

Synthesis, Characterization, Molecular Docking, and Antimicrobial Evaluation of Certain Cyclic Amine-Containing Trisubstituted 1,3,5-Triazine Derivatives

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ABSTRACT

A series of novel tri-substituted 1,3,5-triazine derivatives (5a–l) was synthesized and characterized using standard analytical and spectroscopic techniques including IR, ¹H NMR, ¹³C NMR, and mass spectrometry. The antimicrobial efficacy of the synthesized compounds was evaluated against four bacterial strains (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688, *Staphylococcus aureus* MTCC 96, and *Streptococcus pyogenes* MTCC 442) and three fungal strains (*Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282, and *Aspergillus clavatus* MTCC 1323). Compound 5i exhibited the most potent antibacterial activity against *S. aureus* with a minimum inhibitory concentration (MIC) of 62.5 µg/mL, while compound 5b demonstrated broad-spectrum antibacterial activity with MIC values of 50–100 µg/mL, comparable to ciprofloxacin (MIC 25–50 µg/mL). Compound 5k showed significant antifungal activity against *C. albicans* (MIC 100 µg/mL), surpassing griseofulvin (MIC 500 µg/mL). Molecular docking studies against *S. aureus* DNA gyrase (PDB: 3FY8) revealed that compound 5e exhibited the highest binding affinity (docking score: -7.074 kcal/mol), approaching ciprofloxacin (-7.778 kcal/mol). The compounds demonstrated strong interactions with key active-site residues via hydrophobic contacts, hydrogen bonding with TYR 98, halogen bonding, and electrostatic interactions with ASP 27 and LYS 45, suggesting that DNA gyrase inhibition is the mechanism of antibacterial action. These findings highlight the pharmaceutical potential of 1,3,5-triazine scaffolds as promising lead compounds for the development of new antimicrobial agents with DNA gyrase-inhibitory activity.

Keywords: Heterocyclic, derivatives, Cyclic amine, antimicrobial activities

INTRODUCTION

The important heterocyclic compound s-triazine, also referred to as 1,3,5-triazine, contains nitrogen. ^[1] 1,3,5-triazine, also known as s-triazine, is a well-studied structural motif in medicinal chemistry. ^[2] The pharmaceutical significance of s-triazine derivatives is evidenced by several clinically approved drugs containing this scaffold (Figure 1). Notable examples include 6-azacytosine, an antiviral agent; 6-azauracil, employed in antitumor therapy; tirapazamine, utilized in cancer treatment; lamotrigine, a widely prescribed anticonvulsant; and metribuzin, used as a herbicide. These diverse applications underscore the versatility and therapeutic potential of triazine-based compounds across multiple therapeutic areas.

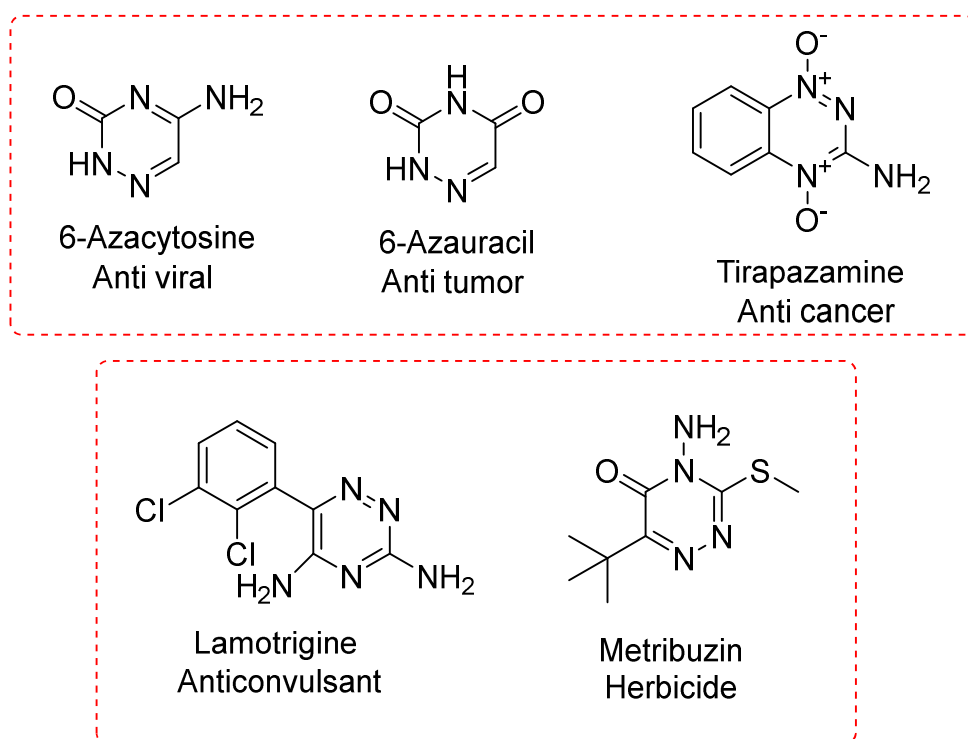


Figure 1. Representative examples of marketed and clinically relevant s-triazine derivatives with diverse therapeutic applications, demonstrating the pharmaceutical importance of the triazine scaffold in drug discovery.

The core for obtaining several s-triazine derivatives is 2,4,6-Trichloro-1,3,5-triazine, due to its low cost and the ease with which its three independent, easily adjustable ring locations can be manipulated. This enables nucleophilic substitution processes to occur sequentially with almost all nucleophiles, including sulfur, oxygen, and nitrogen. ^[3-9] A significant class of chemicals is represented by s-triazine derivatives due to their potential biological activity. They are

recognized as antimicrobials, ^[10] cyclin-dependent kinase modulators, ^[11] estrogen receptor modulators, ^[12] antimalarials, ^[13] anticancer drugs, ^[14] and anti-protozoal. ^[15] Cyanuric chloride is a cheap, readily accessible reagent that has been used extensively in organic reactions because it is less corrosive and hazardous than similar reagents. ^[16] An affordable, commercially available reagent for preparing a range of s-triazine derivatives is cyanuric chloride (1,3,5-triazine). The reagent's usefulness for the production of mono-, di-, and tri-substituted 1,3,5-triazine derivatives at regulated temperatures is increased by the ease with which different nucleophiles can displace the chlorine atoms in cyanuric chloride. ^[17] It shows the order of nucleophile incorporation onto s-triazene. The incorporation of the first nucleophile can be at 0-5° C temperature, while the second one is at room temperature, and the third one at reflux temperature. ^[18-20] In this work, we are presenting the synthesis of new asymmetrical triazine compounds based on O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine and some cyclic amines because of their dominant biological properties, including antibacterial, fungicidal, antimalarial, anticancer, antiviral, antimicrobial, anti-inflammatory, antiamoebic, and antitubercular properties. ^[21] Triazine and its derivatives have long stood at the forefront of chemotherapeutic compound development. The central objectives of organic and medicinal chemistry remain the design, synthesis, and biological evaluation of novel molecules with potential therapeutic value. Among various heterocyclic frameworks, the triazine nucleus has recently emerged as a particularly versatile and biologically active scaffold, exhibiting a broad spectrum of pharmacological properties. ^[22] In the present study, we report the synthesis of a new class of asymmetrical triazinyl compounds derived from O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine. The target molecules were synthesized through nucleophilic substitution reactions involving various amines. Structural elucidation of the synthesized compounds was carried out using comprehensive spectroscopic techniques. Furthermore, their antimicrobial activity was evaluated against the Gram-positive bacterium *Staphylococcus aureus* and the fungal pathogen *Candida albicans*, aiming to assess their potential as bioactive agents. ^[23]

