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Design, synthesis and molecular modeling studies of new series of *s*-triazine derivatives as antimicrobial agents against multi-drug resistant clinical isolates

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ABSTRACT

Three novel series of *s*-triazine derivatives, including thirty-five new compounds **2a-d**, **3a-3p, 4b-d**, **5b-d, 6d-6d**, and **7a-7f** were synthesized comprising a diversity of substituents based on the structure of Astrazeneca arylaminotriazine DNA gyrase B inhibitor. The antimicrobial activity was determined for all compounds against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using the two-fold serial dilution technique and against reference standards Ampicillin for the antibacterial screening and Clotrimazole regarding the antifungal evaluation. The tested compounds showed strong to moderate antibacterial inhibitory action and weak antifungal activity. Compounds **3j** and **6b** were the most potent antibacterial agents against the tested strains and multi-drug resistant (MDR) clinical isolates of *Klebsiella pneumoniae* and methicillin resistant *Staphylococcus aureus* (*MRSA1*) with minimal toxicity in comparison to the reference drugs. *In silico* molecular properties calculations and molecular docking study for **3j** and **6b** revealed that both compounds could be considered as promising antibacterial DNA gyrase B inhibitors.

1. Introduction

Worldwide, microbial infectious diseases caused by pathogenic microorganisms pose danger to the community [1,2]. The misuse of antibiotics had a profound impact on the development of multi-drug microbial resistance against the chemotherapeutic agents available [2]. The resistance developed by the microbial strains urged medicinal chemists to search for both novel antimicrobial agents and antibacterial targets to overcome this issue [3–5]. The *s*-triazine ring has been widely studied for its synthetic availability and diverse broad biological activities such as antiprotozoal [6], anticancer [7], antimalarial [3], antiviral [8], antifungal [9,10], antimicrobial [11], antileishmanial activity [12], MAO inhibitors [13] and drug delivery systems [14]. Cyanuric chloride is the starting reagent for synthesis of various *s*-triazine derivatives. It is a commercially available reagent that has attracted attention to its temperature-controlled substitution of the chlorine atoms by various nucleophiles [3,15–19].

Previous reports demonstrated that *s*-triazines linked to lipophilic parts had remarkable antibacterial and antifungal activities [20–23]. Similarly, hydrazones are important as antimicrobial [24–27], antiviral [25,28], antioxidant [29], and antitumor [30,31] agents. Recently we have demonstrated a series of dimeric *s*-triazine hydrazide derivatives [32] and *s*-triazines containing *p*-aminobenzoic acid moiety [33] as antibacterial and antifungal agents (**I** and **II**, Fig. 1). Many investigations presented DNA gyrase enzyme as a pivotal antibacterial target. The enzyme is essential in overseeing the topological state of DNA during transcription and replication processes [34]. DNA gyrase inhibition results in the disruption of DNA synthesis and leads eventually to bacterial cell death. Astrazeneca arylaminotriazine (**III**, Fig. 1) has been identified as a promising DNA gyrase inhibitor with profound antibacterial activity [35].

2. Rationale and design

Taking into consideration the abovementioned data and in continuation to our dedicated program towards synthesis of new *s*-triazines

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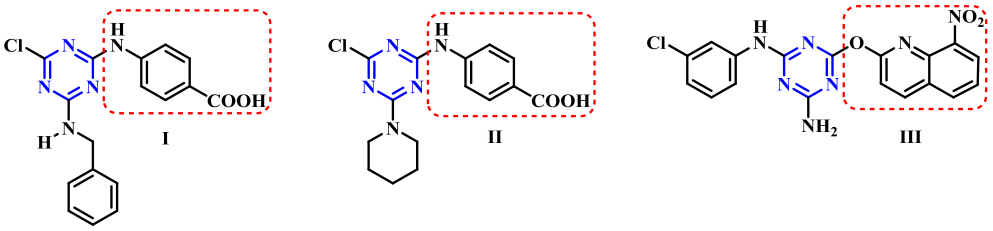


Fig. 1. Structures of reported antibacterial *s*-triazines (compounds **I** and **II**) and gyrase inhibitor Astrazeneca arylaminotriazine **III**.

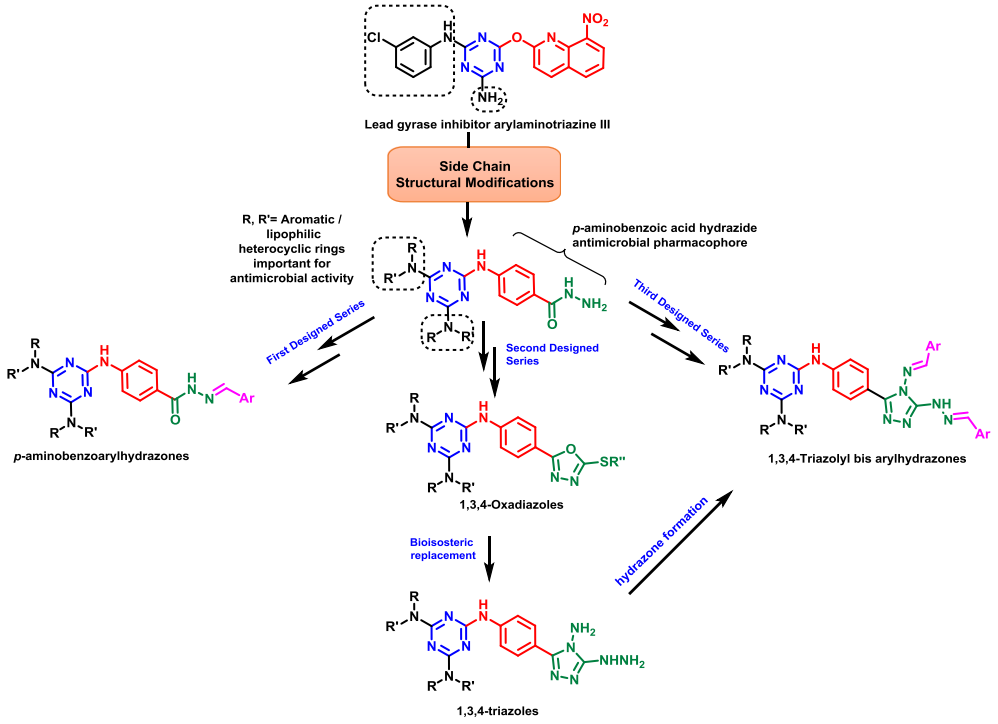


Fig. 2. The different design strategies for obtaining the target compounds based on the lead gyrase inhibitor arylaminotriazine **III.**

[32,33], we aimed at designing new *s*-triazine derivatives based on the important structural features of DNA gyrase B inhibitor arylaminotriazine **III**. The suggested candidates are designed to possess hydrophobic phenyl rings and/ or lipophilic amine moieties linked to the triazine core at the 4- and 6-positions. Such moieties are known to be essential for antimicrobial activity [33]. The triazine 2-position is planned to incorporate 2-aminobenzohydrazide linker which, first, was designed to be in the form of aryl hydrazones that are known to be found in different antimicrobial agents [36]. The second strategy involved cyclization of the benzohydrazide into 1,3,4-oxadiazole ring, followed by isosteric replacement into amino hydrazinyl-1,3,4-triazole. These heterocyclic rings are found in many antimicrobial derivatives [35,37]. Finally, design of different triazolyl bis aryl hydrazones was suggested to investigate their impact on the anticipated antimicrobial activity (Fig. 2). Synthesis of the target compounds were carried out according to outlined synthetic schemes and the derivatives were evaluated for their antibacterial and antifungal activities.

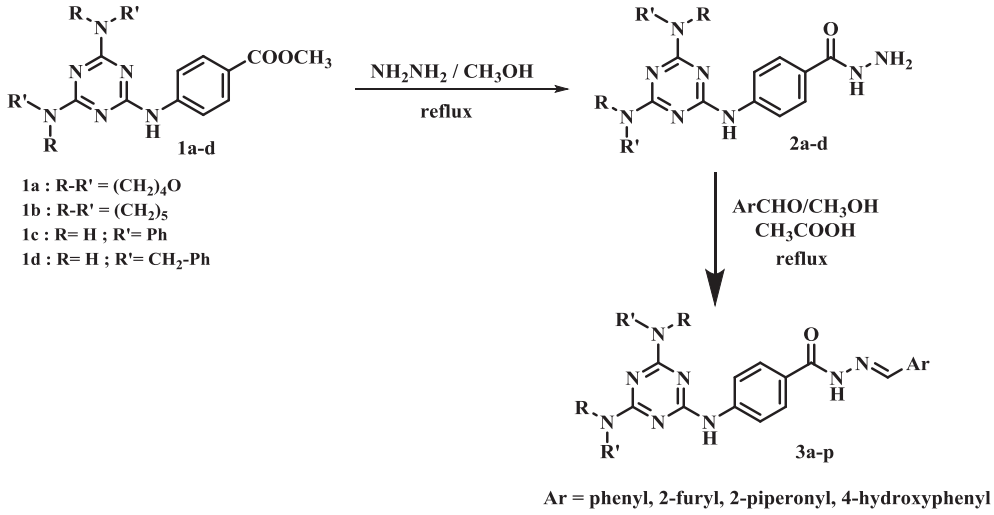
3. Results and discussion

3.1. Chemistry

Synthesis of the new derivatives are outlined in Schemes 1, 2 and 3. Structures of the newly synthesized compounds were determined by spectroscopic methods (IR, ^{1}H NMR and ^{13}C NMR) and elemental analyses. Scheme 1 describes the reaction between 2-aminobenzamido methyl esters **1a-d** [33] and hydrazine hydrate in methanol to yield the corresponding hydrazides **2a-d**. IR spectra of **2a-d** revealed presence of absorption bands due to $NHNH_2$ at 3427–3276 cm^{-1}. While, the absorption bands of carbonyl group appeared at 1620–1604 cm^{-1}. ^{1}H NMR spectra of **2a-d** showed two D_2O exchangeable singlets at δ 9.35–9.63 and 4.40–4.51 ppm corresponding to $NHNH_2$ protons, emphasizing the complete hydrazinolysis of the starting ester. Subsequent condensation of **2a-d** with different aromatic aldehydes in a mixture of methanol containing catalytic amount of glacial acetic acid afforded the corresponding hydrazones **3a-p**.

As observed from ^{1}H NMR spectra of **3a-p**, some hydrazones appeared as *syn* and *anti* isomers (Fig. 3). Taking ^{1}H NMR spectrum of **3c** as example, it showed that the ratio between the two isomers is nearly 5:1. The two isomers differ in the chemical shift and integration of N—H and methine (CH=N) proton. The eight methylene protons of morpholine moieties appeared as multiplet at δ 3.60–3.67 ppm, while the methylene protons of piperonal appeared as singlet at δ 6.06 ppm. Furthermore, the aromatic protons were observed as doublet at δ 6.95, singlet at δ 7.27 and multiplet at δ 7.12–7.13 and 7.80–7.88 ppm. The methine proton appeared as two singlets at δ 8.32 and 8.34 ppm, while the hydrazono NH proton appeared as two D_2O exchangeable singlets at δ 9.45 and 9.61 ppm. In addition, two D_2O exchangeable singlets were observed at δ 11.59 and 11.62 ppm corresponding to aminobenzoyl NH proton.

