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Synthesis, antimicrobial activities, and molecular docking studies of dihydrotriazine derivatives bearing a quinoline moiety

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In this paper, three series of dihydrotriazine derivatives bearing a quinoline moiety (**5a-b**, **8a-c**, and **9a-m**) have been designed, synthesized, and evaluated as antibacterial agents. Compound **8a, 8b** and **8c** were found to be the most potent of all of the compounds tested, with an MIC value of 1 μg/mL against several Gram-positive (*S. aureus* 4220 and MRSA CCARM 3506) and Gram-negative (*E. coli* 1924) strains of bacteria. In addition, compound **8a** showed potent inhibitory activity (MIC = 2 μg/mL) against *Pseudomonas aeruginosa* 2742, indicating that its antibacterial spectrum is similar to those of the positive controls gatifloxacin and moxifloxacin. Structure-activity relationships (SAR) analyses and docking studies implicated the dihydrotriazine group in increasing the antimicrobial potency of the quinoline compounds. *In vitro* enzyme study implied that compound **8a** also displayed DHFR inhibition.

Keywords: antibacterial agents • antifungal activities • quinoline • dihydrotriazine • DHFR inhibition.

Introduction

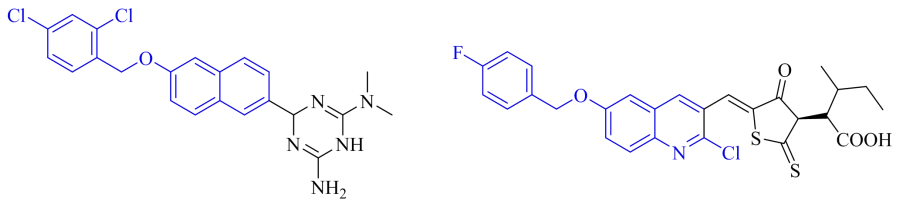
A Gram-negative opportunistic pathogenic bacterium, *Pseudomonas aeruginosa*, is an important causative agent of nosocomial infections[1,2] and is involved in several acute and chronic infections. It is one of the major pathogens causing pneumonia.[3] Moreover, during the past decades, *P. aeruginosa* strains resistant to nearly all established antibiotics have emerged, necessitating alternative therapeutic options.[4,5] Without effective antimicrobials for prevention and treatment of infections, medical procedures such as organ transplantation, chemotherapy, diabetes management, and routine surgery have a significantly higher risk of morbidity and mortality.[6] New antibacterial agents that operate through unique mechanisms of action are of significant importance to combat the emergence of antibiotic-resistant and tolerant bacteria. Bacteria utilize multiple mechanisms to acquire, or gain, resistance to antibiotics following drug exposure during treatment.[7] Recent studies have identified dihydrogen triazine-containing compounds as inhibitors of dihydrofolate reductase (DHFR), a ubiquitous enzyme that catalyses the conversion of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate, which is involved in metabolic reactions such as purine and thymidine nucleotide biosynthesis.[8,9] DHFR is required by all organisms to grow and multiply; however, selective inhibitors of microbial enzymes have been utilized as therapeutic agents.

A lot of compounds bearing a quinoline moiety have been reported in the literature with a variety of different pharmacological activities, including antimicrobial,[10-12] antimalarial,[13] and anticancer activities.[14] By the previous investigations, we found that several rhodanine derivatives bearing a quinoline moiety showed moderate to strong activities against several Gram-positive bacterial strains, including multidrug-resistant clinical isolates, such as compound **B** (MIC = 1 μg/mL). Unfortunately, compounds belonging to this series did not show any bacteriostatic activity against Gram-negative bacteria.[15] We also reported the identification of a series of 1,4-dihydro-1,3,5-triazine derivatives and evaluated their antibacterial activities. Compound **A** was found to be the most potent of all of the compounds tested, with an MIC value of 0.5 μg/mL against several Gram-positive (*S. aureus*

10.1002/cbdv.201900056

4220 and quinolone-resistant *S. aureus* CCARM 3505) and Gram-negative (*E. coli* 1924) strains of bacteria.[16] However, this compound was inactive against *P. aeruginosa* 2742 and *S. typhimurium* 2421 (Fig. 1).

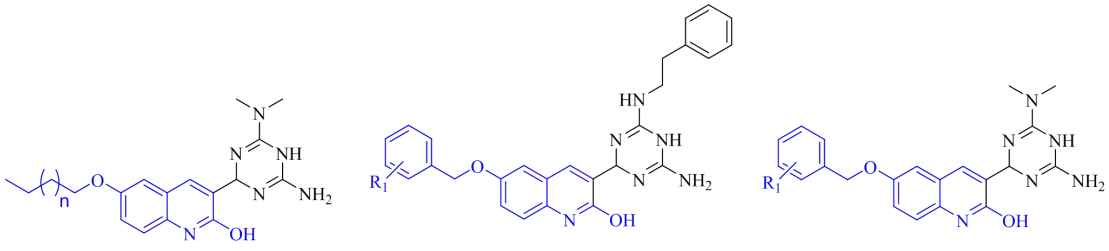
The concept of hybrid molecules has proved to be the most interesting topic in medicinal chemistry, where two or more pharmacophores are linked covalently resulting into one molecule.[17-19] Based on the above considerations, we designed and synthesized a series of dihydrotriazine derivatives using a hybrid strategy, in which the rhodanine moiety was replaced by a dihydrogen triazine scaffold. Thus, three series of 18 novel dihydrotriazine derivatives (Fig. 2) were designed, synthesized and evaluated for their antibacterial and antifungal activities in vitro. The substituents on the hydrocarbon or alkyl phenyl ring were changed simultaneously to investigate their contribution to the biological activity.



