Molecular Diversity
https://doi.org/10.1007/s11030-019-10019-8

ORIGINAL ARTICLE

Synthesis and molecular docking studies of novel pyrimidine derivatives as potential antibacterial agents

Xue-Qian Bai[1] · Chun-Shi Li[2] · Ming-Yue Cui[2] · Ze-Wen Song[1,3] · Xing-Yu Zhou[1] · Chao Zhang[1] · Yang Zhao[1] · Tian-Yi Zhang[1] · Tie-Yan Jiang[4]

Received: 9 September 2019 / Accepted: 21 November 2019

Abstract

The present work describes the in vitro antibacterial evaluation of some new pyrimidine derivatives. Twenty-two target compounds were designed, synthesized and preliminarily explored for their antimicrobial activities. The antimicrobial assay revealed that some target compounds exhibited significantly inhibitory efficiencies toward bacteria and fungal including drug-resistant pathogens. Compound **7c** presented the most potent inhibitory activities against Gram-positive bacteria (e.g., *Staphylococcus aureus* 4220), Gram-negative bacteria (e.g., *Escherichia coli* 1924) and the fungus *Candida albicans* 7535, with an MIC of 2.4 μmol/L. Compound **7c** was also the most potent, with MICs of 2.4 or 4.8 μmol/L against four multidrug-resistant, Gram-positive bacterial strains. The toxicity evaluation of the compounds **7c**, **10a**, **19d** and **26b** was assessed in human normal liver cells (L02 cells). Molecular docking simulation and analysis suggested that compound **7c** has a good interaction with the active cavities of dihydrofolate reductase (DHFR). In vitro enzyme study implied that compound **7c** also displayed DHFR inhibition.

Xue-Qian Bai and Chun-Shi Li have contributed equally to this work.

✉ Tian-Yi Zhang
tianyizhang@126.com

✉ Tie-Yan Jiang
jty781213@sina.com

[1] Jilin Medical University, Jilin 132013, People's Republic of China

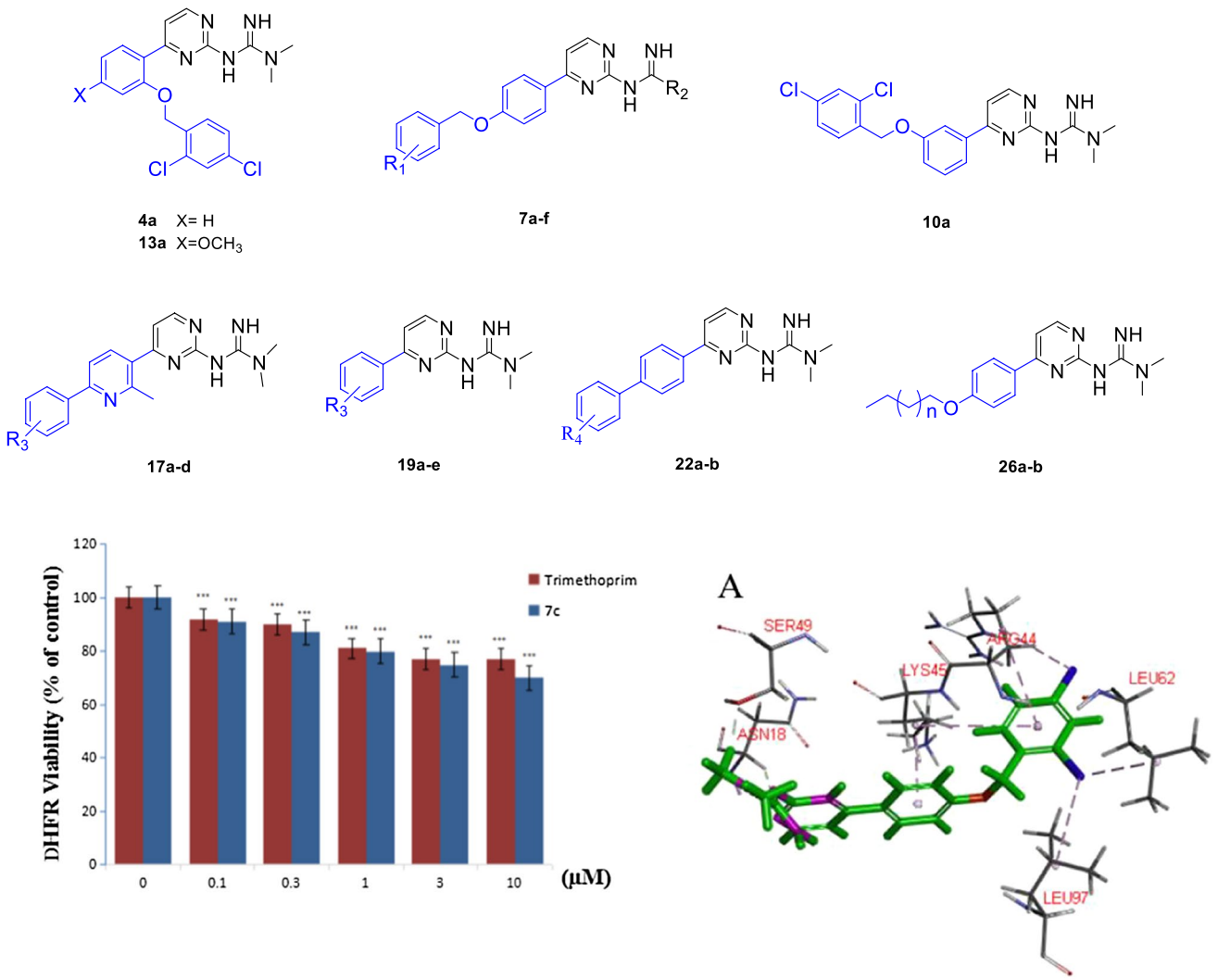
[2] The Third People's Hospital of Dalian, Dalian 116000, People's Republic of China

[3] Department of Pharmary, Yanbian University, Yanji 133002, People's Republic of China

[4] Changning Branch of Shanghai Municipal Public Security Bureau, Shanghai 200336, People's Republic of China

Published online: 02 December 2019

Graphic abstract



Keywords Pyrimidine · Antibacterial activity · Toxicity · Molecular docking · DHFR inhibition

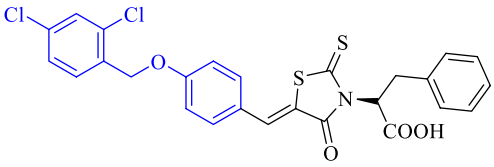
Introduction

Infections caused by bacterial resistance to the major classes of therapeutic drugs have become one of the greatest threats to public health problems [1]. Drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli*, cause great difficulties in the treatment of nosocomial infections [2–5], which severely threaten global public health [6]. Drug-resistant bacteria and the simultaneous decline in efforts by academic laboratories or pharmaceutical companies directed toward the discovery of new antibacterial agents to combat resistant strains now pose a serious threat to the treatment of life-threatening infections [7]. "The cost in terms of lost global production between now and 2050 would be an enormous 100 trillion USD if we do not take action," as a UK Government report states [8]. Furthermore, fungal infections pose a serious threat to human health, especially to immunocompromised patients [9, 10]. The clinical treatment of life-threatening invasive fungal infections (IFIs) is also a significant global challenge [11, 12]. Therefore, this highlights the need for development of new antimicrobial agents that differ from those of existing agents [13].

