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含直链烷烃的胍基腙衍生物的合成及其抗菌活性评价

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摘要 抗生素耐药性的上升和新抗生素发现的减少造成了全球健康危机. 为了获得有效的抗菌剂和缓解细菌耐药性, 设计合成了一系列新型的含直链烷烃的胍基腙, 评价了目标化合物对五株革兰氏阳性菌株、四株革兰氏阴性菌株和四株多药耐药菌株的抗菌活性. 结果显示, 除(*E*)-2-己基亚肼-1-碳酰亚胺(**1a**)之外, 其他所有化合物均表现出不同程度的抗菌性能, 最低抑菌浓度(MIC)为 0.5～64 μg/mL. 其中(*E*)-2-十二烷基亚肼-1-碳酰亚胺(**1d**)、(*E*)-2-十四烷基亚肼-1-碳酰亚胺(**1e**)、(*E*)-2-(4-(庚氧基)亚苄基)肼-1-碳酰亚胺(**2d**)和(*E*)-2-(4-(辛氧基)亚苄基)肼-1-碳酰亚胺(**2e**)对菌株 33693、29212、63501、10104 和 43300 的抗菌活性与青霉素和诺氟沙星相当或更好. 本研究证实化合物 **2d** 还具有优良的杀菌性能, 并且未发现细菌对化合物 **2d** 产生耐药性趋势. 细胞毒性评价显示该系列化合物具有一定细胞毒性, 但抗菌活性浓度下未见毒性反应. 此外, 还进行了 **2d** 与 LpxC 的分子对接, 结果证明化合物 **2d** 与 LpxC 结合良好, 这可能是其产生优良抗菌活性的分子机制之一. 这些发现说明胍腙与直链烷烃偶联可以作为一种潜在骨架, 用于开发新型抗菌药物.

关键词 胍基腙; 抗菌; 直链烷烃; 耐药性

Synthesis and Antibacterial Activity Evaluation of Guanidine Hydrazone Derivatives Containing Linear Alkanes

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Abstract The rise of antibiotic resistance and the declining discovery of new antibiotics have created a global health crisis. To obtain effective antibacterial agents and relieve bacterial resistance, a series of novel guanidine hydrazones containing linear alkanes were designed and synthesized. The antimicrobial activities of these compounds were evaluated against five gram-positive strains, four gram-negative strains and four multidrug-resistant strains. All of the targets except (*E*)-2-hexylidenehydrazine-1-carboximidamide (**1a**) exhibited a different degree of antibacterial properties with minimum inhibitory concentration (MIC) in 0.5～64 μg/mL. Among them, (*E*)-2-dodecylidenehydrazine-1-carboximidamide (**1d**), (*E*)-2-tetradecylidenehydrazine-1-carbox-imidamide (**1e**), (*E*)-2-(4-(heptyloxy)benzylidene)hydrazine-1-carboximidamide (**2d**) and (*E*)-2-(4-(octyloxy)benzylidene)hydrazine-1-carbox-imidamide (**2e**) showed equivalent or better antibacterial activity than penicillin and norfloxacin against strains 33693, 29212, 63501, 10104 and 43300. Excellent bactericidal properties of **2d** and low frequency of bacteria developing resistance toward **2d** were established. The cytotoxicity evaluation showed that the target compounds had certain cytotoxicity, but no cytotoxicity was found under the concentration of antibacterial activity. Molecular

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docking of **2d** with LpxC was performed, which demonstrated that **2d** has a forceful binding with LpxC. These findings strongly support the assumption that guanidine hydrazone coupled with linear alkane is a potential skeleton to develop new antimicrobial agents.

Keywords guanidine hydrazone; antimicrobial; linear alkane; resistance

1 Introduction

The development of bacteria resistance to most of the clinical drugs has aggravated the global threat of infectious diseases.[1-2] Critical issues are caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), or multidrug-resistant Gram-negative bacteria, including *Klebsiella pneumoniae*, *Escherichia coli* (*E. coli*), or *Pseudomonas aeruginosa* (*P. aeruginosa*).[3-4] Higher morbidity and mortality are caused by the infection of multidrug-resistant bacteria, which is increasingly threatening human health.[5] In recent years, although some progresses have been made in the research and development of antibacterial drugs, there are still some defects in clinical application, such as narrow antibacterial spectrum, easy to produce drug resistance and so on.[6-8] Designing new antibiotics with new structures is still the core of attention for medicinal chemists now.

The Zn^{2+}-dependent deacetylase LpxC is an essential enzyme of lipid A biosynthesis in Gram-negative bacteria and a promising target for the development of antibiotics.[9] In the past three decades, researchers from industry and academia have synthesized structurally diverse LpxC inhibitors, like the substrate analog TU-514, the aryloxazoline L-161240, the sulfonamide BB-78485, the *N*-aroyl-*L*-threonine derivative LPC-009 and the cycloprodiynyl derivative ACHN-975 (Figure 1), exhibiting excellent LpxC inhibitory and antibacterial activities.[10-12] Among them, ACHN-975 is the only LpxC inhibitor that has entered clinical research. However, due to the local inflammatory reaction at the injection site, it was terminated in the clinical phase II. Almost all of the highly active LpxC inhibitors have the same structural feature, that is, they contain groups that chelate with the catalytic active center Zn^{2+} (such as hydroxamic acid) and hydrophobic side chains that effectively addressing the hydrophobic tunnel or the uridinediphosphate (UDP) binding site of the enzyme.

