

## Substituted 1,3,5-Triazine Derivatives as Promising Antimicrobial Leads: Synthesis, Spectral Characterization and *In silico* Investigation

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Received: 10<sup>th</sup> May 2026; Revised: 21<sup>st</sup> May 2026; Accepted: 05<sup>th</sup> Jun 2026

**Abstract:** A new series of substituted 1,3,5-triazine derivatives (4a–4f) was successfully synthesized through a two-step nucleophilic aromatic substitution strategy using cyanuric chloride as the key precursor. The synthesized compounds were characterized by melting point determination, TLC, IR, <sup>1</sup>H NMR, LC-MS, and elemental analysis, which confirmed the proposed structures. The *In vitro* antimicrobial activity of the compounds was evaluated against selected Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus anthracis*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*), and fungal strains (*Candida albicans* and *Aspergillus niger*) using the cup plate method. Among the tested derivatives, compounds 4c and 4d exhibited the most promising broad-spectrum antimicrobial activity, showing inhibition zones close to the standard drugs. *In silico* ADME studies using SwissADME indicated acceptable lipophilicity and hydrogen-bonding properties, although high TPSA and molecular weight may limit oral bioavailability. Molecular docking studies against *Mycobacterium tuberculosis* dihydrofolate reductase (PDB ID: 4KM2) and *Staphylococcus aureus* DNA gyrase (PDB ID: 2XCT) revealed favorable binding affinities for all synthesized compounds, with 4c showing the best docking performance against both targets. The combined synthetic, biological, and computational findings suggest that these triazine-based hybrids, particularly 4c and 4d, may serve as promising lead molecules for the development of new antimicrobial agents.

**Keywords:** 1,3,5-Triazine; Antimicrobial activity; Molecular docking; SwissADME; Dihydrofolate reductase; DNA gyrase; Sulfonamide hybrids; Drug-likeness.

**How to cite this article:** Vaishnav P, Parihar N, Tomar R. Substituted 1,3,5-triazine derivatives as promising antimicrobial leads: Synthesis, spectral characterization and in silico investigation. *NextGen Chem Discov*. 2026 Apr-Jun;2(2):20–31.

**Conflict of interest:** None

## Introduction

Heterocyclic compounds occupy a central position in medicinal chemistry because of their broad spectrum of biological activities and their structural compatibility with a wide variety of biological targets [1]. Among the nitrogen-containing heterocycles, 1,3,5-triazines have attracted considerable attention due to their unique electronic properties, synthetic versatility, and remarkable pharmacological potential. The 1,3,5-triazine nucleus, also known as s-triazine, contains three  $sp^2$ -hybridized nitrogen atoms within a six-membered aromatic ring, which imparts a highly electron-deficient character to the scaffold [2]. This feature makes triazines particularly suitable for diverse chemical transformations, especially nucleophilic aromatic substitution reactions, enabling the rapid generation of structurally diverse analogues for biological screening. Owing to these characteristics, triazine derivatives have emerged as privileged scaffolds in the design and development of new therapeutic agents [3].

Over the years, numerous triazine-based compounds have been reported to exhibit a wide range of pharmacological activities, including anticancer, antimicrobial, anti-inflammatory, antimalarial, anticonvulsant, and central nervous system activities [4]. The success of certain clinically relevant triazine-containing drugs has further validated the importance of this heterocyclic core in drug discovery. In medicinal chemistry, the triazine ring is especially valued because its substitution pattern can be easily manipulated to modulate important physicochemical properties such as lipophilicity, polarity, hydrogen bonding capacity, and metabolic stability [5]. Such modifications directly influence receptor binding, target selectivity, membrane permeability, and overall pharmacokinetic behavior. Therefore, triazines continue to serve as useful building blocks for lead generation and optimization in various therapeutic areas [6].

A major advantage of the triazine scaffold lies in its synthetic accessibility, particularly through the use of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) as a key starting material. Cyanuric chloride is a highly reactive electrophilic platform in which the three chlorine atoms undergo stepwise nucleophilic displacement under controlled temperature conditions [7]. This temperature-dependent reactivity enables selective mono-, di-, and trisubstitution, making cyanuric chloride one of the most versatile intermediates in triazine chemistry. Through this approach, a wide variety of amines, phenols, thiols, and other nucleophiles can be incorporated into the triazine ring, providing access to large libraries of substituted derivatives. Such synthetic flexibility has facilitated the construction of pharmacologically enriched molecules in which the triazine core acts as a central linker or bioactive framework [8].