EXPERIMENTAL

The solvents and other chemical reagents needed for the synthetic processes were purchased from reliable vendors, Merck Ltd., SD Fine Chemicals, and Sigma-Aldrich Chemical. Before being used, the reagent-grade compounds were refined by either distillation or recrystallization after being purchased from commercial sources. Thin layer chromatography has been used to

verify the prepared compound's purity. Melting points were measured uncorrected using an electrical digital melting point device VMP-PM-Digital Melting/Boiling point Apparatus. Using TMS as the internal standard, ^1H NMR spectra are recorded in CDCl_3 or DMSO-d_6 on a Bruker Avance neo 500MHz spectrometer. The mass spectra were obtained using a Triple Quadrupole LC-MS/MS(LCMS-8060NX) spectrometer, and the chemical shifts are expressed as parts per million (ppm).

Preparation of O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine (2)

In a 500 ml glass flask with a stirring apparatus, a mixture of (0.15 mol) of methyl acetate, (0.15 mol) of hydroxyl amine in water, and 100 ml of water was prepared. Following this, (0.15 mol) of 4% NaOH solution was added in 60 min and after 2-3 hours, a solution of 1,3-dichloropropene was slowly added drop by drop while simultaneously adding (0.20 mol) of 4% NaOH solution. The reaction temperature was maintained at $0-5^\circ\text{C}$, and the mixture was stirred for approximately 2-4 hours until the starting material disappeared, as confirmed by TLC examination. Subsequently, the reaction mass was heated to 50°C , and (0.45 mol) of concentrated hydrochloric acid solution was added. The mixture was stirred for about 5-6 hours until the starting material disappeared, as confirmed by TLC examination. After that, 200 ml of Carbon tetrachloride was added, and the pH was neutralised with 4% NaOH Solution, and the organic layer was separated. The Carbon tetrachloride was then evaporated under reduced pressure using a rotary evaporator to obtain compound (2). purified by column Distillation. Analysis. Calculated for Molecular Formula: $\text{C}_3\text{H}_6\text{ClNO}$, Formula Weight: 107.53, Composition: C (33.51%) H (5.62%) Cl (32.97%) N (13.03%) O (14.88%), ^1H NMR (100 MHz, CDCl_3): δ 4.26 (2H, d, $J = 6.9$ Hz), 5.75 (1H, dt, $J = 14.0, 6.9$ Hz), 6.22 (1H, d, $J = 14.0$ Hz). ^{13}C NMR (500 MHz, CDCl_3): δ 69.2 (1C, s), 119.3 (1C, s), 131.1 (1C, s).

Preparation of 4,6-dichloro-N-{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazin-2-amine (3)
s-triazine (1) (0.05 mol), 4% NaOH Solution (0.1 mol), and 100 ml Methylbenzene were fed into a 500 ml glass flask equipped with a stirrer. O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine (2) (0.05 mol) dissolved in 50 ml methylbenzene, and the obtained solution was added dropwise into the flask. The reaction temperature was kept at $0-5^\circ\text{C}$, and the mixture was stirred for about 2-4 h until the starting material disappear examined by TLC, the inorganic material was filtered off and the filtrate wash with water and separate out organic layer methylbenzene evaporated under reduced pressure by Rota evaporator to give (3) (yield: 89%), Analysis. Calculated for Molecular

Formula: $C_6H_5Cl_3N_4O$, **Formula Weight:** 255.48, **Composition:** C (28.21%) H (1.97%) Cl (41.63%) N (21.93%) O (6.26%), **1H NMR (500 MHz, $CDCl_3$):** δ 4.54 (2H, d, $J = 6.4$ Hz), 5.82 (1H, dt, $J = 14.0, 6.4$ Hz), 6.22 (1H, d, $J = 14.0$ Hz). **^{13}C NMR (100 MHz, $CDCl_3$):** δ 69.2 (1C, s), 119.3 (1C, s), 131.1 (1C, s), 160.9 (2C, s), 162.8 (1C, s). **IR cm^{-1} :** 3373(N-H), 3208 (C-H), 1565 (C=C), 1419 (C=N), 1171(C-N) and at 752 (Cl-C).

Preparation of 6-chloro- N^2, N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazine-2,4-diamine (4)

4,6-Dichloro-N-{[(2E)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazin-2-amine (3) (0.05 mol), 4% NaOH Solution (0.1 mol), and 100 mL methylbenzene were fed into a 500 mL glass flask equipped with a stirrer. O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine (2) (0.05 mol) dissolved in 50 ml methylbenzene, and the obtained solution was added drop wise into the flask. The reaction temperature was kept at 30-35°C, and the mixture was stirred for about 8-10 h until the starting material disappeared examined by TLC, the inorganic material was filtered off and the filtrate wash with water and separate out organic layer methylbenzene evaporated under reduced pressure by Rota evaporator to give (4) (yield: 93%), Analysis. Calculated for Molecular Formula: $C_9H_{10}Cl_3N_5O_2$, **Formula Weight:** 326.56, **Composition:** C (33.10%) H (3.09%) Cl (32.57%) N (21.45%) O (9.80%), **^{13}C NMR (100 MHz, $CDCl_3$):** δ 69.2 (2C, s), 119.3 (2C, s), 131.1 (2C, s), 160.9 (1C, s), 162.8 (2C, s). **1H NMR (500 MHz, $CDCl_3$):** δ 4.54 (4H, d, $J = 6.4$ Hz), 5.82 (2H, dt, $J = 14.0, 6.4$ Hz), 6.22 (2H, d, $J = 14.0$ Hz). **IR cm^{-1} :** 3361(N-H), 1670 (C=C), 1447 (C=N), 1290 (C-N) and at 761 (Cl-C).

General procedures for the preparation of compounds 5a-l

Preparation of 6-chloro- N^2, N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl]oxy}-1,3,5-triazine-2,4-diamine, Cyclopropylamine derivatives (5a)

In the three-necked 500 ml flask, a solution of compound (4) (0.05 mol) in methylbenzene (100 ml) and 10% NaOH Solution (0.1 mol) were added, a solution of the Cyclopropylamine in methylbenzene (a) (0.05 mol) in 50 ml Methylbenzene was added dropwise to the mixture. The reaction mixture was stirred at reflux for 12-16 h until the starting material had disappeared, as monitored by TLC. Then, ice water (100 ml) was added to the semi-dry precipitate. The solid was collected by vacuum filtration, washed with water, dried and recrystallised from chloroform/n-hexane to give a colourless crystalline product of 5a (yield: 85%). Analysis. Calculated Molecular Formula: $C_{12}H_{16}Cl_2N_6O_2$, **Formula Weight:** 347.2, **Composition:** C

(41.51%) H (4.65%) Cl (20.42%) N (24.21%) O (9.22%), **¹³C NMR (100 MHz, CDCl₃):** δ 6.6 (2C, s), 23.3 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)). **¹H NMR (500 MHz, CDCl₃):** δ 0.45 (4H, dddd, *J* = 8.1, 7.8, 7.5, 6.7 Hz), 2.97 (1H, tt, *J* = 8.1, 7.5 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.22 (2H, d, *J* = 14.0 Hz). **IR cm⁻¹:** 3029 (N-H), 3008 (C-H), 1558 (C=C), 1451 (C=N), 1262 (C-N) and at 803 (Cl-C).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cyclopropylamine (5a).