Aminoguanidine is a functional group with high polarity and capacity for hydrogen bonding with many critical amino acid residues as well as metal ions. Many aminoguanidine derivatives exhibit antitumor activity via the formation of complexes with metal ions.[13-15] In our previous work, some aminoguanidines linked with di-phenyl moieties (**I**, Scheme 1) were found as potent antibacterial agents against Gram-positive bacteria and Gram-negative bacteria including multi-drug resistant (MDR) clinical isolates.[16-17] The binding of these compounds involves a specific interaction with the LpxC.[16] By analyzing the structure-activity relationship (SAR) of these compounds, it could be concluded that aminoguanidine combined with a hydrophobic di-phenyl moiety in the form of azomethine imine is the common structural requirement for their antibacterial activity. This structural feature is meet to the requirement of

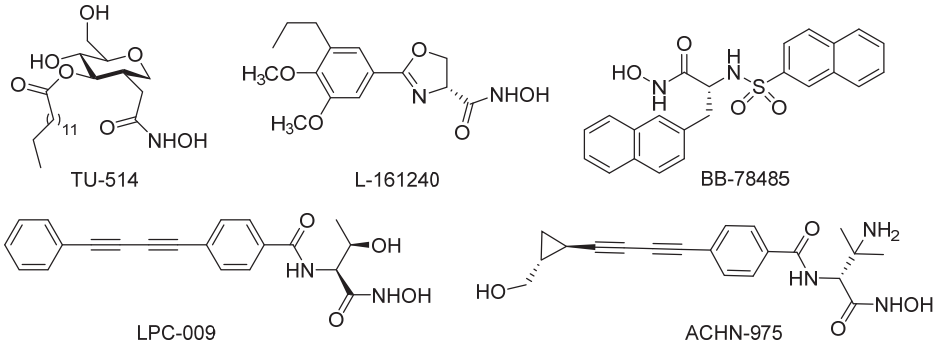
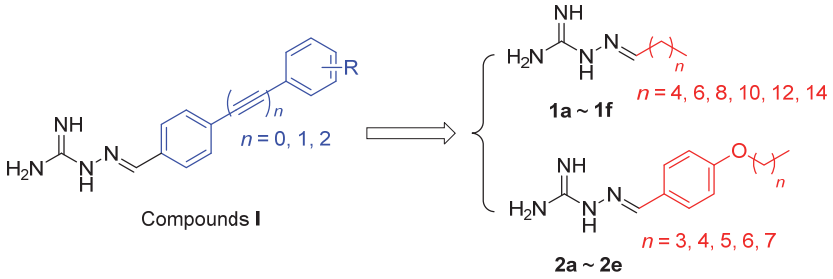


Figure 1 Structures of some representative LpxC inhibitors



Scheme 1 Design of the target compounds **1a**～**1f** and **2a**～**2e**

LpxC inhibiting. The aminoguanidine part of these compounds occupies the active site of LpxC, and the di-phenyl part (phenyldiyne/diphenylbuta-1,3-diyne/diphenyl) occupies the hydrophobic passage to obtain more binding.

In the molecular modeling, it was found the di-phenyl part mostly interacted with the hydrophobic residues in the lipophilic tunnel.[17] This result made us wonder if the aminoguanidine linked with fatty chains would also bind with LpxC well and have a potent antibacterial activity. Another reason that impels us to study this structure is that the natural substrate of LpxC has been identified as UDP-(3-*O*-(*R*-3-hydroxymyristoyl))-*N*-acetylglucosamine (myr-UDP-GlcNAc), which also has a long fatty chain. Therefore, to obtain more effective antibacterial agents and rich the existing SAR, we altered one or both phenyl groups of the di-phenyl moiety in the skeleton **I** to fat chains with different lengths in this study. These compounds were synthesized and screened for their antibacterial activity.

