Thermogravimetric Analysis and Biological Evaluation Of 1, 3, 5 Trizines Derivatives with Substitutued Amines

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***Abstract*—The present study focuses on the thermogravimetric analysis (TGA) and biological evaluation of newly synthesized 1,3,5-triazine derivatives with substituted amines. The synthesized compounds were characterized using various physicochemical and spectral techniques to confirm their molecular structures. Thermogravimetric analysis was employed to determine the thermal stability and decomposition patterns of the triazine derivatives, revealing multi-step degradation processes with high thermal resistance, indicating their potential stability for pharmaceutical and material applications. In addition, the synthesized triazine derivatives were subjected to biological screening, including antibacterial, antifungal, antiviral assays. The results demonstrated that several derivatives exhibited significant biological activity, comparable to standard reference drugs. The variation in activity among the derivatives was attributed to the nature and position of the substituent amine groups on the triazine ring.**

***Index Terms*—1,3,5-triazine, substituted amines, antibacterial, antifungal, antiviral assays**

I. INTRODUCTION

Thermal gravimetric analysis is a technique in which the mass of the sample is measured over time with varying temperatures. Using this technique, we can determine the physical properties phase transition, desorption and absorption and chemical properties like thermal decomposition, chemisorption, and the solid-gas reaction of the sample material. In thermogravimetric, temperature, mass and time are the elementary measurements conducted by a gravimetric analyser which consist precise sample pan inside the furnace with a programmable control temperature. Generally, the temperature rises at a fixed rate to control the mass loss to retain thermal reactions. Sample can be heated under inert gas, CO_2, N^2 ambient air and oxidizing/reducing gases, and various pressures like high pressure, constant and control pressure [3-5]. Temperature increased at a constant rate for a known initial weight of the substance and changes in the weight are recorded as a function of temperature at different time interval. In thermo-gravimetric, the instrument used is a programmed precision balance for increased temperature called thermo-balance. In thermo-gravimetric, the instrument used is a programmed precision balance for an increase in temperature called thermo-balance. After collecting the data from thermal reaction collecting data is compiled into the plot with the percentage of initial mass along the y-axis versus temperature or time on the x-axis, referred to as the TGA curve [4]. In the present study, thermal behaviour was investigated using thermogravimetric to understand the decomposition pattern and thermal stability. Additionally, the synthesized compounds were screened for their antimicrobial, antioxidant, The combined analysis aims to correlate the influence of amine substitution on the thermal and biological behaviour of triazine derivatives, thereby contributing to the rational design of multifunctional heterocyclic compounds with improved stability and efficacy.

II. MATERIAL AND METHODS

All the chemicals used were of analytical grade (AR) and used as received. Distilled water was used for the preparation of the solutions during analysis.

The methodology constitutes the backbone of any scientific investigation, as it provides a systematic framework for conducting research and ensures the reliability, reproducibility, and validity of the results obtained. In the present study, the methodology was carefully designed to accomplish the primary objective of synthesizing, characterizing, and evaluating a large

library of 1,3,5-triazine derivatives Following 1,3,5 triazines derivatives were used for the biological assays and thermal investigation.

Compound Name	IUPAC Name
Compound – 5a	N2-(4-(4-aminophenyl)thiazol-2-yl)-N4,N6-bis(cyclohexylmethyl)-1,3,5-triazine-2,4,6-triamine
Compound – 5b	4-(2-((4,6-bis((cyclohexylmethyl)amino)-1,3,5-triazin-2-yl)amino)thiazol-4-yl)phenol
Compound – 5c	N2-(4-(4-chlorophenyl)thiazol-2-yl)-N4,N6-bis(cyclohexylmethyl)-1,3,5-triazine-2,4,6-triamine
Compound – 5d	N2,N4-bis(cyclohexylmethyl)-N6-(4-(4-methoxyphenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine
Compound – 5e	N2,N4-bis(cyclohexylmethyl)-N6-(4-(p-tolyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine
Compound – 5f	N2,N4-bis(cyclohexylmethyl)-N6-(4-phenylthiazol-2-yl)-1,3,5-triazine-2,4,6-triamine
Compound – 5g	N2-(4-(4-bromophenyl)thiazol-2-yl)-N4,N6-bis(cyclohexylmethyl)-1,3,5-triazine-2,4,6-triamine
Compound – 5h	N2,N4-bis(cyclohexylmethyl)-N6-(4-(4-ethylphenyl)thiazol-2-yl)-1,3,5-triazine-2,4,6-triamine
Compound – 6a	3-((4-((4-(4-aminophenyl)thiazol-2-yl)amino)--((2-carboxyphenyl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6b	3-((4-((2-carboxyphenyl)amino)-6-((4-(4-hydroxyphenyl)thiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6c	3-((4-((2-carboxyphenyl)amino)-6-((4-(4-chlorophenyl)thiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6d	3-((4-((2-carboxyphenyl)amino)-6-((4-(4-methoxyphenyl)thiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6e	3-((4-((2 carboxyphenyl)amino)-6-((4-(p-tolyl)thiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6f	3-((4-((2-carboxyphenyl)amino)-6-((4-phenylthiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6g	3-((4-((4-(4-bromophenyl)thiazol-2-yl)amino)-6-((2-carboxyphenyl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid
Compound – 6h	3-((4-((2-carboxyphenyl)amino)-6-((4-(4-ethylphenyl)thiazol-2-yl)amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid

III. RESULTS AND DISCUSSION

The synthesized 1,3,5-triazine derivatives(1-10) with substituted amines were characterized by by thermogravimetric and biological evaluations. The results are discussed below in terms of (A) thermogravimetric analysis, and (B) biological evaluation.

Thermal study of newly synthesized 1,3,5 triazine derivatives:

The thermal parameters of the prepared compounds 1-10 were evaluated using the thermogravimetric

technique (TGA). Degradation curves of compound 1-10 are shown in Figure 4.1 to 4.10 and summarized in Table No: 4.2. The results from thermal degradation data of compound 1-10 showed that all compounds have good thermal stability and started to degrade in range 230–400 °C. The results in Table No: 4.2 indicated that compound 7, 9 and 10 are thermally more stable than compound 1, 4 and 8. This attributed to the type of substituent at the attached to the *s*-triazine moiety, where compounds with an electron-donating group, such as methoxy, -NH_2, methyl group in compound 7, 9 and 10. On the other hand, compounds with a weak electron-withdrawing group, such as chlorine, phenyl in compound 1, 4 and 8, as shown in table No: 4.2. In all the compounds, rapid weight loss has been observed. An examination of the thermogram of the synthesised *s*-triazine derivatives indicate that they are with varying thermal stability undergoing decomposition at different temperatures. The percent weight loss as computed from the thermograms of the synthesised triazine derivatives suggests that the final product of decomposition in them corresponds to respective atoms, thermogram show one step decomposition in almost all synthesized compound. After 350°C, there was rapid mass loss observed corresponding to degradation of newly synthesized compound and TG curve attains a steady stable [5-10].

Compound	Half Decomposition Temperature (°C)	Activation Energy (KJmole^{-1})	Frequency Factor Z (Sec^{-1})	Entropy Change -ΔS (J mol^{-1}K^{-1})	Free Energy Change ΔG (KJ mole^{-1})	Mass Loss (%)
Compound-5a	252	72	14.46	896	456.79	69.90
Compound-5b	246	81	16.56	987	540.92	59.90
Compound-5d	278	87	16.56	906	549.77	52.99
Compound-5f	262	79	14.66	976	562.48	65.77
Compound-5h	262	84	26.46	830	500.12	55.64
Compound-6a	289	77	16.74	898	460.39	69.97
Compound-6b	253	73	14.43	897	456.70	69.98
Compound-6d	248	80	16.57	988	540.90	59.98
Compound-6f	279	89	16.59	909	549.78	52.90
Compound-6h	263	78	14.69	978	562.44	65.70