¹³C NMR (100 MHz, CDCl₃): δ 6.6 (2C, s), 23.3 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **¹H NMR (500 MHz, CDCl₃):** δ 0.45 (4H, m), 2.97 (1H, d, 7.5 Hz), 4.54 (4H, d, *J* = 6.4 Hz), 5.82 (2H, dt, *J* = 14.0, 6.4 Hz), 6.22 (2H, d, *J* = 14.0 Hz), **IR Spectrum(cm⁻¹):** 3029(N-H), 3008 (C-H), 1558 (C=C), 1451 (C=N),1262(C-N) and at 803 (Cl-C), **Composition:** C (41.51%) H (4.65%) Cl (20.42%) N (24.21%) O (9.22%), **Found:** C (41.52%) H (4.64%) Cl (20.44%) N (24.20%) O (9.23%).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cyclobutylamine (5b).

¹³C NMR (100 MHz, CDCl₃): δ 18.3 (1C, s), 24.5 (2C, s), 48.5 (1C, s), 73.9-77.3 (2C, s), 124.1- (2C, s), 136.2 (2C, s), 165.0-169.1 (3C, 165.0 (s), 168.3 (s)), **¹H NMR (500 MHz, CDCl₃):** δ 1.74-1.93 (6H, 1.82 (d, *J* = 4.5 Hz), 1.85 (d, *J* = 4.5 Hz)), 3.70 (1H, d, *J* = 6.7 Hz), 4.20 (4H, d, *J* = 6.4 Hz), 5.87 (2H, d, *J* = 6.4 Hz), 6.29 (2H, d, *J* = 14.0 Hz), **IR Spectrum(cm⁻¹):** 3347 (N-H), 3165 (C-H), 1633 (C=C), 1465 (C=N),1221(C-N), and at 794(Cl-C), **Composition:** C (43.23%) H (5.02%) Cl (19.63%) N (23.27%) O (8.86%), **Found:** C (43.25%) H (5.04%) Cl (19.60%) N (23.25%) O (8.85%).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cyclopentyl amine (5c).

¹³C NMR (100 MHz, CDCl₃): δ 24.1 (2C, s), 31.3 (2C, s), 50.1 (1C, s), 73.9 (2C, s), 124.1 (2C, s), 131.1 (2C, s), 165.0-169.11 (3C, 165.05 (s), 168.33 (s)), **¹H NMR (500 MHz, CDCl₃):** δ 1.50-1.69 (4H, 1.59 (m, *J* = 13.3, 6.5, 5.4, 4.1 Hz), 1.60 (m, *J* = 13.3, 8.2, 5.3, 3.5 Hz)), 1.71-1.91 (4H, 1.79 (m), 1.81 (m)), 3.82 (1H, m), 4.19 (4H, d, *J* = 6.4 Hz), 5.87 (2H, dt, *J* = 14.0, 6.4 Hz), 6.29 (2H, d, *J* = 14.0 Hz), **IR Spectrum(cm⁻¹):** 3402 (N-H), 3170 (C-H), 1594 (C=C), 1500 (C=N),1245(C-N), and at 831 (Cl-C), **Composition:** C (44.81%) H (5.37%) Cl (18.89%) N (22.40%) O (8.53%), **Found:** C (44.84%) H (5.36%) Cl (18.88%) N (22.41%) O (8.52%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cyclohexylamine (5d).

^{13}C NMR (100 MHz, CDCl_3): δ 25.0 (2C, s), 25.7 (1C, s), 33.5 (2C, s), 51.8 (1C, s), 69.2 (2C, s), 119.3 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz, CDCl_3):** δ 1.29-1.56 (6H, 1.37 (m), 1.48 (m)), 1.76 (4H, dt, $J = 13.3, 6.5$ Hz), 3.69 (1H, m), 4.54 (4H, d, $J = 6.4$ Hz), 5.82 (2H, dt, $J = 14.0, 6.4$ Hz), 6.22 (2H, d, $J = 14.0$ Hz), **IR Spectrum(cm^{-1}):** 3469 (N-H), 3107 (C-H), 1572 (C=C), 1470 (C=N), 1213 (C-N), and at 745 (Cl-C), **Composition:** C (46.28%) H (5.70%) Cl (18.21%) N(21.59%) O(8.22%), **Found:** C (46.30%) H (5.71%) Cl (18.18%) N(21.57%) O(8.24%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cycloheptyl amine (5e).

^{13}C NMR (100 MHz, CDCl_3): δ 23.4 (2C, s), 27.9 (2C, s), 33.8 (2C, s), 57.7 (1C, s), 69.2 (2C, s), 119.3 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz, CDCl_3):** δ 1.45-1.66 (12H, 1.54 (m), 1.54 (m), 1.58 (m)), 3.72 (1H, m), 4.54 (4H, d, $J = 6.4$ Hz), 5.82 (2H, dt, $J = 14.0$ Hz), 6.22 (2H, d, $J = 14.0$ Hz), **IR Spectrum(cm^{-1}):** 3387 (N-H), 3103 (C-H), 1570 (C=C), 1437 (C=N), 1277 (C-N), and at 742 (Cl-C), **Composition:** C (47.65%) H (6.00%) Cl (17.58%) N (20.84%) O (7.93%), **Found:** C (47.66%) H (6.02%) Cl (17.57%) N (20.85%) O (7.91%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, cyclooctyl amine (5f).

^{13}C NMR (100 MHz, CDCl_3): δ 25.1 (2C, s), 25.6 (1C, s), 27.1 (2C, s), 29.2 (2C, s), 69.2 (2C, s), 76.8 (1C, s), 119.3 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz, CDCl_3):** δ 1.36-1.68 (14H, 1.44 (m), 1.48 (m), 1.52 (m), 1.59 (m)), 3.50 (1H, t, $J = 9.6$ Hz), 4.54 (4H, d, $J = 6.4$ Hz), 5.82 (2H, dt, $J = 14.0, 6.4$ Hz), 6.22 (2H, d, $J = 14.0$ Hz), **IR Spectrum(cm^{-1}):** 3468 (N-H), 3108 (C-H), 1570 (C=C), 1476 (C=N), 1277 (C-N), and at 742 (Cl-C), **Composition:** C (48.93%) H (6.28%) Cl (16.99%) N (20.14%) O (7.67%), **Found:** (48.91%) H (6.29%) Cl (16.98%) N (20.16%) O (7.69%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-methylcyclopropanamine (5g).