2 Results and discussion

2.1 Chemistry

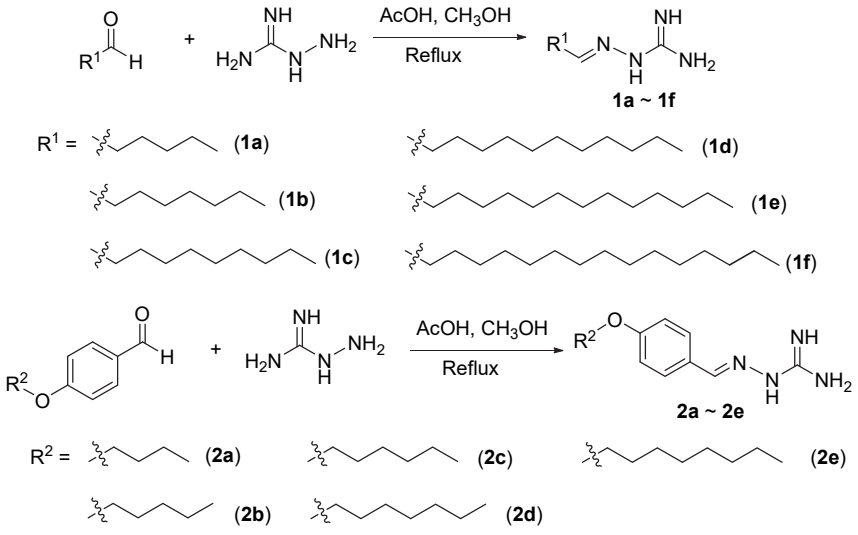
The synthetic route to prepare a new class of aminoguanidine derivatives is depicted in Scheme 2. The target compounds **1a**～**1f** and **2a**～**2e** were prepared by the condensation of different aldehydes with aminoguanidine.[18] Structures of the target compounds were well characterized by ^{1}H NMR, ^{13}C NMR and high-resolution mass spectrometry (HRMS).

Taking compounds **1a** and **2a** as examples in the structure confirmation, ^{1}H NMR, ^{13}C NMR and HRMS were analyzed. In the ^{1}H NMR spectrum of compound **1a**, signals of C—H in pentyl were found at δ 0.87～2.17. A singlet due to N—H of guanidyl was observed at δ 5.41, which was assigned to two NH_2 groups in terminal formed by the tautomerism of guanidyl group leading to the extension of the conjugated chain. The N=CH signal as triplet, influenced by the *ortho* CH_2, was observed at δ 7.31. In the ^{1}H NMR spectrum of compound **2a**, the signal of CH_3 was found at δ 0.97, the signal peaks of CH_2 in the butyl were found at δ 1.46～3.96. A singlet due to N—H of guanidyl was observed at δ 4.93. Two doublets due to =CH in the phenyl group were observed at δ 6.85 and 7.57, respectively. And the signal peak found at δ 8.14 was attributed to the N=CH group. Peaks in the hydrogen spectrum of compounds **1a** and **2a** were completely in conformity with the hydrogen signal in the structures. The ^{13}C NMR spectra also gave accurate information about the structure of the compounds **1a** and **2a**, which involved 7 and 10 kinds of carbon in different chemical environments, respectively. Moreover, the high-resolution mass spectrometry of **1a** and **2a** displayed an $[M+H]^+$ signal at *m*/*z* 157.1446 and 235.1554, respectively, which were corresponding to their molecular weight of 157.1448 and 235.1553, respectively.

2.2 Antimicrobial activity

The *in vitro* antimicrobial activities of the target compounds (**1a**～**1f** and **2a**～**2e**) were evaluated using a serial dilution method to obtain the MIC on a 96-well microtiter plate against different clinical strains. Penicillin and norfloxacin were used as positive standard drugs.

First, two series of targets were screened for their activities against five gram-positive strains and four gram-negative strains. Initial screening results described as MIC values are presented in Table 1. The results showed that most of the targets exhibited a certain degree of antibacterial properties with MICs in 0.5～64 μg/mL. Compound **1a** did not show any inhibitory activity against all of the selected strains at 64 μg/mL. Compound **1f** did not show any inhibitory activity at 64 μg/mL against all the selected Gram-negative organisms. Length of the alkyl chain seems to have a direct effect on their antibacterial activity. From compound **1a** to compound **1f**, as alkyl chain length increased, MICs gradually decreased with compound **1d** (with the dodecyl) and **1e** (with the *n*-tetradecyl) being the most active two. The trend reversed, however, when the alkyl chain



Scheme 2 Synthesis route and structures of compounds **1a**～**1f** and **2a**～**2e**

Table 1 Inhibitory activity of compounds **1a**～**1f** and **2a**～**2e** against Gram-positive and Gram-negative bacteria [MIC/(μg•mL^{-1})]

Compd.	R	Gram-positive strain					Gram-negative strain			
		26003[a]	25923[b]	33693[c]	29212[d]	63501[e]	25922[f]	44568[g]	27853[h]	10104[i]
1a	C_5H_{11}	＞64	＞64	＞64	＞64	＞64	＞64	＞64	＞64	＞64
1b	C_7H_{15}	64	64	64	64	64	64	64	64	64
1c	C_9H_{19}	**0.5**	8	16	8	2	8	8	16	8
1d	$C_{11}H_{23}$	**0.5**	2	2	2	**1**	**2**	**2**	8	**2**
1e	$C_{13}H_{27}$	**0.5**	8	**1**	**1**	**0.5**	**2**	**4**	16	**2**
1f	$C_{15}H_{31}$	4	64	8	16	8	＞64	＞64	＞64	＞64
2a	C_4H_9	4	2	16	16	4	32	16	16	16
2b	C_5H_{11}	4	2	8	4	2	8	8	8	8
2c	C_6H_{13}	2	2	2	2	2	8	4	8	4
2d	C_7H_{15}	**0.5**	**0.5**	**1**	**1**	**1**	4	4	8	**2**
2e	C_8H_{17}	**0.5**	**0.5**	**1**	**1**	**1**	8	8	32	4
Penicillin	—	0.125	0.125	0.125	128	128	128	＞128	＞128	32
Norfloxacin	—	0.125	0.125	16	1	2	0.125	0.125	2	4