Since formation of **3c** involves two geometric isomers as shown in Fig. 3. Therefore, it was of interest to model the compounds using molecular mechanics MM2 calculations. In addition, quantum chemical calculations were carried out with the GAUSSIAN 98 suite. Geometry



Scheme 1. Synthetic pathway for the preparation of the target compounds **2a-d** and **3a-3p.**

optimizations were carried out using the DFT level (B3LYP/6-31G) theory to assess the relative stability of the diastereomeric species. The calculated relative energies of **3c** *syn* and *anti* conformers were 56.7272 kcal/mol and 56.7468 kcal/mol, respectively. The computed energies indicated that the *syn* isomer is 0.0196 kcal/mol more stable than the *anti* counterpart (Fig. 4).

Scheme 2 outlines the cyclization of the hydrazides **2b-d** to the corresponding oxadiazole thiol derivatives **4b-d** using carbon disulfide and aqueous KOH. IR spectra of **4b-d** showed absorption bands due of N—H and SH at 3398–3288 cm^{-1} and 2924–2852 cm^{-1} respectively. ^{1}H NMR spectra of **4b-d** lacked the signals due to $NHNH_2$ and instead revealed presence of D_2O exchangeable singlet at δ 2.94–3.12 ppm corresponding to SH proton. Signals attributed to other aliphatic and aromatic protons appeared at their expected chemical shifts. Further methylation of the oxadiazole thiols **4b-d** with methyl iodide in alcoholic NaOH solution efficiently afforded the corresponding methylsulfanyloxadiazoles **5b-d**. IR spectra of **5b-d** lacked the SH absorption band but showed only absorption bands attributed to N—H at 3402–3379 cm^{-1}. ^{1}H NMR spectra **5b-d** lacked the signals due to SH protons while displayed signals at δ 2.79–3.33 ppm corresponding to SCH_3 protons. Heating **5b-d** with excess hydrazine hydrate in methanol afforded the corresponding aminohydrazinyltriazole derivatives **6b-d**. IR spectra of **6b-d** showed absorption bands for NH at 3412–3340 cm^{-1}. ^{1}H NMR spectra of **6b-d** lacked the signals of the methyl protons and instead displayed D_2O exchangeable singlets at δ 4.39–4.46 and 6.98–7.82 ppm corresponding to NH_2 protons.

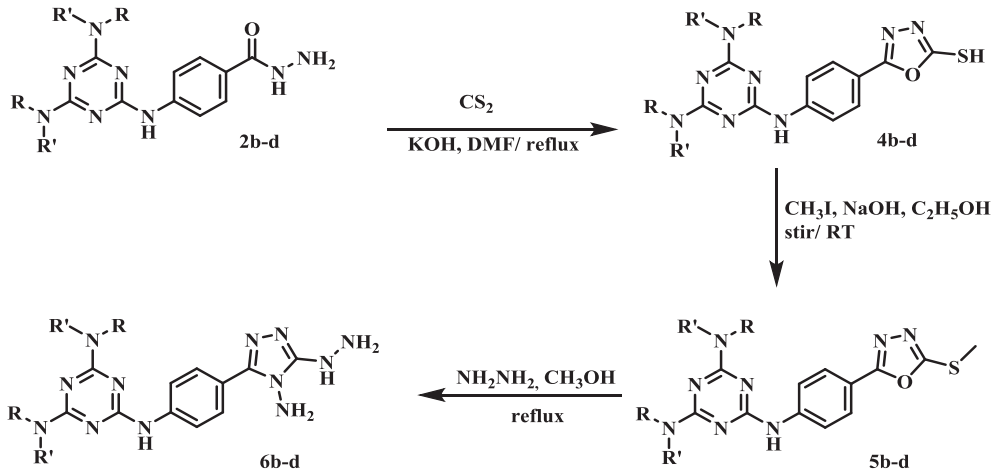
The target hydrazones **7a-f** were obtained by reaction of the amino hydrazinyl triazoles **6b-d** with different aromatic aldehydes in a refluxing mixture of methanol containing catalytic amount of glacial acetic acid (Scheme 3). IR spectra of **7a-f** showed absorption bands due to NH at 3428–3395 cm^{-1}. ^{1}H NMR of **7a-f** lacked the signals attributed to NH_2 and in turn revealed singlets at δ 8.27–8.84 ppm corresponding to methine protons.

3.2. Biological evaluation

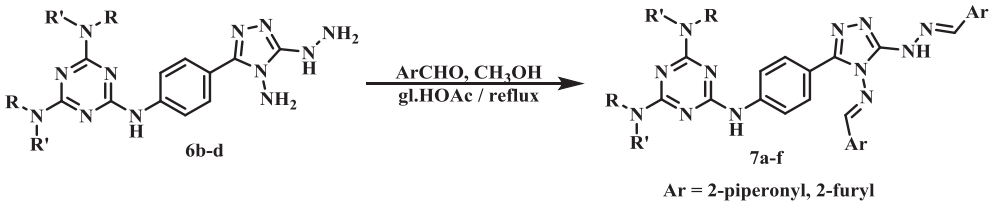
3.2.1. In vitro antimicrobial activity

All synthesized compounds were screened for their *in vitro* antimicrobial activity against *Staphylococcus aureus* (*S. aureus* ATCC 19433) as example of Gram-positive bacteria, *Escherichia coli* (*E. coli* ATCC 25922) as example of Gram-negative bacteria and *Candida albicans* (*C. albicans*) as yeast-like fungus. Individual minimum inhibitory concentration (MIC, μg/mL) values of the screened compounds against the test microbes are listed in Table 1 along with MIC values of reference Ampicillin (for bacteria) and Clotrimazole (for yeast-like fungus).

In general, all compounds exhibited promising antibacterial activity against the bacterial strains tested and weaker antifungal activity in comparison to the reference drugs. Compounds **3j**, **5b**, **6b** and **6c** showed two-fold higher antibacterial activity against *E. coli* than ampicillin with MIC values 12.5 μg/mL. While compounds **3e**, **4b**, **4c**, and



Scheme 2. Synthetic pathway for the preparation of the target compounds **4b-d**, **5b-d** and **6d-6d.**



Scheme 3. Synthetic pathway for the preparation of the target compounds **7a-7f.**

7c showed comparable antibacterial activity to that of ampicillin. Whereas, compounds **2a**, **2c**, **3f**, **3n**, **3p**, **4d**, **5c**, **5d**, **6d**, **7a** and **7d-f** displayed two-fold weaker antibacterial activity than ampicillin against *E. coli*. Compounds **3j**, **4b** and **6b** showed two-fold higher antibacterial activity against *S. aureus* than ampicillin with MIC values 6.25 μg/mL. While compounds **3g**, **4c** and **5b** showed comparable antibacterial activity against *S. aureus* to that of ampicillin. Compounds **3k**, **4d** and **6c, 6d** and **7a** exhibited two-fold weaker antibacterial activity than ampicillin against *S. aureus*. Other compounds displayed weaker antibacterial activities in comparison to the reference drugs. Compounds **3e**, **3g**, **3j**, **4b, 4c**, **5b**, **6b, 6c** and **7c**, which showed promising inhibitory activities, were further screened against multi-drug resistant (MDR) clinical isolates of *Klebsiella pneumonia* (*K. pnuemoniae*) and methicillin-resistant *Staphylococcus aureus* (*MRSA1*) (Table 2). Compounds **3j 5b**, **6b,** and **6c** emerged with MIC value 12.5 μg/mL against MDR clinical isolate of *K. pnuemoniae*. While, compounds **3j** and **6b** emerged with the lowest MIC value 3.125 μg/mL against MDR clinical isolate of *MRSA1*.

3.2.2. In vitro cytotoxicity

The cytotoxicity of the most active compounds **3e**, **3g**, **3j**, **4b**, **4c**, **5b**, **6b**, **6c** and **7c** were tested in a VERO cell line using the Mosmann method with modifications, as described in the literature [38]. The cells were incubated for 72 h with a range of dilutions of the selected compounds, using MTT as a reagent for the detection of cytotoxicity. The 50% cytotoxic concentration (CC50) value together with the selectivity index (SI) for each active compound was calculated (Table 3). Results revealed that these compounds had greater antimicrobial selectivity against K. pneumoniae and MRSA1 compared to mammalian cells, indicating that the test compounds have good safety profile.

3.3. Structure-activity relationship (SAR)

On the basis of SAR studies and from the obtained biological results, the acid hydrazides **2a-d** did not exhibit potential antibacterial activities. Whereas, it was observed that acyl hydrazone **3j** with *N*-phenyl substituent on *s*-triazine and furyl hydrazone possessed the highest antibacterial activity against all tested strains as well as MDR clinical isolates of *K. pneumoniae* and *MRSA1*. Whereas, replacing the phenyl ring with piperidine moiety as in **3g** and **3e** which also bear the bulky piperonal and phenyl hydrazone moieties, respectively, displayed comparable antibacterial activities against some bacterial strains with respect to ampicillin. Cyclization of the acid hydrazides **2b-d** into oxadiazoles **4b-d** and **5b-d** led to **4b** and **5b**, bearing the lipophilic piperidine moieties, with two-fold higher potency against *S. aureus* and *E. coli*, respectively. However, other derivatives possessing the more hydrophobic phenyl substituent retained some comparable antibacterial activities with respect to ampicillin. Bioisosteric replacement of oxadiazole with aminohydrazinyl-1,3,4-triazole ring resulted in improved antibacterial activities represented in **6b** and **6c**, with **6b** (also bearing the piperidine moiety) showing the lowest MIC values against all bacterial and MDR strains in reference to ampicillin. However, the amino triazolyl bis hydrazones **7a-f** were not as potent as expected to be, with compound **7c** exhibiting moderate antibacterial activities against *E. coli* and *K. pneumoniae* MDR clinical isolates. This decrease in potency might be partly attributed to bulkiness which might have hindered its penetration through the bacterial cell membrane and/or resulted in a steric clash with the bacterial enzyme binding pocket. It is worth mentioning that all derivatives bearing the benzyl substituent displayed poor antibacterial activities. It could be concluded from this preliminary SAR study that for obtaining better antibacterial potency, compounds should bear the lipophilic piperidine or hydrophobic phenyl moieties on the *s*-triazine ring. On the other hand, less steric furyl hydrazone as in **3j** or an amino hydrazinyl triazole as in **6b** provides a positive influence on antimicrobial activity.