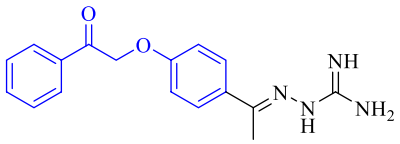
Compounds based on the pyrimidine scaffold are known to exhibit many different biological actions such as antibacterial, antifungal, anti-inflammatory and antitumor activities [14]. Lots of amino pyrimidine-based derivatives have been reported to exhibit antibacterial activities

via inhibiting dihydrofolate reductase (DHFR) [15]. Based on our previous work, several rhodanine or aminoguanidine derivatives bearing (benzyloxy)benzylidene [16, 17] moieties showed moderate activities against several Gram-positive bacterial strains, including multidrug-resistant clinical isolates as exemplified by compounds **A** and **B** (Fig. 1). Furthermore, non-fused pyridines constitute another important unit of heterocycles, which exhibited various biological activities [18]. Mohammed A. Seleem et al. reported that compounds **C** and **D** (Fig. 1) exhibited potent activity against MRSA RCMB 2658 strain (MIC = 0.4 μg/mL or 1.56 μg/mL) in vitro [19]. Herein, we design of new compounds using **A**–**D** as lead compounds, and 22 synthesized compounds were screened for their antibacterial activities with a minimum inhibitory concentration method (Fig. 2).

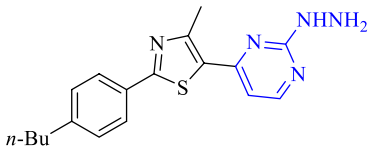
Fig. 1 Several molecules of previously reported antibacterial agents



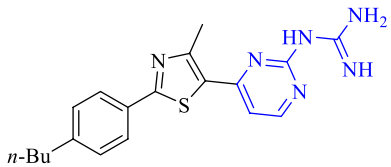
A **MIC** = 2 μg/mL (*S. aureus*)



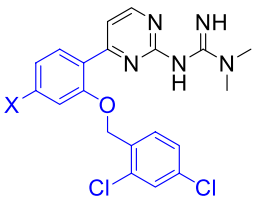
B **MIC** = 16 μg/mL (*S. aureus*)



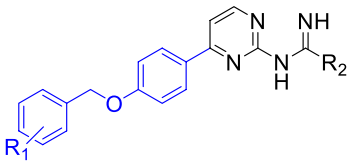
C **MIC** = 0.4 μg/mL (MRSA)



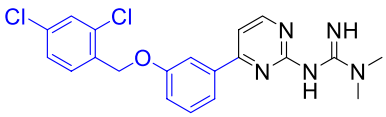
D **MIC** = 1.56 μg/mL (MRSA)



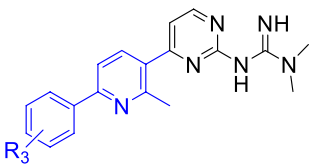
4a X= H
13a X=OCH_3



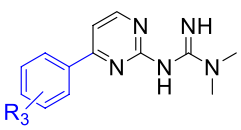
7a R_1=2,6-di-Cl R_2=NMe_2 **7d** R_1=4-Br R_2=NMe_2
7b R_1=4-CH_3 R_2=NMe_2 **7e** R_1=4-CN R_2=NMe_2
7c R_1=2,4-di-Cl R_2=NMe_2 **7f** R_1=2,4-di-Cl R_2=morpholino



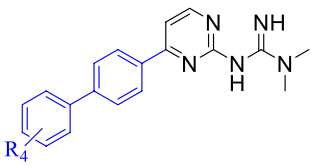
10a



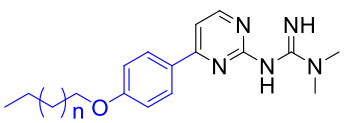
17a R_3=*n*-pentyl
17b R_3=4-CH_3
17c R_3=2,4-di-Cl
17d R_3=4-Br



19a R_3=2,4-di-Cl
19b R_3=phenyl(3,4-fused)
19c R_3=4-Br
19d R_3=*n*-pentyl
19e R_3=NMe_2

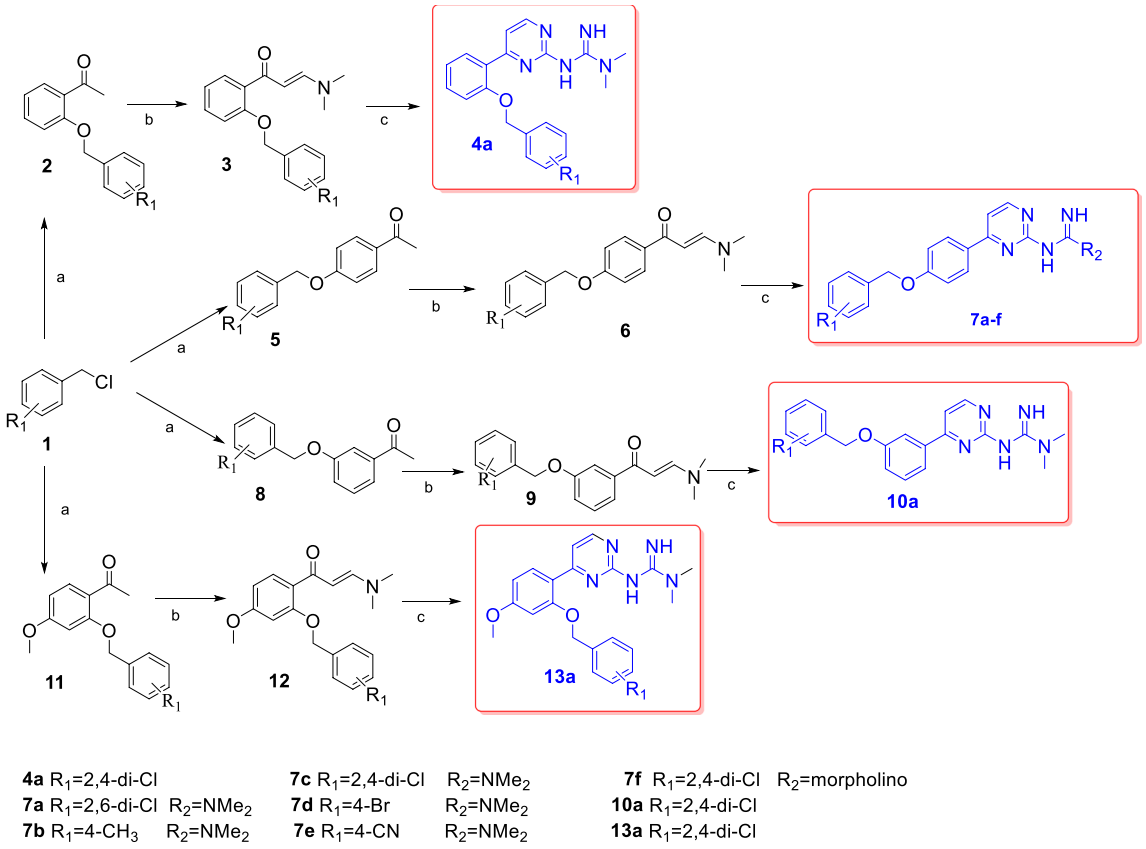


22a R_4=H
22b R_4=2,4-di-Cl



26a n=2
26b n=3

Fig. 2 Synthesis of target compounds



4a R_1=2,4-di-Cl
7a R_1=2,6-di-Cl R_2=NMe_2
7b R_1=4-CH_3 R_2=NMe_2
7c R_1=2,4-di-Cl R_2=NMe_2
7d R_1=4-Br R_2=NMe_2
7e R_1=4-CN R_2=NMe_2
7f R_1=2,4-di-Cl R_2=morpholino
10a R_1=2,4-di-Cl
13a R_1=2,4-di-Cl

Scheme 1 **a** DMF, K_2CO_3, 70–80 °C, reflux, 4–7 h; **b** DMF-DMA, DMF, 80 °C, 12 h; **c** moroxydine hydrochloride or metformin hydrochloride, AcOH, 120 °C, reflux, 8–12 h