[a] *Staphylococcus aureus* CMCC(B)26003; [b] *Staphylococcus aureus* CMCC 25923; [c] *Streptococcus mutans* BNCC 33693; [d] *Enterococcus faecalis* CMCC 29212; [e] *Bacillus subtilis* CMCC 63501; [f] *Escherichia coli* CMCC 25922 [g] *Escherichia coli* CMCC 44568; [h] *Pseudomonas aeruginosa* CMCC 27853. [i] *Pseudomonas aeruginosa* CMCC 10104.

reached sixteen carbon numbers. For the series of **2a**～**2e**, the most active one was compound **2d**, which showed potent anti-bacterial properties against all the selected strains with MICs in 0.5～8 μg/mL.

In the current stage, it is more important to find antibiotics sensitive to drug-resistant bacteria. Therefore, all the target compounds except **1a** were also evaluated for their inhibitory activities against clinical isolates of multi-drug-resistant strains. As shown in Table 2, the performance of test compounds against the drug-resistant bacteria was similar to the above. Compound **1b** exhibited week antibacterial activity against all the test strains with a MIC of 64 μg/mL. As the alkyl chain length increased, the antibacterial activity gradually increased. Compounds **1d** and **1e** were the most active two, which gave the MIC values of 0.5 and 1 μg/mL to the multi-drug resistant *Staphylococcus aureus* ATCC 43300 and ATCC 33591, respectively. Compound **1d** also showed potent inhibitory activity against the multi-drug resistant *Escherichia coli* ATCC BAA-196 and multi-drug resistant *Pseudomonas aeruginosa* ATCC BAA-2111 with the MIC values of 2 and 4 μg/mL, respectively. In the series of **2a**～**2e**, multi-drug resistant *Staphylococcus aureus* ATCC 43300 was most sensitive to compounds **2d** and **2e**, which gave the MIC value of 1 μg/mL. Compounds **2d** and **2e** also presented potent inhibitory activity against the multi-drug resistant *Escherichia coli* ATCC BAA-196,with the MIC value of 2 μg/mL.

Comparing the antibacterial activities of the synthesized compounds with that of the positive drugs, it can be found that the preferred compounds **1d**, **1e**, **2d**, **2e** showed a broader spectrum of antibacterial activity than penicillin, especially against negative bacteria and drug-resistant bacteria. When compared with norfloxacin, compounds **1d**, **1e**, **2d** and **2e** showed equivalent or better antibacterial activity against strains 33693, 29212, 63501, 10104, and 43300.

2.3 Propensity to develop bacterial resistance

In current times, many clinical antibacterial drugs and even newly approved antibiotics have been reported to bacterial resistance.[19-20] Therefore, evaluating the potency

Table 2 Inhibitory activityof compounds **1a**～**1f** and **2a**～**2e** against clinical isolates of multidrug-resistant strains [MIC/(μg•mL^{-1})]

Compd.	R	Multidrug-resistant Gram-positive strain		Multidrug-resistant Gram-negative strain	
		43300[a]	33591[b]	BAA-196[c]	BAA-2111[d]
1b	C_7H_{15}	64	64	64	64
1c	C_9H_{19}	4	4	8	16
1d	$C_{11}H_{23}$	0.5	1	2	4
1e	$C_{13}H_{27}$	0.5	1	1	16
1f	$C_{15}H_{31}$	＞64	4	64	＞64
2a	C_4H_9	4	16	16	32
2b	C_5H_{11}	2	8	8	16
2c	C_6H_{13}	1	4	4	16
2d	C_7H_{15}	0.5	4	2	32
2e	C_8H_{17}	0.5	8	2	64
Penicillin	—	32	＞32	＞32	＞32
Norfloxacin	—	0.5	0.25	0.5	1

[a] Multi-drug resistant *Staphylococcus aureus* ATCC 43300; [b] multi-drug resistant *Staphylococcus aureus* ATCC 33591; [c] Multi-drug resistant *Escherichia coli* ATCC BAA-196; [d] multi-drug resistant *Pseudomonas aeruginosa* ATCC BAA-2111.

of bacterial resistance against these biocides is extremely important. The propensity to develop bacterial resistance of the synthesized compounds was evaluated by using representative compounds **1d** and **2d** against Gram-positive *S. aureus* and Gram-negative *E. coli* (Figure 2).[21-22] Norfloxacin, an antibiotic generally used in clinical, was used as a positive control. These antibacterial agents were repeatedly challenged against bacteria at their sub-MIC values to allow bacteria to develop resistance. Resistance is usually defined as a ＞ 4-fold increase in the original MIC.[23] As shown in Figure 2, little change in the MICs of compounds **1d** and **2d** was observed over 20 generations, while a 64-fold increase in MIC was observed in the case of norfloxacin (MIC value from 0.125 μg/mL to 8 μg/mL). The above results indicated that bacteria did not develop resistance against this type of aminoguanidines within the experimental time period.