In recent years, the concept of molecular hybridization has gained prominence in medicinal chemistry as an efficient strategy for designing multifunctional bioactive compounds. This approach involves the rational combination of two or more pharmacophoric units within a single molecular framework in order to obtain enhanced biological activity, improved selectivity, or dual mechanisms of action [9]. In this context, triazine derivatives are particularly attractive because the triazine ring can be readily functionalized with different aromatic and heteroaromatic groups. Hybrid molecules containing triazine linked with sulfonamides, pyrazoles, triazoles, indoles, coumarins, pyrimidines, and related moieties have been widely investigated for their antimicrobial, anticancer, antimalarial, and anti-inflammatory activities [10]. The incorporation of suitable electron-donating or electron-withdrawing substituents into these systems often leads to improved target interaction and biological potency.

Among the various therapeutic applications of triazines, the search for novel antimicrobial agents remains especially relevant [11]. The emergence of microbial resistance to conventional antibiotics and antifungal drugs has become a serious global health challenge. Pathogenic bacteria and fungi are rapidly acquiring resistance mechanisms that reduce the effectiveness of existing treatments, leading to increased morbidity, mortality, and healthcare burden. In this scenario, the identification of new chemical entities with potent

antimicrobial activity and alternative mechanisms of action has become a pressing scientific need [12]. Heterocyclic scaffolds such as triazines offer significant promise in this field because of their capacity to establish strong interactions with microbial enzymes and other biomolecular targets. Previous reports have demonstrated that appropriately substituted triazine derivatives can display promising inhibitory activity against both Gram-positive and Gram-negative bacteria, as well as various fungal strains [13].

Another important aspect of modern drug discovery is the integration of computational approaches with synthetic and biological evaluation. Structure-based drug design, molecular docking, quantitative structure–activity relationship studies, and *in silico* ADME prediction have become indispensable tools for accelerating lead identification and optimization [14]. Molecular docking, in particular, provides valuable insight into the binding orientation, affinity, and interaction pattern of small molecules within the active site of target proteins. Such studies help in rationalizing experimental activity and identifying the structural features responsible for enhanced binding [15]. Likewise, *in silico* ADME profiling allows early assessment of pharmacokinetic parameters such as molecular weight, lipophilicity, hydrogen-bonding characteristics, topological polar surface area, gastrointestinal absorption, and drug-likeness. Together, these computational methods reduce experimental cost and time while supporting the design of compounds with improved biological and pharmacokinetic profiles [16].

The current study was therefore designed on the basis of these medicinal chemistry considerations. In the present work, a new series of substituted 1,3,5-triazine derivatives (4a–4f) was synthesized using a stepwise nucleophilic aromatic substitution strategy based on cyanuric chloride [17]. The designed compounds incorporate dimethoxyphenyl, substituted phenylamino, and benzenesulfonamide functionalities, which were selected in view of their known contribution to biological activity. Sulfonamide groups are well recognized pharmacophores in antimicrobial drug design because of their ability to participate in hydrogen bonding and enzyme inhibition, while substituted aromatic amines can modulate hydrophobicity, steric profile, and electronic distribution [18]. It was anticipated that the combination of these structural features with the triazine core would generate molecules capable of favorable interaction with microbial targets and thereby exhibit significant antimicrobial activity.

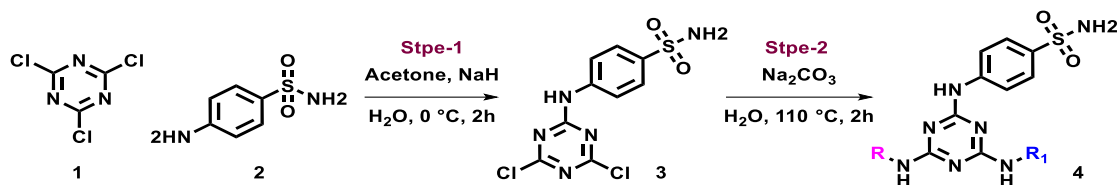
Following synthesis, the obtained compounds were characterized by standard analytical and spectral techniques including melting point determination, TLC, IR spectroscopy, <sup>1</sup>H NMR, LC-MS, and elemental analysis, ensuring confirmation of their chemical structures and purity [19]. The synthesized molecules were then subjected to *In vitro* antimicrobial screening against selected bacterial and fungal strains to evaluate their biological potential. In addition, SwissADME-based *in silico* pharmacokinetic evaluation was performed to determine their drug-likeness and oral bioavailability-related properties. To further understand the possible mechanism of antimicrobial action, molecular docking studies were carried out against two biologically relevant microbial targets: dihydrofolate reductase from *Mycobacterium tuberculosis* (PDB ID: 4KM2) and *Staphylococcus aureus* DNA gyrase complex (PDB ID: 2XCT). These targets were chosen because of their established role in microbial survival and replication, and because inhibition of these enzymes represents an important approach in antimicrobial drug development [20].