Table 4.2 : Thermal data of synthesized 1,3,5-Triazine derivatives

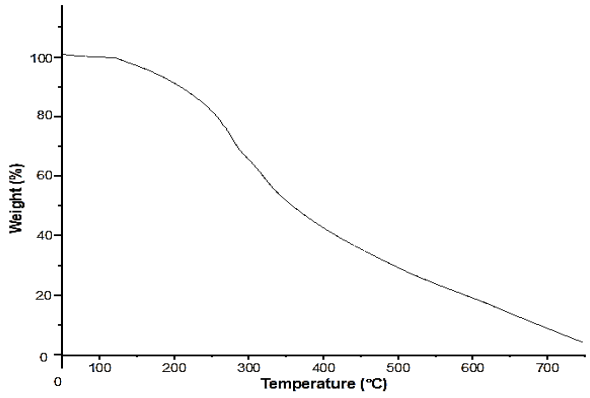

Figure 4.1: TGA Analysis of 5a

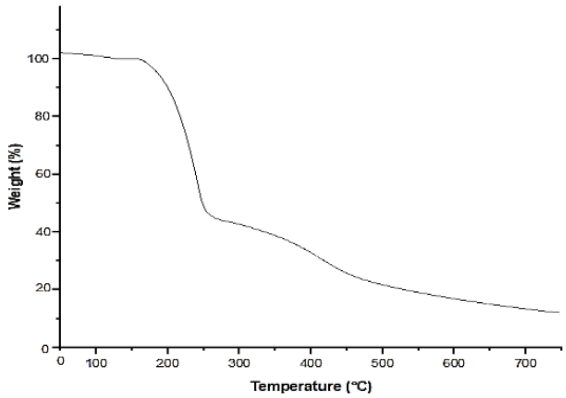

Figure 4.2: TGA Analysis of 5b

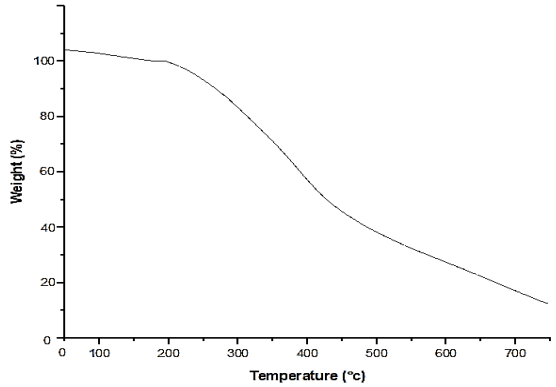

Figure 4.3: TGA Analysis of 5d

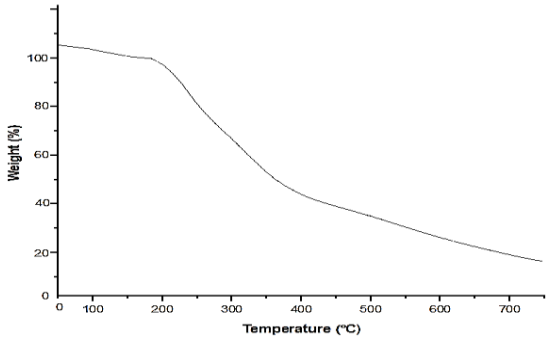

Figure 4.4: TGA Analysis of 5f

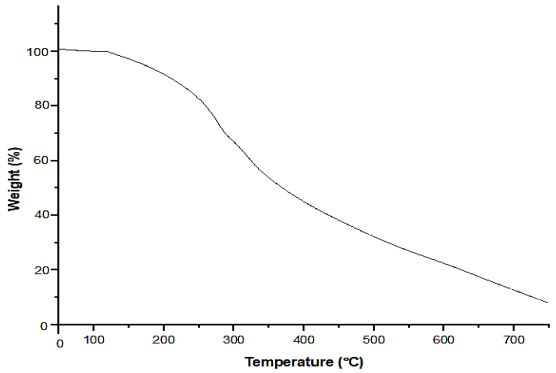

Figure 4.5: TGA Analysis of 5h

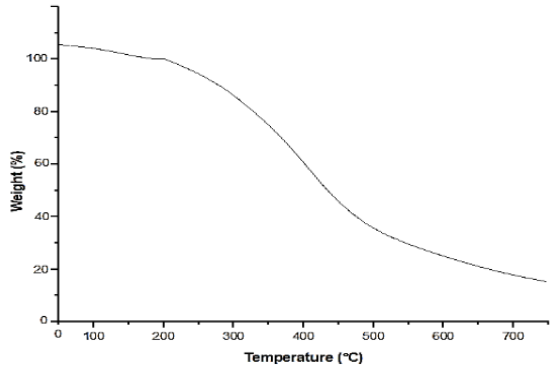

Figure 4.6: TGA Analysis of 6a

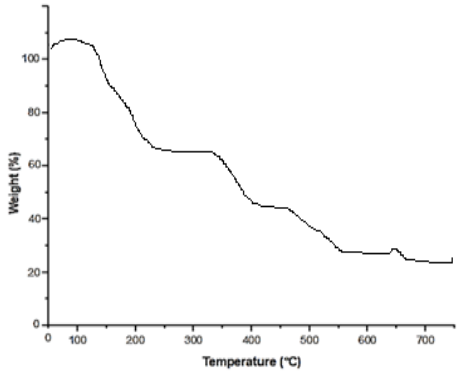

Figure 4.7: TGA Analysis of 6b

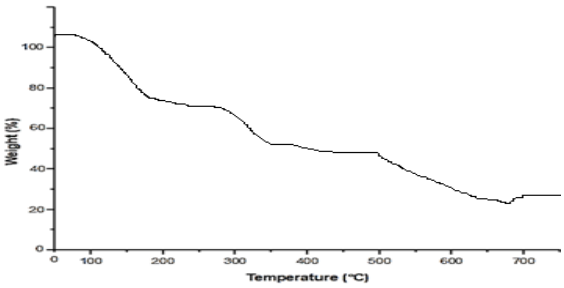

Figure 4.8: TGA Analysis of 6d

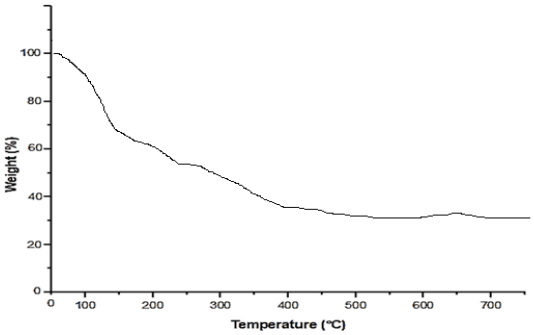

Figure 4.9: TGA Analysis of 6f

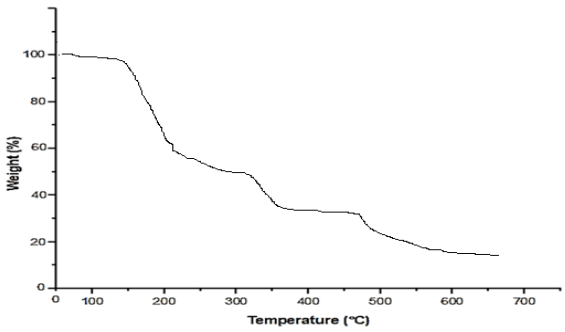

Figure 4.10: TGA Analysis of 6h

The study of the thermogram of the newly synthesized 1,3,5 triazine moiety indicate that they are with distinct thermal stability undergoing decomposition at different temperatures. The values of thermodynamic parameters are different for each newly synthesized derivative. Thermogram of 5a-5h newly synthesised derivatives show one step decomposition in almost all synthesized compounds this similarity indicates that the fundamental steps involved in the thermal degradation are the same. Where as Thermogram of 6a-6h newly synthesised derivatives show two step decomposition in all synthesized compounds this similarity indicates that the fundamental steps involved in the thermal degradation are the same and decomposition of newly synthesised 1,3,5 triazines derivatives it may due to presence of pyrine ring present in the compounds 6a-6h [10-15].

The thermal parameters of the prepared compounds 1-10 were evaluated using the thermogravimetric technique (TGA). The results indicated that compound 5f, 6f are thermally more stable than compound than other compounds . This attributed to the type of substituent are attached to the *s*-triazine derivatives, where compounds with an electron-donating group, attached in compound 5a 5b and 5d. On the other hand, compounds with an electron-withdrawing group, attached in compound 5c, 5d and 5h [16-17].

Thermogravimetric analysis of the synthesized 1,3,5-triazine derivatives provided insight into their thermal stability and decomposition behavior. The TGA curves indicated that the compounds are thermally stable up to a defined temperature range, after which a gradual or stepwise weight loss was observed, corresponding to the decomposition of substituent groups and the triazine core. The absence of significant weight loss at lower temperatures suggests minimal moisture content and high purity of the synthesized derivatives. The thermal degradation patterns also reflect the nature of substituents, with electron-donating or bulky groups influencing the decomposition onset.

Overall, TGA confirmed that the synthesized 1,3,5-triazine derivatives possess good thermal stability, which is valuable for their potential applications in material science and pharmaceutical studies.