3.4. In silico calculation of molecular properties

It is well documented that both molecular weight (Mwt) and octanol-water partition coefficient (*c*Log *P*) parameters greatly influence membrane penetration and permeability of molecules. Molecules with molecular weights < 500 Da are easily transported, diffused and absorbed compared to high Mwt compounds [40]. Compounds having *c*Log *P* in the range 1–5 often cross the cellular membranes efficiently [41]. In the present study, both **3j** and **6b** displayed the highest antibacterial activities among the synthesized compounds. Employing the SwissADME suite [42] for *in silico* calculations of Mwt and *c*Log *P*, it is believed that the promising antibacterial activity of both derivatives is due to their optimal molecular weights (490.52 and 450.54 respectively) and *c*Log *P* values (4.20 and 1.69 respectively), which facilitated their penetration in the lipophilic bacterial membrane. On the other hand, **7c** (as example of the triazolyl bis hydrazone series) was expected to display better antibacterial activities due to presence of bis hydrazone moieties in comparison to the furyl hydrazone **3j**. However, the poor observed activity of **7c** is thought to be due to its bulkiness (Mwt = 730.73) and poor lipophilicity (*c*Log *P* = 5.85), which probably hindered its penetration inside the bacterial cell membrane.

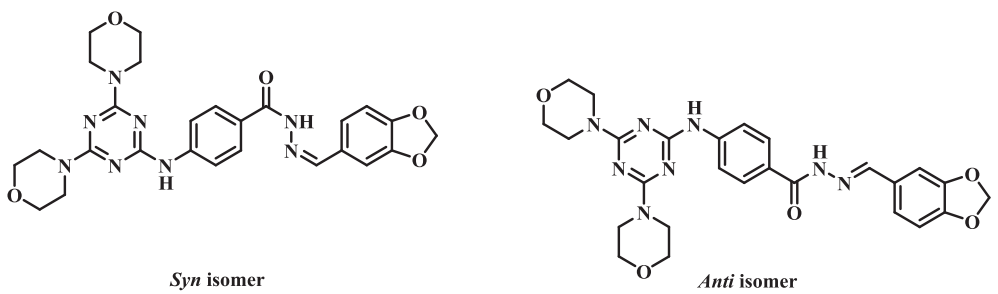


Fig. 3. The expected *syn* and *anti* isomers of **3c.**

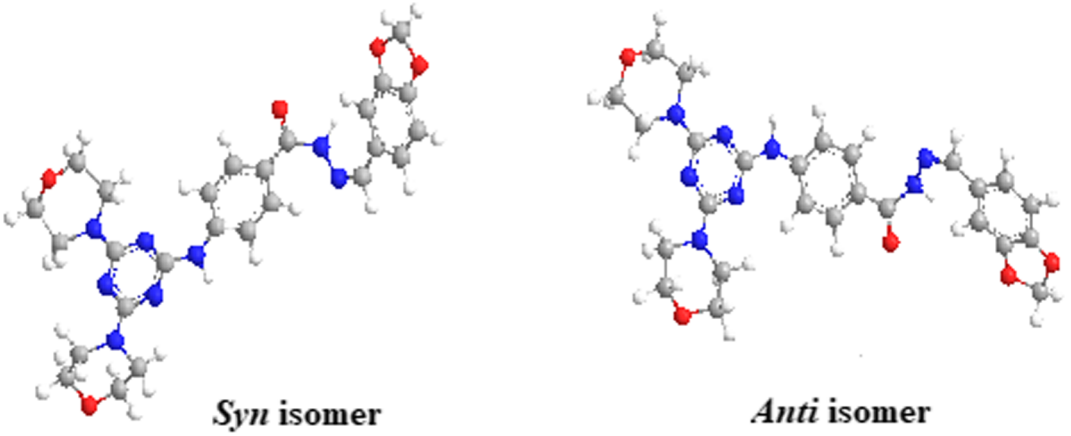


Fig. 4. The 3D structure of the *syn* and *anti* isomers of **3c**.

Table 1
Antibacterial and antifungal screening in terms of MIC (μg/mL) for the tested compounds in reference to Ampicillin and Clotrimazole, respectively.

Compound	R	R'	Ar	MIC (μg/mL)		
				E. coli	*S. aureus*	*C. albicans*
2a	$(CH_2)_4O$			50	100	100
2b	$(CH_2)_5$			100	100	50
2c	H	Ph	–	50	50	50
2d	H	CH_2-Ph	–	100	100	50
3a	$(CH_2)_4O$		Ph	100	100	> 200
3b	$(CH_2)_4O$		2-furyl	100	100	100
3c	$(CH_2)_4O$		2-piperonyl	> 200	> 200	50
3d	$(CH_2)_4O$		4-OH-Ph	> 200	> 200	50
3e	$(CH_2)_5$		Ph	**25**	100	> 200
3f	$(CH_2)_5$		2-furyl	50	100	50
3g	$(CH_2)_5$		2-piperonyl	100	12.5	50
3h	$(CH_2)_5$		4-OH-Ph	100	100	50
3i	H	Ph	Ph	100	100	> 200
3j	H	Ph	2-furyl	**12.5**	6.25	50
3k	H	Ph	2-piperonyl	> 200	25	100
3l	H	Ph	4-OH-Ph	100	100	50
3m	H	CH_2-Ph	Ph	100	100	100
3n	H	CH_2-Ph	2-furyl	50	> 200	100
3o	H	CH_2-Ph	2-piperonyl	> 200	> 200	25
3p	H	CH_2-Ph	4-OH-Ph	50	50	100
4b	$(CH_2)_5$		–	**25**	6.25	100
4c	H	Ph	–	**25**	12.5	100
4d	H	CH_2-Ph	–	50	25	100
5b	$(CH_2)_5$		–	**12.5**	12.5	> 200
5c	H	Ph	–	50	100	100
5d	H	CH_2-Ph	–	50	100	> 200
6b	$(CH_2)_5$		–	**12.5**	6.25	> 200
6c	H	Ph	–	**12.5**	25	> 200
6d	H	CH_2-Ph	–	50	25	> 200
7a	$(CH_2)_5$		2-piperonyl	50	25	> 200
7b	H	Ph	2-furyl	100	100	> 200
7c	H	CH_2-Ph	2-piperonyl	**25**	100	> 200
7d	$(CH_2)_5$		2-furyl	50	100	> 200
7e	H	Ph	2-piperonyl	50	100	> 200
7f	H	CH_2-Ph	2-furyl	50	100	> 200
Ampicillin				25	12.5	–
Clotrimazole				–	–	12.5

The MIC values of the most active compounds are written in Bold.

3.5. Molecular docking study

It was of interest to elucidate the mechanism by which the two active compounds **3j** and **6b** persuaded their antibacterial activities. Since the compounds were designed based on the gyrase B inhibitor arylaminotriazine **III**, the co-crystallized structure of DNA gyrase B of *E. coli* (PDB code: 4DUH) was downloaded from the protein data bank [43] coupled with its ligand inhibitor and used as reference for the essential docking interactions. Docking was performed using Molecular Operating Environment (MOE) software version 2016.0802, Chemical Computing Group, Montreal, Canada [44], to evaluate the 2D and 3D binding modes of the selected derivatives in the enzyme active site. Enzyme preparation and docking protocol were carried out according to a previously reported method [45]. The 2D ligand interactions of the co-crystallized inhibitor (Fig. 5a) showed ionic interactions with amino acid residues Arg 76 and Arg 136. It also displayed hydrogen bond interactions with Gly 101 and Arg 136, in addition to other hydrophobic interactions. For comparison, docking of the gyrase B inhibitor arylaminotriazine **III** displayed free energy of binding ΔG −8.4542 kcal/mol and showed hydrogen bonding interactions with amino acid residues Gly 101 and His 99 in addition to π-H interaction with Asn 46 (Fig. 5b). Docking of **3j** and **6b** displayed ΔG −9.3159 and −9.6475 kcal/mol, respectively, better than arylaminotriazine **III** which suggests that the test compounds are embedded deeply in the enzyme active site. Compound **3j** exhibited hydrogen bond donor interaction between *N* at 2-position of triazine ring and Ala 100, hydrogen bond acceptor interaction between oxygen of the benzohydrazone and His 99 in addition to π-H interaction of *N*-phenyl ring with Lys 103 (Fig. 6). Whereas **6b** displayed mainly hydrogen bond acceptor interactions mainly between the two triazole *N* and Arg 136, besides π-H interaction between the phenyl ring and Pro 79 (Fig. 7). As a comparative study to relate binding mode to potency, docking of the moderately active derivative **7c** was considered. Although the estimated ΔG is −11.9675 kcal/mol, the 2D ligand interaction revealed only one hydrogen bond acceptor between the imine *N* and amino acid residue Lys 103 in addition to π-H interaction of the triazole ring also with Lys 103 (Fig. 8). It is worth mentioning that upon investigating the 3D interaction of **7c** with the enzyme active site it was obvious that the *s*-triazine core completely extended outside the binding pocket which would greatly be the cause of its weak interactions and decreased potency.

4. Conclusion

The present study describes the design and synthesis of thirty-five new s-triazine derivatives as promising antimicrobial agents based on DNA gyrase inhibitor Astrazeneca arylaminotriazine **III**. Among the synthesized derivatives, nine compounds (**3e**, **3g**, **3j**, **4b**, **4c**, **5b**, **6b**, **6c**, **7c**) showed promising antibacterial activities against tested E. *coli* and S. *aureus* bacterial strains more potent than Ampicillin as reference. Further antibacterial screening of these active compounds against MDR clinical isolates of *K. pneumoniae* and *MRSA1* revealed compounds **3j** and **6b** as potent antibacterial agents with minimal toxicity. SAR study pointed out the importance of the *s*-triazine ring substituted with hydrophobic phenyl rings or lipophilic piperidine moieties to the

Table 2
Minimum inhibitory concentrations (MIC, μg/mL) of the most active compounds on MDR clinical isolates of *K. pneumoniae* and *MRSA1*.

MIC (μg/mL) for MDR clinical isolates		
Compound	*K. pneumoniae*	*MRSA1*
3e	50	–
3g	–	25
3j	**12.5**	**3.125**
4b	25	12.5
4c	50	12.5
5b	**12.5**	25
6b	**12.5**	**3.125**
6c	**12.5**	25
7c	50	–
Ampicillin	–	–

The MIC values of the most active compounds are written in Bold.

Table 3
CC_{50} values of the most active compounds against normal VERO cells and their selectivity indices.

