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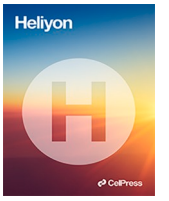

Research article

Design, synthesis and antibacterial activity of novel 7*H*-thiazolo [3,2-*b*]-1,2,4-triazin-7-one derivatives

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ABSTRACT

Based on the observed biological activity of 1,2,4-triazin-5-one derivatives and their cyclic analogues, a novel series of 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives that contain ester moiety compounds **3a-3g,** carboxylic acid moiety compounds **4a-4g** and piperazine amide moiety compounds **5a-5k** at position-3 of the thiazolotriazinone scaffold were synthesized. The intermolecular cyclization occurred regioselectively at N2-position of 1,2,4-triazine ring was characterized by X-ray single-crystal diffraction analysis. The *in vitro* biological activities of the target compounds were assayed against some bacterial strains. Compared with ciprofloxacin, compounds **3g** and **4g** exhibited more excellent antibacterial activity, especially the activity against *Staphylococcus aureus and Escherichia coli*, showing that the fluorine at the para position of the benzyl group would be the best choice. In addition, compounds **4e-4g** with carboxylic acid moiety can enhance the antibacterial activity. Compounds **5g-5k** containing bulky 1-(substituted phenyl) piperazine moiety were found with slightly less biological activity. Similar to ciprofloxacin, the docking result of target compounds with DNA topoisomerase II indicates the carboxyl group of the target compounds with carboxylic acid moiety has a crucial salt bridge interaction with Mg^{2+} in the protein.

1. Introduction

Antibacterial drugs are of key significance to the prevention and control of diseases. Antibacterial drugs can prevent disease infection and ensure the inhibitory effect on pathogens and their activity at a certain concentration [1–3]. However, due to the unreasonable use of antibiotics in countries around the world, the emergence of bacterial resistance to antibiotics has aggravated the global threat of infectious diseases [4,5]. In the past decades, bacterial DNA gyrase has attracted widespread attention as a selected target for finding effective antibacterial agents. Therefore, many synthetic quinolone antibacterial agents have been developed and are now widely used to treat bacterial infectious diseases. Quinolones could inhibit DNA gyrase and topoisomerase IV, and induce

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bacterial cell death [6]. With the widespread application of quinolones, their drug resistance is also increasing rapidly. At present, almost all pathogenic bacteria have appeared quinolone drug-resistant strains, and clinical drug resistance is very common, which has become a major problem faced by such antibacterial drugs [7]. Therefore, discovering antibacterial drugs with a new scaffold structure is still extremely important.

In recent years, some 1,2,4-triazine-5-one derivatives which displayed excellent antibacterial activity [8–10], antiviral [11] and antiproliferative effects [12] were found. For instance, thiadiazolotriazinones [13,14] (**A** and **B**, Fig. 1) and thiazolotriazinones [15] (**C** and **D**, Fig. 1) exhibited the activities against a broad spectrum of gram-negative and certain gram-positive bacteria. In addition, the 3-carboxylic acid moiety is considered the most critical structurally site for quinolone compounds (norfloxacin, ciprofloxacin and gatifloxacin, Fig. 1) acting on DNA gyrases because the carboxylic acid moiety can interact with the metal atom Mg^{2+} [16]. The esters derivatives of these carboxylic acids [17–21] and the piperazine derivatives with N-substituted moieties were alao shown excellent antimicrobial activities [22].

Based on the aforementioned facts, the shared scaffold of the compound **A-D** was taken as the lead scaffold. According to the principle of scaffold hopping, hybridization, the scaffold 4*H*-thiazolo[2,3-c]-1,2,4-triazin-4-one was replaced with 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one and the benzyl substituent on the 1,2,4-triazine ring was retained. On the other hand, in order to achieve better antibacterial activity, the benzene ring on the thiadizole ring or the thiazole ring in **A-D** was chosen to substitution with carboxymethyl to obtain **4a-4g**, and ethyl esters derivatives of these carboxylic acids (**3a-3g**) were simultaneously gained, and then the piperazine derivatives with N-substituted moieties (**5a-5k**) were acquired (Fig. 2). All of these designed compounds were synthesized and screened *in vitro* for their antibacterial activities.

2. Results and discussion

2.1. Synthesis

The synthesis of the intermediates and the target compounds was accomplished according to the steps depicted in Schemes 1. The starting material 6-substituted-3-mercapto-1,2,4-triazin-5-ones **1a-1g** were synthesized as previously described [23–25]. Condensation of equimolar amounts of **1a-1g** with ethyl 4-chloroacetoacetate in DMF in the presence of base afforded the corresponding *S*-alkylated derivatives **2a-2g**. Subsequently, compounds **2a-2g** underwent an intermolecular condensation to give compounds **3a-3g**. Then **3a-3g** underwent hydrolysis and subsequent acidification to give the compounds **4a-4e**. Finally, a series of amide derivatives **5a-5k** were obtained through the reaction of key intermediates **4a-4g** with corresponding 1-arylpiperazines.

Interestingly, compounds **2a-2g** are known to undergo the tautomeric change as a mixture of tautomer 5(2*H*)-one (**2A**) and 5(4*H*)-one (**2B**), mainly in the form of **2A** [26,27] (Scheme 2). One explanation for this is obvious that, in tautomer **2B**, there would be a considerable amount of electron-electron repulsion energy between the unshared electron pairs on *N*-1 and *N*-2. However, this repulsion is easy to relieve through the formation of the tautomer **2A** [28]. In tautomer **2A** the electron density is significantly higher on atom *N*-2. During the reaction, the compounds **2a-2g** are primarily available in form **2A**, and the ring closure occurred at the *N*-2 to form the [3,2-*b*] isomers, rather than the [2,3-*c*] isomers. The β-keto esters **2a**–**2g** were cyclized in polyphosphoric acid (PPA), which yielded the intermediates **3a**–**3g** by the intermolecular condensation [15] (Scheme 1).

2.2. Molecular structure

The direction of regioselective cyclization at N2 of the 1,2,4-triazine ring in compound **3a** was immediately verified based on the X-ray structural analysis data.

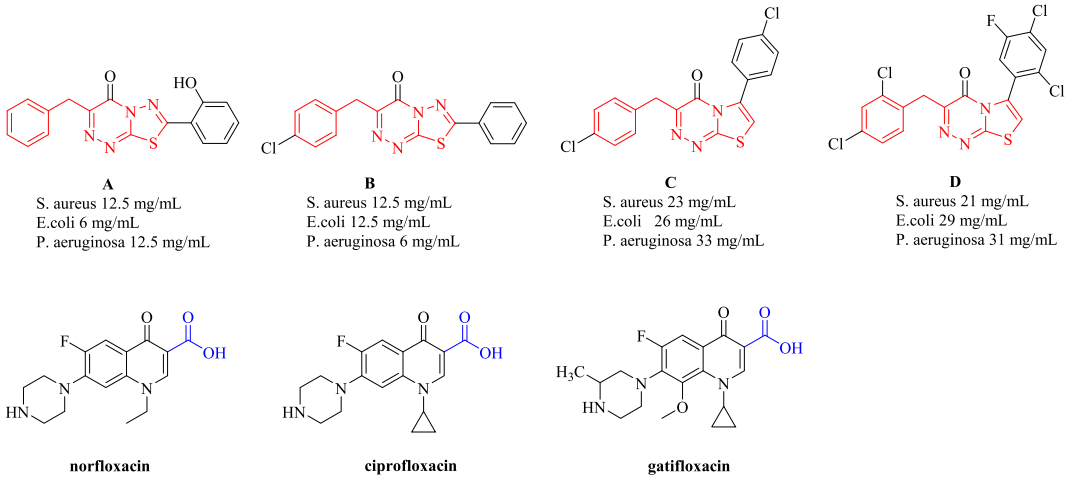


Fig. 1. Structure of reported antimicrobial inhibitors and quinolone antibacterial agents.

