°C)
BT_a	>300°C
BT_b	>300°C
BT_c	>300°C
BT_d	>300°C
BT_e	>300°C

The completion of reaction was monitored by TLC and the dried products were again subjected to TLC using precoated TLC plates to calculate the R_f value of the compounds (Table 05).

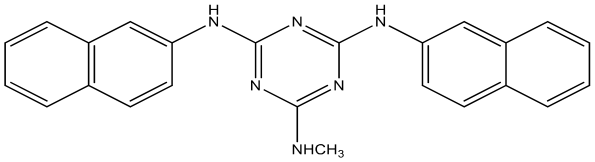
Table 05: Retention factor of benzothiazine derivatives

Compound Code	R_f Value	Developing solvent system
BT_a	0.35	n-hexane: ethyl acetate (8:2)
BT_b	0.68	n-hexane: ethyl acetate (8:2)
BT_c	0.73	n-hexane: ethyl acetate (8:2)
BT_d	0.54	n-hexane: ethyl acetate (8:2)
BT_e	0.56	n-hexane: ethyl acetate (8:2)

Structural study of the synthesized compounds

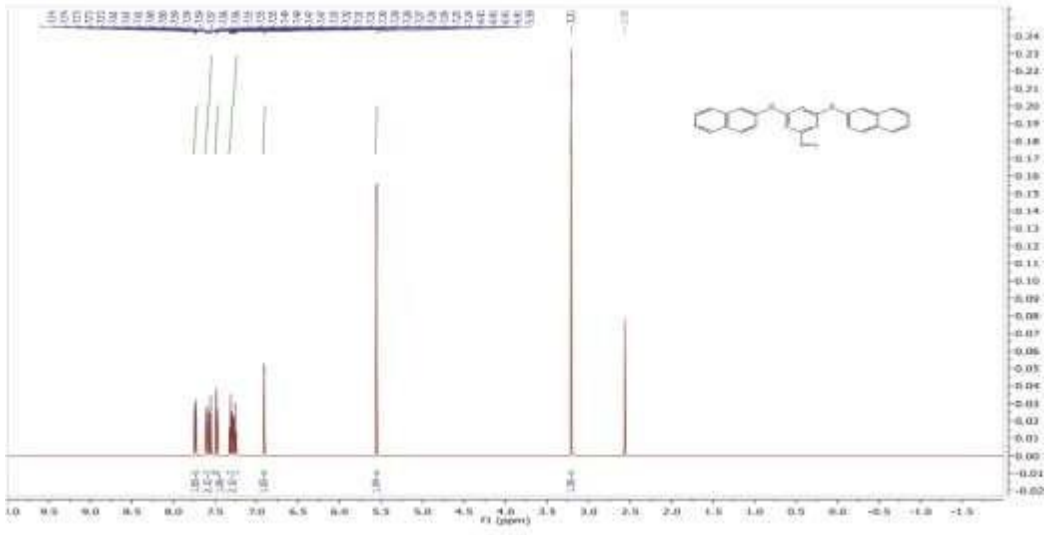
The structure of the synthesized compounds was confirmed by studying the 1H-NMR, and FTIR spectra of the molecules.