Results and discussion

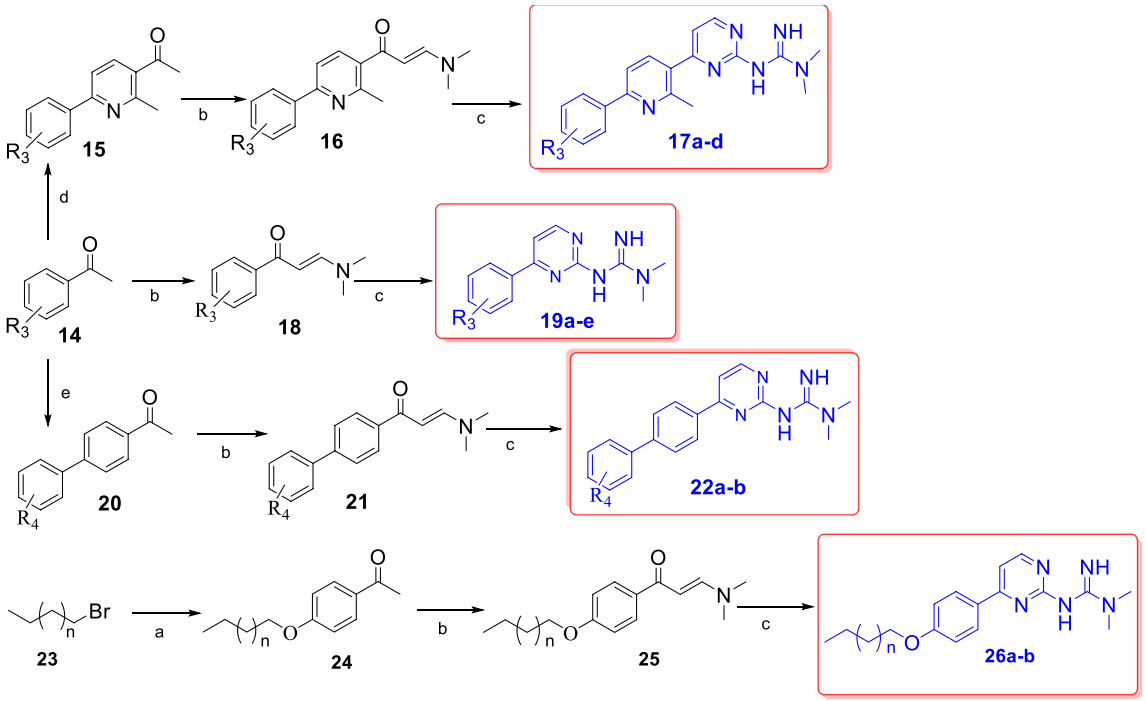
Chemistry

The synthesis of target compounds is outlined in Schemes 1 and 2. Intermediates **2**, **5**, **8**, **11** and **24** were obtained by reacting hydroxyacetophenone or 2′-hydroxy-4′-methoxyacetophenone with the appropriate substituted benzylchlorides or bromine alkane. Intermediates **3**, **6**, **9**, **12** and **25** were prepared using a previously reported method [17]. Then, the intermediates **3**, **6**, **9**, **12**, **16**, **18**, **21** and **25** were reacted with moroxydine hydrochloride or metformin hydrochloride to generate the pyrimidine derivatives **4a**, **7a**–**f**, **10a** and **13a** (Scheme 1) [19]. Intermediate **15** was prepared according to a previously reported method [20]. Intermediate **20** was prepared using Suzuki coupling reactions [21]. Compounds **17a**–**d**, **19a**–**e**, **22a**–**b** and **26a**-**b** were synthesized in the same way as compounds **4a**, **7a**–**f**, **10a** and **13a** (Scheme 2). The structures of the target compounds were confirmed by ^{1}H NMR, ^{13}C NMR, mass spectroscopy and HRMS.

In vitro antibacterial activity evaluation

Totally 22 target compounds were synthesized, and their structures and activities against susceptible and drug-resistant bacteria are listed in Tables 1 and 2, respectively. Gatifloxacin, trimethoprim, fluconazole and itraconazole were used for the antimicrobial activity as positive controls.

The in vitro antimicrobial activity of the target compounds is shown in Table 1. Most of the target compounds exhibited better bactericidal effect against the different bacteria. Compound **7c** had the strongest activity against the Gram-positive bacteria (*S. aureus* 4220), with an MIC value of 2.4 μmol/L. Against the Gram-negative *E. coli* 1924, compound **7c** was more potent than the positive controls trimethoprim, with an MIC of 2.4 μmol/L. Compounds **10a**, **17a** and **22b** were also equipotent potent with the positive controls gatifloxacin and moxifloxacin. Against the fungus *C. albicans* 7535, compound **7c** displayed the highest potency of all of the compounds with an MIC value of 2.4 μmol/L, which was more potent than that of fluconazole (MIC = 3.3 μmol/L). Compounds **7a**, **10a**, **17a**, **22b** and **26a**



17a R_3=*n*-pentyl
17b R_3=4-CH_3
17c R_3=2,4-di-Cl
17d R_3=4-Br
19a R_3=2,4-di-Cl
19b R_3=phenyl(3,4-fused)
19c R_3=4-Br
19d R_3=*n*-pentyl
19e R_3=NMe_2
22a R_4=H
22b R_4=2,4-di-Cl
26a n=2
26b n=3

Scheme 2 **a** DMF, K_2CO_3, 70–80 °C, reflux, 4–7 h; **b** DMF-DMA, DMF, 80 °C, 12 h; **c** metformin hydrochloride, AcOH, 120 °C, reflux, 8–12 h; **d** acetylacetone, AcOH, CH_3COONH_4, 110 °C, reflux, 3 h; **e** orthoboric acid, Na_2CO_3, DME, H_2O, 85 °C, 4 h

also displayed good potency, with MICs ranging from 4.8 to 5.8 µmol/L.

As depicted in Table 2, the target compounds were tested for their inhibitory activities against the clinical isolates of several different multidrug-resistant bacterial strains. Compound **7c** showed potent activity against the MRSA (3167 and 3506) strains, with an MIC value of 2.4 µmol/L. This was equivalent to moxifloxacin (MIC = 2.5 µmol/L) and greater than oxacillin (MIC > 151.2 µmol/L). Compounds **10a** and **17a** showed the equipotent or more potent than the positive controls gatifloxacin (MIC = 5.3 µmol/L) against the MRSA (3167 and 3506) strains. For the QRSA (CCARM 3505 and 3519) strains, compound **7c** also had the strongest inhibitory effect, with an MIC value of 2.4 µmol/L and 4.8 µmol/L. This was equivalent to oxacillin (MIC = 2.4 µmol/L) and stronger than norfloxacin (MIC > 200.4 µmol/L) and gatifloxacin (MIC = 21.3 µmol/L against CCARM 3505 and 10.6 µmol/L against CCARM 3519). Compounds **7a**, **7d** and **10a** were equipotent to the moxifloxacin (MIC = 10.0 µmol/L for QRSA 3505).

Based on the analysis of the activities of the synthesized compounds, the following structure–activity relationships (SARs) were obtained. The position of the 2,4-di-Cl benzyloxy group with respect to the benzene ring significantly influenced the antibacterial activity, with an activity order of *p* > *m* > *o* for compounds **4a**, **7c** and **10a**. Furthermore, the position of the di-Cl substituent on the benzyloxy moiety 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 **7a** and **7c**. A comparison of the potency of compounds **4a** and **13a** revealed that the introduction of an additional 4-OCH_3 moiety to the phenyl ring increased the antimicrobial activity. A comparison of the potency of compounds **7c** and **7f** revealed that the substitution of the dimethylamino group with a morpholino moiety reduced the antimicrobial activity. The inclusion of a *n*-pentyl group at the benzene ring, as exemplified by the compounds **17a** and **17b**, indicated that a suitable length of the molecule was critical for the activity.