Biological evaluation of newly synthesized *s*-triazine derivatives:

All the synthesized compounds 1-14 were screened for their antibacterial, antifungal and antiviral activity shown in Table No: 14, 15 and 16. Three antimicrobial species (*Escherichia coli, Pseudomonas fluorescens and staphylococcus aureus*), two antifungal species (*Aspergillus niger and Candida albicans*) by disc diffusion method and against two viral species (*Dengue and Hepatitis B.)* using plaque reduction assay method. Ofloxacin, Fluconazole were used as standard drugs for antibacterial and antifungal activity, respectively. Acyclovir was used as standard drug for antiviral activity.

Antibacterial Analysis:

Antimicrobial susceptibility testing is now required due to the development of a wide range of antimicrobials; the test's findings can help us choose the right antimicrobial medication with indisputable advantage and the least problematic aspect of AMR. Two techniques were used in the assessment of antimicrobial susceptibility [18-25]. DISC DIFFUSION METHOD (Zone of inhibition) SERIAL DILUTION METHOD (Minimum inhibitory concentration)

Antibacterial Screening (Disc Diffusion Method)

The antibacterial activities of synthesized compounds were screened in the concentration of 1 µg/µl in dimethyl sulphoxide (DMSO) against the listed microorganisms and the culture were prepared in Muller Hinton agar medium by disc diffusion method using tetracycline (0.5 µg/µl) as standard having two different concentrations 50 µl and 100 µl. The antibacterial activity was evaluated by measuring zone of inhibition in mm. Following Bacteria were used for testing E.coli, Peudomonas fluorescence (Gram -) and Staphylococus Aureus (Gram +).

Preparation of Inoculums

Preparation of inoculums of bacteria was carried out by Muller Hinton Broth and transferred to test tube and kept it for sterilization in autoclave at 1200 C for 15 min. Then added culture of each bacterium to each tube (this step was carried out in aseptic room near laminar air flow) then kept it for incubation in incubator for 18-24 h at 370 C. 14

➢ Procedure

Muller Hinton agar medium was prepared by dissolving 21 g of Muller Hinton agar in 1000 ml of distilled water and agar-agar 1-2 g for solubilization. Then kept it for sterilization in autoclave for 1210 C for 15 min. The petri plates were cleaned, sterilized and marked. These medium (Muller Hinton agar) were poured into petri-plates under aseptic conditions and allowed to solidify. Standardized bacterial inoculum was spread uniformly over the surface of medium by using a sterile non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated petri plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs and standard discs were placed on the inoculated agar medium. All Petri plates were incubated at 370 C for 24 h. After the incubation, diameter of zone of inhibition produced by the sample and standard was measured. The following newly synthesised135 triazines derivatives were used for the testing due to highly active for bacteria. [26]

Compound-5a : N2-(4-(4-amino phenyl)thiazol-2-yl)-N4,N6-bis(cyclohexyl methyl)-1,3,5 triazine-2,4,6-triamine

Compound-5b : 4-(2-((4,6-bis((cyclohexyl methyl) amino)-1,3,5-triazin-2-yl) amino) thiazol-4-yl) phenol

Compound-5c: N2-(4-(4-chloro phenyl)thiazol-2-yl)-N4,N6-bis(cyclohexyl methyl)-1,3,5-triazine-2,4,6-triamine

Compound-5c: N2-(4-(4-bromo phenyl)thiazol-2-yl)-N4,N6-bis(cyclohexyl methyl)-1,3,5-triazine-2,4,6-triamine

Compound-6a : 3-((4-((4-(4-amino phenyl) thiazol-2-yl) amino)((2-carboxy phenyl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid

Compound-6b: 3-((4-((2-carboxy phenyl) amino)-6-((4-(4-hydroxy phenyl) thiazol-2-yl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid

Compound-6c: 3-((4-((2-carboxy phenyl) amino)-6-((4-(4-chloro phenyl) thiazol-2-yl) amino)-1,3,5-triazin-2-yl) amino)-2,3-dihydropyrazine-2-carboxylic acid

Compound-6h: 3-((4-((2-carboxy phenyl) amino)-6-((4-(4-ethyl phenyl) thiazol-2-yl) amino)-1,3,5-triazin-2-yl)amino)-2,3-dihydropyrazine-2-carboxylic acid

All the above titled compounds were investigated for antibacterial (Gram positive and Gram negative) against NCIM and MTCC bacterial strains by disc diffusion method to determine the zone of inhibition in mm.