Structure elucidation of BT_a



IUPAC - N^2-methyl-N^4, N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

Color - Off-White

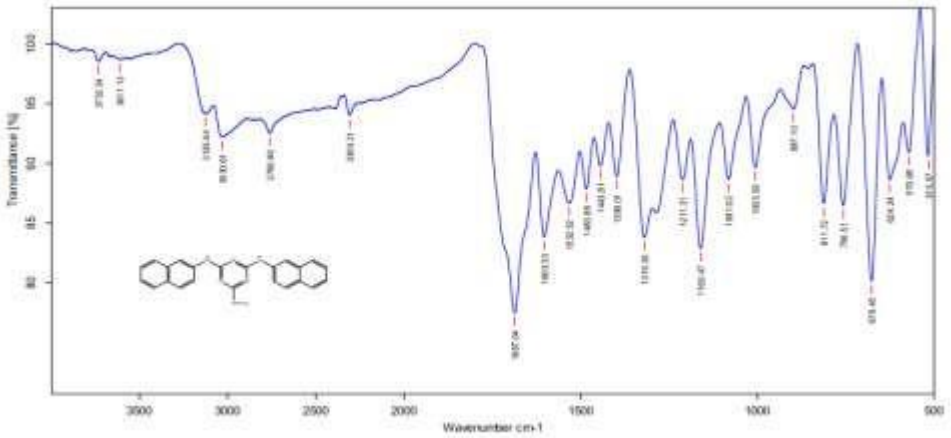


Graph 1 ^{1}H-NMR Spectra of BT$_a$

Table 06: Interpretation of ^{1}H-NMR Spectra of BT$_a$

S. No.	Peak Obtained	Peak occurred due to proton of…
1	6.91-7.74	Aromatic Protons (C-H)
2	3.21 & 5.55	Protons of amine (N-H)
3	2.56	Aliphatic proton (CH_3)

Mass (m/e, calculated) **–** 392.17



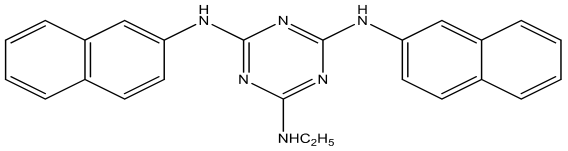
Graph 2 FTIR Spectra of BT$_a$

Table 07: Interpretation of FTIR Spectra of BT$_a$

S. No.	Peak Obtained (cm^{-1})	Reference Range (cm^{-1})	Peak occurred due to functional group
1	3030.61	3080-3030	Ar C-H stretch
2	1603.33	1625-1575	Ar C-C/Ar C=C stretch