		K. pneumoniae		*MRSA1*	
Compound	CC_{50}[a]	MIC[b]	SI[c]	MIC	SI
3e	250	50	5	–	–
3g	250	–	–	25	10
3j	125	12.5	10	**3.125**	40
4b	125	25	5	12.5	10
4c	500	50	10	12.5	40
5b	250	12.5	20	25	10
6b	62.5	12.5	5	**3.125**	20
6c	125	12.5	10	25	5
7c	250	50	5	–	–

The MIC values of the most active compounds are written in Bold.
[a] CC_{50} is the concentration at which 50% of cells survive.
[b] MIC (μg/mL) is the minimum concentration that inhibits bacterial cell growth.
[c] SI is the selectivity index regarding antimicrobial activity against *K. pneumoniae* and *MRSA1*; SI = log [CC_{50}/MIC]. Positive value represents more selectivity against microorganism strains than Vero cells, and negative values indicate higher toxicity to Vero cells and low selectivity to the bacteria [39].

antibacterial potency. In addition, substitution with less steric hydrazone or amino hydrazinyl triazole moieties are essential for optimal antibacterial activities. *In silico* calculation of Mwt and *c*Log *P* values emphasized their impact on the increased antibacterial activities of **3j** and **6b**. Furthermore, docking study of these two derivatives in order to discern the possible mechanism for their antibacterial potency revealed strong binding interactions in DNA gyrase active site. Thus, both **3j** and **6b** could be considered as potential leads for further optimization and development in future work.

5. Experimental section

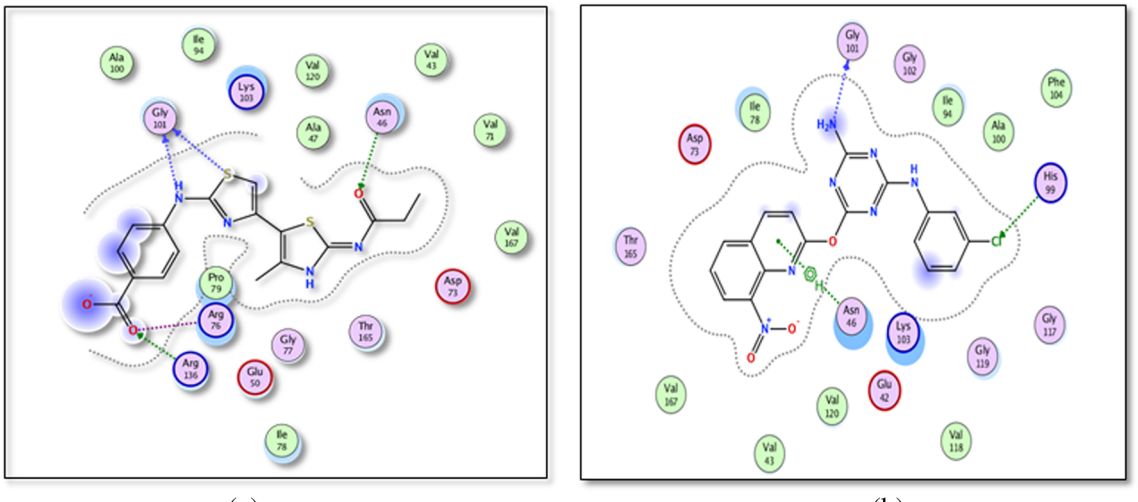
5.1. Materials and methods

All reagents were purchased from commercial suppliers and the solvents used were of HPLC grade. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument using KBr cell and measured by ύ cm^{-1} scale. Nuclear Magnetic resonance spectra (^{1}H NMR and ^{13}C NMR spectra) were recorded on 500 MHz JEOL and 400 MHz BRUKER spectrometers. Chemical shifts were reported in δ values (ppm) relative to trimethylsilane (TMS) as an internal standard and are referenced relative to residual solvent (e.g. $CHCl_3$ at δ H 7.26 ppm for $CDCl_3$ and DMSO at δ H 2.50 ppm for DMSO-d_6). Splitting patterns were designated as follows: s: singlet; br.s: broad singlet; d: doublet; m: multiplet and the coupling constants (*J*) in hertz. Elemental analyses were performed on Perkin-Elmer 2400 elemental analyzer, and the values found were within ± 0.3% of the theoretical values. Follow-up of the reactions and checking the purity of the compounds were done by TLC on silica gel coated aluminum sheets (Type 60 GF254, Merck) and the spots were detected by exposure to UV-lamp at λ. 254 nm for few seconds.

5.1.1. General procedure for the preparation of 4-[(4,6-disubstituted-1,3,5-triazin-2-yl)amino]benzohydrazide (2a-d)

To a solution of the appropriate ester **1a-d** (2 mmol) in methanol (20 mL), hydrazine hydrate (2 mL) was added. The reaction mixture was heated under reflux for 6 h. The precipitated hydrazide products were filtered off and washed with methanol and recrystallized from ethanol.

*5.1.1.1. 4-[(4,6-Dimorpholino-1,3,5-triazin-2-yl)amino]benzohydrazide (**2a**).* White powder. Yield: 0.75 g (94%); mp 218–220 °C. IR (KBr, $ύ_{max}$



(a) (b)

Fig. 5. The 2D binding mode of (**a**) the co-crystallized ligand; (**b**) arylaminotriazine **III** in the binding site of *E. coli* DNA gyrase B (PDB code: 4DUH). Hydrogen bond donor is indicated by blue dotted arrow and hydrogen bond acceptor is indicated by green dotted arrow.

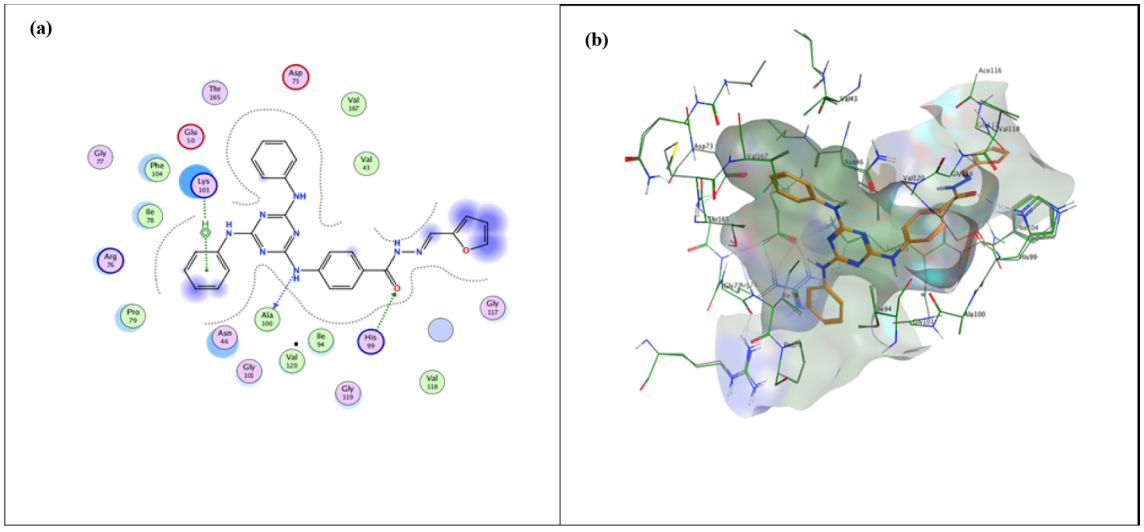


Fig. 6. (**a**) The 2D binding mode and (**b**) the 3D binding mode of **3j** in the active site of *E. coli* DNA gyrase B (PDB code: 4DUH).

cm^{-1}): 3410, 3311 (NH), 1608 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.60–3.74 (m, 16H, 8CH_2), 4.40 (brs, 2H, NH_2, D_2O exchangeable), 7.70–7.74 (m, 4H, Ar—H), 9.35 (s, 1H, NH, D_2O exchangeable.), 9.53 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.75 (4 C—N), 66.42 (4 C—O), 119.39, 119.62, 128.1 (2 C), 132.11, 148.86, 163.03, 164.43, 165.09. Anal. Calcd. for $C_{18}H_{24}N_8O_3$: C, 53.99; H, 6.04; N, 27.98. Found: C, 52.78; H, 5.93; N, 28.11.

5.1.1.2. 4-[(4,6-Di(piperidine eridin-1-yl)-1,3,5-triazin-2-yl)amino] benzohydrazide ***(2b)***. White powder. Yield: 0.7 g (88%); mp 190–192 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3296 (NH), 1620 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.47–1.58 (m, 12H, 6 CH_2, piperidine), 3.34–3.74 (m, 8H, 4 CH_2, piperidine), 4.43 (br.s, 2H, NH_2, D_2O exchangeable), 7.70–7.74 (m, 4H, Ar—H), 9.43 (s, 1H, NH, D_2O exchangeable), 9.51–9.59 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.55, 25.72, 44.25, 118.99, 119.39, 125.59, 128.03, 148.64, 163.06, 164.85, 165.97. Anal. Calcd. for $C_{20}H_{28}N_8O$: C, 60.59; H, 7.12; N, 28.26. Found: C, 60.23; H, 7.01; N, 28.50.

5.1.1.3. 4-[(4,6-Bis(phenylamino)-1,3,5-triazin-2-yl)amino] benzohydrazide ***(2c)***. White powder. Yield: 0.7 g (85.4%); mp 180–182 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3289 (NH), 1604 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 4.45 (br.s, 2H, NH_2, D_2O exchangeable), 6.97–7.00 (m, 2H, Ar—H), 7.27–7.30 (m, 4H, Ar—H), 7.72–7.96 (m, 8H, Ar—H), 9.31–9.47 (m, 3H, NH, D_2O exchangeable), 9.63 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 119.51, 121.06, 122.70, 126.75, 127.89, 128.83, 132.82, 140.23, 143.20, 164.5, 164.61, 166.22. Anal. Calcd. for $C_{22}H_{20}N_8O$: C, 64.07; H, 4.89; N, 27.17. Found: C, 64.92; H, 4.97; N, 27.02.

5.1.1.4. 4-[(4,6-Bis(benzylamino)-1,3,5-triazin-2-yl)amino] benzohydrazide ***(2d)***. White powder. Yield: 0.75 g (85.2%); mp 216–218 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3427, 3276 (NH), 1613 (C═O, amide).^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 4.47–4.51 (m, 6H, 2CH_2 & NH_2, D_2O exchangeable), 7.19–7.31 (m, 8H, Ar—H & NH, D_2O exchangeable), 7.69–7.84 (m, 8H, Ar—H& NH, D_2O exchangeable), 9.60–9.63 (m, 2H, 2NH, D_2O exchangeable.). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.92, 118.85, 126.92, 127.74, 128.57, 141.0, 148.26, 164.09, 164.66, 166.43. Anal. Calcd. for $C_{24}H_{24}N_8O$: C, 65.44; H, 5.49; N, 25.44. Found: C, 65.89; H, 5.86; N, 25.51.