Thus, the present investigation represents a combined synthetic, biological, and computational study directed toward the discovery of new triazine-based antimicrobial agents [21]. The work not only explores the synthetic feasibility of structurally diverse substituted triazines but also attempts to correlate their antimicrobial activity with physicochemical properties and target-binding behavior. By integrating classical organic synthesis with modern *in silico* tools and microbiological evaluation, this study contributes to the growing body of research focused on hybrid heterocyclic systems as promising therapeutic leads. It is expected that the findings of this work will support the further optimization of triazine derivatives and

encourage their continued exploration as scaffolds for the development of safer and more effective antimicrobial agents.

### Experimental Work

Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. The IR spectra were recorded on a Bruker spectrophotometer to identify the characteristic functional groups present in the synthesized molecules [22]. The  $^1\text{H}$  NMR spectra were recorded at 400 MHz to confirm the proton environment and structural features of the compounds. Mass spectra were used to verify the molecular ion peaks, while elemental analysis (C, H, N) was performed to confirm the molecular composition. The obtained analytical values were found to be in close agreement with the calculated values, supporting the proposed structures [23].



Scheme 1: Synthesis of compounds 4a-4f

#### Step-1: General Procedure for the Synthesis of Intermediate (3)

To a stirred solution of compound 2 (2 equiv.) in a mixture of acetone and water, the reaction was cooled to 0 °C. Sodium hydride (NaH) was added portion-wise under controlled conditions to generate the nucleophilic amine species. After complete addition, compound 1 (cyanuric chloride) was slowly introduced to the reaction mixture while maintaining the temperature at 0 °C. The reaction was stirred for 2 hours at this temperature to allow selective mono-substitution on the triazine ring. Upon completion (monitored by TLC), the reaction was quenched carefully with water, and the mixture was worked up by extraction with an appropriate organic solvent [24]. The combined organic layers were dried, filtered, and concentrated to afford intermediate 3, which was used in the next step without further purification or purified if required.

#### Step-2: General Procedure for the Synthesis of Final Product (4)

Intermediate 3 was dissolved in water, and sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added to the solution to provide basic conditions [25]. The reaction mixture was then heated to 110 °C and stirred for 2 hours to promote further nucleophilic substitution of the remaining chloro groups on the triazine ring with the corresponding amines (R and  $\text{R}_1$  substituents). After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature and neutralized if necessary. The product was isolated by filtration or extraction, followed by washing and drying to obtain compound 4 as the desired final product [26]. Further purification (e.g., recrystallization or chromatography) was performed if needed.

4-((4-((2,4-dimethoxyphenyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4a**)

Melting Point: 182-184 °C; Yield: 88 %;  $R_f$  value: 0.77; Solvent system: Benzene: Methanol (9:1); LCMS:  $m/z$  [M]<sup>+</sup> 494.16; IR  $\nu$  ( $\text{cm}^{-1}$ ): 3360 (NH), 1605, 1580 (C=N), 1490, 1445 (C=C); 1320, 1115 (S=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  ppm: 7.78 – 6.53 (m, 12H, Ar-H), 7.30, 4.62 – 3.67 (s, 5H, 3NH,  $\text{NH}_2$ ), 3.83 – 3.80 (s, 6H, 2OCH<sub>3</sub>); Anal. Calcd. for  $\text{C}_{23}\text{H}_{23}\text{N}_7\text{O}_4\text{S}$  (493.55): C, 55.97%; H, 4.70%; N, 19.87%; Found: C, 56.35%; H, 4.56%; N, 19.59%.

4-((4-((2,4-dimethoxyphenyl)amino)-6-(p-tolylamino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4b**)

Melting Point: 218-220 °C; Yield: 81 %;  $R_f$  value: 0.75; Solvent system: Benzene: Methanol (9:1); LCMS:  $m/z$  [M]<sup>+</sup> 507.22; IR  $\nu$  ( $\text{cm}^{-1}$ ): 3560 (NH), 1610, 1575 (C=N), 1455, 1405 (C=C); 1300, 1145 (S=O);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

$\delta$  ppm: 7.91 – 6.53 (m, 10H, Ar-H), 7.26, 4.52 – 3.63 (s, 5H, NH, NH<sub>2</sub>), 3.81 – 3.79 (s, 6H, 2OCH<sub>3</sub>), 2.34 (s, 3H, CH<sub>3</sub>); Anal. Calcd. for C<sub>24</sub>H<sub>25</sub>N<sub>7</sub>O<sub>4</sub>S (507.58): C, 56.79%; H, 4.96%; N, 19.32%; Found: C, 56.96%; H, 4.90%; N, 19.15%.