Toxicity evaluation

We evaluated the toxicity of a normal human liver cell line (L02) using a standard technique. As can be seen from Table 3, compound **19d** exhibited weaker activity than **10a**

Table 1 Inhibitory activity (MIC[a], μmol/L) of compounds **4a**, **7a–f**, **10a**, **13a**, **17a–d**, **19a–e**, **22a–b** and **26a–b** against various bacteria

Compd	$R_1/R_3/R_4$	R_2	Gram-positive strains		Gram-negative strains	Fungus
			S. aureus	*S. mutans*	*E. coli*	*C. albicans*
			4220[b]	3289[c]	1924[d]	7535[e]
4a	2,4-di-Cl	-	> 153.8	> 153.8	> 153.8	> 153.8
7a	2,6-di-Cl	NMe_2	4.8	9.6	9.6	4.8
7b	4-CH_3	NMe_2	11.0	22.1	22.1	22.1
7c	2,4-di-Cl	NMe_2	2.4	4.8	2.4	2.4
7d	4-Br	NMe_2	9.4	18.7	18.7	9.4
7e	4-CN	NMe_2	85.8	171.6	85.8	85.8
7f	2,4-di-Cl	Morpholino	> 139.7	> 139.7	> 139.7	> 139.7
10a	2,4-di-Cl	–	4.8	9.6	4.8	4.8
13a	2,4-di-Cl	–	17.9	> 143.5	35.9	> 143.5
17a	*n*-pentyl	–	5.6	11.2	5.6	5.6
17b	4-CH_3	–	> 184.4	> 184.4	> 184.4	> 184.4
17c	2,4-di-Cl	–	> 159.6	> 159.6	> 159.6	159.6
17d	4-Br	–	77.9	155.7	155.7	155.7
19a	2,4-di-Cl	–	> 206.5	> 206.5	> 206.5	> 206.5
19b	Phenyl(3,4-fused)	–	109.6	219.2	109.6	109.6
19c	4-Br	–	> 199.4	> 199.4	> 199.4	> 199.4
19d	*n*-pentyl	–	25.6	51.3	25.6	25.6
19e	$NHMe_2$	–	> 224.6	> 224.6	> 224.6	> 224.6
22a	H	–	50.3	50.3	50.3	50.3
22b	2,4-di-Cl	–	5.2	5.2	5.2	5.2
26a	*n*-hexyl	–	5.8	11.7	11.7	5.8
26b	*n*-pentyl	–	24.4	48.8	48.8	48.8
Gatifloxacin			0.7	0.7	5.3	1.3
Moxifloxacin			0.6	0.6	5.0	1.2
Trimethoprim			110.2	1.7	13.8	6.9
Itraconazole			n.d[f]	n.d[f]	n.d[f]	0.85
Fluconazole			n.d[f]	n.d[f]	n.d[f]	3.3

[a]MICs were determined by microbroth dilution method for microdilution plates

[b]*Staphylococcus aureus* RN 4220

[c]*Streptococcus mutans* 3289

[d]*Escherichia coli* KCTC 1924

[e]*Candida albicans* 7535

[f]*n.d.* not determined

against the different bacteria, in spite of its greater cytotoxicity than **10a**, comparably indicating that the promising antibacterial activity of these compounds may not be due to their cytotoxicity, but some unknown mechanism of action.

Molecular docking

Molecular docking is a significant computational method used to forecast the binding of the ligand to the receptor binding site by varying position and conformation of the ligand keeping the receptor rigid. To evaluate the antibacterial mechanism of compounds action, a molecular docking investigation was undertaken. Compounds **7c** and **22b** being the most potent were selected as a template molecule. It was also interesting to start a comparative modeling study of the most active compounds **7c** and **22b** against Iclaprim (Fig. 3). The structure data were obtained from the protein data bank (PDB ID: 3fra) [22, 23]. The water molecules and heavy atom in protein were removed, the protein was prepared by adding hydrogen and correcting incomplete residues using Clean Protein tool of DS, and then the protein was refined with CHARMm. The structures of **7c** and **22b** were sketched in 2D and converted into 3D using the DS molecule editor (Fig. 3b, e). Compound **7c** is bound into the active site, in which the benzene ring formed alkyl bond (4.34 Å) with Arg44. The

Table 2 Inhibitory activity (MIC[a], μmol/L) of compounds **4a**, **7a–f**, **10a**, **13a**, **17a–d**, **19a–e**, **22a–b** and **26a–b** against clinical isolates of multidrug-resistant Gram-positive strains

Compd	$R_1/R_3/R_4$	R_2	Multidrug-resistant Gram-positive strains			
			MRSA		QRSA	
			3167[b]	3506[c]	3505[d]	3519[e]
4a	2,4-di-Cl	–	> 153.8	> 153.8	> 153.8	> 153.8
7a	2,6-di-Cl	NMe_2	9.6	9.6	9.6	9.6
7b	4-CH3	NMe_2	22.1	11	22.1	22.1
7c	2,4-di-Cl	NMe_2	2.4	2.4	2.4	4.8
7d	4-Br	NMe_2	9.4	18.7	9.4	9.4
7e	4-CN	NMe_2	85.8	85.8	85.8	85.8
7f	2,4-di-Cl	Morpholino	> 139.7	> 139.7	> 139.7	> 139.7
10a	2,4-di-Cl	–	4.8	4.8	9.6	4.8
13a	2,4-di-Cl	–	35.9	71.7	71.7	143.5
17a	*n*-pentyl	–	5.6	5.6	11.2	11.2
17b	4-CH_3	–	> 184.4	> 184.4	> 184.4	> 184.4
17c	2,4-di-Cl	–	> 159.6	> 159.6	> 159.6	> 159.6
17d	4-Br	–	> 155.7	77.9	> 155.7	77.9
19a	2,4-di-Cl	–	> 206.5	> 206.5	> 206.5	> 206.5
19b	Phenyl(3,4-fused)	–	109.6	109.6	219.2	109.6
19c	4-Br	–	> 199.4	> 199.4	> 199.4	> 199.4
19d	*n*-pentyl	–	25.6	25.6	25.6	26.5
19e	$NHMe_2$	–	> 224.6	> 224.6	> 224.6	> 224.6
22a	H	–	50.3	50.3	100.6	100.6
22b	2,4-di-Cl	–	5.2	5.2	5.2	5.2
26a	*n*-hexyl	–	11.7	5.8	11.7	11.7
26b	*n*-pentyl	–	48.8	24.4	48.8	48.8
Gatifloxacin			5.3	5.3	21.3	10.6
Moxifloxacin			2.5	2.5	10.0	10.0
Norfloxacin			25.1	12.5	> 200.4	> 200.4
Oxacillin			> 151.2	> 151.2	2.4	2.4
Trimethoprim			n.d[f]	13.8	13.8	n.d[f]

[a]The antibacterial testing was carried out three times, and the MICs are average of them

[b]Methicillin-resistant *S. aureus* 3167

[c]Methicillin-resistant *S. aureus* 3506

[d]Quinolone-resistant *S. aureus* 3505

[e]Quinolone-resistant *S. aureus* 3519

[f]*n.d.* not determined

Table 3 Antibacterial activity and cytotoxicity (IC^a_{50} μM) for **7c**, **10a**, **19d** and **26b** against L02 cell

	Test organisms	7c	10a	19d	26b
MIC (μmol/L)	*S. aureus* 4220	2.4	4.8	25.6	24.4
	MRSA 3506	2.4	4.8	25.6	24.4
IC^a_{50} (μM)	L02[b]	18.53	45.06	12.19	18.45

[a]IC_{50} is the concentration of compound required to inhibit the growth of the cells by 50%

[b]Human normal liver cells

NMe_2 atom of **7c** formed carbon hydrogen bond (1.94 Å) with Ser49. The Cl-substituted phenyl ring of **7c** formed alkyl bond (3.97 Å) with Leu62. The NMe_2 atom of **22b** formed carbon hydrogen bond (2.46 Å) with Thr46. The pyrimidine ring of **22b** formed alkyl bond (4.19 Å) with Arg44. Compound **22b** is bound into the active site where benzene ring shows interaction (4.19 and 5.33 Å) with Leu62. Otherwise, the enzyme surface model is shown in Fig. 3c, f, I, which revealed that compounds **7c** and **22b** were well inserted into the active pocket of *S. aureus* DHFR, which were the same to that of Iclaprim. These data provide certain theoretical support for experimental results and the next optimization.