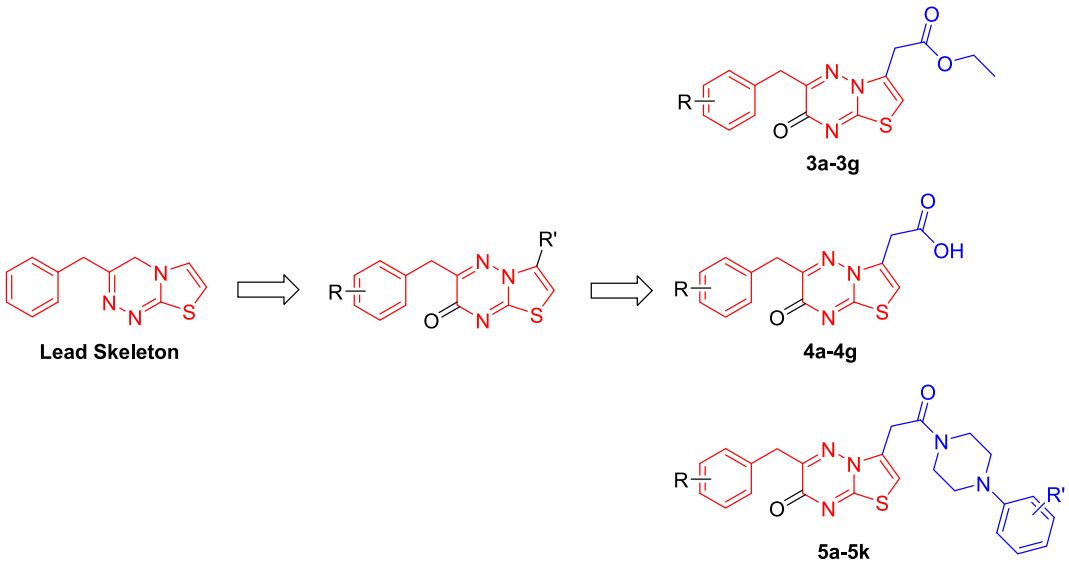
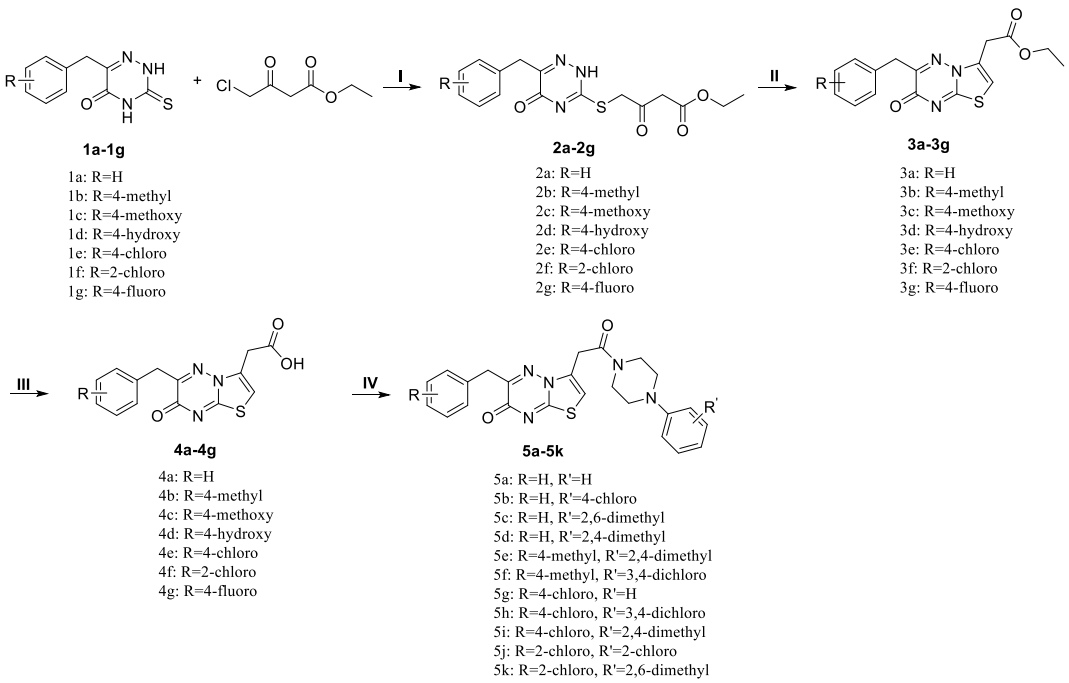


Fig. 2. The designed structures of the target compounds.

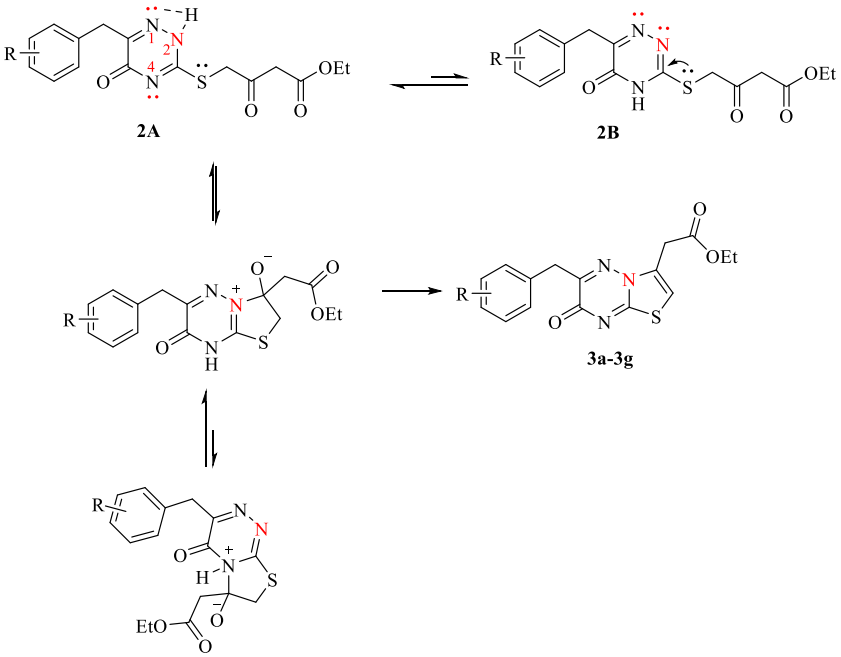


Scheme 1. Synthesis of the target compounds **3a**-**3g**, **4a**-**4g** and **5a**-**5k.** Reagents and Conditions: I: 10%NaOH, DMF, r.t.; II: 85 % PPA, 120 °C; III: 10%NaOH, CH_3OH, r.t.; IV: 1-(Substituted phenyl)piperazine, HOBt, triethylamine, EDCI, CH_2Cl_2.

The crystal structure of compound **3a** (0.22 mm × 0.20 mm × 0.18 mm) was confirmed by X-ray diffraction analysis. The structure was confirmed by direct methods and expanded by difference Fourier techniques with SHELXS-97 program [29]. All of the non-hydrogen atoms were located with successive difference Fourier syntheses. The hydrogen atoms were added according to theoretical models. The crystal and instrumental parameters used in the cell determination, the data collection, and structure refinement parameters were listed in Table 1 while selected bond distances and angles in Table 2.

The molecular structures and crystal packing pictures are drawn by Diamond and Mercury programs [30,31]. One structure unit of **3a** was shown in Fig. 3. The molecular packing for **3a** viewed along the *b* axis is depicted in Fig. 4.

There were some weak interactions (C–H···O, C–H···S and C–H···π interactions) observed in Fig. 5, with the red dashed lines indicating the interactions. The weak interactions are listed in Tables 2–4.



Scheme 2. A plausible mechanistic pathway of the intermidate **3**.

Table 1
Crystal data and structure refinement for compound 3a.

Compound	3a
Chemical formula	$C_{16}H_{15}O_3N_3S$
Formula weight	329.37
Temperature (K)	296 (2)
Wavelength (Å)	0.71073
Color	white
Crystal size (mm)	0.22 × 0.20 × 0.18
Crystal system	monoclinic
Space group	*C*2/c
a (Å)	20.247 (6)
b (Å)	8.818 (2)
c (Å)	19.078 (6)
α (°)	90.00
β (°)	111.936 (11)
γ (°)	90.00
Volume (Å^3)	3159.5 (16)
Z	8
Density (calculated, g·cm^{-3})	1.385
F (000)	1376
Absorption coefficient (mm^{-1})	0.223
□ q range for data collection (°)	2.17/25.00
Index ranges (h, k, l)	−24/23, −10/9, −22/22
Measured reflections	8153
Independent reflections	2653
R_{int}	0.0743
Completeness to $\theta = 25.00°$	99.9 %
Observed reflections [I > 2σ (I)]	1798
Data/restraints/parameters	2779/0/209
Goodness-of-fit on F^2	1.017
R indices (all data)	$R_1 = 0.0878$, w$R_2 = 0.1778$
R indices [I > 2σ (I)]	$R_1 = 0.0571$, w$R_2 = 0.1637$
Largest diffraction peak and hole (e·Å^{-3})	0.314 and −0.240

The dihedral angle between the thiazole ring and the triazine ring was 0.360(92) °, which meant that the two rings were coplanar. It was surprised to find that the dihedral angle between the thiazolo[3,2-*b*]-1,2,4-triazine parent scaffold and the benzene ring was 84.925(85) °, it meant that the two rings were nearly perpendicular to each other.

Table 2
Selected bond lengths (Å) and bond angles (°) for compound 3a.

Bond	Dist.	Bond	Dist.	Bond	Dist.
S(1)–C(11)	1.723(3)	N(1)–N(2)	1.362(3)	C(8)–C(9)	1.477(4)
S(1)–C(10)	1.725(3)	N(2)–C(10)	1.346(3)	C(11)–C(12)	1.334(4)
O(1)–C(9)	1.235(3)	N(2)–C(12)	1.401(3)	C(12)–C(13)	1.473(4)
O(2)–C(14)	1.192(4)	N(3)–C(10)	1.305(3)	C(13)–C(14)	1.493(4)
O(3)–C(14)	1.309(3)	N(3)–C(9)	1.370(3)	C(15)–C(16)	1.343(5)
O(3)–C(15)	1.463(4)	C(4)–C(7)	1.502(4)		
N(1)–C(8)	1.298(3)	C(7)–C(8)	1.499(4)		
Angle	(°)	Angle	(°)	Angle	(°)
C(11)-S(1)-C(10)	90.57(1)	N(1)-C(8)-C(9)	122.8(2)	C(11)-C(12)-N(2)	110.2(2)
C(14)-O(3)-C(15)	115.9(3)	N(1)-C(8)-C(7)	117.8(2)	C(11)-C(12)-C(13)	130.2(2)
C(8)-N(1)-N(2)	115.1(2)	C(9)-C(8)-C(7)	119.4(2)	N(2)-C(12)-C(13)	119.6(2)
C(10)-N(2)-N(1)	122.9(2)	O(1)-C(9)-N(3)	121.9(3)	C(12)-C(13)-C(14)	112.3(2)
C(10)-N(2)-C(12)	115.9(2)	O(1)-C(9)-C(8)	119.8(3)	O(2)-C(14)-O(3)	123.9(3)
N(1)-N(2)-C(12)	121.1(2)	N(3)-C(9)-C(8)	118.4(2)	O(2)-C(14)-C(13)	126.0(3)
C(10)-N(3)-C(9)	115.9(2)	N(3)-C(10)-N(2)	124.9(2)	O(3)-C(14)-C(13)	110.1(2)
C(3)-C(4)-C(7)	121.6(3)	N(3)-C(10)-S(1)	125.4(2)	C(16)-C(15)-O(3)	110.4(4)
C(5)-C(4)-C(7)	120.6(3)	N(2)-C(10)-S(1)	109.7(2)		
C(8)-C(7)-C(4)	114.5(2)	C(12)-C(11)-S(1)	113.6(2)		

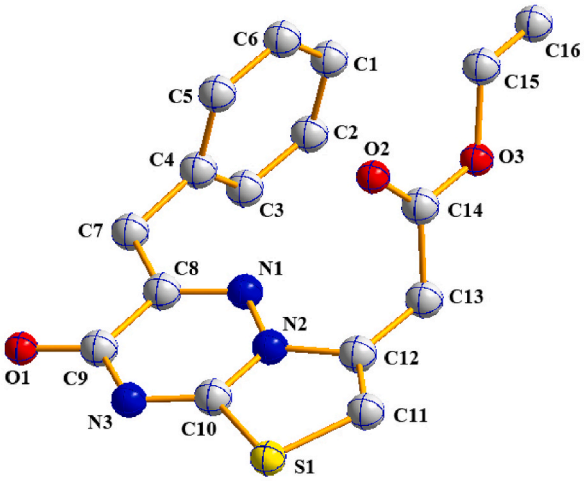


Fig. 3. Structure unit of compound **3a**, showing the atom numbering scheme.