4-((4-((2,4-dimethoxyphenyl)amino)-6-((4-fluorophenyl)amino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4c**)

Melting Point: 246-248 °C; Yield: 82 %; R<sub>f</sub> value: 0.78; Solvent system: Benzene: Methanol (9:1); LCMS: m/z [M]<sup>+</sup> 512.04; IR  $\nu$  (cm<sup>-1</sup>): 3365 (NH), 1595, 1530 (C=N), 1495, 1485 (C=C); 1310, 1145 (S=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 7.90 – 6.53 (m, 11H, Ar-H), 7.29, 4.51 – 3.63 (s, 5H, NH, NH<sub>2</sub>), 3.83 – 3.73 (s, 6H, 2OCH<sub>3</sub>); Anal. Calcd. for C<sub>23</sub>H<sub>22</sub>FN<sub>7</sub>O<sub>4</sub>S (511.54): C, 54.00%; H, 4.34%; N, 19.17%; Found: C, 54.10%; H, 4.55%; N, 19.19%.

4-((4-((2,4-dimethoxyphenyl)amino)-6-((4-isopropylphenyl)amino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4d**)

Melting Point: 212-214 °C; Yield: 79 %; R<sub>f</sub> value: 0.68; Solvent system: Benzene: Methanol (9:1); LCMS: m/z [M]<sup>+</sup> 536.21; IR  $\nu$  (cm<sup>-1</sup>): 3340 (NH), 1605, 1540 (C=N), 1485, 1445 (C=C); 1335, 1150 (S=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 7.93 – 6.66 (m, 11H, Ar-H), 4.58 – 3.93 (s, 5H, NH, NH<sub>2</sub>), 3.83 – 3.78 (s, 6H, 2OCH<sub>3</sub>), 3.06 (m, 1H, CH), 1.35 (s, 6H, 2CH<sub>3</sub>); Anal. Calcd. for C<sub>26</sub>H<sub>29</sub>N<sub>7</sub>O<sub>4</sub>S (535.63): C, 58.30%; H, 5.46%; N, 18.31%; Found: C, 58.48%; H, 5.23%; N, 17.96%.

4-((4-((2,4-dimethoxyphenyl)amino)-6-((4-methoxyphenyl)amino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4e**)

Melting Point: 160-162 °C; Yield: 87 %; R<sub>f</sub> value: 0.71; Solvent system: Benzene: Methanol (9:1); LCMS: m/z [M]<sup>+</sup> 523.26; IR  $\nu$  (cm<sup>-1</sup>): 3345 (NH), 1600, 1535 (C=N), 1485, 1445 (C=C); 1340, 1155 (S=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 7.92 – 6.51 (m, 10H, Ar-H), 7.85, 4.68 – 4.53 (s, 5H, NH, NH<sub>2</sub>), 3.86 – 3.82 (s, 9H, 3OCH<sub>3</sub>); Anal. Calcd. for C<sub>24</sub>H<sub>25</sub>N<sub>7</sub>O<sub>5</sub>S (523.57): C, 55.06%; H, 4.81%; N, 18.73%; Found: C, 55.02%; H, 4.78%; N, 18.70%.

4-((4-((4-cyanophenyl)amino)-6-((2,4-dimethoxyphenyl)amino)-1,3,5-triazin-2-yl)amino)benzenesulfonamide (**4f**)

Melting Point: 148-150 °C; Yield: 82 %; R<sub>f</sub> value: 0.84; Solvent system: Benzene: Methanol (9:1); LCMS: m/z [M]<sup>+</sup> 519.11; IR  $\nu$  (cm<sup>-1</sup>): 3340 (NH), 1595, 1515 (C=N), 1450, 1375 (C=C); 1315, 1125 (S=O); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  ppm:  $\delta$  7.94 – 6.53 (m, 11H, Ar-H), 4.76, 4.55, 4.47 (s, 3H, NH), 4.41 (s, 2H, NH<sub>2</sub>), 3.84 – 3.83 (s, 6H, 2OCH<sub>3</sub>); Anal. Calcd. for C<sub>24</sub>H<sub>22</sub>N<sub>8</sub>O<sub>4</sub>S (518.55): C, 55.59%; H, 4.28%; N, 21.61%; Found: C, 55.55%; H, 4.25%; N, 21.60%.