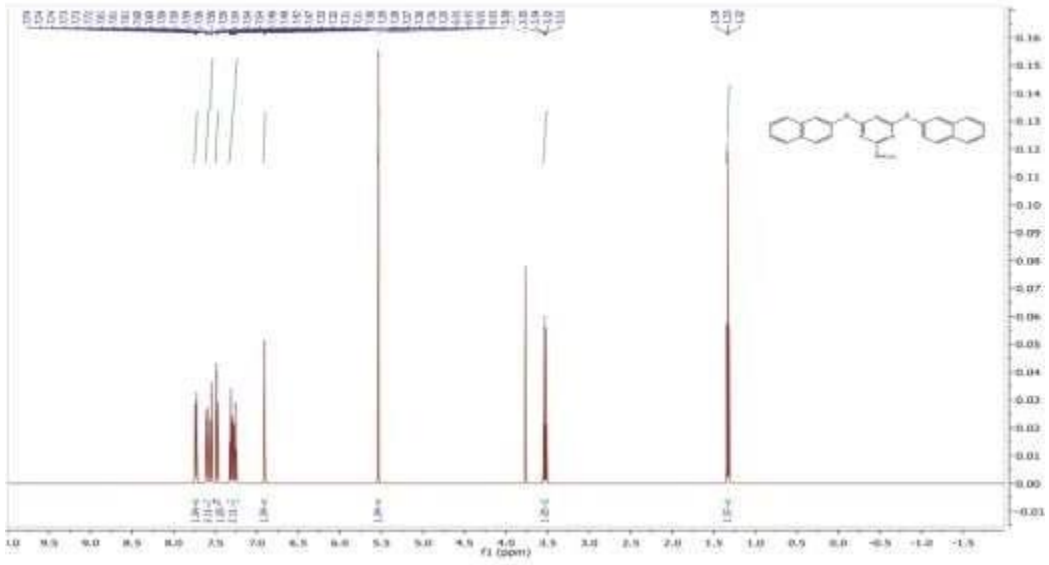
3	897.10	800-900	C-N stretch

Structure elucidation of BT_b



IUPAC - N^2-ethyl-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

Color - Off-White

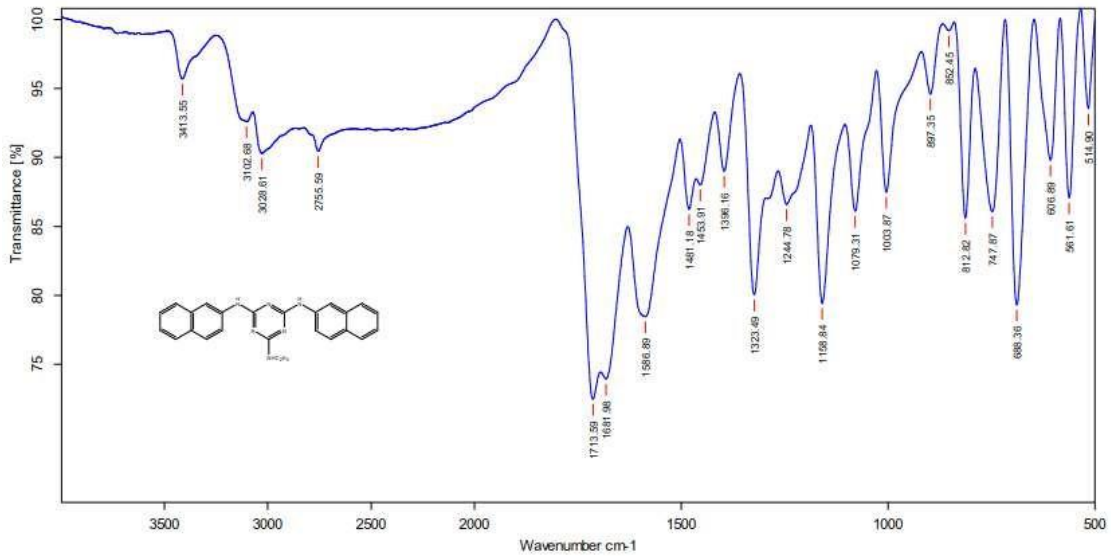


Graph 3 ^{1}H-NMR Spectra of BT_b

Table 8: Interpretation of ^{1}H-NMR Spectra of BT_b

S. No.	Peak Obtained	Peak occurred due to proton of…
1	6.91-7.74	Aromatic Protons (C-H)
2	5.54	Protons of amine (N-H)
3	3.51-3.57	Aliphatic proton (CH_2)
4	1.32-1.34	Aliphatic proton (CH_3)

Mass (m/e, calculated) **–** 406.48

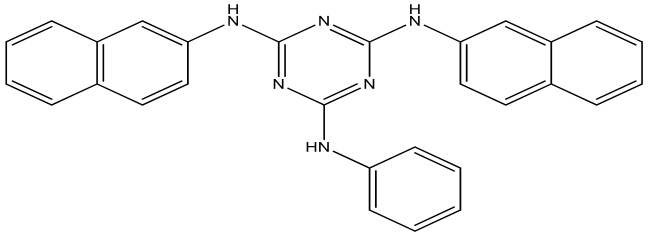


Graph 4 FTIR Spectra of BT_b

Table 09: Interpretation of FTIR Spectra of BT_b

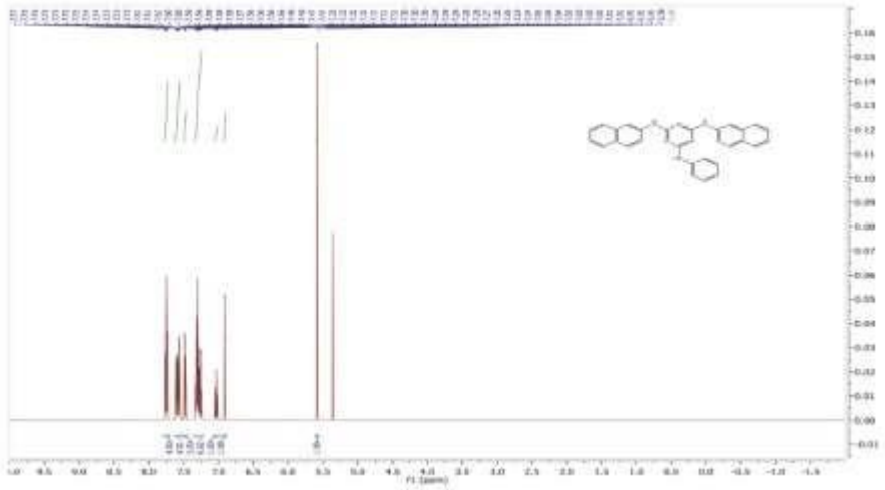
S. No.	Peak Obtained (cm^{-1})	Reference Range (cm^{-1})	Peak occurred due to functional group
1	3028.61, 3102.68	3080-3030	Ar C-H stretch
2	1586.89	1625-1575	Ar C-C/Ar C=C stretch
3	3413.55	3500-3100	N-H stretch
4	852.45, 897.35	800-900	C-N stretch