A MIC = 0.5 μg/mL

B MIC = 1 μg/mL

Figure 1. Chemical structures of some reported compounds



5a n=2
5b n=3

8a-c R_2=phenylethanamine

9a-m R_2=$NHMe_2$

R_1=**8a**: 3-Cl	**9b**: 2,6-di-Cl	**9f**: H	**9j**: 3-CH_3
8b: 2,4-di-Cl	**9c**: 2-Cl	**9g**: 2-F	**9K**: 4-CH_3
8c: 2,6-di-Cl	**9d**: 3-Cl	**9h**: 4-F	**9l**: 2-CN
9a: 2,4-di-Cl	**9e**: 4-Cl	**9i**: 2-Br	**9m**: 4-NO_2

Figure 2. Designed target compounds

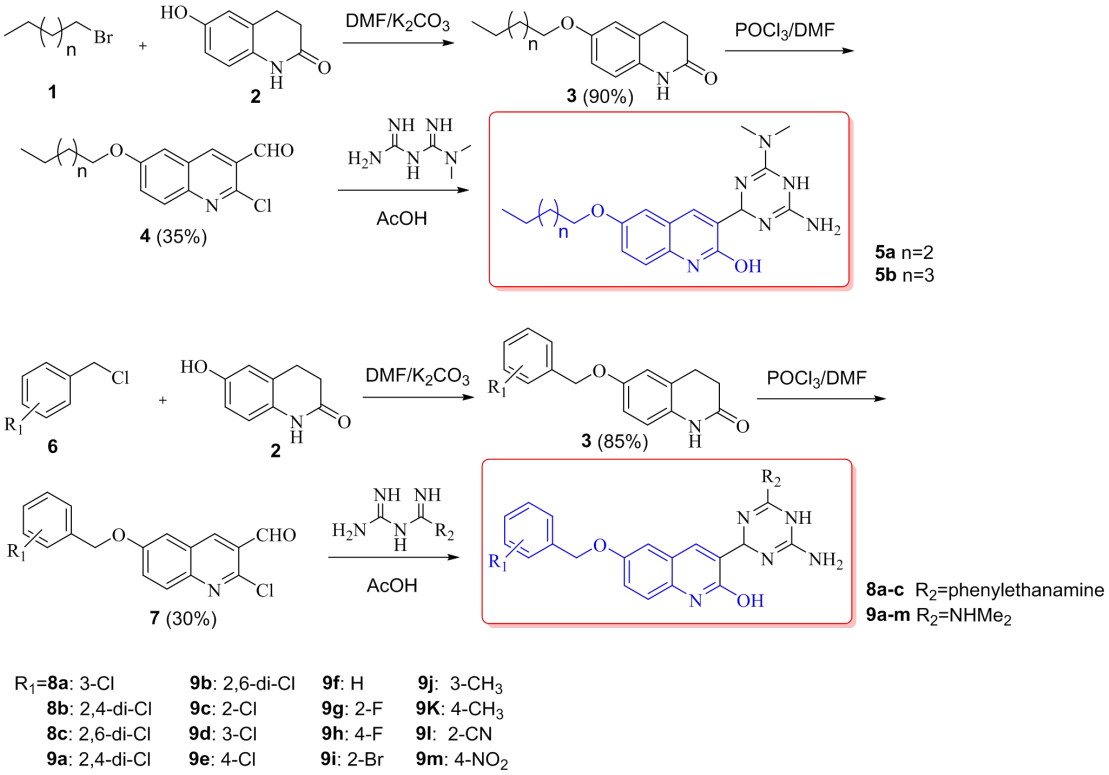
Results and discussion

Chemistry

The target compounds were synthesized as outlined in Scheme 1. Williamson

10.1002/cbdv.201900056

condensation reactions between 6-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (**2**) and a variety of different substituted chloromethylbenzene or bromine alkane compounds (**1** or **6**) afforded the corresponding 6-substituted-3,4-dihydroquinolin-2(1*H*)-ones (**3**), which were subsequently reacted under Vilsmeier-Haack conditions to give the corresponding 6-substituted-2-chloroquinoline-3-carbaldehydes (**4** or **7**). Then, the intermediates (**4** or **7**) were reacted with Phenformin hydrochloride or metformin hydrochloride in acetic acid to generate the target compounds. The target compounds were confirmed by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry.



Scheme 1. Synthetic route for the compounds **5a-b**, **8a-c** and **9a-m**.

Antibacterial activity

The compounds were screened against four Gram-positive strains (*S. aureus* 4220, QRSA CCARM 3505, MRSA CCARM 3506 and *S.mutans* 3289) and Gram-negative strains (*E. coli* 1924, *P. aeruginosa* 2742 and *S. typhimurium* 2421), as well as one fungus (*C. albicans* 7535), and the results are shown in Table 1. Most of the synthesized compounds showed potent inhibitory activities against the different bacteria tested in the current study with MICs ranging from 1 to 64 μg/mL. Almost all

of the compounds in series **8** exhibited potent antibacterial activity with MICs ranging from 1 to 16 μg/mL, except for **8c** against the *S. typhinurium* 2421 and QRSA CCARM 3505 strain (MIC > 64 μg/mL). It is noteworthy that compound **8a** showed potent inhibitory activity (MIC = 2 μg/mL) against *Pseudomonas aeruginosa* 2742, indicating that its antibacterial spectrum is similar to those of the positive controls gatifloxacin and moxifloxacin. Compound **8b** exhibited the greatest activities of all of the compounds prepared in the current study with MIC values in the range of 1-16 μg/mL, making its potency comparable to those gatifloxacin and moxifloxacin. Furthermore, in terms of its activity towards the fungus *C. albicans* 7535, compound **8b** displayed the strongest activity of all of the compounds synthesized in the current study with an MIC value of 2 μg/mL, which was lower than that of fluconazole. For the *S. typhinurium* 2421, however, only compound **8b** exhibited good activity with MIC values of 16 μg/mL.