^{13}C NMR (100 MHz, CDCl_3): δ 11.9 (1C, s), 12.4 (1C, s), 18.4 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz,**

CDCl₃): δ 0.28-0.51 (2H, 0.36 (m), 0.44 (m)), 0.79 (1H, m), 1.21 (3H, d, $J = 6.9$ Hz), 2.94 (1H, m), 4.48-4.60 (4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm⁻¹): 3452 (N-H), 3106 (C-H), 1571 (C=C), 1476 (C=N), 1273 (C-N), and at 746 (Cl-C), **Composition**: C (43.23%) H (5.02%) Cl (19.63%) N (23.27%) O (8.86%), **Found**: C (43.24%) H (5.01%) Cl (19.64%) N (23.26%) O (8.87%).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-ethylcyclopropanamine (5h).

¹³C NMR (100 MHz, CDCl₃): 12.1 (1C, s), 12.4 (1C, s), 17.1 (1C, s), 21.6 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **¹H NMR (500 MHz, CDCl₃)**: 0.29-0.52 (2H, 0.37 (m), 0.44 (m)), 0.93 (3H, dd, $J = 7.1$ Hz), 1.10 (1H, m), 1.52-1.70 (2H, 1.61 (m), 1.61 (m)), 2.90 (1H, m), 4.48-4.60 (4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm⁻¹): 3455 (N-H), 3101 (C-H), 1573 (C=C), 1466 (C=N), 1279 (C-N), and at 746 (Cl-C), **Composition**: C (44.81%) H (5.37%) Cl (18.89%) N (22.40%) O (8.53%), **Found**: C (44.82%) H (5.35%) Cl (18.91%) N (22.42%) O (8.55%).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-Propylcyclopropanamine (5i).

¹³C NMR (100 MHz, CDCl₃): δ 12.4 (1C, s), 13.7 (1C, s), 17.1 (1C, s), 19.6 (1C, s), 32.2 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **¹H NMR (500 MHz, CDCl₃)**: δ 0.30-0.52 (2H, 0.37 (m), 0.45 (m)), 0.89 (3H, dd, $J = 6.6, 6.6$ Hz), 1.09 (1H, m), 1.26-1.55 (4H, 1.35 (m), 1.35 (m), 1.48 (m), 1.48 (m)), 2.91 (1H, m), 4.48-4.60 (4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm⁻¹): 3469 (N-H), 3110 (C-H), 1566 (C=C), 1471 (C=N), 1280 (C-N), and at 746 (Cl-C), **Composition**: C (46.28%) H (5.70%) Cl (18.21%) N (21.59%) O (8.22%), **Found**: C (46.29%) H (5.71%) Cl (18.22%) N (21.62%) O (8.20%).

6-chloro-N², N⁴-bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-isopropyl cyclopropylamine (5j).

¹³C NMR (100 MHz, CDCl₃): δ 12.4 (1C, s), 17.8 (1C, s), 21.0-21.1 (2C, 21.0 (s), 21.0 (s)), 27.9 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **¹H NMR (500 MHz, CDCl₃)**: δ 0.30-0.52 (2H, 0.37 (m), 0.44 (m)), 0.82-0.94 (6H, 0.88 (d, $J = 6.8$ Hz), 0.88 (d, $J = 6.8$ Hz)), 1.09 (1H, m), 1.67 (1H, m), 2.91 (1H, m), 4.48-4.60

(4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm^{-1}): 3459 (N-H), 3113 (C-H), 1570 (C=C), 1459 (C=N), 1293 (C-N), and at 744 (Cl-C), **Composition:** C (46.28%) H (5.70%) Cl (18.21%) N (21.59%) O (8.22%), **Found:** C (46.29%) H (5.71%) Cl (18.24%) N (21.55%) O (8.20%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-tert-butylcyclopropanamine (5k).

^{13}C NMR (100 MHz, CDCl_3): δ 12.4 (1C, s), 21.4 (1C, s), 27.6 (3C, s), 30.7 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz, CDCl_3):** δ 0.28-0.53 (2H, 0.35 (m), 0.45 (m)), 0.91 (9H, s), 1.08 (1H, m), 2.94 (1H, m), 4.48-4.60 (4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm^{-1}): 3441 (N-H), 3116 (C-H), 1572 (C=C), 1459 (C=N), 1274 (C-N), and at 744 (Cl-C), **Composition:** C (47.65%) H (6.00%) Cl (17.58%) N (20.84%) O (7.93%), **Found:** C (47.68%) H (6.01%) Cl (17.61%) N (20.83%) O (7.92%).

6-chloro- N^2 , N^4 -bis{[(2E)-3-chloroprop-2-en-1-yl] oxy}-1,3,5-triazine-2,4-diamine, 2-Methylcyclopropyl-2-propylamine (5l).

^{13}C NMR (100 MHz, CDCl_3): δ 12.4 (1C, s), 17.1 (1C, s), 22.7-22.8 (2C, 22.8 (s), 22.8 (s)), 26.5 (1C, s), 38.5 (1C, s), 49.9 (1C, s), 69.2 (2C, s), 119.9 (2C, s), 131.1 (2C, s), 162.7-162.8 (3C, 162.8 (s), 162.8 (s)), **^1H NMR (500 MHz, CDCl_3):** δ 0.30-0.53 (2H, 0.38 (m), 0.45 (m)), 0.75-0.87 (6H, 0.81 (d, $J = 6.6$ Hz), 0.81 (d, $J = 6.6$ Hz)), 0.99-1.28 (3H, 1.09 (m), 1.21 (dd, $J = 6.9, 6.7$ Hz), 1.21 (dd, $J = 6.9, 6.7$ Hz)), 1.51 (1H, m), 2.92 (1H, m), 4.48-4.60 (4H, 4.54 (d, $J = 6.4$ Hz), 4.54 (d, $J = 6.4$ Hz)), 5.82 (2H, m), 6.23 (2H, d, $J = 14.0$ Hz), **IR Spectrum**(cm^{-1}): 3448 (N-H), 3103 (C-H), 1571 (C=C), 1451 (C=N), 1269 (C-N), and at 747 (Cl-C), **Composition:** C (47.65%) H (6.00%) Cl (17.58%) N (20.84%) O (7.93%), **Found:** C (47.64%) H (6.02%) Cl (17.59%) N (20.82%) O (7.91%).