Structure elucidation of BT_c



IUPAC - N^2,N^4-di(naphthalen-2-yl)-N^6-phenyl-1,3,5-triazine-2,4,6-triamine

Color - Off-White

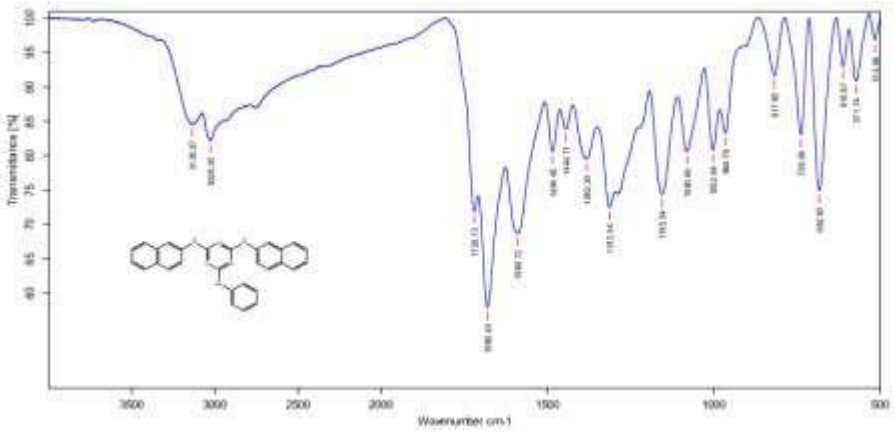


Graph 5 ^{1}H-NMR Spectra of BT_c

Table 10: Interpretation of ^{1}H-NMR Spectra of BT_c

S. No.	Peak Obtained	Peak occurred due to proton of…
1	6.91-7.77	Aromatic Protons (C-H)
2	5.36 & 5.58	Protons of amine (N-H)

Mass (m/e, calculated) **–** 454.53

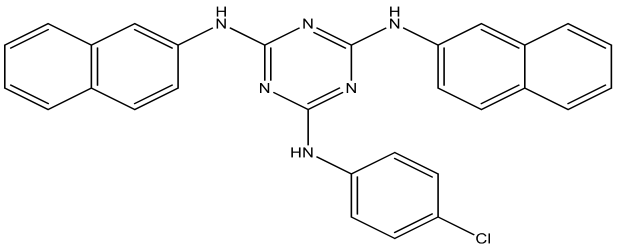


Graph 6 FTIR Spectra of BT_c

Table 11: Interpretation of FTIR Spectra of BT_c

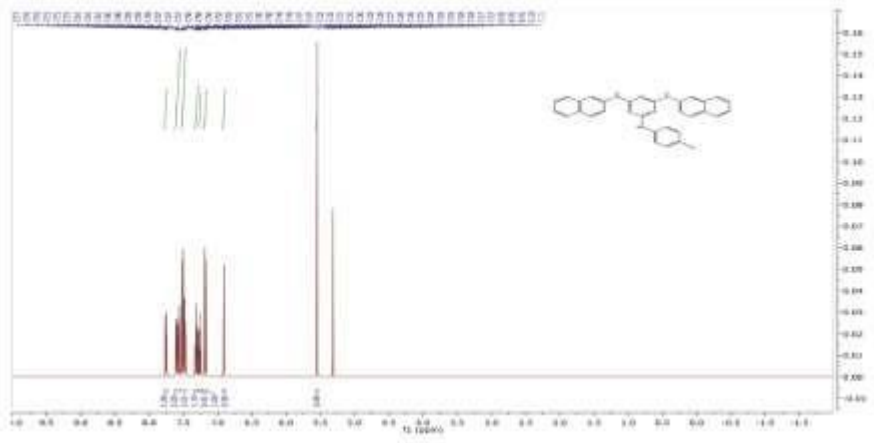
S. No.	Peak Obtained (cm^{-1})	Reference Range (cm^{-1})	Peak occurred due to functional group
1	3026.05	3080-3030	Ar C-H stretch
2	1589.72	1625-1575	Ar C-C/Ar C=C stretch
3	817.65	800-900	C-N stretch