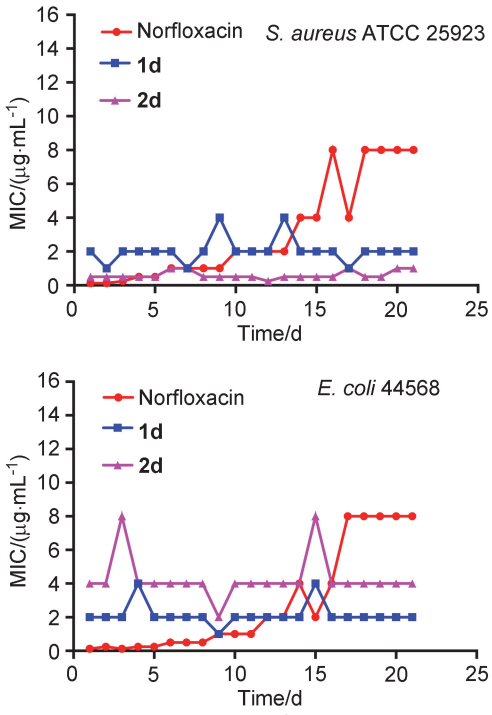


Figure 2 Propensity of the development of bacterial resistance toward compounds **1d** and **2d**

2.4 Bactericidal time-kill kinetics

In vitro time-kill assay was carried out to study the bactericidal activity of the promising compound **2d** against methicillin-resistant *Staphylococcus aureus* (MRSA) at different concentrations (1/2×MIC, 1×MIC, and 2×MIC), while using norfloxacin as a control (Figure 3). The initial bacterial count was $10^{4.0}$ CFU/mL. The number of viable bacteria dropped to 10^{0} CFU/mL after 6 h or 8 h at 2×MIC or 1×MIC of compound **2d** and the bactericidal activity persisted for 12 h. In contrast, norfloxacin did not kill the bacteria after 6 h at the concentration of 2×MIC, its effects were bacteriostatic, not bactericidal. These results obviously indicated the superiority of compound **2d** over the antibiotic norfloxacin.

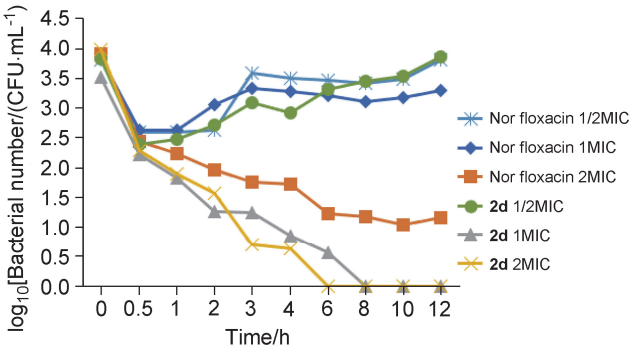


Figure 3 Bactericidal activities of compound **2d** and norfloxacin against *S. aureus* ATCC 43300 (MRSA)

2.5 Cytotoxicity

Amphiphilic molecules usually have high toxicity, which is one of the main reasons limiting the further application of such compounds. To obtained the possible cytotoxicity, compounds **1a**～**1f** and **2a**～**2e** were evaluated their cytotoxic activity against human normal cell line HEK 293T. As shown in the Table 3, compounds **1a**～**1f** and **2a**～**2e** showed certain cytotoxicity with IC_{50} values in the range of 45.3～117.5 μmol/L. However, the cytotoxicity was not found under the concentration of the antibacterial activity.

Table 3 Cytotoxicity of compounds **1a**～**1f** and **2a**～**2e** against normal cell line HEK 293T

Compd.	IC_{50}/(μmol•L^{-1})
1a	100.3
1b	95.8
1c	86.6
1d	57.4
1e	79.2
1f	117.5
2a	56.2
2b	61.8
2c	74.4
2d	45.3
2e	62.5

2.6 Molecular docking

To gain insight into the molecular interactions of these compounds with LpxC, the binding pattern of **1d** with *E. coli* LpxC and *P. aeruginosa* LpxC were obtained computationally. As shown in Figure 4, the fatty chain of compound **1d** inserted into the lipophilic tunnel of LpxC, and the terminal methyl of the fatty chain interacted with the hydrophobic residues of LpxC. Amino acid residues MET195 and PHE212 were involved in the interaction between compound **1d** and *E. coli* LpxC. Amino acid residues ILE197 and VAL216 were involved in the interaction between compound **1d** and *P. aeruginosa* LpxC. The aminoguanidine in compound **1d** offered the major binding

force via forming hydrogen bonds with LpxC. In the interaction of *E. coli* LpxC with compound **1d** (Figure 4, A), the guanidine group of compound **1d** bound to the active site Zn^{2+} ion of *E. coli* LpxC and formed hydrogen bonds with CYS63, GLU78, THR191, ASP242, and HIS265. In the interaction of *P. aeruginosa* LpxC with compound **1d** (Figure 4, B), the aminoguanidine of compound **1d** bound to the active site Zn^{2+} ion of *P. aeruginosa* LpxC and formed hydrogen bonds with MET62, GLU77, THR190, ASP241, and HIS264.