### **In vitro Antimicrobial Activity**

The synthesized compounds (4a–4f) were evaluated for antimicrobial activity by the cup plate method using *Staphylococcus aureus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. Streptomycin and Fluconazole were used as standard antibacterial and antifungal drugs, respectively, while DMSO served as the solvent control. Sterile agar plates were inoculated with the test organisms, and 6 mm wells were made in the medium [27]. The test compounds, standards, and control were added separately into the wells. After diffusion at 4 °C for 1 hour, bacterial plates were incubated at 37 °C for 24 hours and fungal plates at 25 °C for 48 hours. Antimicrobial activity was assessed by measuring the zone of inhibition in millimeters [28].

### **In silico ADME study**

The ADME properties of the synthesized molecules (4a–4f) were predicted using the SwissADME web server by submitting their SMILES structures generated from ChemDraw. Key pharmacokinetic parameters such as molecular weight, H-bond donors/acceptors, TPSA, iLOGP, GI absorption, rotatable bonds, and Lipinski's rule-of-five compliance were recorded to assess drug-likeness and oral bioavailability [29].

### Molecular Docking Studies

Molecular docking studies were carried out to evaluate the binding affinity of the synthesized compounds against selected microbial targets associated with antimicrobial activity [30]. The docking work was performed on a Windows 10 (64-bit) system using PyRx 0.8 in AutoDock Vina Wizard. AutoDock Tools 4.2.6 was used for protein preparation and grid generation, while Open Babel was used for ligand conversion and 3D optimization. The binding interactions were visualized and analyzed using Discovery Studio Visualizer [31]. Two microbial target proteins were selected from the RCSB Protein Data Bank: dihydrofolate reductase of *Mycobacterium tuberculosis* (PDB ID: 4KM2) and *Staphylococcus aureus* DNA gyrase complex (PDB ID: 2XCT) [32].

For protein preparation, water molecules and co-crystallized ligands were removed, followed by the addition of polar hydrogens and Kollman charges, and the structures were saved in PDBQT format [33]. The ligands were converted from SMILES to SDF format, energy-minimized using the UFF force field, and then converted into PDBQT format [34]. Grid boxes were defined around the active sites of the target proteins and adjusted manually in PyRx. Docking was performed with an exhaustiveness value of 8, and the resulting binding poses were analyzed on the basis of docking scores and molecular interactions using Discovery Studio Visualizer [35].

## Results and Discussion

### Chemistry

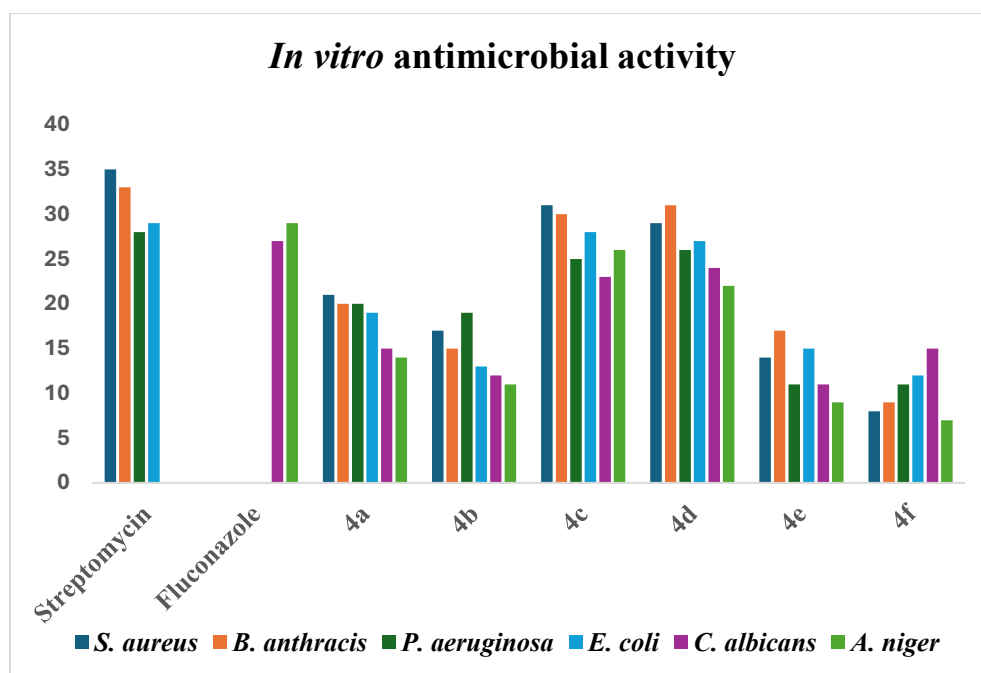
The substituted 1,3,5-triazine derivatives (4a–4f) were successfully synthesized through a two-step nucleophilic aromatic substitution route using cyanuric chloride as the key precursor. The adopted method afforded the target compounds in good to excellent yields (79–88%), indicating the efficiency of the synthetic protocol. The reaction progress and purity of the products were confirmed by TLC, while the melting point ranges suggested good crystalline nature and stability of the synthesized molecules. Structural confirmation was achieved through IR, <sup>1</sup>H NMR, LC-MS, and elemental analysis, which showed characteristic signals for NH, C=N, aromatic protons, methoxy groups, and sulfonamide functionality. Overall, the spectral and analytical data clearly established the successful formation of the designed triazine hybrids.