2.3. Molecular properties

To be effective as an orally available drug, a potent molecule must possess suitable pharmacokinetic and physicochemical properties. A compound that fulfils at least three out of the key parameters of 'rule-of-five' is considered a suitable drug candidate [32]. The values of these properties for the new synthesized compounds were estimated using Discovery Studio 3.5. As shown in Table 5, only the molecular weight of compounds (**5f**, **5h-k**) is slightly higher than the desirable value, and other properties are consistent with the 'rule-of-five'.

2.4. Biological assays and structure-activity relationship analysis

Using a 96-well microtiter plate and a serial dilution method, the minimum inhibitory concentration (MIC) values of the target compounds were assayed against *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) *in vitro*. Table 6 shows the MIC values of the target compounds.

The antimicrobial activity of target compounds is given in Table 6. According to the analysis of the antibacterial activities of target compounds **3a-3g** and **4a-4g**, the distinct activities were due to various substituents on the benzyl group at 6-position of 7*H*-thiazolo [3,2-*b*]-1,2,4-triazin-7-one scaffold. In comparison, the derivatives **3e-3g** and **4e-4g** with electron-withdrawing substituents (e.g. F, Cl) on the benzyl group exhibited more potent antibacterial activity than those **3a-3d** and **4a-4d** with electron-donating substituents (e.g. CH_3, OH, OCH_3). Moreover, compounds with substituents at the para position of the benzyl group(**3e**, **4e**) exhibited a similar antibacterial activity to those at orthro position(**3f**, **4f**), suggesting that the location of the halogen substituent had less significantly

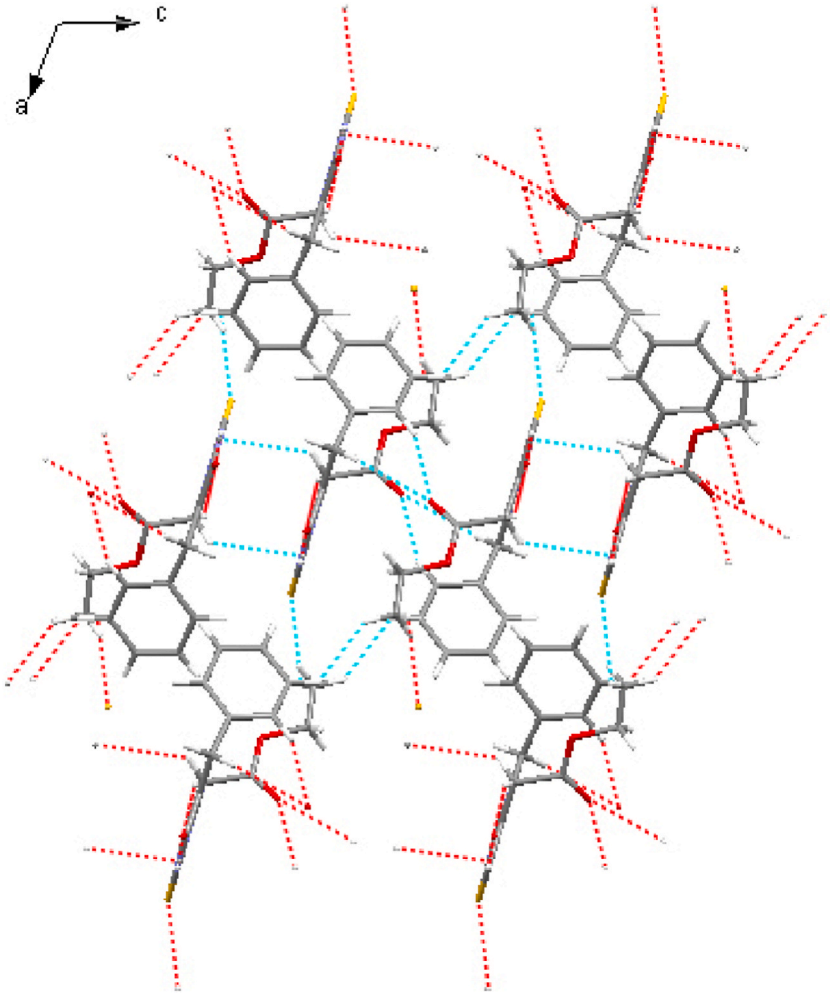


Fig. 4. Molecular packing for compound **3a**.

affected the antibacterial activity. Both **3g** and **4g** had obvious better activity than other tested compounds, showing that the fluorine at the para position of the benzyl group would be the best choice.

Compounds **4e-4g** displayed superior antibacterial activity against all tested bacterial strains compared with the corresponding **3e-3g**, which implied that the introduction of carboxylic acid moiety can enhance the antibacterial activity. Compared with **3e-3g** and **4e-4g**, their corresponding amide derivatives **5g-5k** were found with slightly less biological activity, probably due to bulky 1-(substituted phenyl)piperazine moiety. Furthermore, the activity was only slightly affected by substituents of phenyl ring of 1-(substituted phenyl) piperazine moiety.

In addition, the enzyme inhibitory activity against LeuRS from *M. smegmatis* has shown that the target compound **4e** decreases aminoacylation activity of *M. smegmatis* LeuRS. The residual activity of *M. smegmatis* LeuRS was 66 % at 15 μg/mL, which is more potent than its corresponding amide derivatives **5h** with a percentage inhibition of 31 % at 15 μ g/mL. This is completely consistent with the aforementioned structure-activity relationship.

2.5. Molecular modeling

The crystal structure of the target enzyme with PDB ID 5BTC was downloaded from RCSB Protein Date Bank (RCSB PDB, http://www.rcsb.org/). 5BTC is a co-crystal structure of ciprofloxacin and topoisomerase II. Using the Protein Preparation Wizard in Schrodinger Suite 2016 [33–35], after deleting the co-crystallized water and other molecular fragments existing in the crystal structure, we docked ciprofloxacin into the active site of this protein. The binding pose is exactly the same as the original ciprofloxacin ligand, which showed the credibility of Schrodinger software. Then all the target compounds into the protein were docked, and these docking scores of all the target compounds were listed in Table 7 and the possible binding modes of protein-ligand interaction of the target compounds **4a-4g** were listed in Table 8, these results showed that all the compounds have similar binding modes with ciprofloxacin.

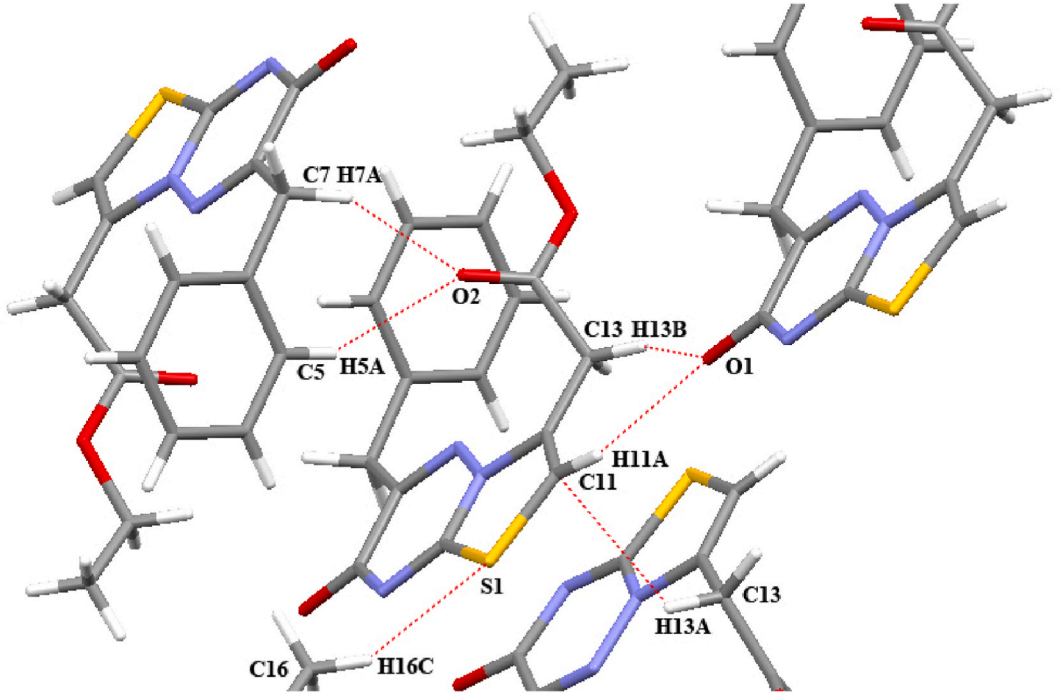


Fig. 5. Part of the crystal structure of compound **3a**, C–H···O, C–H···S, and C–H···π interactions are showed with red dashed lines

Table 3
Weak C–H···O and C–H···S interactions of compound 3a.