Based on the analysis of the activities of the synthesized compounds, the following structure-activity relationships (SARs) were obtained. The inclusion of a phenethyl group at the *N*-position of the dihydrotriazine ring, as exemplified by the compounds in series **8** and **9**, resulted in a significant difference in the activity, which indicated that the substituent of the aromatic nucleus was critical to the activities of these compounds. It is also noteworthy that compounds **8b** and **9a**, bearing a 2,4-di-Cl substituted phenyl ring, showed excellent antimicrobial activities with MICs ranging from 1 to 16 μg/mL and 8-32 μg/mL, respectively, against most of the bacteria strains and the fungus tested in this study (except *S. typhimurium* 2421). Furthermore, the position of the di-Cl substituent on the phenyl ring also affected the activity of the compounds with an activity order of 2,4-di-Cl > 2,6-di-Cl, as exemplified by a comparison of the results for the compounds **8b** and **8c**. These results therefore provide further evidence to suggest that the 2,4-di-Cl substituted phenyl ring plays a critical role in the activity of these compounds, which is consistent with the results obtained for a previously reported series of rhodanine and dihydrotriazine derivatives.[20,21]

Table 1. Antibacterial data as MIC[a] (μg/mL) for target compounds **5a-b**, **8a-c** and **9a-m**.

			Gram-positive strains			Fungus		Gram-negative strains		
	R_1	R_2	*S. aureus*	QRSA	MRSA	*S.mutans*	*C. albicans*	*E.coli*	*P. aeruginosa*	*S. typhinurium*
			4220[b]	3505[c]	3506[d]	3289[e]	7535[f]	1924[g]	2742[h]	2421[i]
5a	—	$NHMe_2$	32	>64	32	64	64	32	64	>64
5b	—	$NHMe_2$	8	>64	16	32	32	16	>64	>64
8a	3-Cl	Phenylethan amine	1	16	1	2	4	1	2	64
8b	2,4-di-Cl	Phenylethan amine	1	4	1	1	2	1	4	16
8c	2,6-di-Cl	Phenylethan amine	1	>64	1	2	4	1	16	>64
9a	2,4-di-Cl	$NHMe_2$	8	8	32	8	16	8	16	>64
9b	2,6-di-Cl	$NHMe_2$	64	16	>64	32	64	8	64	>64
9c	2-Cl	$NHMe_2$	64	64	>64	>64	>64	64	32	>64
9d	3-Cl	$NHMe_2$	32	16	>64	64	64	16	16	>64
9e	4-Cl	$NHMe_2$	32	16	>64	32	64	16	16	>64
9f	H	$NHMe_2$	>64	>64	>64	>64	>64	>64	>64	>64
9g	2-F	$NHMe_2$	>64	>64	>64	>64	>64	64	>64	>64
9h	4-F	$NHMe_2$	>64	64	>64	>64	>64	64	64	>64
9i	2-Br	$NHMe_2$	32	32	>64	64	64	32	32	>64
9j	3-CH_3	$NHMe_2$	64	64	>64	64	64	64	64	>64
9k	4-CH_3	$NHMe_2$	64	32	>64	64	64	32	64	>64
9l	2-CN	$NHMe_2$	>64	>64	>64	>64	>64	>64	>64	>64
9m	4-NO_2	$NHMe_2$	32	>64	64	>64	>64	64	>64	>64
Gatifloxacin			0.25	8	2	0.25	nd	2	1	0.5
Moxifloxacin			0.25	4	1	0.25	nd	2	1	0.5
Fluconazole			nd[j]	nd	nd	nd	1	nd	nd	nd
Trimethoprim			32	4	4	0.5	2	4	32	2

[a] MICs were determined by micro broth dilution method for microdilution plates. [b] *S. aureus* 4220. [c] QRSA 3505. [d] MRSA 3506. [e] *S. mutans* 3289. [f] *Candida albicans* 7535. [g] *E. coli* 1924. [h] *Pseudomonas aeruginosa* 2742. [i] *Salmonella typhimurium* 2421. [j] nd: Not determined.

In vitro cytotoxic activity

Compound **8a** was assessed on its cytotoxic effect against two human cell lines: HCT116 and L02 to determine whether the compound **8a** has inhibitory influence on host cells. As shown in Table 2, the result indicated that the compound **8a** showed no appreciable cytotoxic activity (IC_{50} > 100 μmol/L against HCT116 and L02 cells), suggesting that the promising starting point for further optimisation.

Table 2. Cytotoxicity activity (IC_{50}[a] μmol/L) of compound **8a** against HCT116 and L02 cells.

Compound	In vitro cytotoxicity activity (IC_{50}[a] μmol/L)	
	HCT116[b]	L02[c]
8a	> 100	> 100

[a] IC_{50} is defined as the concentration to inhibit the cell growth by 50%.

[b] Human colon cancer cells.

[c] Human normal hepatic cells.

Molecular docking

It has reported that dihydrotriazine and its derivatives could effectively interact with dihydrofolate reductase (DHFR). The preferred coordination modes of **8a** and **9a** with the *S. aureus* DHFR protein are presented in Fig. 3. The structure data were obtained from the protein data bank (PDB ID: 3fra).[22-24] The output poses of the ligands generated were analysed based on the LibDockScore function. The structures of **8a** and **9a** were sketched in 2D and converted into 3D using the DS molecule editor (Fig. 3B, E). Compound **8a** is bound into the active site, in which the benzene ring formed pi-pi T-shaped bond with Phe92. The thiazine N atom of **8a** formed conventional hydrogen bond with Ile14 and the quinolin ring of **8a** formed conventional hydrogen bond with the backbone of Gln19. The 3-Cl substituted phenyl ring of **8a** formed pi-alkyl bond with Arg44. Compound **9a** is bound into the active site where the thiazine N atom shows interaction with Gln19 and Ile14. Furthermore, the enzyme surface model is shown in Figure 3C and 3F, which revealed that compound **8a** was well inserted into the active pocket of *S. aureus* DHFR, comparably indicated its more potent activity than that of **9a**. To summarise, our docking results suggested that **8a**, the compound with the most therapeutic potential, strongly interacted with the critical active-site residues of DHFR.