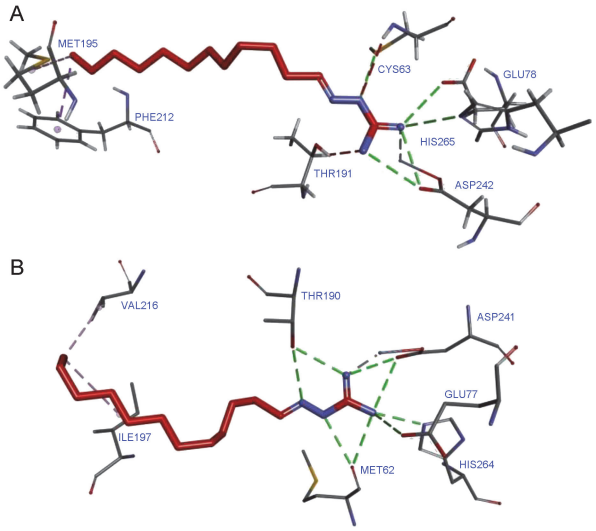


Figure 4 Interactions of compound **1d** with *E. Coli* LpxC (A) and *P. aeruginosa* LpxC (B)

LPC-009 is a classical LpxC inhibitor assembled by a hydroxamate and a linear diacetylene motif. It shows potent antibacterial activity against a variety of clinically important Gram-negative pathogens, including *E. coli*, *P. aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurium*, *Burkholderia cepacia*, and so on. For comparison, the interactions of compound **1d** with *E. coli* LpxC and *P. aeruginosa* LpxC were superimposed on the co-crystallised LPC-009. As seen in Figure 5, compound **1d** (red) showed similar interactions as LPC-009 (green) and overlay each other. This suggests that the binding of **1d** to LpxC is likely to be responsible for its antibacterial activity.

3 Conclusions

In the present work, a series of novel aminoguanidines containing linear alkanes were designed, synthesized and characterized. The antimicrobial activities of these compounds were evaluated. All of the targets except **1a** exhibited different degree of antibacterial properties with MICs in 0.5～64 μg/mL. The derivatives **2a**～**2e** kept one phenyl group in the skeleton showed the similar antibacterial properties and SARs to another series of derivatives **1a**～**1f**, which have no phenyl group in their structures. The best alkyl chain length is twelve (**1d**) or thirteen (**1e**) carbon atoms for the series of **1a**～**1f**, and seven (**2d**) for the series of **2a**～**2e**.

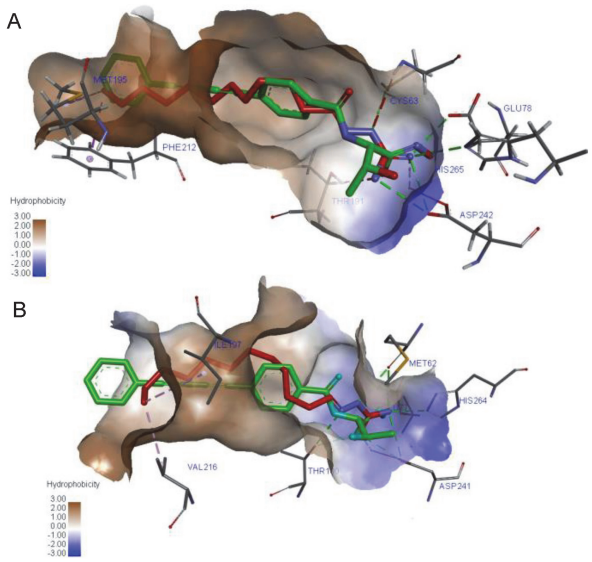


Figure 5 Overlay of **1d** and LPC-009 binding to *E. Coli* LpxC (A) and *P. aeruginosa* LpxC (B)

Compounds **1d**, **1e**, **2d** and **2e** showed a broader spectrum of antibacterial activity than penicillin, especially against negative bacteria and drug-resistant bacteria. And they showed equivalent or better antibacterial activity against strains 33693, 29212, 63501, 10104 and 43300 when compared to norfloxacin. The propensity of the development of bacterial resistance of compound **2d** indicated that the resistance of *S. aureus* and *E. coli* toward **2d** is not easily developed. Bactericidal time-kill kinetics of compound **2d** confirmed the rapidly bactericidal properties of **2d**. To understand the binding pattern for their antibacterial activity, molecular docking of representative **2d** was performed, which demonstrated that compound **2d** has a forceful binding with LpxC. The above findings strongly support the assumption that guanidine hydrazone coupled with linear alkane is a potential skeleton to develop new antimicrobial agents.