D–H···A	d (D–H) (Å)	d (H···A) (Å)	d (D···A) (Å)	Angle (D–H···A) (°)	Symmetry codes
C(5)–H(5A)···O(2)$^{(i)}$	0.9299(29)	2.6026(24)	3.4489(38)	151.577(223)	(i): –1–*x*, 1–*y*, –*z*
C(7)–H(7A)···O(2)$^{(ii)}$	0.9696(27)	2.6680(23)	3.4232(36)	135.023(174)	(ii): 1–*x*, –1–*y*, –*z*
C(11)–H(11A)···O(1)$^{(iii)}$	0.9304(27)	2.3414(19)	3.1423(33)	144.034(170)	(iii): *x*, 1 + *y*, *z*
C(13)–H(13B)···O(1)$^{(iii)}$	0.9697(27)	2.5182(20)	3.3473(35)	143.441(168)	(iii): *x*, 1 + *y*, *z*
C(16)–H(16C)···S(1)$^{(iv)}$	0.9598(69)	2.9846(11)	3.5278(51)	117.172(324)	(iv): −1/2 + *x*, 1/2 + *y*, *z*

Table 4
Weak C–H···π interactions of compound 3a.

D–H···A	d_{atm} (D–H) (Å)	d_{atm} (H···A) (Å)	d_{atm} (D···A) (Å)	Angle (D–H···A) (°)	Symmetry codes
C(13)–H(13A)···C(11)$^{(v)}$	0.9700(24)	2.8781(32)	3.5068(41)	123.394(167)	(v): –1–*x*, *y*, 1/2–*z*

The docking scores shown in Table 7, indicate that compounds **4a-4g** with carboxylic acid group exhibit better activity. Ulteriorly, the results of molecular docking studies (Table 8) showed that most of the compounds in **4a-4g** can form Pi-anion interactions with Asp536, hydrogen bonds with Gly483 and Gly484, and metal-acceptor interactions between the oxygen atoms on the carboxyl group with Mg^{2+}. In other words, the carboxyl groups in **4a-4g** can effectively form salt bridge interaction with Mg^{2+}, which is consistent with our original design idea (shown in Figs. 6 and 7,**4e-4g** as examples). The docking scores of **4e-4g** with carboxylic acid group were better than their corresponding amide derivative **5g-5k**. This difference was also embodied in the antibacterial activity profiles. Although the carbonyl group on the scaffold ring of **5g-5k** can also form coordination interaction with Mg^{2+} in proteins, for example in Fig. 8 showed compound **5h** could coordinate with Mg^{2+} in the protein, the bulky steric hindrance of arylpiperazine may affect the binding conformation between the molecules and proteins, resulting in relatively weak binding ability.

3. Materials and methods

3.1. General methods

Common commercial suppliers provided all solvents and chemicals, which were employed without further purification. The melting points of each compound were taken in open capillary tubes and are reported uncorrected. FT-IR 920 spectrophotometer

Table 5
Molecular properties calculated for the synthesized compounds.

No.	MW[a]	HBA[b]	HBD[c]	AlogP[d]	RB[e]	PSA[f]
	≦500	≦ 10	≦ 5	≦5	2–8	≦140Å^2
3a	329.374	7	0	2.147	6	69.530
3b	343.400	7	0	2.633	6	69.530
3c	359.400	8	0	2.130	7	78.460
3d	345.373	8	1	1.905	6	90.346
3e	363.819	7	0	2.811	6	69.530
3f	363.819	7	0	2.811	6	69.530
3g	347.364	7	0	2.352	6	69.530
4a	301.320	7	1	1.572	4	81.416
4b	315.347	7	1	2.058	4	81.416
4c	331.346	8	1	1.556	5	90.346
4d	317.320	8	2	1.330	4	102.231
4e	335.766	7	1	2.237	4	81.416
4f	335.766	7	1	2.237	4	81.416
4g	319.311	7	1	1.778	4	81.416
5a	445.537	7	0	2.986	5	67.305
5b	479.982	7	0	3.650	5	67.305
5c	473.590	7	0	3.958	5	67.305
5d	473.590	7	0	3.958	5	67.305
5e	487.616	7	0	4.444	5	67.305
5f	528.453	7	0	4.801	5	67.305
5g	479.982	7	0	3.650	5	67.305
5h	548.872	7	0	4.979	5	67.305
5i	508.035	7	0	4.623	5	67.305
5j	514.427	7	0	4.315	5	67.305
5k	508.035	7	0	4.623	5	67.305

[a] MW, Molecular Weight.
[b] HBA, number of Hydrogen Bond Acceptors.
[c] HBD, number of Hydrogen Bond Donors.
[d] AlogP Log value of the octanol–water partition coefficient.
[e] RB, number of Rotatable Bonds.
[f] PSA, Polar Surface Area, Å^2.

Table 6
Antibacterial activity of the target compounds.

No.	Antibacterial activity MIC value(μg/mL)			
	S. aureus	B.subtilis	E.coli	*P. aeruginosa*
3a	800	>800	400	800
3b	800	>800	800	>800
3c	>800	>800	>800	>800
3d	>800	>800	>800	>800
3e	200	200	200	400
3f	200	400	50	800
3g	50	50	50	100
4a	400	800	400	800
4b	800	800	400	>800
4c	>800	>800	800	>800
4d	>800	>800	>800	>800
4e	50	50	50	400
4f	100	50	50	200
4g	50	50	50	100
5a	>800	>800	>800	>800
5b	>800	>800	200	400
5c	200	>800	400	>800
5d	>800	>800	>800	>800
5e	200	>800	>800	>800
5f	>800	>800	>800	>800
5g	200	100	200	400
5h	200	200	100	400
5i	100	100	200	200
5j	200	200	200	400
5k	100	100	100	200
ciprofloxacin	25	100	25	50

Table 7
The docking score of the synthesized compounds.

No.	docking score	No.	docking score	No.	docking score
3a	−5.996	4a	−7.354	5a	−6.584
3b	−6.610	4b	−7.793	5b	−7.624
3c	−6.731	4c	−7.447	5c	−5.982
3d	−7.52	4d	−8.12	5d	−6.058
3e	−5.889	4e	−6.947	5e	−5.837
3f	−6.683	4f	−8.036	5f	−6.657
3g	−6.35	4g	−7.461	5g	−7.056
				5h	−6.316
				5i	−6.205
				5j	−6.079
				5k	−6.580

Table 8
The binding modes of protein-ligand interaction of the target compounds 4a-4g.

No.	Hydrogen bond	Hydrophobic interaction	Pi-cation/anion	Metal-acceptor
4a	Ser98,Gly88			
4b	Ser90, Arg128	Lys484,Ile540	Asp536	
4c	Gly483, Asp536,Asp534		Asp536,Arg128	Mg601, Mg701
4d	Asp532,Glu459, Gly483		Asp536	Mg601,Mg701
4e	Lys484	Lys484	Asp536	Mg601,Mg701
4f	Gly483	Lys484	Trp129	Mg601
4g	Lys484, Ser90, Gly88	Lys484		Mg601

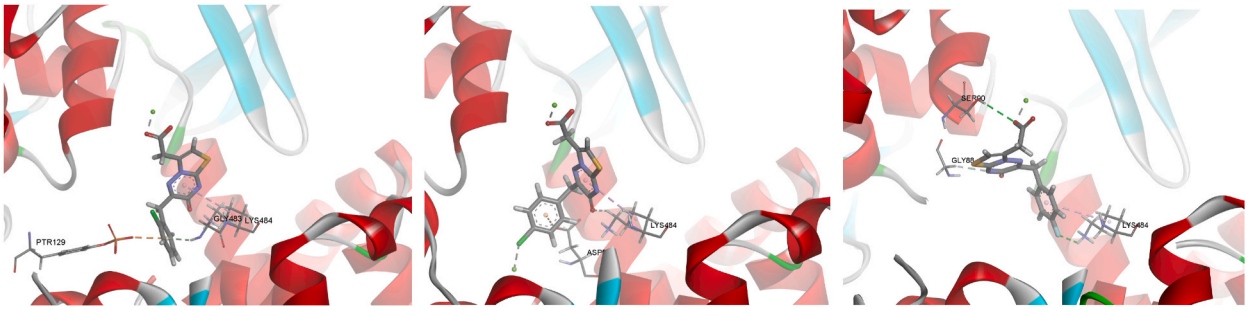


Fig. 6. The view of **4e-4g** (left to right) docking into the protein 5BTC.

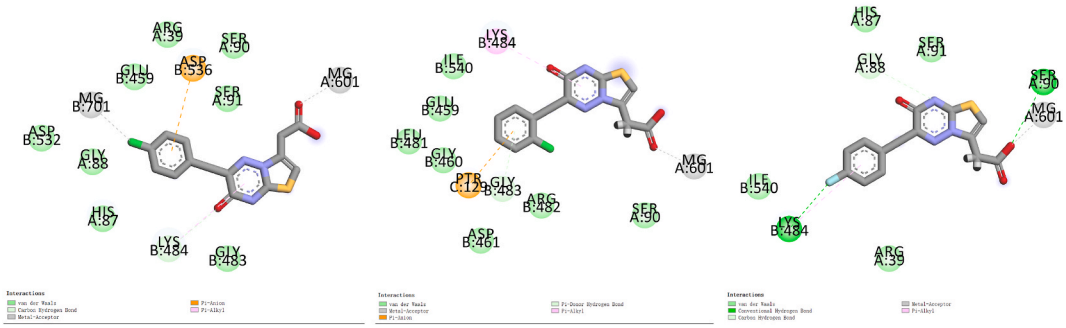


Fig. 7. The ligand interaction of **4e-4g** (left to right) with the active site of 5BTC.