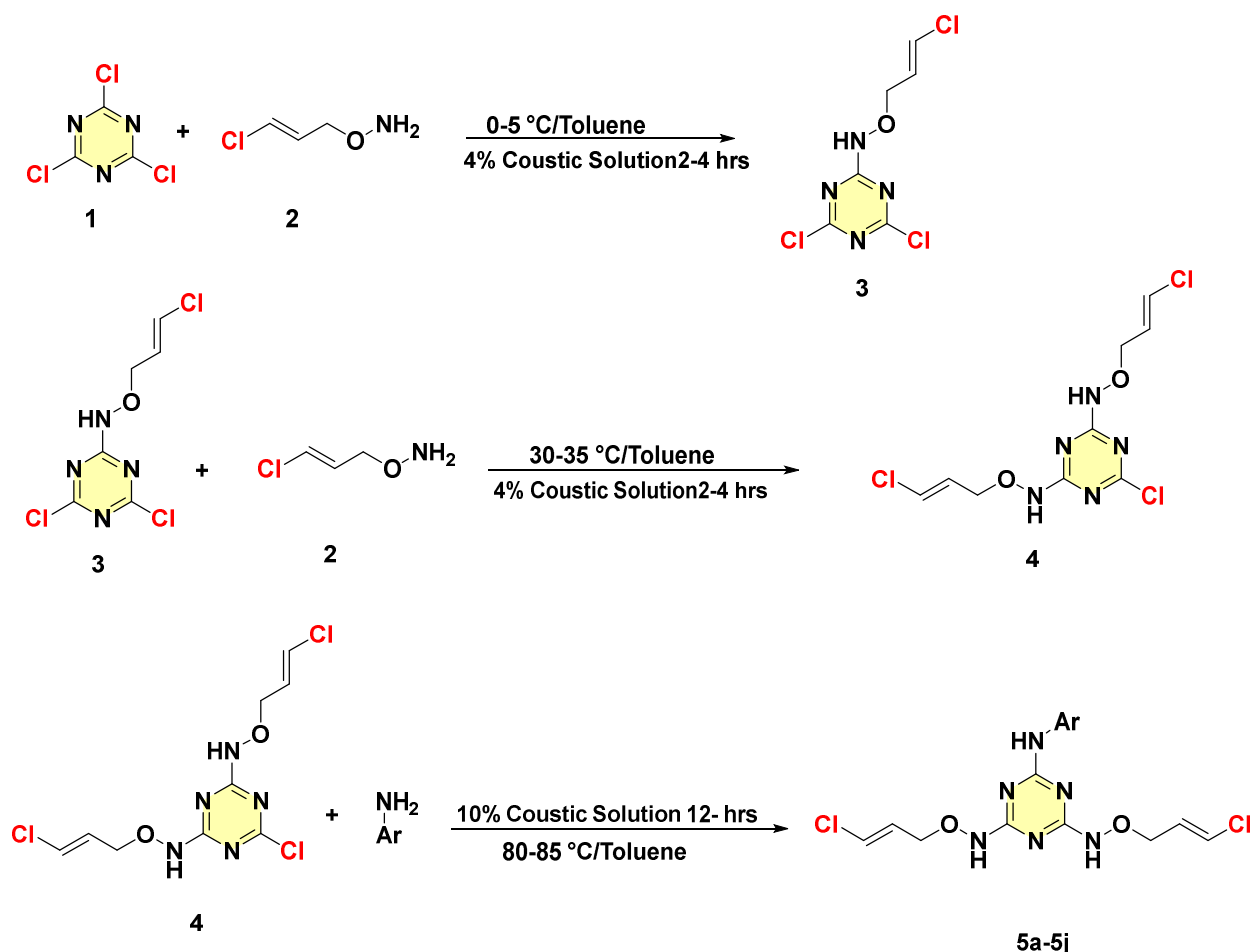
Molecular docking studies

Molecular docking studies were conducted using the Glide module of Schrödinger Suite (Schrödinger, LLC, New York, NY) to assess the binding affinity of the test compound with the Co Crystal structure of *S. Aureus* (PDB ID: 3FY8). The crystal structure was retrieved from the RCSB Protein Data Bank and prepared using the Protein Preparation Wizard, which included hydrogen atom addition, bond order assignment, and optimization of the hydrogen bonding network using PROPKA at pH 7.4. Water molecules beyond 5 Å from heteroatoms were deleted,

and the structure was energy-minimized using the OPLS4 force field until RMSD convergence to 0.30 Å. The ligand was prepared via LigPrep with ionization states generated at pH 7.4 ± 2.0 using Epik, followed by tautomer and stereoisomer generation. A receptor grid of $20 \text{ \AA} \times 20 \text{ \AA} \times 20 \text{ \AA}$ was centered on the ATP-binding pocket of 1M17, with van der Waals scaling set to 1.0 for atoms with partial charges <0.25 . Glide XP (Extra Precision) docking was performed with a flexible ligand and rigid receptor. The optimal binding pose was selected based on GScore, Emodel, and interaction analysis. Binding interactions and free energies were evaluated using the Glide XP Visualizer and MM-GBSA calculations in Prime. [29-32]

RESULTS AND DISCUSSION

Scheme 1 depicts the synthetic route for the compounds 5a-5l, in which a nucleophilic substitution reaction was used to form the intermediates 3 and 4, followed by reaction with cyclic amines to prepare the target compounds. (Reaction scheme-1, Table-1)



Reaction scheme-1 Synthetic route for the preparation of tri-substituted 1,3,5-triazine derivatives (5a–5j).

The intermediate **2** was then reacted with cyanuric chloride *via* substitution reaction to obtain the desired (**3**). another molecule of O-[(2E)-3-chloroprop-2-en-1-yl] hydroxylamine (**2**) reacting with cyclic amines (**5a-5l**) with in the presence of sodium hydroxide solution in methyl benzene solvent at reflux temperature.

Table 1. Physical and analytical data of compounds 5a–5l.

Compound	Molecular formula	Mol. Wt.	M.P. (°C)	Yield (%)
5a	C ₁₂ H ₁₆ Cl ₂ N ₆ O ₂	347.2	112	85
5b	C ₁₃ H ₁₈ Cl ₂ N ₆ O ₂	361.22	122	85
5c	C ₁₄ H ₂₀ Cl ₂ N ₆ O ₂	375.25	127	87
5d	C ₁₅ H ₂₂ Cl ₂ N ₆ O ₂	389.28	121	83
5e	C ₁₆ H ₂₄ Cl ₂ N ₆ O ₂	403.30	117	81
5f	C ₁₇ H ₂₆ Cl ₂ N ₆ O ₂	417.33	147	78
5g	C ₁₃ H ₁₈ Cl ₂ N ₆ O ₂	361.22	137	68
5h	C ₁₄ H ₂₀ Cl ₂ N ₆ O ₂	375.25	107	88
5i	C ₁₅ H ₂₂ Cl ₂ N ₆ O ₂	389.28	116	78
5j	C ₁₅ H ₂₂ Cl ₂ N ₆ O ₂	461.49	119	88
5k	C ₂₇ H ₂₆ FN ₃ O ₄	475.52	161	71
5l	C ₂₅ H ₂₁ FN ₄ O ₄	460.47	149	80

The synthesized compounds (Scheme 1) were characterized through mass spectrometry, ¹H NMR, and ¹³C NMR analysis. Physical properties of compounds 5a-l are detailed in Table 1. A detailed description of the synthetic methodologies, accompanied by complete characterization data, is provided in the experimental section.