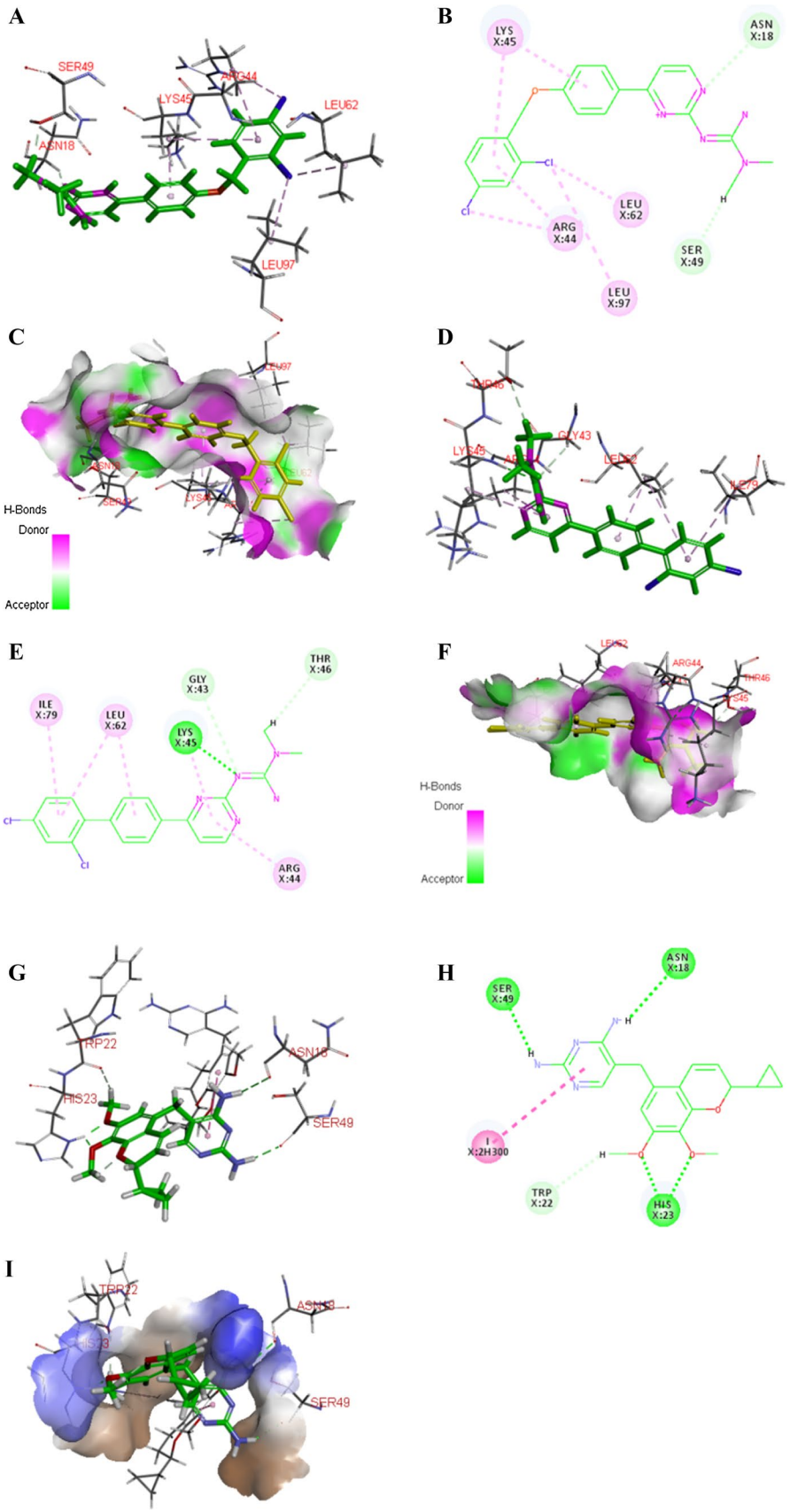


Fig. 3 **a** 3D conformation of **7c** docked in DHFR complex. **b** Predicted interactions between **7c** and the amino acids of 3fra. **c** Proposed pose of **7c** in the binding pocket of DHFR. **d** 3D conformation of **22b** docked in DHFR complex. **e** Predicted interactions between **22b** and the amino acids of 3fra. **f** Proposed pose of **22b** in the binding pocket of DHFR. **g** 3D conformation of **Iclaprim** docked in DHFR complex. **h** Predicted interactions between **Iclaprim** and the amino acids of 3fra. **i** Proposed pose of **Iclaprim** in the binding pocket of DHFR

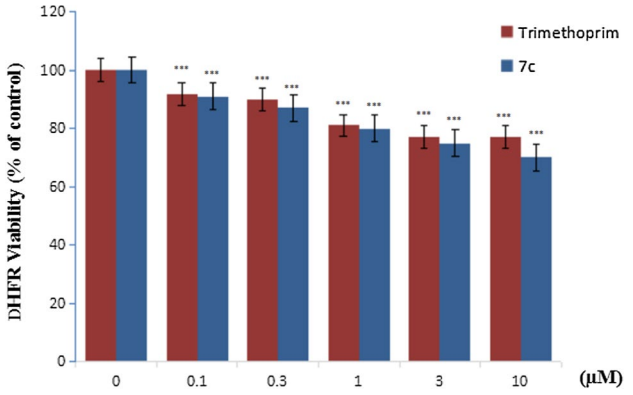


Fig. 4 DHFR inhibition assay. Data are represented as the mean ± standard deviation of three independent experiments. *$p < 0.05$, significant with respect to the control

DHFR inhibition assay

We performed in vitro enzyme assays to test the inhibitory effect of compound **7c** and standard drugs (**trimethoprim**) on DHFR activity (Fig. 4). The results indicated that compound **7c** was potent DHFR inhibitors when compared to **trimethoprim**. Furthermore, at concentration of 10 μmol/L, compound **7c** decreased DHFR activity to 70% compared with the negative control. These results imply that compound **7c** possibly displays their antibacterial activity through DHFR protein inhibition.

Conclusions

In summary, totally 22 new analogues and hybrids bearing the pyrimidine scaffold were synthesized and their in vitro antibacterial activities were investigated comprehensively. Compounds **7c** and **22b** presented the most potent inhibitory activity against Gram-positive bacteria and Gram-negative bacteria, with MICs ranging from 2.4 to 5.2 μmol/L. Furthermore, compound **7c** showed the most potent antimicrobial activity (MIC = 2.4 μmol/L) against selected MRSA strains. In vitro enzyme study implied that compound **7c** possibly displayed their antibacterial activity through DHFR protein inhibition. These results suggested that the pyrimidine derivatives bearing a benzyloxybenzaldehyde moiety, which play a critical role in increasing the antibacterial properties of the compounds, represented promising lead compounds for the development of novel antibacterial agents.