### *In vitro* Antimicrobial Activity

The synthesized triazine derivatives (4a–4f) were screened for *In vitro* antimicrobial activity against selected Gram-positive, Gram-negative, and fungal strains using the cup plate method. The results, expressed as zone of inhibition in mm, revealed significant variation in activity among the tested compounds. Among the series, compounds 4c and 4d exhibited the most potent and broad-spectrum antimicrobial activity. Compound 4c showed excellent antibacterial activity against *S. aureus* (31 mm), *B. anthracis* (30 mm), and *E. coli* (28 mm), while compound 4d displayed strong inhibition against *B. anthracis* (31 mm), *P. aeruginosa* (26 mm), and *E. coli* (27 mm). These values were found to be close to those of the standard antibacterial drug Streptomycin. Compounds 4a and 4b showed moderate antibacterial and antifungal activity, whereas 4e and 4f were comparatively less active. In antifungal screening also, compounds 4c and 4d demonstrated better inhibition against *C. albicans* and *A. niger* than the other synthesized compounds. The enhanced activity of 4c and 4d may be attributed to favorable electronic and lipophilic substituent effects on the triazine scaffold. Although none of the compounds exceeded the activity of the standard drugs, the overall findings indicate that 4c and 4d are promising lead molecules for further antimicrobial optimization.

**Table 1:** *In vitro* antimicrobial activity of compounds 4a-4f

| Compound (1000 µg/ml) | Zone of Inhibition (mm) |                     |                      |                |                    |                 |
|-----------------------|-------------------------|---------------------|----------------------|----------------|--------------------|-----------------|
|                       | <i>S. aureus</i>        | <i>B. anthracis</i> | <i>P. aeruginosa</i> | <i>E. coli</i> | <i>C. albicans</i> | <i>A. niger</i> |
| Streptomycin          | 35                      | 33                  | 28                   | 29             | -                  | -               |
| Fluconazole           | -                       | -                   | -                    | -              | 27                 | 29              |
| 4a                    | 21                      | 20                  | 20                   | 19             | 15                 | 14              |
| 4b                    | 17                      | 15                  | 19                   | 13             | 12                 | 11              |
| 4c                    | 31                      | 30                  | 25                   | 28             | 23                 | 26              |
| 4d                    | 29                      | 31                  | 26                   | 27             | 24                 | 22              |
| 4e                    | 14                      | 17                  | 11                   | 15             | 11                 | 09              |
| 4f                    | 08                      | 09                  | 11                   | 12             | 15                 | 07              |

Figure 1: *In vitro* antimicrobial activity of compounds 4a-4f***In silico* ADME study**

The *in silico* ADME properties of compounds 4a-4f were evaluated using SwissADME to assess their drug-likeness and pharmacokinetic behavior. All synthesized derivatives showed molecular weights in the range of 493.54–535.62 g/mol, with most compounds slightly exceeding the ideal limit for oral drugs. The compounds possessed acceptable hydrogen bond donor and acceptor values along with moderate lipophilicity (iLOGP 2.97–4.21), indicating favorable interaction potential with biological targets. However, all derivatives exhibited high TPSA values (161.76–185.55 Å<sup>2</sup>), which resulted in low predicted gastrointestinal absorption. Lipinski analysis showed that compound 4a had only one violation, whereas compounds 4b-4f showed two violations each. Overall, compound 4a displayed comparatively better pharmacokinetic properties, while the remaining compounds may require structural optimization to improve oral bioavailability.

Table 2: *In silico* ADME study of Compounds 4a-4f

| Co mp | Molecular Weight | Rotatable bonds | H-bond acceptors | H-bond donors | TPSA   | iLO GP | GI absorption | Lipinski violations |
|-------|------------------|-----------------|------------------|---------------|--------|--------|---------------|---------------------|
| 4a    | 493.54           | 9               | 8                | 4             | 161.76 | 3.27   | Low           | 1                   |
| 4b    | 507.56           | 9               | 8                | 4             | 161.76 | 3.52   | Low           | 2                   |
| 4c    | 511.53           | 9               | 9                | 4             | 161.76 | 3.24   | Low           | 2                   |
| 4d    | 535.62           | 10              | 8                | 4             | 161.76 | 4.21   | Low           | 2                   |
| 4e    | 523.56           | 10              | 9                | 4             | 170.99 | 3.61   | Low           | 2                   |
| 4f    | 518.55           | 9               | 9                | 4             | 185.55 | 2.97   | Low           | 2                   |

### Molecular Docking Studies

#### Docking results against PDB ID: 4KM2

The synthesized triazine derivatives (4a–4f) showed good to excellent binding affinity against Mycobacterium tuberculosis dihydrofolate reductase (PDB ID: 4KM2).