5.1.2. General procedure for the preparation of the target compounds ***(3a-3p)***

The appropriate hydrazide **2a-d** (1 mmol) and aromatic aldehyde (1 mmol) in a mixture of methanol (60 mL) and acetic acid (2 mL) was refluxed for 8 h. Excess solvent was removed under vacuum. The

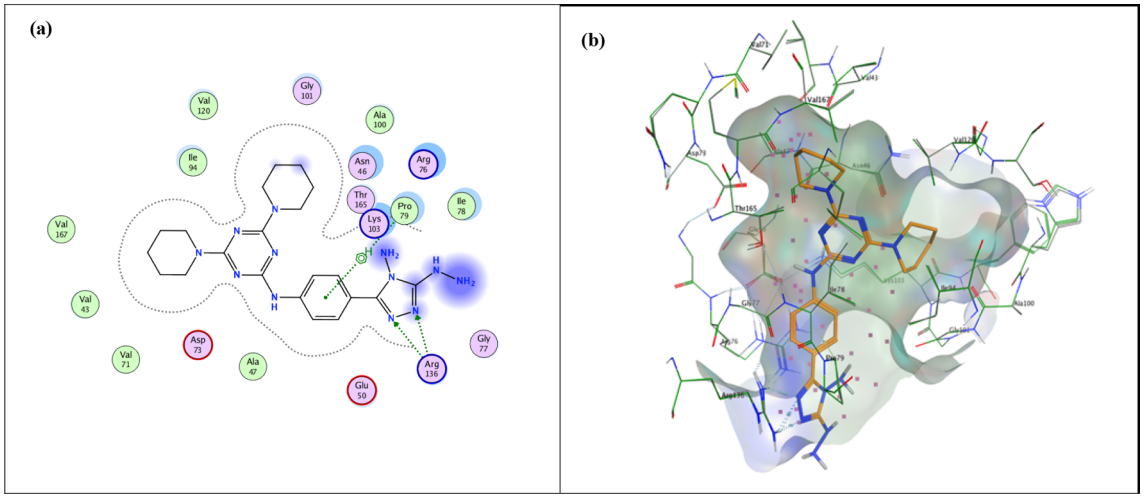


Fig. 7. (**a**) The 2D binding mode and (**b**) the 3D binding mode of **6b** in the active site of *E. coli* DNA gyrase B (PDB code: 4DUH).

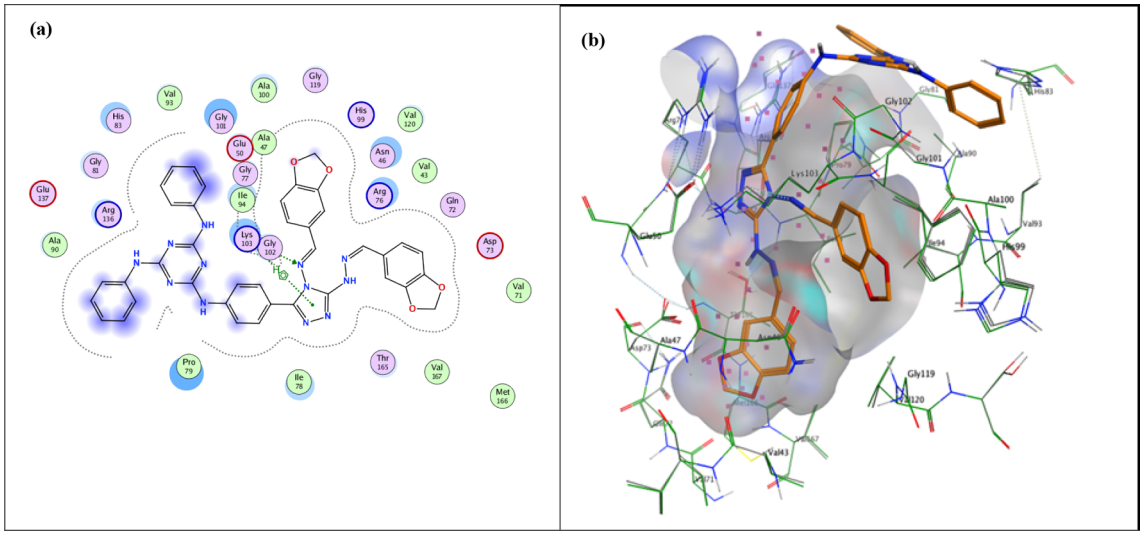


Fig. 8. (**a**) The 2D binding mode and (**b**) the 3D binding mode of **7c** in the active site of *E. coli* DNA gyrase B (PDB code: 4DUH).

remaining residue was diluted with ice water and the precipitated product was filtered off and washed with water to afford the pure products.

5.1.2.1. N'-Benzylidene-4-[(4,6-dimorpholino-1,3,5-triazin-2-yl)amino] Benzohydrazide ***(3a)***. White powder. Yield: 0.41 g (83.7%); mp 286–288 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3218 (NH), 1646 (C=O, amide). ^{1}H NMR (500 MHz, DMSO-d_6) **(Isomer A: 80%):** δ 3.60–3.69 (m, 16H, 8 CH_2), 7.42–7.43 (m, 3H, Ar–H), 7.69–7.89 (m, 6H, Ar–H), 8.42 (s, 1H, CH=N), 9.46 (s, 1H, NH, D_2O exchangeable), 11.69 (s, 1H, NH, D_2O exchangeable). **(Isomer B: 20%)** δ 3.60–3.69 (m, 16H, 8 CH_2), 7.42–7.43 (m, 3H, Ar–H), 7.69–7.89 (m, 6H, Ar–H), 8.44 (s, 1H, CH=N), 9.62 (s, 1H, NH, D_2O exchangeable), 11.69 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.74, 66.44, 119.20, 127.73, 128.89, 129.32, 130.86, 134.30, 144.09, 148.85, 164.10, 164.41, 165.05. Anal. Calcd. for $C_{25}H_{28}N_8O_3$: C, 61.46; H, 5.78; N, 22.94. Found: C, 61.95; H, 5.17; N, 22.83.

5.1.2.2. 4-[(4,6-Dimorpholino-1,3,5-triazin-2-yl)amino]-N'-(2-furylmethylidene)benzohydrazide ***(3b)***. Yellow powder. Yield: 0.38 g (79%); mp 204–206 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3410 (NH), 1609 (C=O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**: (Isomer A: 71%)** δ 3.61–3.68 (m, 16H, 8 CH_2), 6.61 (s, 1H, Ar–H), 6.89 (s, 1H, Ar–H), 7.71–7.88 (m, 5H, Ar–H), 8.30 (s, 1H, CH=N), 9.45 (s, 1H, NH, D_2O exchangeable), 11.63 (s, 1H, NH, exchangeable), **(Isomer B: 29%)** δ 3.61–3.68 (m, 16H, 8 CH_2), 6.61 (s, 1H, Ar–H), 6.89 (s, 1H, Ar–H), 7.71–7.88 (m, 5H, Ar–H), 8.32 (s, 1H, CH=N), 9.62 (s, 1H, NH, D_2O exchangeable), 11.68 (s, 1H, NH, exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.82, 66.44, 112.75, 113.32, 113.82, 119.23, 119.65, 128.91, 132.25, 144.06, 148.82, 163.80, 164.48, 165.17. Anal. Calcd. for $C_{23}H_{26}N_8O_4$: C, 57.73; H, 5.48; N, 23.42. Found: C, 57.38; H, 5.03; N, 23.65.

5.1.2.3. 4-[(4,6-Dimorpholino-1,3,5-triazin-2-yl)amino]-N'-[(2H-1,3-benzodioxol-5-yl)methylidene)benzohydrazide ***(3c)***. White powder. Yield: 0.41 g (77%); mp 254–256 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3369 (NH), 1656 (C=O, amide). ^{1}H NMR (500 MHz,DMSO-d_6)**: (Isomer A: 83%)** δ 3.60–3.67 (m, 16H, 8 CH_2), 6.06 (s, 2H, piperonal CH_2), 6.95 (d, 1H, *J* = 7.6 Hz, Ar–H), 7.12–7.13 (m, 1H, Ar–H), 7.27 (br.s, 1H, Ar–H), 7.80–7.88 (m, 4H, Ar–H), 8.32 (s, 1H, CH=N), 9.45 (s, 1H, NH, D_2O exchangeable.), 11.59 (d, 1H, NH, D_2O exchangeable). **(Isomer B: 17%)** δ 3.60–3.67 (m, 16H, 8 CH_2), 6.06 (s, 2H, piperonal CH_2), 6.95 (d, 1H, *J* = 7.6 Hz, Ar–H), 7.12–7.13 (m, 1H, Ar–H), 7.27 (br.s, 1H, Ar–H), 7.80–7.88 (m, 4H, Ar–H), 8.34 (s, 1H, CH=N), 9.61 (s, 1H, NH, D_2O exchangeable.), 11.62 (d, 1H, NH, D_2O exchangeable.). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.88, 66.47, 101.96, 105.59, 108.89, 118.96, 119.39, 123.67, 126.20, 126.67, 128.74, 129.38, 143.78, 144.12, 147.44, 148.44, 149.43, 163.14, 164.49, 165.20. Anal. Calcd. for $C_{28}H_{28}N_8O_5$: C, 60.42; H, 5.07; N, 20.13. Found: C, 59.02; H, 5.52; N, 20.01.

5.1.2.4. 4-((4,6-Dimorpholino-1,3,5-triazin-2-yl)amino)-N'-(4-hydroxybenzylidene) benzohydrazide ***(3d)***. Brown powder. Yield: 0.41 g (82%); mp 250–252 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3349 (OH), 3255 (NH), 1647 (C=O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 3.60–3.69 (m, 16H, 8 CH_2), 6.79 (d, 1H, *J* = 7 Hz, Ar–H), 7.07 (d, 1H, *J* = 7 Hz, Ar–H), 7.16–7.32 (m, 2H, Ar–H), 7.80–7.84 (m, 4H, Ar–H), 8.31 (s, 1H, CH=N), 9.46, 9.64 (2 s, each 1H, 2NH, D_2O exchangeable), 11.64 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.93, 66.47, 113.27, 119.02, 119.12, 119.43, 126.24, 126.71, 128.84, 130.27, 136.25, 143.80, 144.14, 147.76, 158.10, 163.30, 164.53, 165.27. Anal. Calcd. for $C_{25}H_{28}N_8O_4$: C, 59.51; H, 5.59; N, 22.21. Found: C, 59.99; H, 5.98; N, 22.30.

5.1.2.5. N'-Benzylidene-4-[(4,6-di(piperidin-1-yl)-1,3,5-triazin-2-yl) Amino]benzohydrazide ***(3e)***. White powder. Yield: 0.38 g (79%); mp 282–284 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3233 (NH), 1650 (C=O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.51–1.60 (m, 12H, 6 CH_2), 3.34–3.74 (m, 8H, 4 CH_2), 7.42–7.43 (m, 3H, Ar–H), 7.69–7.85 (m, 7H, Ar–H & NH, D_2O exchangeable), 8.42 (s, 1H, CH=N), 11.73 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.33, 25.75, 45.10, 79.53, 119.77, 127.53, 129.31, 130.49, 134.85, 148.10. Anal. Calcd. for $C_{27}H_{32}N_8O$: C, 66.92; H, 6.66; N, 23.12. Found: C, 66.12; H, 6.11; N, 23.92.