Structure elucidation of BT_d



IUPAC - N^2-(4-chlorophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

Color - Pinkish-White

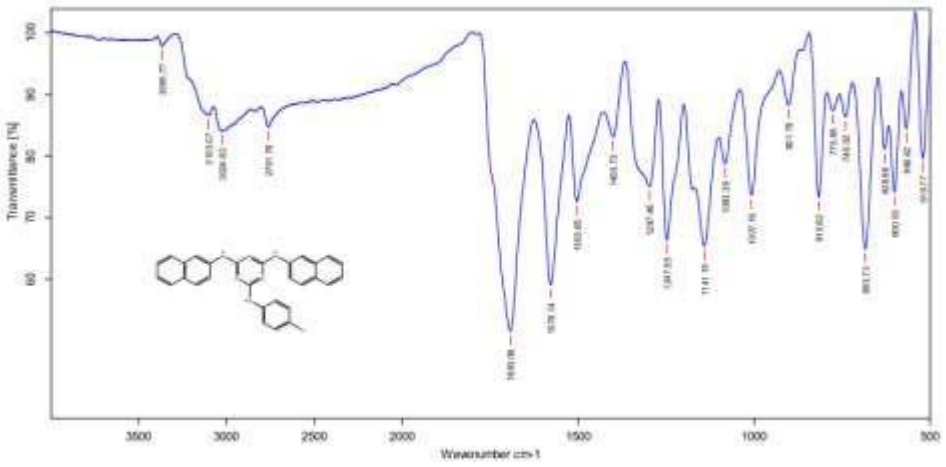


Graph 7 ^{1}H-NMR Spectra of BT$_d$

Table 12: Interpretation of ^{1}H-NMR Spectra of BT$_d$

S. No.	Peak Obtained	Peak occurred due to proton of…
1	6.91-7.77	Aromatic Protons (C-H)
2	5.32 & 5.55	Protons of amine (N-H)

Mass (m/e, calculated) – 488.97

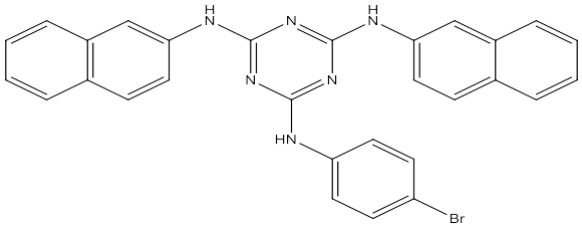


Graph 8 FTIR Spectra of BT$_d$

Table 13: Interpretation of FTIR Spectra of BT$_d$

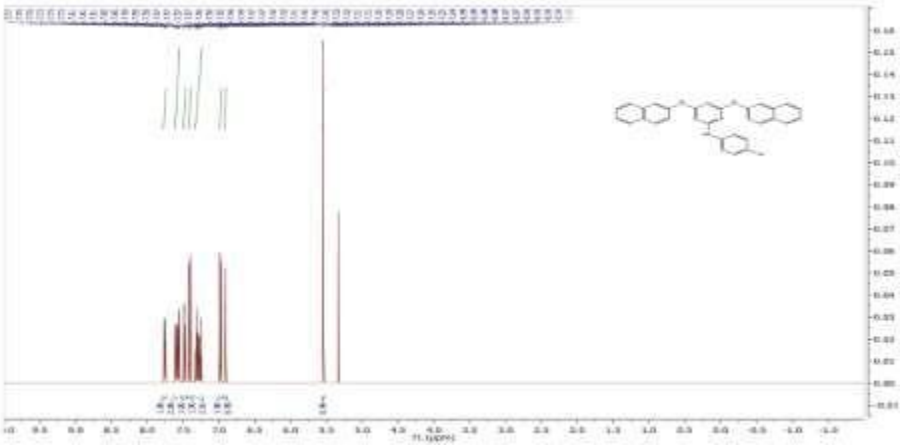
S. No.	Peak Obtained (cm^{-1})	Reference Range (cm^{-1})	Peak occurred due to functional group
1	3024.82, 3103.07	3080-3030	Ar C-H stretch
2	1578.14	1625-1575	Ar C-C/Ar C=C stretch
3	3365.77	3500-3100	N-H stretch
4	815.82	900-800	C-N stretch
5	1082.39	1100-1020	C-Cl stretch