Biological evaluation

Antimicrobial activity

The synthesized compounds 5a-l were evaluated for their *in vitro* antimicrobial activity against a panel of bacterial and fungal strains using the broth microdilution method. The minimum inhibitory concentration (MIC) values are presented in Table 2, expressed in µg/mL. Ciprofloxacin and griseofulvin were used as standard antibacterial and antifungal agents, respectively. [24-28]

Antibacterial Activity

The antibacterial screening revealed that all synthesized compounds exhibited moderate to good activity against the tested bacterial strains, with MIC values ranging from 50 to 250 $\mu\text{g/mL}$. Compound 5b demonstrated the most potent broad-spectrum antibacterial activity, showing MIC values of 50 $\mu\text{g/mL}$ against both *E. coli* (MTCC 443) and *P. aeruginosa* (MTCC 1688), and 50 $\mu\text{g/mL}$ against *S. pyogenes* (MTCC 442), which is comparable to the standard drug ciprofloxacin (MIC: 25-50 $\mu\text{g/mL}$). Compound 5i showed remarkable activity against *S. pyogenes* with an MIC value of 62.5 $\mu\text{g/mL}$, representing the lowest MIC among all tested compounds for this strain. Against Gram-negative bacteria, compounds 5a, 5f, 5g, and 5h displayed good activity against *E. coli* and *P. aeruginosa* with MIC values of 100-125 $\mu\text{g/mL}$. For Gram-positive bacteria, compound 5e exhibited uniform activity against both *S. aureus* (MTCC 96) and *S. pyogenes* with MIC values of 100 $\mu\text{g/mL}$, while compounds 5c, 5d, 5h, 5i, 5j, and 5l demonstrated MIC values of 100 $\mu\text{g/mL}$ against *S. aureus*.

Antifungal Activity

The antifungal evaluation revealed that most compounds exhibited moderate antifungal activity with MIC values ranging from 100 to 1000 $\mu\text{g/mL}$. Compound 5k displayed the most potent antifungal activity against *C. albicans* (MTCC 227) with an MIC of 100 $\mu\text{g/mL}$, which is five-fold more potent than the standard drug griseofulvin (MIC: 500 $\mu\text{g/mL}$). Against *A. niger* (MTCC 282), compound 5b showed moderate activity with an MIC value of 250 $\mu\text{g/mL}$, though less potent than griseofulvin (MIC: 100 $\mu\text{g/mL}$). For *A. clavatus* (MTCC 1323), compound 5b demonstrated the best activity with an MIC of 100 $\mu\text{g/mL}$, matching the potency of griseofulvin. Compounds 5a, 5g, and 5j showed selective antifungal activity against *C. albicans* with MIC values of 250-500 $\mu\text{g/mL}$ while maintaining moderate antibacterial properties.

Table 2. Minimum inhibitory concentrations (MIC, $\mu\text{g/mL}$) of synthesized triazine derivatives (5a–5l) against bacterial and fungal strains.

Compound Code	<i>E. coli</i> (MTCC 443)	<i>P. aeruginosa</i> (MTCC 1688)	<i>S. aureus</i> (MTCC 96)	<i>S. pyogenes</i> (MTCC 442)	<i>C. albicans</i> (MTCC 227)	<i>Niger</i> (MTCC 282)	<i>A. clavatus</i> (MTCC 1323)
5a	125	100	200	250	500	500	500
5b	50	50	100	50	500	250	100
5c	200	250	125	100	500	1000	1000
5d	200	100	100	200	1000	500	500

5e	250	200	100	100	500	500	1000
5f	100	100	200	125	1000	500	500
5g	100	100	250	250	250	500	500
5h	125	100	100	125	500	1000	1000
5i	125	200	62.5	100	1000	500	500
5j	200	250	100	100	250	500	500
5k	250	200	250	250	100	500	1000
5l	200	100	100	250	500	1000	1000
Ciprofloxacin	25	25	50	50	-	-	-
Grieseofulvin	-	-	-	-	500	100	100

Molecular docking results:

Molecular docking studies against *S. aureus* DNA gyrase (PDB: 3FY8) revealed that all synthesized compounds (5a-l) exhibited favorable binding interactions within the active site, with docking scores ranging from -4.381 to -7.074 kcal/mol (Table 3). Compound 5e demonstrated the highest binding affinity (-7.074 kcal/mol), approaching the docking score of the reference antibiotic ciprofloxacin (-7.778 kcal/mol), while compound 5g showed the weakest interaction (-4.381 kcal/mol).

The binding mode analysis revealed that all compounds occupied the same binding pocket and formed extensive interactions with key active-site residues. Hydrophobic interactions predominantly involved VAL 6, ALA 7, LEU 5, ILE 14, PHE 92, TYR 98, and GLY 93/94, which formed a stable hydrophobic core essential for ligand stabilization. Polar residues including THR 46, THR 96, GLN 19, GLN 95, ASN 18, and SER 49 contributed to the binding stability through van der Waals contacts and electrostatic interactions.

Notably, several compounds formed critical hydrogen bonds with TYR 98 (5a, 5c, 5d, 5g, 5j), a catalytically important residue in the DNA gyrase active site, similar to ciprofloxacin. This interaction pattern suggests a potential mechanism of enzyme inhibition comparable to established fluoroquinolone antibiotics. Compound 5e, despite lacking direct hydrogen bonding, compensated through halogen bonding with LEU 97 (Cl) and extensive hydrophobic contacts, explaining its superior docking score. Halogen bonding interactions were observed in compounds 5a, 5c, 5d, 5e, 5k, and 5l, providing additional stabilization to the protein-ligand complex.

Charged interactions with ASP 27 (negative) and LYS 45 (positive) were observed in multiple compounds (5a, 5c, 5d, 5e, 5j, 5l), further anchoring the ligands within the binding pocket. The

presence of these electrostatic interactions, combined with hydrogen bonding and halogen bonding, contributed to the enhanced binding affinity of the most potent compounds.

Interestingly, griseofulvin, included as a reference antifungal agent, exhibited the weakest binding affinity (-3.691 kcal/mol) and a distinct binding orientation, engaging primarily peripheral residues (PHE 42, PHE 47, PHE 122) rather than core active-site residues. This distinct interaction profile correlates with its lack of antibacterial activity against *S. aureus*.

The structure-activity relationship analysis revealed that compounds bearing electron-withdrawing halogen substituents (5c, 5e, 5k, 5l) generally exhibited superior binding affinities compared to their non-halogenated counterparts, likely due to enhanced halogen bonding capabilities and favorable electronic effects. These computational findings corroborate the in vitro antibacterial activity results and provide molecular-level insights into the mechanism of action of the synthesized compounds as potential DNA gyrase inhibitors. (Figure 2,3,4)

Table 3. Molecular docking results of synthesized triazine derivatives (5a–5l) with *S. aureus* DNA gyrase (PDB ID: 3FY8).