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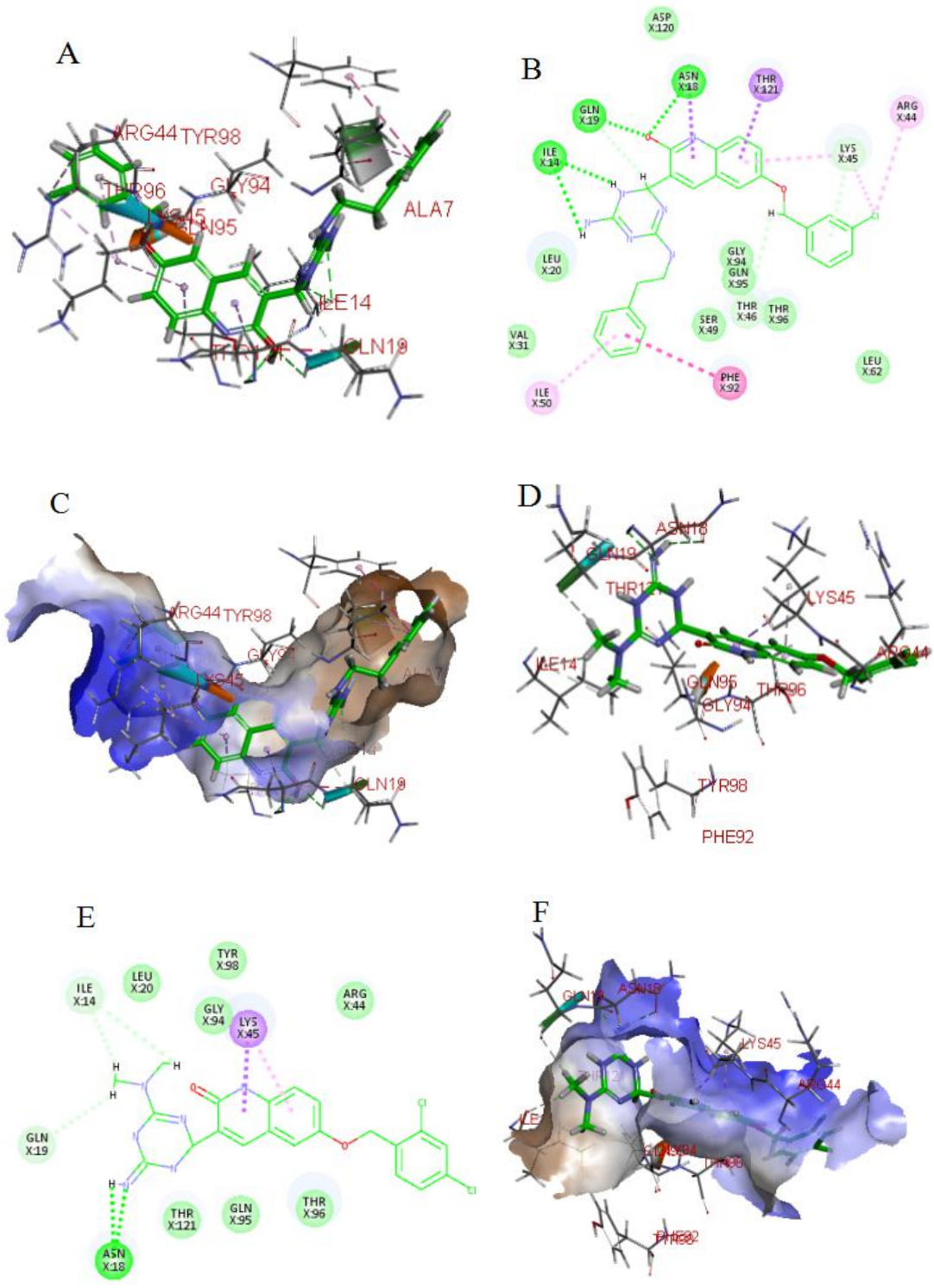

Figure 3. (A) Three-dimensional conformation of compound **8a** docked in DHFR complex. (B) 2D molecular docking modeling of compound **8a** with 3fra. (C) Interaction of **8a** with DHFR inside the binding pocket. (D) Three-dimensional conformation of compound **9a** docked in DHFR complex. (E) 2D molecular docking modeling of compound **9a** with 3fra. (F) Interaction of **9a** with DHFR inside the binding pocket.

Inhibition studies of compound 8a with DHFR

To verify the if compound **8a** does indeed bind to and block the active site of DHFR, we performed in vitro enzyme assays to test the inhibitory effect of compound **8a** (MIC of 1 μg/mL) on DHFR activity (Figure 4). At concentrations of 50 μmol/L, compound **8a** decreased DHFR activity by 81% compared with the negative control. These results confirm that compound **8a** also displayed DHFR inhibition.