Structure elucidation of BT_e



IUPAC - N^2-(4-bromophenyl)-N^4,N^6-di(naphthalen-2-yl)-1,3,5-triazine-2,4,6-triamine

Color - Pinkish-White

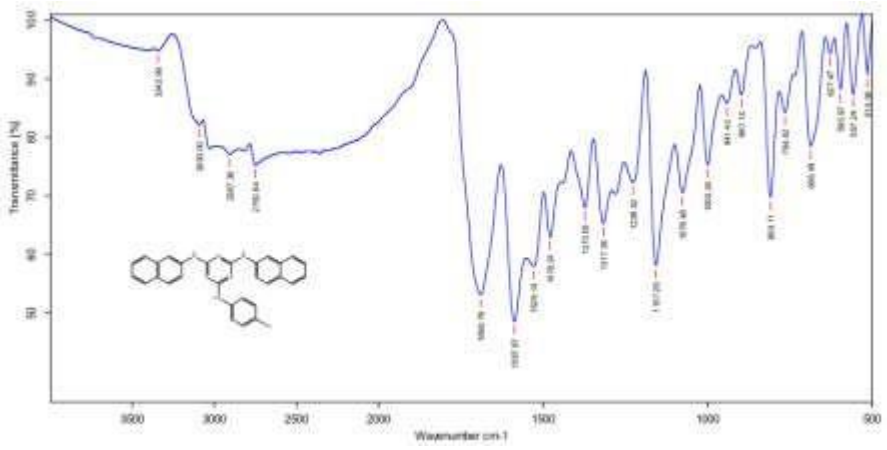


Graph 9 ^{1}H-NMR Spectra of BT_e

Table 14: Interpretation of ^{1}H-NMR Spectra of BT_e

S. No.	Peak Obtained	Peak occurred due to proton of…
1	6.91-7.77	Aromatic Protons (C-H)
2	5.33 & 5.54	Protons of aromatic amine (N-H)

Mass (m/e, calculated) **–** 533.42

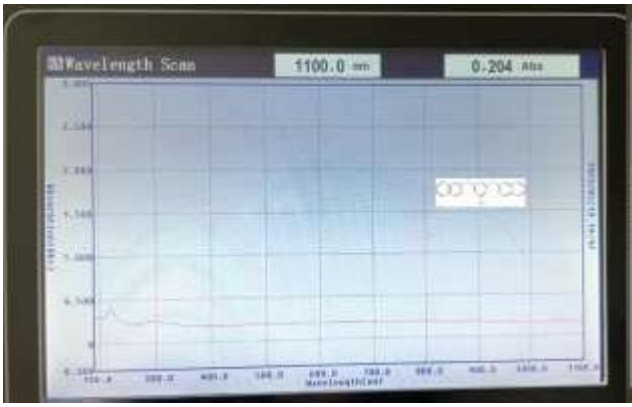


Graph 10 FTIR Spectra of BT_e

Table 15: Interpretation of FTIR Spectra of BT_e

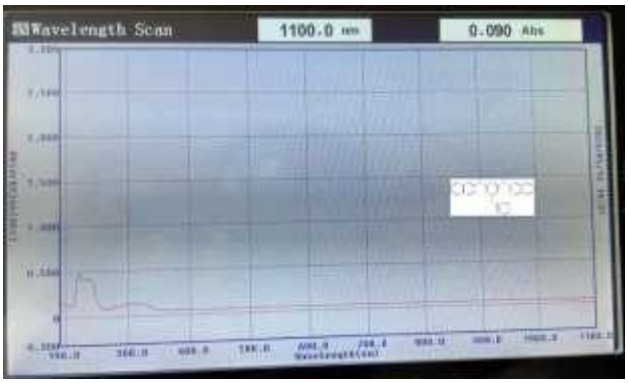
S. No.	Peak Obtained (cm^{-1})	Reference Range (cm^{-1})	Peak occurred due to functional group
1	3093.00	3080-3030	Ar C-H stretch
2	1587.97	1625-1575	Ar C-C/Ar C=C stretch
3	3342.99	3500-3100	N-H stretch
4	897.14, 809.11	900-800	C-N stretch
5	597.97	600-500	C-Br stretch