Co Crystal structure of <i>S. Aureus</i> (3FY8)					
Compound code	Docking score (kcal/mol)	Interacting amino acids			
		Hydrophobic interactions	Polar interactions	H-bond	Others
5a	-5.247	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, ILE 14, ILE 50, PHE 92, TYR 98, TRP 22, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 111, THR 121, GLN 19, GLN 95, ASN 18, SER 49	TYR 98 (H-bond with O), GLY 94 (halogen bond with Cl)	ASP 27 (charged negative), LYS 45 (charged positive)
5b	-5.897	VAL 6, ALA 7, LEU 5, LEU 20, LEU 97, ILE 14, ILE 50, PHE 92, TYR 98, TRP 22, GLY 15, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	ASN 18 (H-bond with NH)	LYS 45 (charged positive)
5c	-6.357	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, ILE 14, ILE 50, PHE 92, TYR 98, GLY 15, GLY 93,	THR 121, GLN 19, GLN 95, ASN 18, SER 49	TYR 98 (H-bond with cyclopentyl NH), LEU 20	ASP 27 (charged negative), LYS 45 (charged positive)

		GLY 94		(halogen bond with Cl)	
5d	-5.811	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, ILE 14, ILE 50, PHE 92, TYR 98, GLY 15, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	TYR 98 (H-bond with THR 96), LEU 20 (halogen bond with Cl)	ASP 27 (charged negative), LYS 45 (charged positive)
5e	-7.074	VAL 6, VAL 31, ALA 7, LEU 5, LEU 28, ILE 14, ILE 50, PHE 92, TYR 98, TRP 22, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	LEU 97 (halogen bond with Cl)	ASP 27 (charged negative), LYS 45 (charged positive)
5f	-6.815	VAL 6, VAL 31, ALA 7, LEU 5, LEU 28, LEU 54, ILE 14, PHE 92, TYR 98, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	-	LYS 45 (charged positive)
5g	-4.381	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, ILE 14, ILE 50, PHE 92, TYR 98, GLY 15, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 111, GLN 19, GLN 95, ASN 18, SER 49	TYR 98 (H-bond with NH), GLY 94 (halogen bond with Cl)	ASP 27 (charged negative), LYS 45 (charged positive)
5h	-6.219	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, ILE 14, PHE 92, GLY 15, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 95, ASN 18, SER 49	-	LYS 45 (charged positive)
5i	-5.342	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, LEU 97, ILE 14, PHE 92, TYR 98, TRP 22, GLY 15, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	GLN 95 (H-bond with O)	LYS 45 (charged positive)
5j	-6.081	VAL 6, VAL 31, ALA 7, LEU 5, LEU 28, ILE 14, PHE 92, TYR 98, GLY 15, GLY 93, GLY 94	THR 46, THR 96, THR 111, THR 121, GLN 19, GLN 95, ASN 18, SER 49	TYR 98 (H-bond with O)	ASP 27 (charged negative), LYS 45 (charged positive)
5k	-6.773	VAL 6, VAL 31, ALA 7,	THR 46, THR 96,	LEU 97	LYS 45 (charged

		LEU 5, LEU 97, ILE 14, ILE 50, PHE 92, TYR 98, TRP 22, GLY 15, GLY 43, GLY 93, GLY 94	THR 121, GLN 19, GLN 95, ASN 18, SER 49	(halogen bond with Cl)	positive)
5I	-6.642	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, LEU 54, LEU 97, ILE 14, ILE 50, PHE 92, TYR 98, GLY 15, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 19, GLN 95, ASN 18, SER 49	LEU 97 (halogen bond with Cl)	ASP 27 (charged negative), LYS 45 (charged positive)
Ciprofloxacin	-7.778	VAL 6, VAL 31, ALA 7, LEU 5, LEU 20, LEU 28, LEU 54, ILE 14, ILE 50, PHE 92, TYR 98, TRP 22, PRO 21, GLY 15, GLY 93, GLY 94	THR 46, GLN 19, ASN 18, SER 49	TYR 98 (H-bond with OH)	-
Griseofulvin	-3.691	ILE 14, LEU 97, PHE 42, PHE 47, PHE 122, MET 42, GLY 43, GLY 93, GLY 94	THR 46, THR 96, THR 121, GLN 95, ASN 18	THR 46 (H-bond with O)	ASP 120 (charged negative), GLU 17 (charged negative), GLU 99 (charged negative), LYS 45 (charged positive), ARG 44 (charged positive)

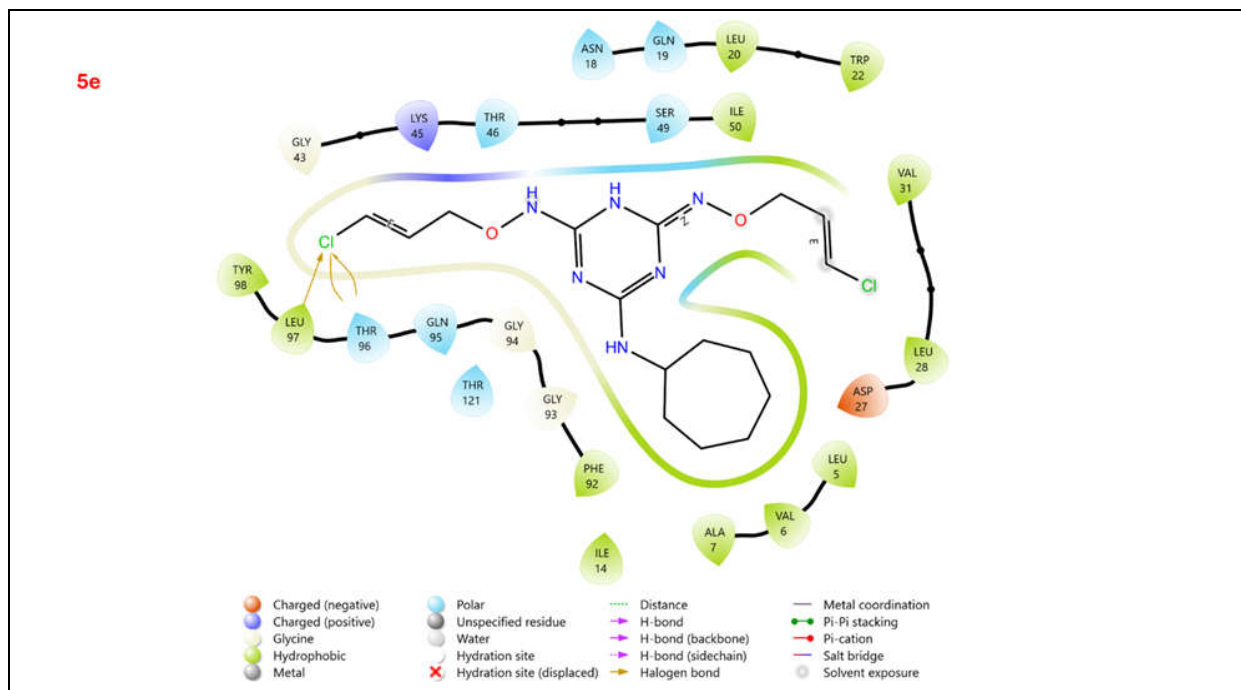


Figure 2. Two-dimensional interaction diagram of compound 5e with *S. aureus* DNA gyrase (PDB ID: 3FY8).