The docking scores ranged from –7.8 to –9.3 kcal/mol, which were better than the reference ligand trimethoprim (–7.1 kcal/mol) and mostly comparable to or better than ciprofloxacin (–8.0 kcal/mol).

Among all compounds, 4c exhibited the highest binding affinity (–9.3 kcal/mol), followed by 4d (–9.2 kcal/mol) and 4b (–9.0 kcal/mol).

Compound 4a also showed good interaction with a docking score of –8.5 kcal/mol, whereas 4f and 4e displayed scores of –8.3 and –7.8 kcal/mol, respectively.

The active derivatives were well accommodated in the binding cavity and interacted with key amino acid residues such as ARG 32, GLN 28, PHE 31, TRP 6, ILE 14, ILE 20, LEU 57, ARG 60, and TYR 100.

Compound 4c formed extensive hydrophobic and polar interactions, indicating formation of a highly stable protein–ligand complex.

Compounds 4d and 4f additionally formed hydrogen bonding interactions with ILE 14, which may contribute to improved binding stability.

The triazine ring along with aromatic and sulfonamide substituents appears to support favorable hydrophobic, van der Waals, and  $\pi$ – $\pi$  interactions within the active site.

The better docking scores of 4b, 4c, and 4d may be due to the presence of lipophilic and electron-modulating substituents that enhance receptor accommodation.

Overall, the docking study suggests that compound 4c is the most promising inhibitor of M. tuberculosis DHFR, followed by 4d and 4b, indicating their potential for further antitubercular investigation.

#### Docking results against PDB ID: 2XCT

The synthesized triazine derivatives (4a–4f) were also docked against *Staphylococcus aureus* DNA gyrase complex (PDB ID: 2XCT) to assess their antibacterial target affinity.

The docking scores ranged from –6.9 to –8.4 kcal/mol, indicating moderate to good binding interactions with the enzyme–DNA complex.

Among the tested compounds, 4c again showed the highest binding affinity with a score of –8.4 kcal/mol, which was better than ciprofloxacin (–6.4 kcal/mol) and trimethoprim (–5.9 kcal/mol).

Compounds 4b and 4d exhibited comparable docking scores of –7.3 kcal/mol, while 4a showed a score of –7.2 kcal/mol.

The least active compounds were 4e and 4f, both showing docking scores of –6.9 kcal/mol.

Compound 4c formed multiple interactions with important residues such as HID B:1081, SER B:1085, ARG D:1122, PHE D:1123, and ARG B:1033, along with DNA bases.

A crucial hydrogen bond with ASP B:512 was observed for 4c, which may play a significant role in stabilizing the ligand within the active pocket.

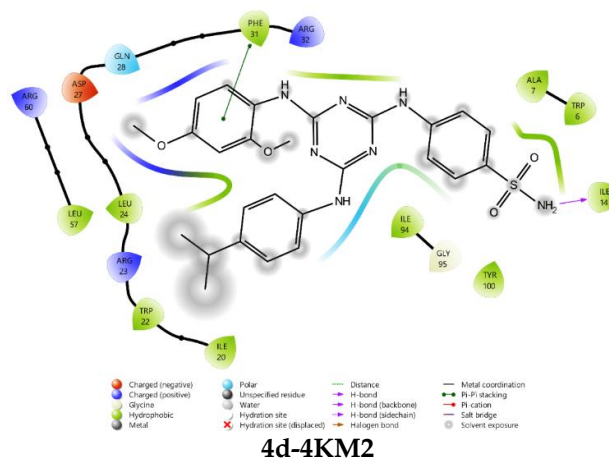
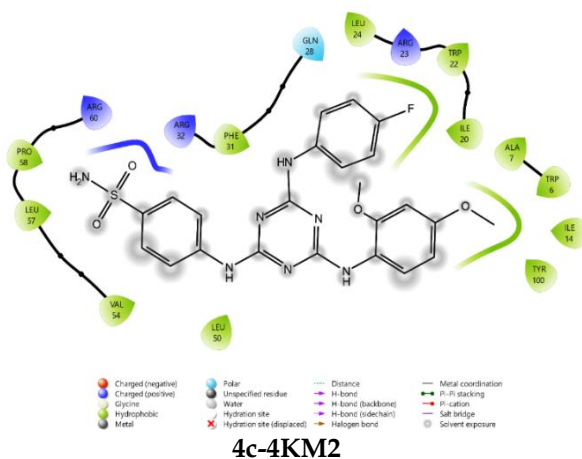
Compound 4b also formed a hydrogen bond with ASP B:512, whereas 4d showed extensive hydrophobic and electrostatic interactions with several active-site residues.