UV Spectra of synthesized triazine compounds

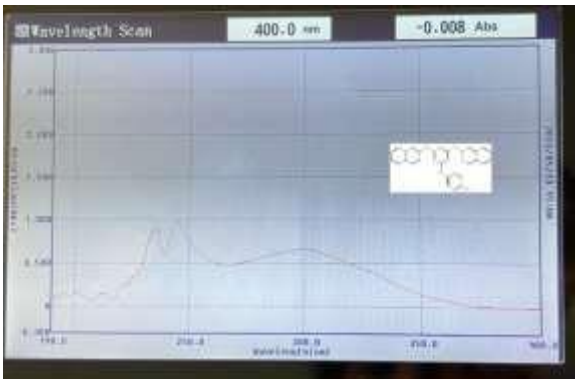


Graph 11. UV Spectra of BT_a

Graph 12. UV Spectra of BT_b



Graph 13. UV Spectra of BT_c



Graph 14. UV Spectra of BT_d

Graph 15. UV Spectra of BT_e

The absorption maxima of each compound was recorded from the spectra. The absorption maxima were found to be 217 nm, 218 nm, 220 nm, 245 nm and 246 nm for BT_a to BT_e in sequence respectively.

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 conjugates were tested for antibacterial action. Norfloxacin was used as the standard drug for antibacterial action (Table 16).

Table 16: Zone of inhibition exhibited by compounds

Compound Code	Zone of Inhibition (mm)*							
	S. aureus				*E. coli*			
	25µg	50µg	75µg	100µg	25µg	50µg	750µg	100µg
BT_a	-	-	16	18	-	-	12	13
BT_b	-	-	15	16	-	-	16	19

BT_c	-	-	17	**22**	-	-	19	**24**
BT_d	-	-	15	17	-	-	16	17
BT_e	-	-	14	15	-	-	13	15
Norfloxacin	**23**	-	-	-	**24**	-	-	-

(a) **(b)**

Figure 01: (a) Culture plate of BT_a exhibiting zone of inhibition against *S. aure*us, ***(b)*** Culture plate of BT_a exhibiting zone of inhibition against *E. coli*

5.1.4.2 Minimum Inhibitory Concentration (MIC)

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. The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Table 17 & 18).

Table 17: Optical density of test samples against *E. coli.*

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**Control**
Concentration (µg/mL)	100	10	1	0.1	0.01	0.001	-
	Optical Density at 600 nm						
BT_a	0.611	0.724	0.867	0.911	0.936	0.949	0.963
BT_b	0.541	0.657	0.793	0.818	0.842	0.85	
BT_c	0.329	0.484	0.591	0.713	0.772	0.845	
BT_d	0.543	0.662	0.798	0.836	0.855	0.869	
BT_e	0.625	0.737	0.899	0.927	0.953	0.961	
Norfloxacin	0.234	0.308	0.419	0.547	0.695	0.721	

Table 18: Optical density of test samples against *S. aureus*

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**Control**
Concentration (µg/mL)	100	10	1	0.1	0.01	0.001	
	Optical Density at 600 nm						

BT_a	0.761	0.847	0.936	1.025	1.087	1.171	1.178
BT_b	0.706	0.781	0.902	0.968	1.005	1.049	
BT_c	0.564	0.617	0.693	0.771	0.834	0.908	
BT_d	0.715	0.789	0.908	0.977	1.021	1.058	
BT_e	0.775	0.853	0.951	1.041	1.099	1.175	
Norfloxacin	0.144	0.197	0.304	0.438	0.579	0.625	