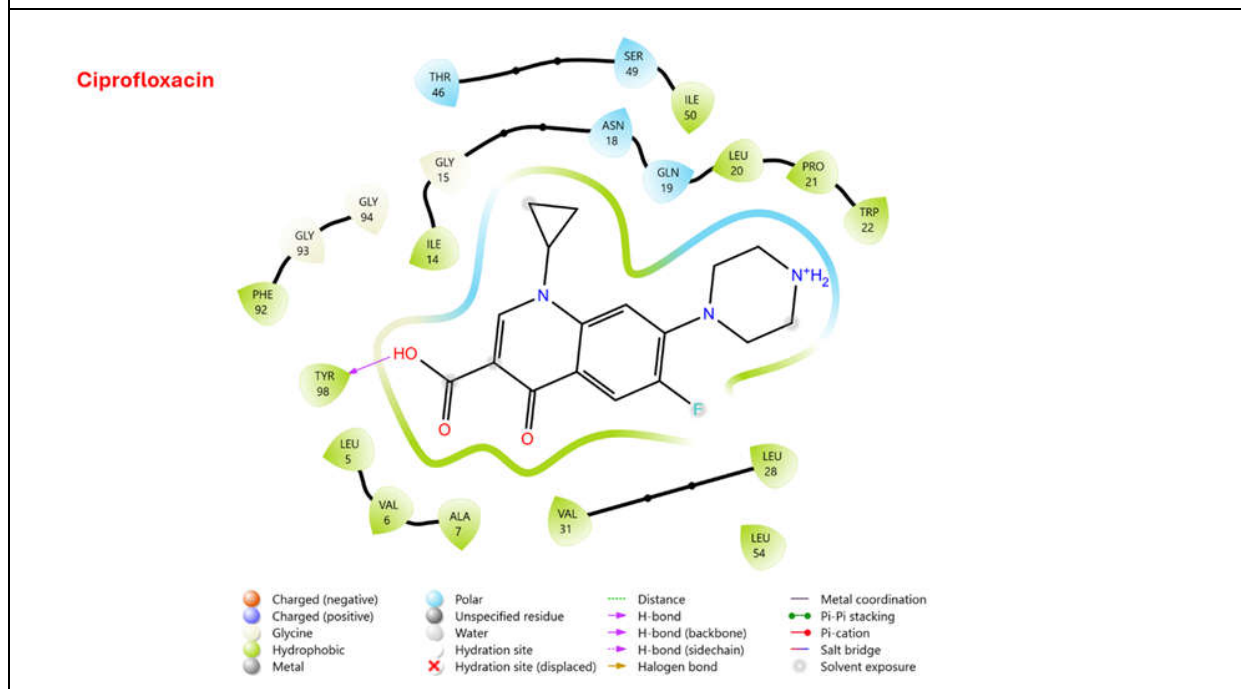
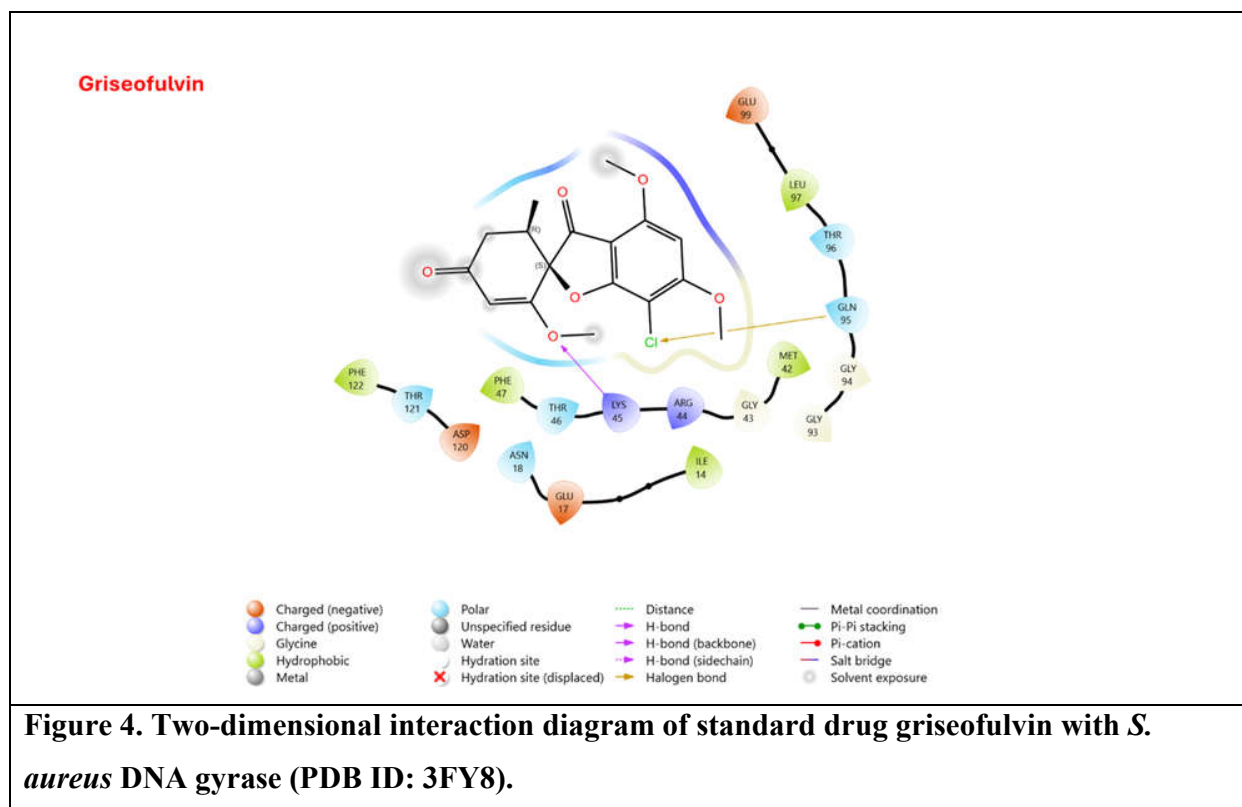


Figure 3. Two-dimensional interaction diagram of standard drug ciprofloxacin with *S. aureus* DNA gyrase (PDB ID: 3FY8).



CONCLUSION

The sequential nucleophilic substitution of cyanuric chloride provides an efficient, operationally simple synthetic route to a diverse array of trisubstituted s-triazine derivatives. Antimicrobial evaluation revealed that compound 5i exhibited potent activity against *S. aureus* (MIC 62.5 $\mu\text{g/mL}$), while compound 5b demonstrated broad-spectrum antibacterial efficacy (MIC 50–100 $\mu\text{g/mL}$) comparable to ciprofloxacin. Compound 5k showed superior antifungal activity against *C. albicans* (MIC 100 $\mu\text{g/mL}$) compared to griseofulvin. Molecular docking studies against *S. aureus* DNA gyrase indicated that compound 5e exhibited the highest binding affinity (-7.074 kcal/mol), with key interactions involving TYR 98, halogen bonding, and electrostatic contacts, suggesting DNA gyrase inhibition as the mechanism of action. These findings establish the synthesized triazine derivatives as promising lead compounds for the development of novel antimicrobial agents.

Declarations

All data generated and analysed during this study are included in this published article as supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization and design of the project: N.D.; Synthesis, workup, and purification: N.D., N.P.; Biological data collection, data processing: N.P.; Characterization: N.D.; Preparation of Manuscript: N.D. All of the authors read and approved the final version of the manuscript.

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