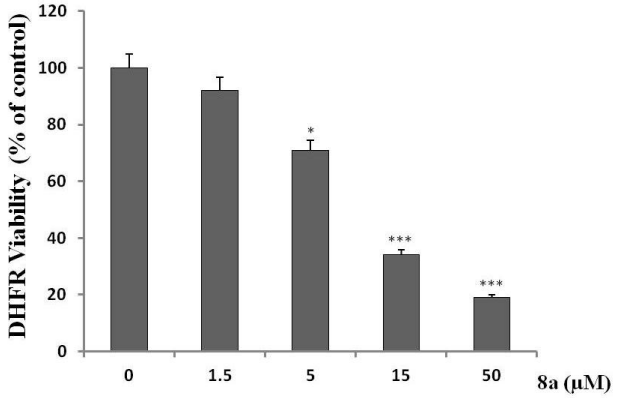

Figure 4. Inhibition of DHFR activities of compound **8a**.

Conclusions

We have synthesized three series of dihydrotriazine derivatives containing a quinoline moiety and evaluated their antibacterial and antifungal activities. The results indicated that the majority of the target compounds exhibited moderate to good levels of antibacterial activity against Gram-positive bacteria. In particular, all of the compounds in series **8** exhibited good levels of antibacterial activities against Gram-negative strains (*E. coli* 1924, *P. aeruginosa* 2742, *S. typhinurium* 2421) and antifungal activity (*C. albicans* 7535). These results suggested that the dihydrotriazine derivatives bearing a quinoline moiety, which plays a critical role in increasing the antibacterial properties of the compounds. *In vitro* enzyme study implied that compound **8a** also displayed DHFR inhibition. These findings suggest that these compounds are a good starting point for the rational development of novel antibacterial agents.

Experimental section

All chemicals, reagents, and solvents were purchased from Aladdin (Shanghai, China) or Macklin Reagent (Shanghai, China), and were used directly as purchased without further purification. Chemical shifts in ^{1}H NMR and ^{13}C NMR spectra are reported in parts per million (ppm) relative to tetramethylsilane (TMS). ^{1}H and ^{13}C-NMR spectra were performed on an AV-300 or AV-500 spectrometer (Bruker, Zurich, Switzerland) operating at 300 MHz for ^{1}H and 126 MHz for ^{13}C and using DMSO-d_6 as solvent. High Resolution Mass Spectrometry was measured on a Thermo Scientific LTQ Orbitrap XL spectrometer. Distilled water was self-prepared in our laboratory.

General procedures for the synthesis of 5a-b, 8a-c, and 9a-m

Intermediates **4** and **7** were synthesized using the reported procedure.[15] A solution of intermediate compounds (1mmol) in glacial acetic acid (7mL), Metformin hydrochloride or phenformin hydrochloride (1mmol) were added successively, and the mixture was stirred at 120 ℃ for 4-6h. The solvent was removed under diminished pressure, and the residue was purified by column chromatography using 20:1 dichloromethane/methanol to obtain target compounds.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-(pentyloxy)quinolin-2-ol (5a):

White solid, Yield 40%; m.p. 129-131℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.12 (s, 1H, NH), 8.67 (s, 2H, NH_2), 7.73 (s, 1H, Ar-H), 7.38-7.31 (m, 2H, Ar-H), 7.19 (dd, J = 9.0, 2.7 Hz, 1H, Ar-H), 5.85 (s, 1H, CH), 3.99 (t, J = 6.5 Hz, 2H, CH_2), 3.12 (s, 6H, CH_3), 1.77-1.69 (m, 2H, CH_2), 1.06 (t, J = 7.0 Hz, 3H, CH_3), 0.90 (t, J = 7.0 Hz, 4H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.69, 157.81, 156.43, 154.33, 135.04, 133.30, 131.23, 121.25, 116.98, 110.89, 68.41, 59.20, 56.47, 37.48 (2C), 28.83, 28.19, 22.35, 14.38. HRESI-MS m/z Anal. for $C_{19}H_{27}N_6O_2$: Calc. Mass 371.21900. Found 371.21942.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-(hexyloxy)quinol

in-2-ol (5b):

Yellow solid, Yield 40%; m.p. 64-66℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.15 (s, 1H, NH), 8.64 (s, 2H, NH_2), 7.73 (s, 1H, Ar-H), 7.35 (dd, J = 13.5, 5.8 Hz, 2H, Ar-H), 7.19 (dd, J = 9.0, 2.7 Hz, 1H, Ar-H), 5.85 (s, 1H, CH), 3.99 (t, J = 6.5 Hz, 2H, CH_2), 3.12 (s, 6H, CH_3), 1.83-1.65 (m, 4H, CH_2), 1.42 (t, J = 7.0 Hz, 3H, CH_3), 0.88 (t, J = 7.0 Hz, 4H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.67, 156.39, 154.36, 135.06, 131.17, 121.28, 119.52, 116.93, 110.90, 68.42, 59.15, 56.42, 48.15, 37.45 (2C), 31.45, 29.11, 25.66, 22.53, 14.35. HRESI-MS m/z Anal. for $C_{20}H_{29}N_6O_2$: Calc. Mass 385.23465. Found 385.23516.

3-(4-Amino-6-(phenethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((3-chlorobenzyl)oxy)quinolin-2-ol (8a):

White solid, Yield 48%; m.p. 110-112℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.66 (s, 1H), 7.51 (s, 1H, Ar-H), 7.45-7.37 (m, 4H, Ar-H), 7.35-7.31 (m, 2H, Ar-H), 7.28-7.15 (m, 5H, Ar-H), 5.84 (s, 1H, CH), 5.17 (s, 2H, CH_2), 3.50 (t, J = 7.0 Hz, 2H, CH_2), 3.14 (dd, J = 7.7, 6.1 Hz, 1H, NH), 2.80 (d, J = 6.5 Hz, 2H, CH_2), 1.81 (s, 1H, NH). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.63, 158.24, 157.07, 153.84, 139.90, 139.21, 134.42, 133.65, 133.46, 131.46 , 130.72, 129.07, 128.69, 128.17, 127.64, 126.61, 126.46, 121.40, 119.37, 117.05, 111.42, 69.20, 58.87, 56.41, 41.73, 35.44, 18.70. HRESI-MS m/z Anal. for $C_{27}H_{26}ClN_6O_2$: Calc. Mass 501.18003. Found 501.18036.