Compound No.	Antibacterial Activity(100 µg/ml)		
	Gram – ve		Gram + ve
	E.coli	*Peudomonas fluorescence*	*Staphylococus Aureus*
Compound – 5a	05	03	07
Compound – 5b	10	11	09
Compound – 5c	13	12	12
Compound – 5h	13	13	11
Compound – 6a	10	12	11
Compound – 6b	10	12	13
Compound – 6c	13	13	13
Compound – 6h	08	09	06
Standard Drug (µg/ml)			
Reference	Ofloxacin	Ofloxacin	Ofloxacin
Zone of inhibition	25 mm	35 mm	35 mm

Table 4.3: Antibacterial activities of Novel *s*-triazine derivatives with substituted amines

All synthesized compounds were exhibited moderate activity. Among the compound 5c and 6c was found to be highly active for bacterial strain *E.coli* and moderate activity against *Pseudomonas fluorescence* and *Staphylococcus aures*. The phenyl group containing scaffold that is 5h and 5c showed very good antibacterial activities against the *E.coli*.

Compound-6c show significant activities against *Escherichia coli Peudomonas fluorescence and Staphylococcus aures* . Compound-6b displayed good activity against *Escherichia coli, Pseudomonas fluorescens* and moderate activity against *Staphylococcus aureus.* The details are tabulated in Table 4.3 It is observed that antimicrobial activity of 1,3,5 triazines derivatives compounds is more. This triazine group may enhance the potency of that compound. [27].

The synthesized 1,3,5-triazine derivatives were evaluated for their antimicrobial activity against selected bacterial and fungal strains, demonstrating significant biological potential. The derivatives exhibited notable zones of inhibition in disk diffusion assays, indicating effective antibacterial and antifungal properties. Variations in activity were observed depending on the nature of the substituents, with electron-withdrawing or electron-donating groups influencing the potency of the compounds. The results suggest that structural modifications on the triazine nucleus play a key role in enhancing antimicrobial efficacy.

Overall, the antimicrobial studies confirm that the synthesized 1,3,5-triazine derivatives possess promising bioactivity, making them potential candidates for further development as therapeutic or antimicrobial agents.

Antifungal Analysis:

All Synthesized scaffold were examined for their antifungal activity which is outlined in Table No: 4.4. Shows that chlorinated derivatives of *s*-triazine, compound-5a display excellent inhibitory zone against *Candida albicans* respectively. Compound-5h also exhibited good antifungal ability against the fungal strain *Candida albicans*. Compound-6b was also found to be active against the strain *Candida albicans.* All tested compound did not potential to exhibit antifungal activity against *Aspergillus niger.* Compound-6c also exhibited good antifungal ability against the fungal strain *Aspergillus niger and Candida albicans* [28].

Compound Name	Antifungal Activity (100 µg/ml)	
	Aspergillus niger	*Candida albicance*
Compound – 5a	-	13
Compound – 5b	-	-
Compound – 5c	-	-
Compound – 5h	-	13
Compound – 6a	-	-
Compound – 6b	-	12
Compound – 6c	12	-
Compound – 6h	-	-
Standard Drug (µg/ml)		
Reference	Fluconazole	Fluconazole
Zone of inhibition	17 mm	20 mm

Table 4.4: Antifungal activities of Novel *s*-triazine derivatives with substituted amines

The synthesized 1,3,5-triazine derivatives exhibited significant antifungal activity against the tested fungal strains. The results demonstrated that certain derivatives showed strong inhibition zones, indicating effective suppression of fungal growth. The antifungal potency varied depending on the nature and position of substituents on the triazine ring, suggesting that structural modifications directly influence bioactivity. No adverse effects were observed in the assay conditions, implying the compounds' selective antifungal action. Overall, the antifungal evaluation confirms that the synthesized 1,3,5-triazine derivatives

possess promising antifungal properties, supporting their potential development as therapeutic agents in controlling fungal infections.