4 Experimental section

4.1 Chemistry

Melting points were determined in open capillary-tubes and were uncorrected (Jiahang melting point apparatus). ^{1}H NMR and ^{13}C NMR spectra were recorded on an NMR spectrometer (Bruker, Switzerland), and all chemical shifts were given relative to tetramethylsilane. High resolution mass spectra (HRMS) were measured on an MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany). All the reagents and solvents were purchased from Aladdin (Shanghai, China) or Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and were used as received.

4.2 General procedure for the preparation of compounds **1a**～**1f** and **2a**～**2e**

To a flask containing 50 mL of methanol and 1 mL of AcOH, aldehyde and aminoguanidine bicarbonate were added equally and stirred at 80 ℃ for 12 h. The solvents

were removed and the residue was purified by column chromatography on silica gel, eluting with 6% methanol and 3% ammonia in dichloromethane (DCM) to give the target compounds.

(*E*)-2-Hexylidenehydrazine-1-carboximidamide (**1a**): Oil, 55% yield. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.87 (t, J=6.8 Hz, CH_3, 3H), 1.25～1.33 (m, 4H, $(CH_2)_2$), 1.39～1.46 (m, 2H, CH_2), 2.10～2.17 (m, 2H, CH_2), 5.41 (s, 4H, guanidyl-H), 7.31 (t, J=5.5 Hz, =CH, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 159.22, 148.06, 32.56, 31.47, 26.84, 22.43, 14.37; ESI-HRMS calcd for $C_7H_{17}N_4$ $[M+H]^+$ 157.1448, found 157.1446.

(*E*)-2-Octylidenehydrazine-1-carboximidamide (**1b**): Oil, 56% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J=6.2 Hz, CH_3, 3H), 1.27～1.38 (m, 8H, $(CH_2)_4$), 1.49～1.54 (m, 2H, CH_2), 2.21～2.28 (m, 2H, CH_2), 4.70 (s, 4H, guanidyl- H), 7.55 (t, J=5.4 Hz, =CH, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.08, 153.28, 32.80, 31.75, 29.26, 29.11, 26.82, 22.62, 14.07; ESI-HRMS calcd for $C_9H_{21}N_4$ $[M+H]^+$ 185.1761, found 185.1765.

(*E*)-2-Decylidenehydrazine-1-carboximidamide (**1c**): Gel, 78% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J=6.3 Hz, CH_3, 3H), 1.28～1.37 (m, 12H, $(CH_2)_6$), 1.47～1.56 (m, 2H, CH_2), 2.22～2.28 (m, 2H, CH_2), 4.61 (s, 4H, guanidyl-H), 7.55 (t, J=5.5 Hz, =CH, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.95, 153.34, 32.80, 31.88, 29.51, 29.46, 29.30, 29.30, 26.79, 22.67, 14.10; ESI-HRMS calcd for $C_{11}H_{25}N_4$ $[M+H]^+$ 213.2074, found 213.2077.

(*E*)-2-Dodecylidenehydrazine-1-carboximidamide (**1d**): White solid, 80% yield. m.p. 67～69 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J=6.1 Hz, CH_3, 3H), 1.28～1.36 (m, 16H, $(CH_2)_8$), 1.46～1.56 (m, 2H, CH_2), 2.19～2.30 (m, 2H, CH_2), 4.76 (s, 4H, guanidyl-H), 7.55 (t, J=6.1 Hz, =CH, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.09, 153.32, 32.82, 31.92, 29.65, 29.62, 29.56, 29.48, 29.34, 29.32, 26.82, 22.69, 14.12; ESI-HRMS calcd for $C_{13}H_{29}N_4$ $[M+H]^+$ 241.2387, found 241.2306.

(*E*)-2-Tetradecylidenehydrazine-1-carboximidamide (**1e**): White solid, 74% yield. m.p. 82～83 ℃; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.89 (t, J=6.5 Hz, CH_3, 3H), 1.27～1.36 (m, 20H, $(CH_2)_{10}$), 1.46～1.54 (m, 2H, CH_2), 2.21～2.28 (m, 2H, CH_2), 4.25 (s, 4H, guanidyl-H), 7.56 (t, J=5.6 Hz, =CH, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 158.15, 153.51, 32.82, 31.92, 29.76, 29.68, 29.66, 29.65, 29.56, 29.48, 29.35, 29.32, 26.85, 22.68, 14.11; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 269.2700, found 269.2702.

(*E*)-2-Hexadecylidenehydrazine-1-carboximidamide (**1f**): White solid, 65% yield. m.p. 98～99 ℃; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J=6.2 Hz, CH_3, 3H), 1.24～1.46 (m, 26H, $(CH_2)_{13}$), 2.14～2.21 (m, 2H, CH_2), 6.63 (s, 4H, guanidyl-H), 7.36～7.42 (m, 1H, =CH); ^{13}C NMR (101 MHz, DMSO-d_6) δ: 157.16, 150.49, 32.48, 32.43, 31.78, 29.53, 29.50, 29.46, 29.44, 29.34, 29.33, 29.20, 28.26, 26.77, 26.62, 22.58, 14.43; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 297.3013, found 297.3014.