3-(4-Amino-6-(phenethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2,4-dichlorobenzyl)oxy)quinolin-2-ol (8b):

White solid, Yield 48%; m.p. 165-167℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.32 (s, 1H, NH), 8.41 (s, 2H, NH_2), 7.73-7.62 (m, 4H, Ar-H), 7.52-7.45 (m, 2H, Ar-H), 7.35 (d, J = 2.7 Hz, 1H, Ar-H), 7.22 (dd, J = 17.5, 7.6 Hz, 5H, Ar-H), 5.85 (s, 1H, CH), 5.20 (s, 2H, CH_2), 3.50 (s, 2H, CH_2), 3.17 (d, J = 3.8 Hz, 1H, NH), 2.79 (s, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.72, 158.36, 157.24, 153.70, 139.30, 134.59, 134.05, 133.89, 133.78, 131.81, 131.68, 129.41, 129.18, 128.77, 128.03, 126.69,

121.43, 119.43, 117.22, 111.55, 67.33, 59.02 , 56.49, 49.05, 41.92, 35.53, 19.03. HRESI-MS m/z Anal. for $C_{27}H_{25}Cl_2N_6O_2$: Calc. Mass 535.14106. Found 535.14160.

6-((2,6-Dichlorobenzyl)oxy)-3-(4-hydroxy-6-(phenethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)quinolin-2-ol (8c):

White solid, Yield 55%; m.p. 234-236℃. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 12.17 (s, 1H, NH), 8.49 (s, 2H, NH_2), 7.72 (s, 1H, NH_2), 7.54 (ddd, *J* = 17.1, 8.8, 1.8 Hz, 4H, Ar-H), 7.29 (ddd, *J* = 23.7, 18.4, 9.7 Hz, 7H, Ar-H), 5.86 (s, 1H, CH), 5.29 (s, 2H, CH_2), 4.05 (s, 2H, CH_2), 2.80 (s, 2H, CH_2). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.73, 158.37, 157.23, 154.18, 139.30, 136.53, 134.54, 133.83, 132.15, 132.02, 131.68, 129.29, 129.18, 128.77, 126.69, 121.43, 119.42, 117.21, 111.41, 66.03, 58.98, 56.49, 41.91, 35.53, 19.03. HRESI-MS m/z Anal. for $C_{27}H_{25}Cl_2N_6O_2$: Calc. Mass 535.14106. Found 535.14148.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2,4-dichlorobenzyl)oxy)quinolin-2-ol (9a):

White solid, Yield 42%; m.p. 212-214℃. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 12.15 (s, 1H, NH), 8.75 (s, 1H, NH_2), 8.42 (s, 1H, NH_2), 7.77 (s, 1H, Ar-H), 7.71 (d, *J* = 2.0 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.56-7.48 (m, 2H, Ar-H), 7.35 (dd, *J* = 10.5, 5.7 Hz, 2H, Ar-H), 5.76 (s, 1H, CH), 5.19 (s, 2H, CH_2), 3.12 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.72, 157.72, 156.42, 153.67, 135.01, 134.06, 133.89, 131.83, 131.38, 129.41, 128.05, 121.37, 119.50, 117.12, 111.71, 67.31, 59.20, 56.48, 55.39, 37.48, 19.03. HRESI-MS m/z Anal. for $C_{21}H_{21}Cl_2N_6O_2$: Calc. Mass 459.10976. Found 459.11075.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2,6-dichlorobenzyl)oxy)quinolin-2-ol (9b):

White solid, Yield 42%; m.p. 286-290℃. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 12.17 (s, 1H, NH), 8.82 (s, 1H, NH_2), 8.45 (s, 1H, NH_2), 7.76 (s, 1H, Ar-H), 7.64-7.56 (m, 3H, Ar-H), 7.52-7.47 (m, 1H, Ar-H), 7.36 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.29 (dd, *J* = 9.0, 2.5 Hz, 1H, Ar-H), 5.87 (s, 1H, CH), 5.28 (s, 2H, CH_2), 3.15 (s, 6H, CH_3). ^{13}C NMR (126

MHz, DMSO-d_6) δ 160.74, 157.74, 156.43, 154.11, 136.52, 134.95, 133.86, 132.16, 132.00, 131.39, 129.29, 121.39, 119.47, 117.13, 111.53, 65.99, 59.15, 56.47, 49.04, 37.49, 19.03. HRESI-MS m/z Anal. for $C_{21}H_{21}Cl_2N_6O_2$: Calc. Mass 459.10976. Found 459.11066.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2-chlorobenzyl) oxy)quinolin-2-ol (9c):

White solid, Yield 46%; m.p. 252-254℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H, NH), 8.81 (s, 1H, NH_2), 8.45 (s, 1H, NH_2), 7.77 (s, 1H, Ar-H), 7.66-7.62 (m, 1H, Ar-H), 7.57-7.51 (m, 2H, Ar-H), 7.43-7.39 (m, 2H, Ar-H), 7.37-7.31 (m, 2H, Ar-H), 5.86 (s, 1H, CH), 5.20 (s, 2H, CH_2), 3.13 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.72, 157.75, 156.42, 153.84, 135.04, 134.64, 133.74, 133.14, 131.36, 130.67, 129.88, 121.38, 119.51, 117.11, 111.59, 67.89, 59.19, 56.47, 49.04, 37.49, 19.02. HRESI-MS m/z Anal. for $C_{21}H_{22}ClN_6O_2$: Calc. Mass 425.14873. Found 425.14960.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((3-chlorobenzyl) oxy)quinolin-2-ol (9d):