5.1.2.6. 4-[(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)amino]-N'-[(2-furyl) methylidene] benzohydrazide ***(3f)***. Yellow powder. Yield: 0.39 g (83%); mp 200–202 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3418 (NH), 1651 (C=O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**: (Isomer A: 70%)** δ 1.47–1.59 (m, 12H, 6 CH_2, piperidine), 3.69–3.77 (m, 8H, 4 CH_2, piperidine), 6.61 (s, 1H, Ar–H), 6.88 (s, 1H, Ar–H), 7.81–7.89 (m, 5H, Ar–H), 8.29 (s, 1H, CH), 9.31 (s, 1H, NH, D_2O exchangeable), 11.62 (s, 1H, NH, D_2O exchangeable), **(Isomer B: 30%)** δ 1.47–1.59 (m, 12H, 6 CH_2), 3.69–3.77 (m, 8H, 4CH_2), 6.61 (s, 1H, Ar–H), 6.88 (s, 1H, Ar–H), 7.81–7.89 (m, 5H, Ar–H), 8.32 (s, 1H, CH), 9.56 (s, 1H, NH, D_2O exchangeable), 11.67 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR ((125 MHz, DMSO-d_6): δ 24.57, 25.79, 44.21, 56.83, 114.56, 119.40, 119.44, 128.84, 145.61, 149.50, 164.71, 164.74. Anal. Calcd. for $C_{25}H_{30}N_8O_2$: C, 63.27; H, 6.37; N, 23.61. Found: C, 63.01; H, 6.09; N,

23.84.

*5.1.2.7. 4-[(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)amino]- N'-[(2H-1,3-benzodioxol-5-yl)methylidene)benzohydrazide (**3g**)*. White powder. Yield: 0.40 g (75.7%); mp 230–232 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3313 (NH), 1711 (C═O, amide). ^{1}H NMR (500 MHz,DMSO-d_6)**:** δ 1.47–1.59 (m, 12H, 6 CH_2), 3.69–3.77 (m, 8H, 4 CH_2), 6.06 (s, 2H, CH_2 piperonal), 6.96 (d, 1H, J = 6.85 Hz, Ar−H), 7.11–7.13 (m, 1H, Ar−H), 7.27 (s, 1H, Ar−H), 7.72–7.90 (m, 4H, Ar−H), 8.32 (s, 1H, CH═N), 9.29, 11.57 (2 s, each 1H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.83, 25.85, 44.16, 101.97, 105.57, 108.91, 118.63, 120.43, 123.57, 125.88, 127.93, 128.69, 129.44, 144.49, 147.28, 148.45, 149.41, 163.04, 164.61, 164.94. Anal. Calcd. for $C_{30}H_{32}N_8O_3$: C, 65.20; H, 5.84; N, 20.28. Found: C, 65.01; H, 5.65; N, 20.87.

*5.1.2.8. 4-[(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)amino]-N'-(4 hydroxybenzylidene)benzohydrazide (**3h**)*. White powder. Yield: 0.41 g (82%); mp 212–214 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3600–3200 (OH), 3279 (NH), 1647 (C═O, amide) cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.51–1.63 (m, 12H, 6 CH_2, piperidine), 3.73–3.82 (m, 8H, 4 CH_2, piperidine, 6.82–6.84 (m, 1H, Ar−H), 7.10–7.28 (m, 3H, Ar−H), 7.76–7.93 (m, 5H, Ar−H & NH, D_2O exchangeable), 8.37 (s, 1H, CH═N), 9.64 (s, 1H, NH, D_2O exchangeable), 11.64–11.75 (m, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 24.79, 25.82, 44.22, 114.21, 118.33, 118.91, 119.16, 121.21, 125.91, 126.84, 128.86, 131.27, 136.98, 145.50, 148.98, 159.12, 164.66, 165.03. Anal. Calcd for $C_{27}H_{32}N_8O_2$: C, 64.78; H, 6.44; N, 22.38. Found: C, 64.69; H, 6.35; N, 22.29.

*5.1.2.9. N'-Benzylidene-4-[(4,6-bis(phenylamino)-1,3,5-triazin-2-yl) amino]benzohydrazide (**3i**)*. White powder. Yield: 0.36 g (72%); mp is > 300 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3399 (NH), 1647 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 7.22–7.40 (m, 5H, Ar−H), 7.70–7.97 (m, 14H, Ar−H), 8.16 (s, 1H, CH═N), 9.62 (br.s, 3H, 3NH, D_2O exchangeable), 11.72–11.74 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 119.89, 121.12, 122.21, 125.36, 127.54, 127.89, 129.29, 129.47, 134.24, 141.28, 144.16, 148.22, 164.29, 164.43, 166.41. Anal. Calcd for $C_{29}H_{24}N_8O$: C, 69.59; H, 4.83; N, 22.39. Found: C, 69.76; H, 5.00; N, 22.11.

*5.1.2.10. 4-[(4,6-Bis(phenylamino)-1,3,5-triazin-2-yl)amino]-N'-(2-furylmethylidene) benzohydrazide (**3j**)*. Brown powder. Yield: 0.37 g (75.5%); mp is 210–212 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3283 (NH), 1646 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 6.62 (s, 1H, Ar−H), 6.90 (s, 1H, Ar−H), 6.98–7.00 (m, 1H, Ar−H), 7.17–7.29 (m, 3H, Ar−H), 7.78–8.07 (m, 11*H*, Ar−H), 8.33 (s, 1H CH═N), 9.50–9.84 (m, 3H, 3NH, D_2O exchangeable), 11.68 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 112.65, 113.61, 119.47, 120.23, 121.09, 122.79, 128.86, 140.18, 145.49, 150.07, 164.5, 164.62. Anal. Calcd. for $C_{27}H_{22}N_8O_2$: C, 66.11; H, 4.52; N, 22.84. Found: C, 66.01; H, 4.42; N, 22.97.

*5.1.2.11. 4-[(4,6-Bis(phenylamino)-1,3,5-triazin-2-yl)amino]-N'-[(2H-1,3-benzodioxol-5-yl)methylidene)benzohydrazide (**3k**)*. Brown powder. Yield: 0.43 g (79.0%); mp is > 300 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3294 (NH), 1712 (C═O, amide). ^{1}H NMR (400 MHz: DMSO-d_6)**:** δ 6.10 (s, 2H, CH_2), 6.95–7.04 (m, 6H, Ar−H), 7.33–7.80 (m, 12H, 11 Ar−H & CH═N), 9.6 (s, 3H, 3 NH, D_2O exchangeable), 11.52 (s, 1H, 1 NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 101.16, 106.24, 108.92, 115.98, 117.71, 118.93, 120.22, 120.45, 120.56, 121.21, 121.47, 128.80, 128.88, 129.08, 129.27, 143.63, 147.73, 148.50, 148.50, 148.95, 163.81, 163.86, 165.94. Anal. Calcd for $C_{32}H_{24}N_8O_3$: C, 67.60; H, 4.25; N, 19.71. Found: C, 67.55; H, 4.20; N, 19.96.

*5.1.2.12. 4-[(4,6-Bis(phenylamino)-1,3,5-triazin-2-yl)amino]-N'-(4-hydroxybenzylidene) benzohydrazide (**3l**)*. Grey powder. Yield: 0.40 g (78%); mp is 182–184 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3500–3215 (OH), 3385 (NH), 1576 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 6.82 (s, 1H, Ar−H), 7.00–7.09 (m, 3H, Ar−H), 7.20–7.29 (m, 6H, Ar−H), 7.78–7.98 (m, 8H, Ar−H), 8.36 (s, 1H, CH═N), 9.35 (s, 1H, NH, D_2O exchangeable), 9.58–9.65 (m, 3H, 3NH, D_2O exchangeable), 11.70 (s, 1H, OH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 113.34, 115.16, 117.85, 119.20, 119.56, 121.17, 122.33, 122.91, 126.64, 126.85, 128.87, 130.33, 130.75, 136.19, 140.06, 143.82, 148.08, 150.32, 158.05, 163.58, 164.55, 164.67. Anal. Calcd for $C_{29}H_{24}N_8O_2$: C, 67.43; H, 4.68; N, 21.69. Found: C, 67.58; H, 4.83; N, 21.84.

*5.1.2.13. N'-Benzylidene-4-[(4,6-bis(benzylamino)-1,3,5-triazin-2-yl) amino]benzohydrazide (**3m**)*. White powder. Yield: 0.39 g (73%); mp is 290–292 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3417 (NH), 1618 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** (**Isomer A: 72%**): δ 4.54–4.41 (m, 4H, 2 CH_2), 7.22–7.50 (m, 12H, Ar−H), 7.70–7.86 (m, 7H, Ar−H), 8.43 (s, 1H, CH═N), 9.2–9.61 (m, 3H, 3 NH, D_2O exchangeable), 10.70–11.71 (s, 1H, NH, D_2O exchangeable). **(Isomer B: 28%):** δ 4.41–4.54 (m, 4H, 2 CH_2), 7.22–7.50 (m, 12H, Ar−H), 7.70–7.86 (m, 7H, Ar−H), 8.47 (s, 1H, CH═N), 9.2–9.61 (m, 3H, 3 NH, D_2O exchangeable), 10.70 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.94, 119.13, 119.88, 125.15, 127.63, 128.71, 128.88, 129.26, 130.39, 144.33, 148.38, 164.64, 165.32, 166.02. Anal. Calcd for $C_{31}H_{28}N_8O$: C, 70.44; H, 5.34; N, 21.20. Found: C, 70.29; H, 5.19; N, 21.55.

*5.1.2.14. 4-[(4,6-Bis(benzylamino)-1,3,5-triazin-2-yl)amino]-N'-(2-furylmethylidene) benzohydrazide (**3n**)*. Yellow powder. Yield: 0.41 g (78.8%); mp is 206–208 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3283 (NH), 1643 (C═O, amide). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 4.41–4.53 (m, 4H, 2 CH_2,), 6.60 (s, 1H, Ar−H), 6.89 (s, 1H, Ar−H), 7.18–7.32 (m, 7H, Ar−H), 7.51–7.95 (m, 8H, Ar−H), 8.29 (s, 1H, CH═N), 9.21–9.98 (br.s, 3H, NH, D_2O exchangeable), 11.61–11.69 (m, 1H, 1 NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.91, 112.73, 114.11, 114.23, 119.03, 119.34, 120.28, 127.12, 127.52, 128.69, 128.94, 138.03, 140.61, 145.56, 149.81, 163.63, 164.49, 164.71. Anal. Calcd for $C_{29}H_{26}N_8O_2$: C, 67.17; H, 5.05; N, 21.61. Found: C, 67.29; H, 5.17; N, 21.13.