The interaction pattern indicates that the triazine nucleus and substituted aromatic groups favor binding through hydrogen bonding, hydrophobic contacts, and  $\pi$ - $\pi$  stacking with DNA bases.

Overall, the docking study confirms that compound 4c is the most promising DNA gyrase inhibitor among the synthesized series, and its strong docking result correlates well with its observed *In vitro* antimicrobial activity.

**Table 3:** Molecular Docking of Compounds 4a-4f against PDB id: 4KM2 and PDB ID: 2XCT

| Ligand        | Affinity (Kcal/mol) |              |
|---------------|---------------------|--------------|
|               | PDB id: 4KM2        | PDB ID: 2XCT |
| 4a            | -8.5                | -7.2         |
| 4b            | -9                  | -7.3         |
| 4c            | -9.3                | -8.4         |
| 4d            | -9.2                | -7.3         |
| 4e            | -7.8                | -6.9         |
| 4f            | -8.3                | -6.9         |
| Ciprofloxacin | -8                  | -6.4         |
| Trimethoprim  | -7.1                | -5.9         |



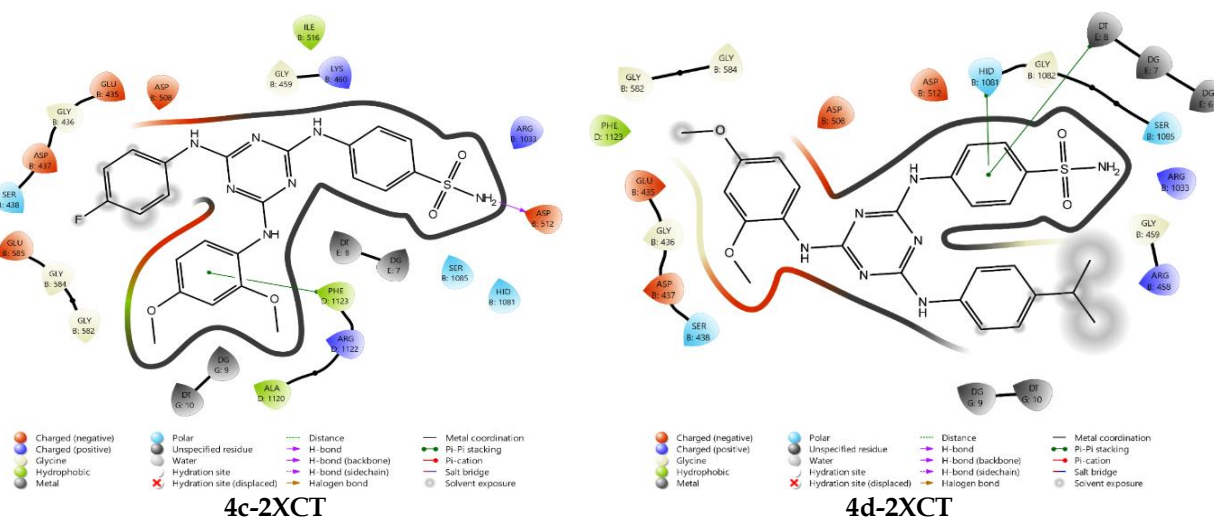


Figure 2: Binding Interaction Diagram

## Conclusion

The present study successfully described the synthesis of a series of novel substituted 1,3,5-triazine derivatives (4a–4f), which were confirmed by spectral and analytical techniques. Biological evaluation revealed that compounds 4c and 4d exhibited the most promising broad-spectrum antimicrobial activity against both bacterial and fungal strains. The *in silico* ADME analysis indicated acceptable lipophilicity and hydrogen-bonding characteristics, although high polarity and molecular weight may limit oral bioavailability. Molecular docking studies against PDB ID: 4KM2 and 2XCT demonstrated strong binding affinities, particularly for compound 4c, which showed the best interaction profile among the synthesized derivatives. Overall, the combined experimental and computational findings suggest that these triazine-based hybrids, especially 4c and 4d, may serve as promising lead molecules for the development of new antimicrobial agents and warrant further structural optimization and biological investigation.

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