(Tianjin Tuopu Instrument Co., Ltd., Tianjin, China) was used to record the IR spectra with KBr pellets. Agilent 400/54 Premium Shielded NMR Magnet System (Agilent Technologies, Santa Clara, CA, USA) was used to record the ^{1}H-NMR and ^{13}C-NMR spectra with TMS as internal standard. Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System B.05.01. (B5125, Agilent Technologies, Santa Clara, CA, USA) were used to record the mass spectra. Bruker SMART APEX II CCD diffractometer (Bruker AXS GMBH, Germany) was used to determine X-ray single-crystal structure data.

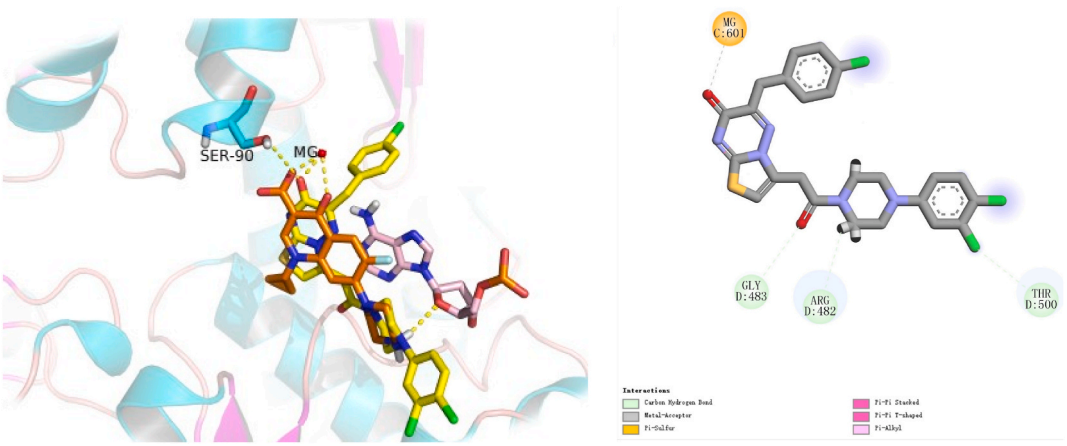


Fig. 8. The view of **5h** docking into the protein 5BTC (left) and interaction with active site (right).

The original figures of ^{1}H-NMR and ^{13}C-NMR specta of all the target compounds and the key intermediates as supplementary materials are available online.

3.2. Chemistry

3.2.1. General procedure for the synthesis of 6-arylmethyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-ones (1a–1g)

A mixture of arylpyruvic acid (0.20 mol), thiosemicarbazide (0.25 mol), ethanol (20 mL), and conc. NaOH aqueous solution (15 mL) was refluxed for 7 h, cooled to room temperature, treated with HCl until pH 2, and the solid was collected to give 6-arylmethyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one.

6-Benzyl-3-thioxo-3,4-dihydro-1,2,4-triazin-5(2H)-one ***(1a)***. A white solid, 93 % yield; mp: 185–187 °C (lit. [36] mp: 188 °C).

6-[(4-Methylphenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1b)***. A white solid, 87 % yield; mp: 138–141 °C (lit. [36] mp: 143–145 °C).

6-[(4-Methoxyphenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1c)***. A white solid, 92 % yield; mp: 166–167 °C (lit. [36] mp: 167–168 °C).

6-[(4-Hydroxyphenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1d)***. A white solid, 89 % yield; mp: 230–232 °C (lit. [37] mp: 230–231 °C.

6-[(4-Chlorophenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1e)***. A white solid, 90 % yield; mp: 220–222 °C (lit. [38] mp:202 °C.

6-[(2-Chlorophenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1f)***. A white solid, 88 % yield; mp: 197–198 °C (lit. [38] mp: 198–199 °C.

6-[(4-Fluorophenyl)methyl]-3,4-dihydro-3-thioxo-1,2,4-triazin-5(2H)-one ***(1g)***. A white solid, 84 % yield; mp: 218–219 °C (lit. [39] mp: 218–219 °C.

3.2.2. General procedure for the synthesis of the β-keto esters 2a–2g

A 10 % aqueous solution of KOH (5.6 mL, 0.01 mol) was added dropwise with stirring to a suspension of compound **1** (0.01 mol) in DMF (5 mL). Then ethyl 4-chloroacetoacetate (1.65 g, 0.01 mol, 1 equiv) was added and the reaction mixture was stirred at room temperature for 30 min. Then the reaction mixture was poured into cold H_2O (100 mL), the resulting precipitate was collected by filtration and washed sequentially with H_2O, then dried to afford the corresponding crude product, which was purified by recrystallized from ethyl acetate.

Ethyl 4-[(6-benzyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*)thio]-3-oxobutanoate* ***(2a)***. A light yellow solid; Yield: 83.20 %; mp: 144.2–145.2 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37–7.25 (m, 4H), 7.28–7.18 (m, 1H), 6.67 (s, 1H), 4.26–4.09 (m, 2H), 4.00 (d, J = 14.2 Hz, 1H), 3.91 (d, J = 14.1 Hz, 1H), 3.69 (d, J = 12.1 Hz, 1H), 3.52 (d, J = 12.1 Hz, 1H), 3.16 (d, J = 16.2 Hz, 1H), 3.09 (d, J = 16.2 Hz, 1H), 1.28 (t, J = 7.1 Hz, 3H); HRMS (*m/z*): calcd. for $C_{16}H_{18}N_3O_4S$ [(M + H)$^+$] 348.10180, found 348.10123.

Ethyl 4-{[6-(4-methylbenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2b)***. A light yellow solid; Yield: 84.36 %; mp: 150.3–150.6 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.28 (s, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.10 (d, J = 7.8 Hz, 2H), 6.46 (s, 1H), 4.29–4.11 (m, 2H), 3.95 (d, J = 14.1 Hz, 1H), 3.86 (d, J = 14.1 Hz, 1H), 3.66 (d, J = 12.1 Hz, 1H), 3.52 (d, J = 12.1 Hz, 1H), 3.19–3.01 (m, 2H), 2.32 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H); HRMS (*m/z*): calcd. for $C_{17}H_{20}N_3O_4S$ [(M + H)$^+$] 362.11745, found 362.11691.

Ethyl 4-{[6-(4-methoxybenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2c)***. A light yellow solid; Yield: 87.41 %; mp: 150.3–150.6 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.20–7.13 (m, 2H), 6.88–6.81 (m, 2H), 4.09–3.94 (m, 3H), 3.83 (d, J = 14.1 Hz, 1H), 3.73 (d, J = 11.2 Hz, 3H), 3.51 (d, J = 12.4 Hz, 1H), 3.34 (s, 1H), 3.23 (d, J = 5.0 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H); HRMS (*m/z*): calcd. for $C_{17}H_{20}N_3O_5S$ [(M + H)$^+$] 378.11237, found 378.10754.

Ethyl 4-{[6-(4-Hydroxybenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2d)***. A light yellow solid; Yield: 71.61 %;

mp: 164.7–165.1 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.25 (s, 1H), 7.81 (s, 1H), 7.08–7.00 (m, 2H), 6.71–6.62 (m, 2H), 4.05 (q, J = 7.1 Hz, 2H), 3.99 (d, J = 12.3 Hz, 1H), 3.78 (d, J = 14.1 Hz, 1H), 3.67 (d, J = 14.0 Hz, 1H), 3.50 (d, J = 12.4 Hz, 1H), 3.30–3.16 (m, 2H), 1.15 (t, J = 7.1 Hz, 3H); HRMS (*m/z*): calcd. for $C_{16}H_{18}N_3O_5S$ [(M + H)$^+$] 364.09672, found 364.09300.

Ethyl 4-{[6-(4-chlorobenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2e)***. A light yellow solid; Yield: 88.23 %; mp: 137.6–137.9 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41–7.30 (m, 2H), 7.34–7.20 (m, 2H), 4.49 (s, 1H), 4.33 (dq, J = 25.6, 7.2 Hz, 2H), 4.10–3.98 (m, 1H), 4.01–3.91 (m, 1H), 1.98 (s, 2H), 1.83 (s, 1H), 1.35 (dt, J = 17.3, 7.2 Hz, 3H); HRMS (*m/z*): calcd. for $C_{16}H_{17}ClN_3O_4S$ [(M + H)$^+$]382.06283, found 382.06230.

Ethyl 4-{[6-(2-chlorobenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2f)***. A light yellow solid; Yield: 86.89 %; mp: 127.5–128.2 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36 (ddd, J = 9.5, 7.8, 3.5 Hz, 2H), 7.27–7.17 (m, 2H), 6.50 (s, 1H), 4.23–4.06 (m, 3H), 4.10–4.00 (m, 1H), 3.73 (d, J = 12.1 Hz, 1H), 3.55 (d, J = 12.1 Hz, 1H), 3.12–2.97 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H); HRMS (*m/z*): calcd. for $C_{16}H_{17}ClN_3O_4S$ [(M + H)$^+$] 382.06283, found 382.06116.

Ethyl 4-{[6-(4-fluorobenzyl)-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl*]thio}-3-oxobutanoate* ***(2g)***. A light yellow solid; Yield:85.24 %; mp: 118.9–119.4 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.33–7.25 (m, 2H), 6.98 (m, 2H), 6.63 (s, 1H), 4.25–4.11 (m, 2H), 3.95 (d, J = 14.3 Hz, 1H), 3.87 (dd, J = 14.3, 2.9 Hz, 1H), 3.55 (dd, J = 11.9, 4.6 Hz, 2H), 3.21–3.04 (m, 2H), 1.34–1.22 (m, 3H) HRMS (*m/z*): calcd. for $C_{16}H_{17}FN_3O_4S$ [(M + H)$^+$] 366.09238, found 382.094415.