*5.1.2.15. 4-[(4,6-Bis(benzylamino)-1,3,5-triazin-2-yl)amino]-N'-[(2H-1,3-benzodioxol-5-yl)methylidene)benzohydrazide (**3o**)*. White powder. Yield: 0.47 g (82.1%); mp is 204–206 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3412 (NH), 1687 (C═O, amide). ^{1}H NMR (400 MHz: DMSO-d_6)**:** δ 4.53–4.61 (m, 4H, 2 CH_2), 6.11 (s, 2H, CH_2, piperonal), 7.01–7.03 (m, 2H, Ar−H), 7.15–7.17 (m, 11*H*, Ar−H), 7.78–7.85 (m, 4H, Ar−H), 8.37 (s, 1H, CH═N), 8.56 (s, 2H, 2 NH, D_2O exchangeable), 8.97–9.04 (m, 1H, NH, D_2O exchangeable), 11.37–11.41 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 44.02,101.93, 102.12, 105.73, 106.70, 108.98, 118.93, 123.35, 125.29, 126.94, 127.63, 128.57, 128.95, 129.61, 140.45, 148.48, 149.42, 150.48, 160.66, 164.71, 166.51 Anal. Calcd for $C_{34}H_{28}N_8O_3$: C, 68.44; H, 4.73; N, 18.78. Found: C, 68.38; H, 4.66; N, 18.71.

*5.1.2.16. 4-[(4,6-Bis(benzylamino)-1,3,5-triazin-2-yl)amino]-N'-(4-hydroxybenzylidene) benzohydrazide (**3p**)*. Brown powder. Yield: 0.42 g (77.7%); mp is 164–166 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3500–3200 (OH), 3409 (NH), 1647 (C═O, amide). ^{1}H NMR (500 MHz,DMSO-d_6)**:** δ 4.43–4.55 (m, 4H, 2 CH_2), 6.80 (s, 1H, Ar−H), 7.06–7.30 (m, 13H, Ar−H), 7.74–7.89 (m, 5H, Ar−H & NH, D_2O exchangeable), 8.34 (br.s, 1H, CH═N), 9.18–9.26 (m, 1H, NH, D_2O exchangeable), 9.60–9.69 (m, 2H, OH & NH, D_2O exchangeable), 11.59–11.71 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.89, 113.13, 117.73, 118.78, 119.13, 120.38, 126.98, 127.46, 128.63, 130.32, 136.24, 140.95, 148.11, 158.10, 161.75, 164.60, 166.33. Anal. Calcd. for $C_{31}H_{28}N_8O_2$: C, 68.37; H, 5.18; N, 20.58. Found: C, 68.24; H, 5.05; N, 20.45.

*5.1.3. General procedure for the preparation of 1,3,4-oxadiazole-2-thiol derivatives (**4b-d**)*

To a solution of the appropriate **2b-d** (2.5 mmol) in dimethylformamide (15 mL), carbon disulphide (0.15 mL, 2.5 mmol) and potassium hydroxide (0.14 g, 2.5 mmol, in 2 mL water) were added while stirring. The reaction mixture was then heated under reflux for 12 h. Excess solvent was removed under vacuum, and the remaining residue was diluted with ice water. The crude products obtained were filtered off, washed with water, dried, and recrystallized from ethanol.

*5.1.3.1. 5-[4-((4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)amino)phenyl]-1,3,4-oxadiazole-2-thiol (**4b**)*. White powder, Yield: 0.80 g (72.7%); mp 290–292 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}):3288 (NH), 2852 (SH), 1606 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.57–1.68 (m, 12H, 6 CH_2, piperidine), 3.12 (s, 1H, SH, D_2O exchangeable), 3.78–3.86 (m, 8H, 4 CH_2, piperidine), 7.80–7.97 (m, 4H, Ar−H), 9.23–9.49 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 25.81, 44.21, 52.06, 119.65, 122.15, 128.56, 129.17, 131.89, 145.84, 161.31, 164.94, 166.59. Anal. Calcd. for $C_{21}H_{26}N_8OS$: C, 57.51; H, 5.98; N, 25.55; O, 3.65. Found: C, 57.42; H, 5.76; N, 25.43.

*5.1.3.2. 5-(4-((4,6-Bis(phenylamino)-1,3,5-triazin-2-yl)amino)phenyl)-1,3,4-oxadiazole-2-thiol (**4c**)*. White powder. Yield: 0.85 g (77.2.5%); mp 160–162 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3398 (NH), 2924 (SH), 1604 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 2.94 (s, 1H, SH, D_2O exchangeable.), 6.97–7.01 (m, 2H, Ar−H), 7.27–7.50 (m, 4H, Ar−H), 7.63–8.10 (m, 9H, Ar−H & 1NH, D_2O exchangeable), 9.29–9.68 (m, 2H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 120.35, 121.16, 122.83, 127.04, 128.13, 128.84, 129.78, 131.96, 140.16, 164.62. Anal. Calcd. for $C_{23}H_{18}N_8OS$: C, 60.78; H, 3.99; N, 24.65. Found: C, 60.61; H, 3.80; N, 24.36.

*5.1.3.3. 5-(4-((4,6-Bis(benzylamino)-1,3,5-triazin-2-yl)amino)phenyl)-1,3,4-oxadiazole-2-thiol (**4d**)*. White powder. Yield: 0.88 g (85.4%); mp 138–140 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3398 (NH), 2924 (SH), 1609 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 3.01 (s, 1H, SH, D_2O exchangeable), 4.40–4.47 (m, 4H, 2CH_2), 7.17–7.29 (m, 10*H*, Ar−H), 7.63–8.00 (m, 6H, Ar−H & 2NH, D_2O exchangeable), 9.35–9.41 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.92, 118.57, 126.14, 126.92, 127.74, 128.63, 151.57, 159.63, 164.63, 164.42, 165.06. Anal. Calcd. for $C_{25}H_{22}N_8OS$: C, 62.22; H, 4.60; N, 23.22. Found: C, 62.02; H, 4.40; N, 23.01.

*5.1.4. General procedure for the preparation of target derivatives (**5b-d**)*

Sodium hydroxide (0.096 g, 2.4 mmol) was added while stirring at room temperature to a solution of the appropriate **4b-d** (1 mmol) in ethanol (20 mL). Methyl iodide was then added (0.22 mL, 3.5 mmol) over a period of 1 h and the reaction mixture was stirred overnight at room temperature. The precipitated solid was filtered off, washed with water and dried to afford the pure products.

*5.1.4.1. N-{4-[5-(Methylsulfanyl)-1,3,4-oxadiazol-2-yl]phenyl}-4,6-di(piperidin-1-yl)-1,3,5-triazin-2-amine (**5b**)*. White powder. Yield: 0.41 g (95.3%); mp 234–236 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3379 (NH), 1607 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.47–1.58 (m, 12H, 6 CH_2, piperidine), 3.23–3.33 (m, 3H, CH_3), 3.68 (s, 8H, 4 CH_2, piperidine), 7.81–7.87 (m, 4H, Ar−H), 9.40 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 15.95, 24.73, 24.81, 44.25, 118.82, 119.41, 126.26, 127.88, 128.49, 130.36, 164.71, 165.16, 168.14. Anal. Calcd. for $C_{22}H_{28}N_8OS$: C, 58.39; H, 6.24; N, 24.76. Found: C, 58.28; H, 6.13; N, 24.65.

*5.1.4.2. N^2-{4-[5-(Methylsulfanyl)-1,3,4-oxadiazol-2-yl]phenyl}-N^4,N^6-diphenyl-1,3,5-triazine-2,4,6-triamine (**5c**)*. White powder. Yield: 0.36 g (80%); mp 170–172 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}):3397 (NH), 1588 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 2.79 (s, 3H, CH_3), 7.02–7.06 (m, 2H, Ar−H), 7.30–7.34 (m, 3H, Ar−H), 7.71–7.81 (m, 7H, Ar−H) 8.07 (s, 2H, Ar−H), 9.10–9.15 (m, 2H, 2NH, D_2O exchangeable), 9.47 (s, 1H, 1NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.91, 116.63, 120.59, 121.33, 122.84, 127.28, 128.77, 140.19, 144.05, 164.63, 164.80, 165.73. Anal. Calcd. for $C_{24}H_{20}N_8OS$: C, 61.52; H, 4.30; N, 23.92. Found: C, 61.64; H, 4.42; N, 40.04.

*5.1.4.3. N^2,N^4-Dibenzyl-N^6-{4-[5-(methylsulfanyl)-1,3,4-oxadiazol-2-yl]phenyl}-1,3,5-triazine-2,4,6-triamine (**5d**)*. White powder, Yield: 0.40 g (87%); mp 254–256 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}):3402 (NH), 1572 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 2.99 (s, 3H, CH_3), 4.39–4.45 (m, 4H, 2CH_2), 7.18–7.27 (m, 12H, Ar−H), 7.66–7.90 (m, 4H, Ar−H & 2 NH, D_2O exchangeable), 9.12–9.22 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): δ 14.88, 39.67, 127.24, 127.43, 127.73, 128.02, 128.61, 128.86, 140.84, 164.53, 166.11. Anal. Calcd. for $C_{26}H_{24}N_8OS$: C, 62.89; H, 4.87; N, 22.56. Found: C, 62.61; H, 4.79; N, 22.38.

*5.1.5. General procedure for the preparation of target derivatives (**6b-d**)*

A solution of the appropriate **5b-d** (1 mmol) and hydrazine hydrate (3 mL) in absolute ethanol (5 mL) was heated under reflux for 6 h. The reaction mixture was then cooled to room temperature and the precipitated product was filtered off, washed with cold ethanol to afford the pure products.

*5.1.5.1. N-{4-[4-Amino-5-hydrazinyl-4H-1,2,4-triazol-3-yl]phenyl}-4,6-di(piperidin-1-yl)-1,3,5-triazin-2-amine (**6b**)*. White powder. Yield: 0.42 g (93.3%); mp 140–142 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3340 (NH), 1600 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 1.46–1.59 (m, 12H, 6 CH_2 piperidine), 3.68 (br.s, 8H, 4 CH_2 piperidine), 4.39 (s, 2H, NH_2, D_2O exchangeable), 7.69–7.71 (m, 6H, Ar -H & NH_2, D_2O exchangeable), 9.20 (s, 1H, NH, D_2O exchangeable), 9.52 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 24.75, 25.81, 44.19, 118.65, 118.78, 128.57, 130.53, 164.62, 164.97. Anal. Calcd. for $C_{21}H_{30}N_{12}$: C, 55.98; H, 6.71; N, 37.31. Found: C, 56.12; H, 6.85; N, 37.55.