White solid, Yield 46%; m.p. 169-171℃. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.15 (d, J = 5.9 Hz, 1H, NH), 8.85 (d, J = 30.3 Hz, 1H, NH_2), 8.48 (d, J = 16.5 Hz, 1H, NH_2), 7.73 (s, 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.50 (d, J = 2.6 Hz, 1H, Ar-H), 7.45-7.35 (m, 4H, Ar-H), 7.31 (dd, J = 8.9, 2.2 Hz, 1H, Ar-H), 5.88-5.84 (m, 1H, CH), 5.17 (s, 2H, CH_2), 3.12 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.70, 157.73, 156.42, 153.74, 139.94, 134.97, 133.63, 131.34, 130.87, 128.29, 127.80, 126.68, 121.36, 119.47, 117.06, 111.72, 69.26, 59.21, 56.48, 37.47, 19.03. HRESI-MS m/z Anal. for $C_{21}H_{22}ClN_6O_2$: Calc. Mass 425.14873. Found 425.14819.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((4-chlorobenzyl) oxy)quinolin-2-ol (9e):

White solid, Yield 44%; m.p. 188-190℃. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.14 (s, 1H, NH), 8.79 (d, J = 3.3 Hz, 1H, NH_2), 8.45 (s, 1H, NH_2), 7.73 (s, 1H, Ar-H), 7.48 (d,

J = 2.8 Hz, 5H, Ar-H), 7.38-7.28 (m, 2H, Ar-H), 5.86 (s, 1H, CH), 5.15 (s, 2H, CH_2), 3.14 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.69, 157.75, 156.41, 153.79, 136.40, 134.98, 133.62, 132.94, 131.32, 130.00, 128.93, 121.39, 119.47, 117.05, 111.71, 69.36, 59.20, 56.48, 55.39, 37.47, 19.02. HRESI-MS m/z Anal. for $C_{21}H_{22}ClN_6O_2$: Calc. Mass 425.14873. Found 425.14877.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-(benzyloxy)quinolin-2-ol (9f):

Yellow solid, Yield 55%; m.p. 212-214℃. ^1H NMR (300 MHz, DMSO-d_6) δ 12.14 (s, 1H, NH), 8.83 (s, 1H, NH_2), 8.47 (s, 1H, NH_2), 7.74 (s, 1H, Ar-H), 7.45 (ddd, *J* = 15.8, 12.0, 5.0 Hz, 6H, Ar-H), 7.35-7.26 (m, 2H, Ar-H), 5.76 (s, 1H, CH), 5.14 (s, 2H, CH_2), 3.12 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.69, 157.77, 156.42, 153.99, 137.33, 135.01, 133.55, 131.29, 128.92, 128.37, 128.21, 121.42, 119.48, 117.03, 111.59, 70.21, 59.20, 56.47, 55.40, 37.49, 19.03. HRESI-MS m/z Anal. for $C_{21}H_{23}N_6O_2$: Calc. Mass 391.18770. Found 391.18710.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2-fluorobenzyl)oxy)quinolin-2-ol (9g):

Yellow solid, Yield 44%; m.p. 265-267℃. ^1H NMR (300 MHz, DMSO-d_6) δ 12.16 (s, 1H, NH), 8.85 (s, 1H, NH_2), 8.47 (s, 1H, NH_2), 7.76 (s, 1H, Ar-H), 7.58 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.44 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.37 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.31 (d, *J* = 2.6 Hz, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 5.86 (s, 1H, CH), 5.18 (s, 2H, CH_2), 3.13 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.71, 157.75, 156.42, 153.82, 135.01, 133.70, 131.33, 131.11, 131.08, 130.94, 130.88, 121.40, 119.49, 117.07, 111.62, 64.55, 59.20, 56.48, 49.04, 37.49, 19.02. HRESI-MS m/z Anal. for $C_{21}H_{22}FN_6O_2$: Calc. Mass 409.17828. Found 409.17871.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((4-fluorobenzyl)oxy)quinolin-2-ol (9h):

Yellow solid, Yield 52%; m.p. 199-201℃. ^1H NMR (300 MHz, DMSO-d_6) δ 12.14 (s, 1H, NH), 8.70 (s, 2H, NH_2), 7.73 (s, 1H, Ar-H), 7.55-7.49 (m, 3H, Ar-H), 7.35-7.28

(m, 2H, Ar-H), 7.23 (t, *J* = 8.7 Hz, 2H, Ar-H), 5.85 (s, 1H, CH), 5.12 (s, 2H, CH_2), 3.11 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.73, 157.82, 156.49, 153.88, 135.03, 133.58, 131.43, 130.51, 130.44, 121.41, 119.50, 117.03, 115.83, 115.66, 111.62, 69.50, 59.32, 56.48, 55.39, 37.45, 19.02. HRESI-MS m/z Anal. for $C_{21}H_{22}FN_6O_2$: Calc. Mass 409.17828. Found 409.17862.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((2-bromobenzyl) oxy)quinolin-2-ol (9i):

Yellow solid, Yield 42%; m.p. 284-286℃. ^1H NMR (300 MHz, DMSO-*d*$_6$) δ 7.78 (s, 1H, Ar-H), 7.69 (d, *J* = 7.9 Hz, 1H, Ar-H), 7.62 (d, *J* = 7.6 Hz, 1H, Ar-H), 7.55 (s, 1H, Ar-H), 7.43 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.37-7.29 (m, 3H, Ar-H), 5.86 (s, 1H, CH), 5.16 (s, 2H, CH_2), 3.12 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.62, 157.58, 156.34, 153.87, 136.21, 135.06, 133.13, 130.83, 130.69, 128.40, 123.41, 121.40, 119.48, 117.05, 111.59, 70.11, 59.15, 56.49, 56.37, 37.44, 18.96. HRESI-MS m/z Anal. for $C_{21}H_{22}BrN_6O_2$: Calc. Mass 469.09821. Found 469.09894.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((3-methylbenzy l)oxy)quinolin-2-ol (9j):