3.2.3. General procedure for the synthesis of ethyl 6-arylmethyl-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazine-3-acetates ***(3a–3g)***

A suspension of compound **2** (1.0 g) in polyphosphoric acid (5 g, 85 %) was heated in an oil bath at 120 °C for 40–60 min, the solution was poured with stirring into cold water. The solid that formed was collected, washed with water, and crystallized from ethyl acetate.

Ethyl 2-(6-benzyl-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*)acetate* ***(3a)***. A white solid; Yield: 68.38 %; mp: 156.5–158.0 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.42–7.34 (m, 2H), 7.39–7.27 (m, 2H), 7.30–7.21 (m, 1H), 6.80 (d, J = 1.0 Hz, 1H), 4.24–4.11 (m, 4H), 3.80 (d, J = 1.1 Hz, 2H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.39, 164.24, 159.05, 154.56, 135.34, 131.89, 129.53, 128.46, 126.95, 106.33, 61.86, 37.01, 32.62, 14.10; HRMS (*m/z*): calcd. for $C_{16}H_{16}N_3O_3S$ [(M + H)$^+$] 330.09124, found 330.09136.

Ethyl 2-[6-(4-methylbenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3b)***. A white solid; Yield: 76.57 %; mp: 125.8–126.2 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.30–7.23 (m, 2H), 7.12 (d, J = 7.8 Hz, 2H), 6.79 (s, 1H), 4.20 (q, J = 7.2 Hz, 2H), 4.08 (s, 2H), 3.81 (d, J = 1.1 Hz, 2H), 2.33 (s, 3H), 1.28 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 170.37, 166.72, 163.52, 154.04, 148.81, 131.65, 131.21, 128.77, 128.53, 105.67, 61.21, 35.99, 32.00, 20.41, 13.47; HRMS (*m/z*): calcd. for $C_{17}H_{18}N_3O_3S$ [(M + H)$^+$] 344.10689, found 344.10707.

Ethyl 2-[6-(4-methoxybenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3c)***. A yellow solid; Yield: 61.32 %; mp: 143.2–144.8 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.84 (s, 1H), 7.20–7.13 (m, 2H), 6.88–6.81 (m, 2H), 4.05 (q, J = 7.1 Hz, 2H), 3.99 (d, J = 12.4 Hz, 1H), 3.83 (d, J = 14.2 Hz, 1H), 3.73 (d, J = 11.2 Hz, 3H), 3.51 (d, J = 12.4 Hz, 1H), 3.34 (s, 1H), 3.23 (d, J = 5.0 Hz, 2H), 1.14 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.03, 168.44, 164.48, 158.48, 153.93, 132.37, 130.64, 127.99, 114.08, 108.12, 61.40, 55.43, 36.06, 32.52, 14.37; HRMS (*m/z*): calcd. for $C_{17}H_{18}N_3O_4S$ [(M + H)$^+$] 360.10180, found 360.09807.

Ethyl 2-[6-(4-hydroxybenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3d)***. A white solid; Yield: 57.36 %; mp: 186.4–187.1 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.24 (s, 1H), 7.28 (s, 1H), 7.04 (d, J = 8.1 Hz, 2H), 6.65 (d, J = 8.1 Hz, 2H), 4.06 (q, J = 7.1 Hz, 2H), 3.92 (s, 2H), 3.80 (s, 2H), 1.15 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 168.46, 164.44, 158.74, 156.56, 154.03, 132.38, 130.55, 126.11, 115.44, 108.09, 61.40, 36.04, 32.53, 14.40; HRMS (*m/z*): calcd. for $C_{16}H_{16}N_3O_4S$ [(M + H)$^+$] 346.08615, found 346.08660.

Ethyl 2-[6-(4-chlorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3e)***. A pale green solid; Yield: 74.35 %; mp: 184.1–184.2 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.35–7.25 (m, 4H), 6.81 (s, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.09 (s, 2H), 3.80 (d, J = 1.0 Hz, 2H), 1.29 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.35, 164.32, 158.89, 154.13, 133.79, 132.86, 131.84, 130.93, 128.55, 106.45, 61.90, 36.43, 32.60, 14.11; HRMS (*m/z*): calcd. for $C_{16}H_{15}ClN_3O_3S$ [(M + H)$^+$]364.05226, found 364.05258.

Ethyl 2-[6-(2-chlorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3f)***. A white solid; Yield: 78.22 %; mp: 167.0–167.5 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.43 (d, J = 7.6 Hz, 1H), 7.35 (dd, J = 7.1, 2.1 Hz, 1H), 7.28 (dd, J = 8.5, 5.8 Hz, 3H), 4.10 (s, 2H), 3.91 (q, J = 7.1 Hz, 2H), 3.74 (s, 2H), 1.09 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 167.15, 164.22, 159.04, 153.39, 134.76, 133.41, 131.98, 131.77, 129.36, 128.48, 126.77, 106.24, 61.79, 35.01, 32.44, 14.04; HRMS (*m/z*): calcd. for $C_{16}H_{15}ClN_3O_3S$ [(M + H)$^+$] 364.05226, found 364.05343.

Ethyl 2-[6-(4-fluorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetate* ***(3g)***. A white solid; Yield: 75.16 %; mp: 156.0–156.1 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 1.0 Hz, 1H), 4.19 (q, J = 7.1 Hz, 2H), 4.10 (s, 2H), 3.82–3.70 (m, 2H), 1.33–1.23 (m, 3H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 167.36, 164.30, 163.14, 160.70, 158.96, 154.37, 131.86, 131.14, 131.06, 115.37, 115.15, 106.42, 61.88, 36.29, 32.59, 14.08; HRMS (*m/z*): calcd. for $C_{16}H_{15}FN_3O_3S$ [(M + H)$^+$] 348.08182, found 348.08254.

3.2.4. General procedure for the synthesis of 6-arylmethyl-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazine-2-acetic acids ***(4a–4e)***

The reaction mixture of ester derivative **3** (5 mmol), NaOH (1.0 g, 25 mmol) in CH_3OH (10 mL) and H_2O (10 mL) was stirred for 2 h at room temperature. The MeOH was removed in vacuo, then the concentrated reaction mixture was acidified to pH 4 with concentrated HCl. The product was collected by filtration, washed with water, and dried.

2-(6-Benzyl-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*)acetic acid* ***(4a)***. A white solid; Yield: 93.79 %; mp: 164.6–165.6 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 12.84 (s, 1H), 7.30–7.24 (m, 5H), 7.21 (m, 1H), 3.94 (s, 2H), 3.85 (s, 2H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.02, 164.60, 158.70, 153.53, 136.24, 133.05, 129.57, 128.73, 127.06, 107.75, 37.06, 32.64; HRMS (*m/z*): calcd. for $C_{14}H_{12}N_3O_3S$

[(M + H)$^+$] 302.05994, found 302.09723.

2-[6-(4-Methylbenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4b**)*. A white solid; Yield: 93.54 %; mp: 182.2–183.7 °C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.82 (s, 1H), 7.28 (s, 1H), 7.18 (d, J = 7.8 Hz, 2H), 7.09 (d, J = 7.8 Hz, 2H), 3.89 (d, J = 9.4 Hz, 4H), 2.26 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.02, 164.57, 158.67, 153.63, 136.09, 133.14, 133.04, 129.43, 129.30, 107.71, 36.65, 32.65, 21.09; HRMS (*m/z*): calcd. for $C_{15}H_{14}N_3O_3S$ [(M + H)$^+$] 316.07559, found 316.07572.

2-[6-(4-Methoxybenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4c**)*. A white solid; Yield: 94.77 %; mp: 205.8–207.0 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 12.84 (s, 1H), 7.26 (s, 1H), 7.19 (d, J = 8.3 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 3.86 (d, J = 8.0 Hz, 4H), 3.70 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.03, 164.54, 158.69, 158.46, 153.72, 133.05, 130.65, 127.93, 114.14, 107.69, 55.42, 36.18, 32.66; HRMS (*m/z*): calcd. for $C_{15}H_{14}N_3O_4S$ [(M + H)$^+$] 332.07050, found 332.06674.

2-[6-(4-Hydroxybenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4d**)*. A white solid; Yield: 89.21 %; mp: 241.2–242.5 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 9.26 (s, 1H), 7.26 (s, 1H), 7.06 (d, J = 8.3 Hz, 2H), 7.01 (s, 1H), 6.65 (d, J = 8.4 Hz, 2H), 3.86 (s, 2H), 3.80 (s, 2H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 170.04, 164.51, 158.69, 156.58, 153.80, 133.11, 130.54, 126.03, 115.53, 107.65, 36.18, 32.73; HRMS (*m/z*): calcd. for $C_{14}H_{12}N_3O_4S$ [(M + H)$^+$] 318.05485, found 318.05478.

2-[6-(4-Chlorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4e**)*. A white solid; Yield: 95.12 %; mp: 194.8–195.8 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 12.78 (s, 1H), 7.31 (d, J = 8.3 Hz, 4H), 7.27 (s, 1H), 3.82 (s, 2H), 3.30 (s, 2H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 169.97, 164.61, 158.69, 153.26, 135.26, 133.04, 131.75, 131.50, 128.61, 107.80, 36.36, 32.61; HRMS (*m/z*): calcd. for $C_{14}H_{11}ClN_3O_3S$ [(M + H)$^+$] 336.02096, found 336.02062.

2-[6-(2-Chlorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4f**)*. A white solid; Yield: 95.37 %; mp: 215.3–215.6 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 12.66 (s, 1H), 7.42 (dd, J = 7.4, 1.8 Hz, 1H), 7.33 (dd, J = 7.1, 2.2 Hz, 1H), 7.29–7.23 (m, 3H), 4.09 (s, 2H), 3.69 (s, 2H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 169.69, 164.57, 158.71, 152.69, 134.01, 133.96, 132.97, 131.78, 129.57, 129.02, 127.42, 107.89, 34.71, 32.38; HRMS (*m/z*): calcd. for $C_{14}H_{11}ClN_3O_3S$ [(M + H)$^+$] 336.02096, found 336.02117.