*5.1.5.2. N^2-{4-[4-amino-5-hydrazinyl-4H-1,2,4-triazol-3-y])phenyl}-N^4,N^6-diphenyl-1,3,5-triazine-2,4,6-triamine (**6c**)*. White powder. Yield: 0.46 g (92.5%); mp 152–154 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}):3396 (NH), 1605 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 4.40 (br., 2H, NH_2, D_2O exchangeable), 6.98 (s, 2H, NH_2, D_2O exchangeable), 7.26–7.27 (m, 5H, Ar−H), 7.71–7.76 (m, 6H, Ar−H), 7.87–7.98 (m, 3H, Ar−H), 9.31 (br.s, 2H, 2NH, D_2O exchangeable), 9.47, 9.64 (2br.s, each 1H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 119.52, 121.07, 122.73, 127.94, 128.84, 140.21, 164.61. Anal. Calcd. for $C_{23}H_{22}N_{12}$: C, 59.22; H, 4.75; N, 36.03 Found: C, 59.10; H, 4.63; N, 35.91.

*5.1.5.3. N2-(4-(4-amino-5-hydrazinyl-4H-1,2,4-triazol-3-yl)phenyl)-N4,N6-dibenzyl-1,3,5-triazine-2,4,6-triamine (**6d**)*. White powder. Yield: 0.43 g (89.6%); mp 160–162 °C. IR (KBr, $\acute{\upsilon}_{max}$ cm^{-1}): 3412 (NH), 1611 (C=N). ^{1}H NMR (500 MHz, DMSO-d_6)**:** δ 4.40–4.46 (m, 6H, 2CH_2 & NH_2, D_2O exchangeable), 7.19–7.28 (m, 12H, Ar−H), 7.61–7.82 (m, 6H, Ar−H, 2NH & NH_2, D_2O exchangeable), 9.08–9.17 (m, 1H, NH, D_2O exchangeable), 9.55 (br.s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 43.02, 91.81, 123.30, 128.32, 129.10, 130.14, 132.07, 132.99, 134.66, 143.40, 161.46, 163.53, 163.81. Anal. Calcd. for $C_{25}H_{26}N_{12}$: C, 60.71; H, 5.30; N, 33.99. Found: C, 60.56; H, 5.15; N, 33.84.

*5.1.6. General procedure for the preparation of target derivatives (**7a-7f**)*

A solution of the appropriate **6a-f** (0.2 g, 0.44 mmol) and the aromatic aldehyde (0.44 mmol) in a mixture of methanol (20 mL) and acetic acid (2 mL) was heated under reflux for 10 h. Excess solvent was removed under vacuum and the crude product was then diluted with ice water. The precipitated solid was filtered off and washed with water

to afford the pure products.

*5.1.6.1. N-{4-[4-((2H-1,3-Benzodioxol-5-ylmethylidene)amino)-5-(2-(2H-1,3-benzodioxol-5-ylmethylidene)hydrazinyl)-4H-1,2,4-triazol-3-yl] phenyl}-4,6-di(piperidin-1-yl)-1,3,5-triazin-2-amine (**7a**)*. White powder. Yield: 0.25 g (79.6%); mp 204–206 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3422 (NH), 1499 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.47–1.58 (m, 12H, 6 CH_2, piperidine), 3.68 (s, 8H, 4 CH_2, piperidine), 6.08 (s, 4H, 2 CH_2, piperonal), 7.00–7.02 (m, 3H, Ar−H), 7.31–7.37 (m, 4H, Ar−H & NH, D_2O exchangeable), 7.70–7.80 (m, 4H, Ar−H), 8.56 (s, 2H, 2 CH═N) 9.30 (br.s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 24.83, 25.85, 44.17, 102.17, 106.53, 109.04, 118.63, 125.51, 128.82, 148.45, 150.50, 161.04, 164.94. Anal. Calcd. for $C_{37}H_{38}N_{12}O_4$: C, 62.17; H, 5.36; N, 23.52. Found: C, 62.33; H, 5.52; N, 23.78.

*5.1.6.2. N-{4-[4-((2-Furylmethylidene)amino)-5-(2-(furan-2-ylmethylene)hydrazinyl)-4H-1,2,4-triazol-3-yl]phenyl}-4,6-di(piperidin-1-yl)-1,3,5-triazin-2-amine (**7b**)*. White powder. Yield: 0.22 g (81.5%); mp 98–100 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3428 (NH), 1497 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.47–1.59 (m, 12H, 6 CH_2, piperidine), 3.91 (br.s, 8H, 4 CH_2, piperidine), 6.54–7.11 (m, 4H, Ar−H), 7.54–7.93 (m, 6H, Ar−H), 8.54 (s, 2H, 2 CH═N), 9.11–9.54 (m, 2H, 2 NH, D_2O exchangeable).^{13}C NMR (125 MHz, DMSO-d_6): 25.82, 44.17, 61.91, 74.54, 80.49, 114.33, 117.97, 1189.69, 121.41, 129.33, 133.34, 145.44, 149.36, 164.90. Anal. Calcd. for $C_{31}H_{34}N_{12}O_2$: C, 61.37; H, 5.65; N, 27.70. Found: C, 61.20; H, 5.48; N, 27.53.

*5.1.6.3. N^2-{4-[4-((2H-1,3-Benzodioxol-5-ylmethylidene)amino)-5-(2-(2H-1,3-benzodioxol-5-ylmethylidene)hydrazinyl)-4H-1,2,4-triazol-3-yl]phenyl}-N^4,N^6-diphenyl-1,3,5-triazine-2,4,6-triamine (**7c**)*. White powder. Yield: 0.19 g (95%); mp 237–239 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3395 (NH), 1495 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.06–6.08 (m, 4H, 2 CH_2, piperonal), 6.96–6.99 (m, 4H, Ar−H), 7.14–7.15 (m, 1H, Ar−H), 7.29–7.30 (m, 6H, Ar−H), 7.77–7.83 (m, 7H, Ar−H), 7.96 (br.s, 2H, Ar−H), 8.35, 8.56 (2 s, each 1H, 2 CH═N), 9.34 (s, 2H, 2 NH, D_2O exchangeable), 9.56, 11.63 (2 s, each 1H, 2NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 101.98, 102.16, 105.61, 106.53, 108.94, 109.04, 119.48, 121.08, 122.77, 123.63, 125.51, 126.71, 128.85, 129.40, 140.19, 147.61, 148.45, 149.45, 150.50, 161.04, 164.51, 164.62. Anal. Calcd. for $C_{39}H_{30}N_{12}O_4$: C, 64.10; H, 4.14; N, 23.00. Found: C, 63.89; H, 3.93; N, 22.79.

*5.1.6.4. N^2-{4-[4-((2-Furylmethylidene)amino)-5-(2-(2-furylmethylidene) hydrazinyl)-4H-1,2,4-triazol-3-yl]phenyl}-N^4,N^6-diphenyl-1,3,5-triazine-2,4,6-triamine (**7d**)*. White powder. Yield: 0.17 g (85%); mp 140–142 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3399 (NH), 1495 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.62 (s, 2H, Ar−H), 6.97–6.98 (m, 2H, Ar−H), 7.24–7.32 (m, 6H, Ar−H), 7.76–7.82 (m, 10*H*, Ar−H), 8.27–8.36 (m, 2H, 2 CH═N), 9.22–9.56 (m, 3H, 3NH, D_2O exchangeable), 11.61–11.69 (m, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 113.11, 117.50, 117.77, 119.46, 121.07, 122.81, 128.86, 140.14, 145.88, 147.05, 149.56, 150.86, 164.61. Anal. Calcd. for $C_{33}H_{26}N_{12}O_2$: C, 63.66; H, 4.21; N, 26.99. Found: C, 63.41; H, 4.06; N, 26.84.

*5.1.6.5. N^2-{4-[4-((2H-1,3-Benzodioxol-5-ylmethylidene)amino)-5-(2-(2H-1,3-benzodioxol-5-ylmethylidene)hydrazinyl)-4H-1,2,4-triazol-3-yl]phenyl}-N^4,N^6-dibenzyl-1,3,5-triazine-2,4,6-triamine (**7e**)*. White powder. Yield: 0.27 g (81.8%); mp 220–222 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3415 (NH), 1502 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.42–4.47 (m, 4H, 2CH_2), 6.06–6.08 (m, 4H, 2 CH_2, piperonal), 7.00–7.02 (m, 3H, Ar−H), 7.18–7.20 (m, 5H, Ar−H), 7.31–7.33 (m, 8H, Ar−H), 7.37 (s, 3H, Ar−H), 7.74–7.86 (m, 3H, Ar−H & 2 NH, D_2O exchangeable), 8.56 (s, 2H, 2 CH═N), 9.15–9.30 (m, 1H, NH, D_2O exchangeable), 11.68 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 40.33, 102.14, 106.53, 109.07, 118.83, 125.61, 127.02, 127.62, 128.13, 128.65, 148.41, 161.11. Anal. Calcd. for $C_{41}H_{34}N_{12}O_4$: C, 64.90; H, 4.52; N, 22.15. Found: C, 65.10; H, 4.72; N, 22.35.

*5.1.6.6. N^2,N^4-Dibenzyl-N^6-(4-(4-((2-furylmethylidene)amino)-5-(2-(2-furylmethylidene)hydrazinyl)-4H-1,2,4-triazol-3-yl)phenyl)-1,3,5-triazine-2,4,6-triamine (**7f**)*. White powder. Yield: 0.19 g (72.2%); mp 186–188 °C. IR (KBr, ύ$_{max}$ cm^{-1}): 3414 (NH), 1511 (C═N). ^{1}H NMR (500 MHz, DMSO-d_6): δ 4.37–4.47 (m, 4H, 2CH_2), 6.60 (s, 2H, Ar−H), 6.87 (s, 2H, Ar−H), 7.20–7.29 (m, 13H, Ar−H), 7.64–7.92 (m, 5H, Ar−H & 2NH, D_2O exchangeable), 8.32, 8.84 (2 s, each 1H, 2 CH═N), 9.12–9.28 (m, 1H, NH, D_2O exchangeable), 11.68 (s, 1H, NH, D_2O exchangeable). ^{13}C NMR (125 MHz, DMSO-d_6): 43.89, 118.76, 126.96, 127.46, 127.62, 128.11, 128.61, 129.10, 131.96, 132.18, 140.44, 140.99, 164.60, 166.41, 167.41. Anal. Calcd. for $C_{35}H_{30}N_{12}O_2$: C, 64.60; H, 4.65; N, 25.83. Found: C, 64.36; H, 4.41; N, 25.69.

5.2. In vitro antimicrobial evaluation

The *in vitro* antimicrobial screening was carried out according to Hawkey et al. [46] and Murray et al. [47], Supporting Information (S1).

5.3. Molecular docking

Molecular operating environment (MOE) software package version 2016.0802 was used to perform docking simulations, Supporting Information (S2).

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary material

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