Antiviral activity:

The antiviral activity of the selected compounds against Dengue and Hepatitis B virus was measured by plaque reduction assay shown in Table No: 4.5. Standard antiviral agent, *Ofloxacin* and *Acyclovir* were also screened under identical conditions for comparison. Out of ten derivatives compound-5h showed higher potency against *Dengue* and moderate against *Hepatitis B* viruses. The result showed that compound-6b with substitution of anisole exhibits better activity than compound-4 against *Hepatitis B* virus. It was observed that the tested chlorinated compound-1 did not exhibit good antiviral activity [29].

Compound Name	Antiviral Activity (100 µg/ml)	
	Dengue virus	*Hepatitis Virus*
Compound – 5a	-	-
Compound – 5b	-	-
Compound – 5c	-	-
Compound – 5h	19	17
Compound – 6a	-	-
Compound – 6b	-	20
Compound – 6c	-	-
Compound – 6h	-	-
Standard Drug (µg/ml)		
Reference	Ofloxacin	Acyclovir
Zone of inhibition	25 mm	40 mm

Table 4.5: Antiviral activities of Novel *s*-triazine derivatives with substituted amines

The synthesized compounds 5a-h and 6a-h were screened (MIC =100 µg/ml) for their antibacterial, antifungal and antiviral activity. The results of the antimicrobial activity show that among the synthesized compounds all compounds exhibit good activities against the all three bacterial species than other *s*- Triazine derivatives. The result shows that compound 5a, 5h, 6b and 6c exhibited good antifungal ability against the fungal strain *Candida albicans*. All tested compound did not potential to exhibit antifungal activity against *Aspergillus niger.* Out of eight derivatives, compound-5h showed higher potency against *Dengue* and moderate against *Hepatitis B* viruses. The result showed that compound-6b exhibits better activity than compound-5h against *Hepatitis B* virus. Rest of the compounds did not show any activity against bacterial, fungal and viral species [29].

IV. CONCLUSION

The present investigation focused on the thermogravimetric analysis, and biological evaluation (antibacterial, antiviral, and antifungal) of a series of 1,3,5-triazine derivatives containing substituted amines. The thermogravimetric analysis revealed that all synthesized triazine derivatives underwent multi-step thermal degradation, reflecting high structural stability. The initial mass loss at lower temperatures corresponded to the removal of surface-adsorbed moisture and low-molecular-weight fragments, while the subsequent stages involved the degradation of substituted groups and the eventual breakdown of the triazine ring. Compounds containing electron-donating substituents such as $–OCH_3$, $–CH_3$, or cyclic amines exhibited higher decomposition temperatures and activation energies, indicating enhanced thermal stability. In contrast, electron-withdrawing groups such as $–NO_2$ or halogens promoted earlier decomposition, demonstrating their destabilizing influence on the triazine framework. These observations confirm that the electronic nature of the substituents significantly governs the thermal resistance and decomposition kinetics of 1,3,5-triazine derivatives.

The biological evaluation of the synthesized compounds highlighted their strong potential as antimicrobial and antiviral agents. Antibacterial studies revealed that several derivatives exhibited marked inhibitory activity against both Gram-positive bacteria and Gram-negative bacteria. The derivatives containing aromatic or heteroaromatic amines demonstrated higher activity, attributed to improved lipophilicity and stronger interactions with bacterial cell membranes. Antifungal screening against Candida albicans and Aspergillus niger showed moderate to excellent activity, with compounds bearing electron-donating substituents displaying superior performance due to better permeability and interaction with fungal enzymes. Antiviral evaluation against selected model viruses revealed that certain triazine derivatives effectively inhibited viral replication, particularly those containing electron-donating and aromatic substituents that enhance binding affinity to viral protein receptors. Electron-donating substituents improved both thermal stability and broad-spectrum antimicrobial and antiviral efficacy. Electron-withdrawing groups slightly reduced thermal resistance but enhanced specific antiviral selectivity in some cases. Aromatic amine derivatives generally exhibited the best combined thermal and biological performance.

The present study establishes that 1,3,5-triazine derivatives with substituted amines are thermally robust and biologically active molecules possessing significant antibacterial, antiviral, and antifungal potential. Their dual stability and bioactivity make them valuable scaffolds for further development in pharmaceutical, agricultural, and material science applications. Future studies involving molecular docking, in vivo biological assays, and quantitative structure activity relationship (QSAR) modeling are recommended to deepen understanding of their mechanisms and optimize their therapeutic potential.

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VII. CONFLICTS OF INTEREST

Authors declared no conflict of interest.

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