Yellow solid, Yield 52%; m.p. 154-156℃. ^1H NMR (300 MHz, DMSO-*d*$_6$) δ 12.43 (s, 1H, NH), 8.62 (s, 2H, NH_2), 7.74 (s, 1H, Ar-H), 7.49 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.29 (dd, *J* = 12.1, 7.6 Hz, 5H, Ar-H), 7.15 (d, *J* = 7.2 Hz, 1H, Ar-H), 5.85 (s, 1H, CH), 5.10 (s, 2H, CH_2), 3.11 (s, 6H, CH_3), 2.32 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.71, 157.77, 156.49, 154.05, 138.09, 137.23, 135.06, 133.47, 131.38, 129.01, 128.80, 125.30, 121.40, 119.50, 116.99, 111.54, 70.24, 59.33, 56.48, 37.43, 21.46, 19.01. HRESI-MS m/z Anal. for $C_{22}H_{25}N_6O_2$: Calc. Mass 405.20335. Found 405.20380.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((4-methylbenzy l)oxy)quinolin-2-ol (9k):

Yellow solid, Yield 48%; m.p. 159-161℃. ^1H NMR (300 MHz, DMSO-*d*$_6$) δ 12.12 (s, 1H, NH), 8.74 (s, 1H, NH_2), 8.41 (s, 1H, NH_2), 7.73 (s, 1H, Ar-H), 7.50 (d, *J* = 2.4 Hz,

1H, Ar-H), 7.38-7.31 (m, 3H, Ar-H), 7.27 (dd, *J* = 9.0, 2.5 Hz, 1H, Ar-H), 7.21 (d, *J* = 8.0 Hz, 2H, Ar-H), 5.86 (s, 1H, CH), 5.09 (s, 2H, CH_2), 3.12 (s, 6H, CH_3), 2.31 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.70, 157.71, 156.42, 154.03, 137.63, 135.01, 134.27, 133.49, 131.24, 129.47, 128.31, 121.48, 119.48, 116.99, 111.63, 70.13, 59.23, 56.48, 55.39, 37.47, 21.25, 19.03. HRESI-MS *m/z* Anal. for $C_{22}H_{25}N_6O_2$: Calc. Mass 405.20335. Found 405.20364.

2-(((3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-2-hydroxyquinolin-6-yl)oxy)methyl)benzonitrile (9l):

White solid, Yield 48%; m.p. 260-262℃. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 7.90 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.79-7.73 (m, 3H, Ar-H), 7.58 (dd, *J* = 9.7, 5.8 Hz, 2H, Ar-H), 7.39-7.28 (m, 2H, Ar-H), 5.87 (s, 1H, CH), 5.28 (s, 2H, CH_2), 3.11 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.78, 157.76, 156.53, 153.72, 140.30, 135.04, 133.96, 133.85, 133.82, 131.56, 130.19, 129.69, 121.31, 119.51, 117.71, 117.11, 111.83, 68.58, 59.40, 56.48, 37.43, 19.03. HRESI-MS *m/z* Anal. for $C_{22}H_{22}N_7O_2$: Calc. Mass 416.18295. Found 416.18335.

3-(4-Amino-6-(dimethylamino)-2,5-dihydro-1,3,5-triazin-2-yl)-6-((4-nitrobenzyl)oxy)quinolin-2-ol (9m):

Yellow solid, Yield 52%; m.p. 220-222℃. ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 12.13 (s, 1H, NH), 8.57 (s, 2H, NH_2), 8.27 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.74 (d, *J* = 9.0 Hz, 3H, Ar-H), 7.51 (s, 1H, Ar-H), 7.34 (d, *J* = 2.5 Hz, 2H, Ar-H), 5.85 (s, 1H, CH), 5.33 (s, 2H, CH_2), 3.11 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 160.72, 157.77, 156.46, 153.56, 147.52, 145.28, 134.95, 133.76, 131.49, 128.73, 124.11, 121.31, 119.49, 117.13, 111.79, 68.99, 59.26, 56.48, 49.05, 37.45, 19.02. HRESI-MS *m/z* Anal. for $C_{21}H_{22}N_7O_4$: Calc. Mass 436.17278. Found 436.17273.

In vitro inhibition of bacterial and fungal growth

Determination of minimal inhibitory concentrations (MIC) was performed as described previously.[16] Testing was performed by the standard broth microdilution method with trimethoprim, moxifloxacin and gatifloxacin. All stock solutions of the

compounds were dissolved in DMSO. A two-fold serial dilution technique was used to obtain final concentrations of 64-0.25 μg/mL.

Molecular modeling

All docking runs were carried out using Discovery Studio v17.1.0.16143. The crystal structure data (*S. aureus* DHFR) were obtained from the protein data bank (PDB ID: 3fra). The water molecules and heavy atom in protein were removed and the protein was prepared by adding hydrogen and correcting incomplete residues using Clean Protein tool of DS, then the protein was refined with CHARMm.

Inhibition of DHFR activities in vitro

Solid-phase antibody was prepared by coating the microtiter plate wells with purified human dihydrofolate reductase (DHFR) antibody. Determination of DHFR activities was performed as described previously.[16] All experiments were performed in triplicates and results were presented as the average of three independent measurements.

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Author Contribution Statement

T. Y. Zhang and B. Feng designed the experiments, performed the separation experiments and structural characterization, wrote the article. X. Q. Bai performed the bioactivity assay and docking study. Y. Chen provided the guidance and revised the manuscript. Z. Liu and L. H. Zhao performed the synthesis of compounds.

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