Materials and methods

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. The reactions were monitored by thin layer chromatography (TLC). The ^{1}H NMR spectra were recorded on 300 MHz spectrometers using DMSO as a solvent. The ^{13}C NMR spectra were recorded on 126 MHz instruments using DMSO as a solvent. Mass spectra were measured on an MALDI-TOF (Shimadzu, Japan). High-resolution mass spectrometry was measured on a Thermo Scientific LTQ Orbitrap XL spectrometer. The other raw materials and solvents were purchased from their respective suppliers and underwent no further purification. Purifications by column chromatography were conducted over silica gel (200–300 mesh).

General procedures for the synthesis of 4a, 7a–f, 10a and 13a

A mixture of hydroxyacetophenone (5 mmol) or paeonol (5 mmol) and K_2CO_3 (5 mmol) in dry DMF (10 mL), the corresponding substituted benzyl chloride (5 mmol) was added to the stirred solution 4–7 h at 80 °C. After the reaction is completed, the mixture was poured into ice water. The resulting precipitate was filtered and recrystallized with ethanol to obtain crude products (intermediates **2**, **5**, **8** and **11**) which were purified by column chromatography (dichloromethane/petroleum ether = 10:1). To compounds **2**, **5**, **8** and **11** (10 mmol), DMF-DMA (20 mmol) was added, and the reaction mixture was heated at 80 °C for 12 h to yield the desired products (intermediates **3**, **6**, **9** and **12**). To a solution of compounds **3**, **6**, **9** and **12** (10 mmol) in acetic acid (10 mL), proper moroxydine hydrochloride or metformin hydrochloride (10 mmol) was added. The reaction mixture was heated at reflux for 12 h, and acetic acid was evaporated under reduced pressure to obtain crude products (compounds **4a**, **7a–f**, **10a** and **13a**) which were purified by column chromatography (dichloromethane/methanol = 15:1).

3-(4-(2-(2,4-Dichlorobenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (4a) Yield 47%, m.p. 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 9.04 (br s, 2H, NH), 8.64 (d, J = 5.3 Hz, 1H, Ar–H), 7.86 (dd, J = 7.7, 1.7 Hz, 1H, Ar–H), 7.69 (d, J = 2.1 Hz, 1H, Ar–H), 7.60 (dd, J = 6.8, 4.4 Hz, 2H, Ar–H), 7.53–7.57 (m, 1H, Ar–H), 7.47 (dd, J = 8.3, 2.1 Hz, 1H, Ar–H), 7.34 (d, J = 8.4 Hz, 1H, Ar–H), 7.17 (t, J = 7.5 Hz, 1H, Ar–H), 5.29 (s, 2H, CH_2), 3.14 (s, 6H, CH_3). MS (MALDI-TOF) m/z 416 ($M^+ + H$).

3-(4-(4-(2,6-Dichlorobenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (7a) Yield 47%, m.p. 169–170 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.89 (br s, 2H, NH), 8.63 (d, J = 5.3 Hz, 1H, Ar–H), 8.16 (d, J = 8.8 Hz, 2H, Ar–H), 7.63 (d, J = 5.4 Hz, 1H, Ar–H), 7.59 (d, J = 8.0 Hz, 2H, Ar–H), 7.50 (dd, J = 8.7, 7.5 Hz, 1H, Ar–H), 7.25 (d, J = 8.8 Hz, 2H, Ar–H), 5.35 (s, 2H, CH_2), 3.14 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.74, 161.35, 158.54, 156.69, 136.55, 132.21, 131.81, 129.70, 129.25, 115.40, 109.35, 65.62, 38.32. MS (MALDI-TOF) m/z 416 (M^+ + H).

3-(4-(4-(4-Methylbenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (7b) Yield 42%, m.p. 141–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.90 (br s, 2H, NH), 8.62 (d, J = 5.4 Hz, 1H, Ar–H), 8.12 (d, J = 8.8 Hz, 2H, Ar–H), 7.63 (d, J = 5.4 Hz, 1H, Ar–H), 7.36 (d, J = 7.9 Hz, 2H, Ar–H), 7.21 (d, J = 7.9 Hz, 2H, Ar–H), 7.17 (d, J = 8.8 Hz, 2H, Ar–H), 5.16 (s, 2H, CH_2), 3.13 (s, 6H, CH_3), 2.31 (s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.62, 161.25, 158.42, 156.83, 137.70, 134.12, 129.49, 129.26, 129.16, 129.06, 128.35, 115.62, 69.82, 38.21, 31.15. MS (MALDI-TOF) m/z 362 (M^+ + H).

3-(4-(4-(2,4-Dichlorobenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (7c) Yield 45%, m.p. 133–135 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 11.25 (s, 1H), 7.79 (s, 3H), 7.58–7.40 (m, 3H), 7.23 (dd, J = 9.8, 6.0 Hz, 2H), 6.90 – 6.53 (m, 2H), 5.16 (s, 2H), 3.80 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.36, 162.91, 160.37, 158.53, 158.12, 134.12, 133.78, 131.92, 130.77, 129.48, 128.81, 128.07, 115.43, 106.76, 66.92, 37.28. HRMS (ESI) m/z calcd for $C_{20}H_{20}Cl_2N_5O$ (M^+ + H): 416.10394, found 416.10397.

3-(4-(4-(4-Bromobenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (7d) Yield 45%, m.p. 242–243 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 9.10 (br s, 2H, NH), 8.68 (d, J = 5.4 Hz, 1H, Ar–H), 8.17 (d, J = 8.6 Hz, 2H, Ar–H), 7.76 (d, J = 5.4 Hz, 1H, Ar–H), 7.62 (d, J = 8.2 Hz, 2H, Ar–H), 7.45 (d, J = 8.0 Hz, 2H, Ar–H), 7.19 (d, J = 8.6 Hz, 2H, Ar–H), 5.22 (s, 2H, CH_2), 3.16 (s, 6H, CH_3). MS (MALDI-TOF) m/z 427 (M^+ + H).

3-(4-(4-((4-Cyanobenzyl)oxy)phenyl)pyrimidin-2-yl)-1,1-dimethylguanidine (7e) Yield 38%, m.p. 159–161 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.46 (br s, 2H, NH), 8.06 (s, 2H, Ar–H), 7.89 (d, J = 8.1 Hz, 2H, Ar–H), 7.69 (s, 3H, Ar–H), 7.28 (s, 1H, Ar–H), 7.17 (s, 2H, Ar–H), 5.32 (s, 2H, CH_2), 3.04 (s, 6H, CH_3). MS (MALDI-TOF) m/z 373 (M^+ + H).

N-(4-(4-(2,4-Dichlorobenzyloxy)phenyl)pyrimidin-2-yl)morpholine-4-carboxamidine (7f) Yield 45%, m.p. 128–130 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.70 (br s, 2H, NH), 8.53 (s, 1H, Ar–H), 8.09 (d, J = 7.9 Hz, 2H, Ar–H), 7.69 (d, J = 24.9 Hz, 2H, Ar–H), 7.52 (s, 2H, Ar–H), 7.18 (d, J = 7.9 Hz, 2H, Ar–H), 5.25 (s, 2H, CH_2), 3.04 (d, J = 21.3 Hz, 8H, CH_2). MS (MALDI-TOF) m/z 458 (M^+ + H).

3-(4-(3-(2,4-Dichlorobenzyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (10a) Yield 42%, m.p. 116–117 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.60 (br s, 2H, NH), 7.65–7.70 (m, 3H, Ar–H), 7.50–7.54 (m, 3H, Ar–H), 7.24 (s, 1H, Ar–H), 6.62 (s, 2H, Ar–H), 5.24 (s, 2H, CH_2), 3.08 (s, 6H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 172.18, 163.77, 136.94, 136.83, 135.41, 134.19, 133.89, 132.91, 125.21, 122.29, 118.41, 71.76, 70.24, 35.23, 23.87. HRMS (ESI) m/z calcd for $C_{20}H_{20}Cl_2N_5O$ (M^+ + H): 416.10394, found 416.10394.