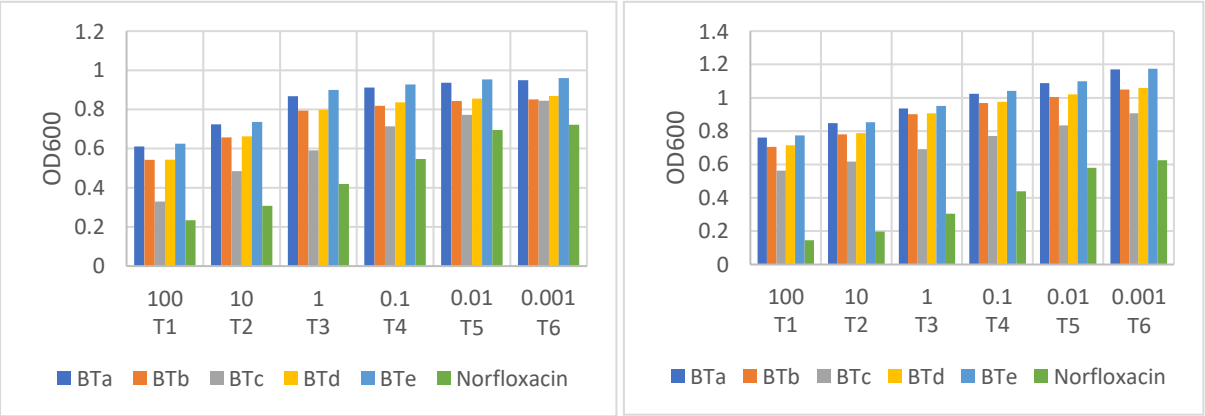


Figure 02: Plot of optical density for *E. coli* and Plot of optical density for *S. aureus*

Table 19: Calculated IC_{50} values of the test samples

Test sample	IC50 (µg/mL)	
	E. coli	*S. aureus*
BT_a	140.14	156.45
BT_b	117.66	139.55
BT_c	55.37	82.44
BT_d	117.43	142.83
BT_e	144.17	162.01
Norfloxacin	0.89	0.01

Discussion

The synthesis of the trisubstituted triazine was achieved in a single pot reaction by varying the temperature of the reaction. In the reaction, nucleophilic substitution of the three chloro groups of the cyanuric chloride occurs in succession at varying temperatures. The attack of the first nucleophile occurs at 0°C leading to formation of a mono substituted compound. This lowers the reactivity of the other chloro groups of the compound and hence the second nucleophilic attack occurs at room temperature. The formation of the disubstituted triazine derivative (bisnaphthylamine substituted in our compouds) further lowers the reactivity of the chloro group and the third nucleophile reacts at an elevated temperature (70-80°C) leading to the formation of the trisubstituted derivative. The products were soluble in water (BT_b, BT_c) and chlroform (BT_d, BT_e) and were obtained in 67-76% 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 while the backbone of the compounds was confirmed by the UV spectroscopic analysis. 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 shifts presence of protons of aromatic ring (6.7-7.7 ppm), amine hydrogen (5.55 ppm) in all compounds. The chemical shifts of the aliphatic protons (2.5-3.7 ppm) was found in BT_a and BT_b. 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 **BT_c** against both gram-positive (88.24 µg/mL) and gram-negative bacteria (55.37 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 **BT_d** (IC_{50} 142.83 µg/mL (*E. coli*) and 117.43 µg/mL (*S. aureus*)) and **BT_e** (IC_{50} 162.01 µg/mL (*E. coli*) and 144.17 µg/mL (*S. aureus*).

Conclusion

The primary objective of the work was to achieve the substitution of the three chloro groups of cyanuric chloride in a single pot reaction without separating the products after each substitution to obtain a trisubstituted triazine. Naphthylamine was used to replace two chlorine atoms while the third was substituted with various amines. The results obtained from this work suggested that the objective was successfully achieved and the compounds were synthesized in good yield (67-76%) in the one pot conditions. The antibacterial potential of the synthesized compounds was also evaluated to conclude that increasing the lipophilicity of the molecules increased antibacterial action whereas the presence of electron withdrawing group on the compounds decreased the antibacterial potential.

Conflict of Interest; The authors declare that the review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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