2-[6-(4-Fluorobenzyl)-7-oxo-7H-thiazolo[3,2-b]-1,2,4-triazin-3-yl*]acetic acid (**4g**)*. A white solid; Yield: 88.89 %; mp: 160.7–161.1 °C; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 12.82 (s, 1H), 7.33–7.28 (m, 2H), 7.26 (s, 1H), 7.12–7.05 (m, 2H), 3.94 (s, 2H), 3.82 (s, 2H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 169.98, 164.59, 162.73, 160.32, 158.70, 153.47, 133.04, 132.30, 132.27, 131.56, 131.48, 115.51, 115.30, 107.78, 36.21, 32.60; HRMS (*m/z*): calcd. for $C_{14}H_{11}FN_3O_3S$ [(M + H)$^+$] 320.05052, found 320.05070.

*3.2.5. General procedure for the synthesis of the target compounds **5a–5j***

1-(Substituted phenyl)piperazine (1.0 mmol) was added to a solution of compound **4** (1.0 mmol), triethylamine (0.34 g, 3.3 mmol), 1-hydroxybenzotriazole (HOBt, 0.15 g, 1.1 mmol), and *N*'-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDCI, 0.21g, 1.1 mmol) in CH_2Cl_2 (30 mL). The solution was then stirred at room temperature for 16 h. The reaction was diluted with water (30 mL), and the organic layer was separated and sequentially washed with 1 mol L^{-1} HCl (10 mL), saturated sodium bicarbonate aqueous solution (10 mL), and brine (10 mL), dried (Na_2SO_4). Filtration and evaporation of the solvent gave the crude product which was purified by chromatography (V_{CH2Cl2}/V_{CH3OH} = 40/1).

6-Benzyl-3-[2-oxo-2-(4-phenylpiperazin-1-yl*)ethyl]-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (**5a**)*.

A white solid; Yield: 72.20 %; mp: 167.1–167.6 °C; IR (KBr): υ 3109, 2957, 2816, 2818, 1639, 1599, 1485, 1454, 1387, 758, 702 cm^{-1};^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39–7.17 (m, 7H), 6.98 (dd, J = 7.7, 3.6 Hz, 3H), 6.80 (s, 1H), 4.14 (s, 2H), 3.80 (d, J = 8.4 Hz, 4H), 3.59 (t, J = 5.1 Hz, 2H), 3.24–3.16 (m, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.89, 164.25, 159.11, 154.59, 135.41, 132.96, 129.68, 129.60, 129.35, 128.43, 126.90, 120.92, 116.72, 106.14, 49.71, 49.28, 45.66, 41.94, 37.13, 31.39; HRMS (*m/z*): calcd. for $C_{24}H_{24}N_5O_2S$ [(M + H)$^+$] 446.16507, found 446.16707.

6-Benzyl-3-{2-[4-(4-chlorophenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (**5b**)*. A white solid; Yield: 65.10 %; mp: 160.8–161.9 °C; IR (KBr): υ 3111, 2976, 2955, 2824, 1641, 1597, 1568, 1454, 1386, 815, 752, 702 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.38–7.17 (m, 7H), 6.92–6.83 (m, 2H), 6.79 (s, 1H), 4.13 (s, 2H), 3.83–3.73 (m, 4H), 3.57 (t, J = 5.1 Hz, 2H), 3.15 (dd, J = 6.2, 4.2 Hz, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.95, 164.23, 159.11, 154.45, 149.20, 135.40, 133.01, 129.64, 129.14, 128.41, 126.88, 125.52, 117.80, 106.29, 49.52, 49.10, 45.50, 41.79, 37.12, 31.44; HRMS (*m/z*): calcd. for $C_{24}H_{23}ClN_5O_2S$ [(M + H)$^+$] 480.12610, found 480.12806.

6-Benzyl-3-{2-[4-(2,6-dimethylphenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (**5c**)*. A white solid; Yield: 68.20 %; mp: 231.1–233.9 °C; IR (KBr): υ 3073, 3003, 2918, 2816, 1655, 1606, 1576, 1487, 1454, 1441, 1377, 779, 762, 710 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ7.39–7.22 (m, 5H), 7.10–6.98 (m, 2H), 6.91 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 0.9 Hz, 1H), 4.16 (s, 2H), 3.79 (dd, J = 19.4, 3.0 Hz, 4H), 3.57 (t, J = 4.9 Hz, 2H), 2.90 (q, J = 5.0 Hz, 4H), 2.34 (d, J = 13.7 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.06, 164.25, 159.13, 154.43, 147.35, 136.65, 135.45, 133.23, 129.66, 129.15, 128.41, 126.84, 125.74, 106.15, 50.02, 49.43, 47.29, 43.50, 37.17, 31.58, 19.65; HRMS (*m/z*): calcd. for $C_{26}H_{28}N_5O_2S$ [(M + H)$^+$] 474.19637, found 474.19808.

6-Benzyl-3-{2-[4-(2,4-dimethylphenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (**5d**)*. A white solid; Yield: 71.60 %; mp: 210.1–220.7 °C; IR (KBr): υ 3115, 2957, 2918, 2806, 1649, 1572, 1476, 1447, 1373, 814, 752, 696 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.39–7.22 (m, 5H), 7.10–6.98 (m, 2H), 6.91 (d, J = 8.1 Hz, 1H), 6.80 (d, J = 0.9 Hz, 1H), 4.16 (s, 2H), 3.79 (m, 4H), 3.57 (t, J = 4.9 Hz, 2H), 2.90 (q, J = 5.0 Hz, 4H), 2.34 (d, J = 13.7 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.01, 164.25, 159.12, 154.54, 147.90, 135.44, 133.64, 133.11, 132.55, 132.03, 129.69, 128.45, 127.21, 126.89, 119.05, 106.07, 52.15, 51.72, 46.29, 42.60, 37.15, 31.45, 20.73, 17.69; HRMS (*m/z*): calcd. for $C_{26}H_{28}N_5O_2S$ [(M + H)$^+$] 474.19637, found 474.19865.

3-{2-[4-(2,4-Dimethylphenyl)piperazin-1-yl*]-2-oxoethyl}-6-(4-methylbenzyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one (**5e**)*. A white solid; Yield: 69.90 %; mp: 199.3–212.3 °C; IR (KBr): υ 3109, 3007, 2918, 2808, 1638, 1572, 1481, 1445, 1373, 813, 796 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.24 (d, J = 7.8 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H), 7.08 (s, 1H), 7.05–6.99 (m, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.80 (s, 1H), 4.11 (s, 2H), 3.85–3.81 (m, 2H), 3.79 (s, 2H), 3.60 (t, J = 4.8 Hz, 2H), 2.92 (q, J = 4.8 Hz, 4H), 2.36 (s, 3H), 2.32 (d, J =

2.9 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.00, 164.21, 159.12, 154.71, 136.40, 133.70, 133.06, 132.53, 132.33, 132.04, 129.53, 129.13, 127.22, 119.05, 105.95, 52.18, 51.78, 46.30, 42.58, 36.74, 31.45, 21.10, 20.72, 17.70; HRMS (*m/z*): calcd. for $C_{27}H_{30}N_5O_2S$ [(M + H)$^+$] 488.21202, found 488.21439.

3-{2-[4-(3,4-Dichlorophenyl)piperazin-1-yl*]-2-oxoethyl}-6-(4-methylbenzyl)-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5f)***. A white solid; Yield: 68.10 %; mp: 174.8–174.8 °C; IR (KBr): υ 3117, 2953, 2918, 2825, 1649, 1595, 1568, 1489, 1458, 1375, 816, 797 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.8 Hz, 1H), 7.21 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 2.7 Hz, 1H), 6.82 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.80 (s, 1H), 4.10 (s, 2H), 3.82 (s, 2H), 3.79 (d, *J* = 5.5 Hz, 2H), 3.60 (s, 2H), 3.18 (d, *J* = 5.3 Hz, 4H), 2.30 (s, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 165.00, 164.22, 159.08, 154.73, 149.87, 136.41, 133.02, 132.79, 132.30, 130.69, 129.51, 129.14, 123.50, 117.91, 115.91, 106.16, 49.09, 48.73, 45.36, 41.63, 36.71, 31.33, 21.07; HRMS (*m/z*): calcd. for $C_{25}H_{24}Cl_2N_5O_2S$ [(M + H)$^+$] 528.10278, found 528.10558.

6-(4-Chlorobenzyl)-3-[2-oxo-2-(4-phenylpiperazin-1-yl*)ethyl]-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5g)***. A white solid; Yield: 73.10 %; mp: 192.3–194.6 °C; IR (KBr): υ 3107, 2919, 2951, 1639, 1598, 1570, 1484, 1385, 808, 758 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.36–7.32 (m, 2H), 7.27 (d, *J* = 2.5 Hz, 4H), 6.99 (d, *J* = 7.9 Hz, 3H), 6.82 (d, *J* = 1.0 Hz, 1H), 4.11 (s, 2H), 3.80 (d, *J* = 4.5 Hz, 4H), 3.62 (t, *J* = 5.0 Hz, 2H), 3.22 (t, *J* = 4.9 Hz, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.85, 164.31, 158.99, 154.12, 150.57, 133.91, 132.94, 132.72, 131.06, 129.32, 128.51, 120.87, 116.75, 106.37, 49.71, 49.26, 45.73, 42.02, 36.54, 31.44; HRMS (*m/z*): calcd. for $C_{24}H_{23}ClN_5O_2S$ [(M + H)$^+$] 480.12610, found 480.12832.