3-(4-(2-(2,4-Dichlorobenzyloxy)-4-methoxyphenyl)pyrimidin-2-yl)dimethylguanidine (13a) Yield 40%, m.p. 181–183 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 9.02 (br s, 2H, NH), 8.58 (s, 1H, Ar–H), 7.92 (s, 1H, Ar–H), 7.74 (s, 1H, Ar–H), 7.62 (s, 2H, Ar–H), 7.52 (s, 1H, Ar–H), 6.88 (s, 1H, Ar–H), 6.76 (dd, J = 8.8, 2.2 Hz, 1H), 5.33 (s, 2H, CH_2), 3.87 (s, 3H, CH_3), 3.13 (s, 6H, CH_3). MS (MALDI-TOF) m/z 446 (M^+ + H).

General procedures for the synthesis of 17a–d

To a stirred solution of the corresponding substituted acetophenone (2 mmol), acetylacetone (2.2 mmol) and ammonium acetate (16 mmol) in glacial acetic acid (10 mL) were added, and the reaction mixture was stirred under reflux for 3 h. After the completion of reaction, the reaction mixture was poured into water and the obtained precipitate was filtered.

Dimethyl-3-(4-(2-methyl-6-(4-pentylphenyl)pyridin-3-yl) pyrimidin-2-yl)guanidine (17a) Yield 47%, m.p. 135–137 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.50 (d, J = 5.0 Hz, 1H, Ar–H), 8.24 (br s, 2H, NH), 8.04 (d, J = 8.2 Hz, 2H, Ar–H), 7.87 (q, J = 8.1 Hz, 2H, Ar–H), 7.32 (d, J = 8.2 Hz, 2H, Ar–H), 6.94 (d, J = 5.0 Hz, 1H, Ar–H), 3.02 (s, 6H, CH_3), 2.60–2.65 (m, 5H, CH_2CH_3), 1.57–1.65(m, 2H, CH_2), 1.27–1.34 (m, 4H, CH_2), 0.86 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.44, 165.19, 159.63, 158.24, 155.73, 144.25, 138.42, 136.09, 129.18, 127.09, 117.75, 111.66, 37.40, 36.06, 35.32, 31.34, 30.95, 24.10, 22.41, 14.37. MS m/z: 358 (M^+ + H). HRMS (MALDI-TOF) calcd for $C_{17}H_{20}N_5O_2$ (M^+ + H): 358.1510, found: 358.1515.

Dimethyl-3-(4-(2-methyl-6-p-tolylpyridin-3-yl)pyrimidin-2-yl) guanidine (17b) Yield 42%, m.p. 174–176 °C. ^{1}H NMR

(300 MHz, DMSO-d_6, ppm): δ 8.61 (d, J = 5.0 Hz, 1H, Ar–H), 8.50 (br s, 2H, NH), 8.06 (d, J = 8.1 Hz, 2H, Ar–H), 7.92 (dd, J = 18.8, 8.1 Hz, 2H, Ar–H), 7.33 (d, J = 8.1 Hz, 2H, Ar–H), 7.16 (d, J = 4.9 Hz, 1H, Ar–H), 3.07 (s, 6H, CH_3), 2.65 (s, 3H, CH_3), 2.38 (s, 3H, CH_3). MS (MALDI-TOF) m/z 347 ($M^+ + H$).

3-(4-(6-(2,4-Dichlorophenyl)-2-methylpyridin-3-yl)pyrimidin-2-yl)dimethylguanidine (17c) Yield 45%, m.p. 135–137 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.58 (d, J = 5.0 Hz, 1H, Ar–H), 8.37 (br s, 2H, NH), 7.95 (d, J = 8.0 Hz, 1H, Ar–H), 7.77 (d, J = 2.0 Hz, 1H, Ar–H), 7.66 (t, J = 8.3 Hz, 2H, Ar–H), 7.58 (dd, J = 8.3, 2.0 Hz, 1H, Ar–H), 7.08 (d, J = 5.0 Hz, 1H, Ar–H), 3.05 (s, 6H, CH_3), 2.61(s, 3H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.05, 158.47, 157.66, 155.84, 155.05, 137.82, 134.43, 133.43, 133.20, 132.69, 129.86, 128.11, 122.52, 112.55, 37.76, 31.14, 23.83. MS (MALDI-TOF) m/z 401 ($M^+ + H$).

3-(4-(6-(4-Bromophenyl)-2-methylpyridin-3-yl)pyrimidin-2-yl)dimethylguanidine (17d) Yield 45%, m.p. 182–184 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.56 (s, 1H, Ar–H), 8.34 (br s, 2H, NH), 8.12 (d, J = 8.5 Hz, 2H, Ar–H), 7.95 (s, 1H, Ar–H), 7.22 (d, J = 8.4 Hz, 2H, Ar–H), 7.05 (s, 1H, Ar–H), 3.04 (s, 6H, CH_3), 2.64 (s, 3H, CH_3). MS (MALDI-TOF) m/z 411 ($M^+ + H$).

3-(4-(2,4-Dichlorophenyl)pyrimidin-2-yl)dimethylguanidine (19a) Yield 42%, m.p. 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.51 (d, J = 4.8 Hz, 1H, Ar–H), 8.22 (br s, 2H, NH), 7.78 (s, 1H, Ar–H), 7.59 (q, J = 8.3 Hz, 2H, Ar–H), 6.94 (d, J = 4.9 Hz, 1H, Ar–H), 3.02 (s, 6H, CH_3). MS (MALDI-TOF) m/z 310 ($M^+ + H$).

Dimethyl-3-(4-(naphthalen-3-yl)pyrimidin-2-yl)guanidine (19b) Yield 38%, m.p. 131–132 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). MS (MALDI-TOF) m/z 292 ($M^+ + H$).

3-(4-(4-Bromophenyl)pyrimidin-2-yl)dimethylguanidine (19c) Yield 40%, m.p. 144–146 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). MS (MALDI-TOF) m/z 321 ($M^+ + H$).

Dimethyl-3-(4-(4-pentylphenyl)pyrimidin-2-yl)guanidine (19d) Yield 42%, m.p. 129–131 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.75, 163.37, 158.57, 157.62, 145.97, 134.78, 129.27, 127.25, 108.47, 37.79, 35.39, 31.34, 30.88, 22.40, 14.36. MS (MALDI-TOF) m/z 312 ($M^+ + H$).

3-(4-(4-(Dimethylamino)phenyl)pyrimidin-2-yl)dimethylguanidine (19e) Yield 42%, m.p. 221–222 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.21, 164.84, 159.42, 157.43, 155.70, 153.04, 129.01, 112.08, 38.91, 31.15. MS (MALDI-TOF) m/z 285 ($M^+ + H$).

3-(4-([1,1′-Biphenyl]-4-yl)pyrimidin-2-yl)-1,1-dimethylguanidine (22a) Yield 45%, m.p. 175–177 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.51 (s, 1H, NH), 8.36 (s, 1H, NH), 8.15 (d, J = 7.7 Hz, 2H, Ar–H), 7.79 (dd, J = 19.9, 7.7 Hz, 4H, Ar–H), 7.29–7.59 (m, 4H, Ar–H), 6.60 (s, 1H, Ar–H), 3.05 (s, 6H, CH_3). MS (MALDI-TOF) m/z 318 ($M^+ + H$).

3-(4-(2′,4′-Dichloro-[1,1′-biphenyl]-4-yl)pyrimidin-2-yl)-1,1-dimethylguanidine (22b) Yield 38%, m.p. 107–109 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). HRMS (ESI) m/z calcd for $C_{20}H_{20}Cl_2N_5O$ ($M^+ + H$): 386.09338, found 386.09348.