6-(4-Chlorobenzyl)-3-{2-[4-(3,4-dichlorophenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5h)***. A white solid; Yield: 68.40 %; mp: 127.4–127.7 °C; IR (KBr): υ 3117, 2953, 2911, 2824, 1647, 1593, 1568, 1477, 1449, 1377, 854, 806 cm^{-1}; ^{1}H-NMR (600 MHz, DMSO-d_6) δ 7.41 (d, *J* = 8.9 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 4H), 7.19 (s, 1H), 7.16 (d, *J* = 2.9 Hz, 1H), 6.95 (dd, *J* = 9.0, 2.9 Hz, 1H), 3.93 (d, *J* = 13.3 Hz, 4H), 3.60–3.51 (m, 4H), 3.23 (d, *J* = 5.6 Hz, 2H), 3.16 (t, *J* = 5.3 Hz, 2H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.85, 164.29, 158.95, 154.18, 149.91, 133.88, 132.97, 132.76, 132.68, 131.11, 130.66, 128.49, 123.41, 117.91, 115.94, 106.41, 49.01, 48.69, 45.34, 41.66, 36.55, 31.40; HRMS (*m/z*): calcd. for $C_{24}H_{21}Cl_3N_5O_2S$ [(M + H)$^+$] 548.04815, found 548.05106.

6-(4-Chlorobenzyl)-3-{2-[4-(2,4-dimethylphenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5i)***. A white solid; Yield: 65.40 %; mp: 167.3–168.6 °C; IR (KBr): υ 3113, 2953, 2918, 1634, 1572, 1481, 1447, 1373, 856, 808 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.37–7.30 (m, 4H), 7.22 (s, 1H), 6.96–6.87 (m, 3H), 3.99 (s, 2H), 3.92 (s, 2H), 3.58–3.56 (m, 4H), 2.85–2.71 (m, 4H), 2.25 (d, *J* = 19.8 Hz, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.86, 164.31, 158.99, 154.11, 147.98, 133.89, 133.57, 133.09, 132.72, 132.63, 132.00, 131.10, 128.49, 127.18, 119.06, 106.22, 52.14, 51.72, 46.32, 42.66, 36.58, 31.58, 20.72, 17.68; HRMS (*m/z*): calcd. for $C_{26}H_{27}ClN_5O_2S$ [(M + H)$^+$] 508.15740, found 508.15974.

6-(2-Chlorobenzyl)-3-{2-[4-(2-chlorophenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5j)***. A white solid; Yield: 72.40 %; mp: 211.1–212.6 °C; IR (KBr): υ 3094, 2968, 2916, 2845, 1659, 1589, 1576, 1487, 1438, 1379, 766, 755 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.47–7.41 (m, 2H), 7.38 (dd, *J* = 5.7, 3.6 Hz, 1H), 7.34–7.23 (m, 3H), 7.09 (t, *J* = 7.5 Hz, 2H), 6.80 (s, 1H), 4.32 (s, 2H), 3.72 (d, *J* = 19.7 Hz, 4H), 3.47 (d, *J* = 5.2 Hz, 2H), 3.04 (s, 4H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.92, 164.22, 159.10, 153.23, 148.30, 134.86, 133.71, 133.06, 132.01, 130.80, 129.32, 128.90, 128.59, 127.77, 126.93, 124.51, 120.45, 106.15, 51.36, 50.67, 45.79, 42.20, 35.17, 31.34; HRMS (*m/z*): calcd. for $C_{24}H_{22}Cl_2N_5O_2S$ [(M + H)$^+$] 514.08713, found 514.08943.

6-(2-Chlorobenzyl)-3-{2-[4-(2,6-dimethylphenyl)piperazin-1-yl*]-2-oxoethyl}-7H-thiazolo[3,2-b]-1,2,4-triazin-7-one* ***(5k)***. A white solid; Yield: 70.10 %; mp: 213.6–214.3 °C; IR (KBr): υ 3102, 2955, 2907, 2810, 1651, 1580, 1477, 1439, 1371, 771, 754, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.41 (ddd, *J* = 9.4, 4.8, 2.5 Hz, 2H), 7.28–7.23 (m, 2H), 7.04 (d, *J* = 2.2 Hz, 3H), 6.82 (s, 1H), 4.32 (s, 2H), 3.71 (s, 2H), 3.67 (s, 2H), 3.39 (t, *J* = 4.9 Hz, 2H), 3.09 (t, *J* = 4.7 Hz, 4H), 2.36 (s, 6H); ^{13}C-NMR (101 MHz, $CDCl_3$) δ 164.89, 164.21, 159.11, 153.15, 147.35, 136.61, 134.89, 133.78, 133.16, 132.02, 129.24, 129.16, 129.13, 128.37, 126.84, 125.75, 106.11, 49.94, 49.34, 47.05, 43.40, 35.12, 31.38, 19.65; HRMS (*m/z*): calcd. for $C_{26}H_{27}ClN_5O_2S$ [(M + H)$^+$] 508.15740, found 508.15946.

3.3. Crystal structure determination

The crystals were grown by slow evaporation from methanol solution for **3a**. Data collections were performed on the Bruker AXS Smart APEX II CCD X–diffractometer using filtered Mo-*Kα* radiation (λ = 0.71073 Å) at 296 ± 2 K. The crystal structure was solved by direct method and refined by full–matrix least squares fitting on F^2 by SHELXS–97. The non–hydrogen atoms were refined anisotropically. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, reference number CCDC1444192. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; E-mail: deposit@ccdc.cam.ac.uk).

3.4. Determination of MIC for bacterial strains

The antibacterial and antitubercular activity of target compounds against *Escherichia coli* [CMCC (B) 44102], *Pseudomonas aeruginosa* [CMCC (B) 10104], *Staphylococcus aureus* [CMCC (B) 26003] *Bacillus subtilis* [CMCC (B) 63501] and *Mycobacterium smegmatis* [CGMCC 12621] *in vitro* were determined.

All of the strains we used in antibacterial and antitubercular tests were purchased from the National Centre for Medical Culture Collection. In brief, a standard inoculum (1.5 × 10^7 c.f.u/mL 0.5 McFarland standards) was inoculated onto the surface of sterile agar plates. Then a sterile glass spreader was used to evenly distribute of the inoculum. The discs measuring 6.0 mm in diameter were prepared from Whatman No. 1 filter paper and sterilized by dry heat at 170 °C for an hour. The tested compounds were dissolved in MeOH to get the solution of 2 mg/mL concentration. The inhibition zones were measured in (mm) at the end of an incubation period of 24 h at 37 °C. MeOH showed no inhibition zones. Minimum inhibitory concentration (MIC) was determined using a series dilution

method. A 96-well microtiter plate (200μL/well) was filled with the diluted compounds in Mueller Hinton Broth (M − H Broth), and then a 5×10^5 c.f.u/mL aliquot of bacterial culture was added to each well (200 μL/well) with the final concentration within the range of 1–800 μg/mL. The MIC value was determined to be the lowest concentration required to arrest the growth of bacteria after 24 h of incubation at 37 °C. The MIC values were calculated with the help of GraphPad 'PRISM' software (version 4.0).

3.5. Aminoacylation assay

The vector pET28a(+) in *Escherichia coli* BL21(DE3) was employed to clone and express the LeuRS gene from *M. smegmatis*. One activity unit (1U) was determined to be the amount of *M. smegmatis* LeuRS necessary for catalyzing the production of AMP from 1 μmol of ATP in 1 min at 37 °C. Purification of the expression product was conducted in accordance with the literature [40].

The aminoacylation assay was performed in accordance with the literature [41]. The CH_3OH was used to dissolved all the test compounds. Typically, the reaction blend was composed of 50 mM HEPES-KOH (pH 8.0), 1 mM dithiothreitol, 13 μM L-[^{14}C]leucine (306 mCi/mmol), 30 mM $MgCl_2$, 30 mM KCl, 15 μM E. *coli* tRNA, 0.2 pM *M. smegmatis* LeuRS, 4 mM ATP, and 0.02 % bovine serum albumin (wt/vol). After the addition of 4 mM ATP, the reaction mixture was enriched with the suitable concentrations of test compounds, and then the aminoacylation reactions were incubated at 37 °C for 7 min. Subsequently, 10 % trichloroacetic acid (wt/vol) was added to quench the aliquots. The inhibitory activity of the aminoacylation of *M. smegmatis* LeuRS was obtained with liquid scintillation counting.

4. Conclusions

In the present study, a series of novel 7*H*-thiazolo[3,2-*b*]-1,2,4-triazin-7-one derivatives were synthesized, and their antimicrobial activities were evaluated. To verify the regioselectivity of the intramolecular cyclization step, an X-Ray single crystal diffraction analysis of compound **3a** was tested and analyzed. Most of the target compounds with chlorine or fluorine substituents at benzyl group at 6-position of the scaffold exhibited obvious antibacterial activities. Moreover, compounds **4e-4g** exhibited better antibacterial activity than **3e-3g**, and their corresponding amide derivatives **5g-5k** exhibited slightly less biological activity, probably due to bulky 1-(substituted phenyl)piperazine moiety. Furthermore, the antibacterial activity was only slightly affected by substituents of phenyl ring of 1-(substituted phenyl)piperazine moiety. The molecular docking result of the target compounds with DNA topoisomerase II demonstrated the carboxyl group in carboxylic acid moiety compounds **4a-4g** possesses a crucial salt-bridge interaction with Mg^{2+} in the protein.

CRediT authorship contribution statement

Shicheng Hou: Writing – original draft, Investigation, Data curation. **Tai Li:** Writing – original draft, Investigation. **Jiangqing Yan:** Investigation. **Dong Cai:** Writing – original draft, Investigation, Data curation. **Yang Peng:** Investigation. **Haibo Zhang:** Investigation. **Feng Tong:** Project administration, Conceptualization. **Haiming Fan:** Project administration, Data curation. **Xiaoping Liu:** Writing – review & editing, Investigation. **Chun Hu:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24589.

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