3-(4-(4-(Hexyloxy)phenyl)pyrimidin-2-yl)dimethylguanidine (26a) Yield 42%, m.p. 140–142 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.30, 161.29, 158.15, 158.00, 129.64, 128.78, 115.09, 107.36, 68.14, 37.55, 31.48, 29.07, 25.64, 22.54, 14.37. MS (MALDI-TOF) m/z 342 ($M^+ + H$).

Dimethyl-3-(4-(4-(pentyloxy)phenyl)pyrimidin-2-yl)guanidine (26b) Yield 48%, m.p. 159–160 °C. ^{1}H NMR (300 MHz, DMSO-d_6, ppm): δ 8.29 (s, 2H), 7.28 (t, J = 6.0 Hz, 6H), 6.48 (ddd, J = 16.3, 10.5, 4.1 Hz, 2H), 5.00 (s, 2H), 3.73 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.76, 168.08, 165.94, 163.13, 162.96, 134.59, 133.46, 123.63, 119.82, 72.86, 42.11, 33.55, 32.91, 27.12, 19.13. MS (MALDI-TOF) m/z 328 ($M^+ + H$).

Biological evaluation

In vitro antibacterial and antifungal activity

MIC assay for each test compound was performed as described previously [24]. Testing was performed by the

standard broth microdilution method with trimethoprim and gatifloxacin. All stock solutions of the compounds were dissolved in DMSO. Bacteria growth was determined by measuring the absorption at 630 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader.

Toxicity evaluation

Toxicity test was performed using MTT assay, where all instructions were performed typically to our previous work [24]. For this study, all compounds were tested against a normal human liver cell line (L02) for 48 h at 37 °C.

Molecular modeling

Molecular docking protocol was followed according to the reported method [24]. All docking runs were carried out using Discovery Studio v17.1.0.16143. The 3D structure of 3FRA in docking study was downloaded from Protein Data Bank. For protein preparation, the hydrogen atoms were added, and water and impurities were removed.

DHFR inhibition assay

Solid-phase antibody was prepared by coating the microtiter plate wells with purified human dihydrofolate reductase (DHFR) antibody. To see the effect of lead inhibitor (**7c**) on DHFR activities, ELISA assay was performed as described previously [24]. All assays were performed in triplicate.

Acknowledgements This work was supported by the Doctoral Foundation of Jilin Medical University (No. JYBS2018007), the Health Department of Jilin Province (2018ZC034) and the Department of Education of Jilin Province (No. JJKH20191070KJ).

References

1. Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10:S122–S129
2. Bi Y, Liu XX, Zhang HY, Yang X, Liu ZY, Lu J, Lewis PJ, Wang CZ, Xu JY, Meng QG, Ma C, Yuan CS (2017) Synthesis and antibacterial evaluation of novel 3-substituted ocotillol-type derivatives as leads. Molecules 22:E590
3. Carrel M, Perencevich EN, David MZ (2015) USA300 methicillin-resistant *Staphylococcus aureus*, United States, 2000–2013. Emerg Infect Dis 21:1973–1980
4. Hvistendahl M (2012) Public health. China takes aim at rampant antibiotic resistance. Science 336:795
5. Yezli S, Li H (2012) Antibiotic resistance amongst healthcare-associated pathogens in China. Int J Antimicrob Agents 40:389–397
6. Azeredo da Silveira S, Perez A (2015) Liposomes as novel anti-infectives targeting bacterial virulence factors? Expert Rev Anti Infect Ther 13:531–533
7. Taylor PW, Stapleton PD, Paul Luzio J (2002) New ways to treat bacterial infections. Drug Discov Today 7:1086–1091
8. O'Neill J (2016) Review on antimicrobial resistance, Government of the UK. http://apo.org.au/node/63983
9. Wirnsberger G, Zwolanek F, Asaoka T, Kozieradzki I, Tortola L, Wimmer RA, Kavirayani A, Fresser F, Baier G, Langdon WY, Ikeda F, Kuchler K, Penninger JM (2016) Inhibition of CBLB protects from lethal Candida albicans sepsis. Nat Med 22:915–923
10. Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 20:133–163
11. Denning DW, Bromley MJ (2015) How to bolster the antifungal pipeline. Science 347:1414–1416
12. Brown GD, Denning DW, Levitz SM (2012) Tackling human fungal infections. Science 336:647
13. Khalafi-Nezhad A, Soltani Rad MN, Mohabatkar H, Asrari Z, Hemmateenejad B (2005) Design, synthesis, antibacterial and QSAR studies of benzimidazole and imidazole chloroaryloxyalkyl derivatives. Bioorg Med Chem 13:1931–1938
14. Seenaiah D, Reddy PR, Reddy GM, Padmaja A, Padmavathi V, Krishna NS (2014) Synthesis, antimicrobial and cytotoxic activities of pyrimidinyl benzoxazole, benzothiazole and benzimidazole. Eur J Med Chem 77:1–7
15. Desai NC, Kotadiya GM, Trivedi AR (2014) Studies on molecular properties prediction, antitubercular and antimicrobial activities of novel quinoline based pyrimidine motifs. Bioorg Med Chem Lett 24:3126–3130
16. Jin X, Zheng CJ, Song MX, Wu Y, Sun LP, Li YJ, Yu LJ, Piao HR (2012) Synthesis and antimicrobial evaluation of L-phenylalanine-derived C5-substituted rhodanine and chalcone derivatives containing thiobarbituric acid or 2-thioxo-4-thiazolidinone. Eur J Med Chem 56:203–209
17. Zhang TY, Li C, Li YR, Li XZ, Sun LP, Zheng CJ, Piao HR (2016) Synthesis and antimicrobial evaluation of aminoguanidine and 3-amino-1,2,4-triazole derivatives as potential antibacterial agents. Lett Drug Des Discov 13:1063–1075
18. Davari AS, Abnous K, Mehri S, Ghandadi M, Hadizadeh F (2014) Synthesis and biological evaluation of novel pyridine derivatives as potential anticancer agents and phosphodiesterase-3 inhibitors. Bioorg Chem 57:83–89
19. Seleem MA, Disouky AM, Mohammad H, Abdelghany TM, Mancy AS, Bayoumi SA, Elshafeey A, El-Morsy A, Seleem MN, Mayhoub AS (2016) Second-generation phenylthiazole antibiotics with enhanced pharmacokinetic properties. J Med Chem 59:4900–4912
20. Eldehna WM, Altoukhy A, Mahrous H, Abdel-Aziz HA (2015) Design, synthesis and QSAR study of certain isatin-pyridine hybrids as potential anti-proliferative agents. Eur J Med Chem 90:684–694
21. Li B, Pai R, Di M, Aiello D, Barnes MH, Butler MM, Tashjian TF, Peet NP, Bowlin TL, Moir DT (2012) Coumarin-based inhibitors of Bacillus anthracis and *Staphylococcus aureus* replicative DNA helicase: chemical optimization, biological evaluation, and antibacterial activities. J Med Chem 55:10896–10908
22. Lam T, Hilgers MT, Cunningham ML, Kwan BP, Nelson KJ (2014) Structure-based design of new dihydrofolate reductase antibacterial agents: 7-(benzimidazol-1-yl)-2,4-diaminoquinazolines. J Med Chem 57:651–668
23. Oefner C, Bandera M, Haldimann A, Laue H, Schulz H (2009) Increased hydrophobic interactions of iclaprim with *Staphylococcus aureus* dihydrofolate reductase are responsible for the increase in affinity and antibacterial activity. J Antimicrob Chemother 63:687–698
24. Zhang TY, Zheng CJ, Wu J, Sun LP, Piao HR (2019) Synthesis of novel dihydrotriazine derivatives bearing 1,3-diaryl pyrazole moieties as potential antibacterial agents. Bioorg Med Chem Lett 29:1079–1084