(*E*)-2-(4-Butoxybenzylidene)hydrazine-1-carboximidamide (**2a**): White solid, 76% yield. m.p. 171～172 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.97 (t, J=7.4 Hz, CH_3, 3H), 1.46～1.52 (m, 2H, CH_2), 1.73～1.79 (m, 2H, CH_2), 3.96 (t, J=6.5 Hz, CH_2), 4.93 (s, 4H, guanidyl-H), 6.85 (d, 2H, J=8.5 Hz, ArH), 7.57 (d, J=8.5 Hz, ArH, 2H), 8.14 (s, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.1, 159.3, 148.5, 128.5, 128.4, 114.5, 67.7, 31.3, 19.2, 13.9; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 235.1553, found 235.1554.

(*E*)-2-(4-(Pentyloxy)benzylidene)hydrazine-1-carboximidamide (**2b**): White solid, 76% yield. m.p. 177～178 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.93 (t, J=7.5 Hz, CH_3, 3H), 1.38～1.46 (m, 4H, $(CH_2)_2$, 1.77～1.80 (m, 2H, CH_2), 3.97 (t, J=7.2 Hz, CH_2), 5.24 (s, 4H, guanidyl-H), 6.88 (d, J=8.7 Hz, Ar-H, 2H), 7.60 (d, J=8.7 Hz, Ar-H, 2H), 8.16 (s, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.1, 158.9, 148.9, 128.5, 128.4, 114.5, 68.1, 28.9, 28.2, 22.5, 14.0; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 249.1710, found 249.1711.

(*E*)-2-(4-(Hexyloxy)benzylidene)hydrazine-1-carboximidamide (**2c**): White solid, 68% yield. m.p. 162～164 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90 (t, J=6.5 Hz, CH_3, 3H), 1.28～1.34 (m, 4H, $(CH_2)_2$, 1.41～1.47 (m, 2H, CH_2), 1.73～1.79 (m, 2H, CH_2), 3.93 (t, J=6.6 Hz, CH_2), 5.20 (s, 4H, guanidyl-H), 6.83 (d, J=8.2 Hz, Ar-H, 2H), 7.55 (d, J=8.2 Hz, Ar-H, 2H), 8.11 (s, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.2, 158.9, 148.5, 128.5, 128.1, 114.5, 68.1, 31.6, 29.2, 25.7, 22.6, 14.1; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 263.1866, found 263.1867.

(*E*)-2-(4-(Heptyloxy)benzylidene)hydrazine-1-carboximidamide (**2d**): White solid, 69% yield. m.p. 167～169 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, J=6.1 Hz, CH_3, 3H), 1.29～1.38 (m, 6H, $(CH_2)_3$, 1.41～1.47 (m, 2H, CH_2), 1.74～1.80 (m, 2H, CH_2), 3.95 (t, J=6.4 Hz, CH_2), 4.63 (s, 4H, guanidyl-H), 6.85 (d, J=8.2 Hz, Ar-H, 2H), 7.57 (d, J=8.2 Hz, Ar-H, 2H), 8.13 (s, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.2, 158.8, 148.7, 128.4, 128.3, 114.5, 68.1, 31.8, 29.3, 29.1, 26.0, 22.6, 14.1; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 277.2023, found 277.2022.

(*E*)-2-(4-(Octyloxy)benzylidene)hydrazine-1-carboximidamide (**2e**): White solid, 71% yield. m.p. 168～169 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, J=6.1 Hz, CH_3, 3H), 1.26～1.36 (m, 8H, $(CH_2)_4$, 1.41～1.47 (m, 2H, CH_2), 1.74～1.79 (m, 2H, CH_2), 3.94 (t, J=6.6 Hz, CH_2), 4.94 (s, 4H, guanidyl-H), 6.84 (d, J=8.2 Hz, Ar-H, 2H), 7.56 (d, J=8.2 Hz, Ar-H, 2H), 8.12 (s, 1H, =CH); ^{13}C NMR (126 MHz, $CDCl_3$) δ: 160.2, 158.9, 148.6, 128.5, 128.2, 114.5, 68.1, 31.8, 29.4, 29.3, 29.2, 26.0, 22.7, 14.1; ESI-HRMS calcd for $C_{15}H_{33}N_4$ $[M+H]^+$ 291.2179, found 291.2178.

4.3 Evaluation of anti-bacterial activity *in vitro*

The anti-bacterial activity *in vitro* was evaluated using a two-fold serial dilution technique, and the final concentrations of compounds obtained were in the range of 0.5～128 μg/mL. Test bacteria were grown to mid-log phase in Mueller-Hinton broth (MHB) or Tryptone Soya Broth (TSB) and diluted 1000-fold in the same medium. The bacteria of 10^5 CFU/mL were inoculated into MHB or TSB and dispensed at 0.2 mL/well in a 96-well microtiter plate.

As positive controls, gatifloxacin, moxifloxacin, norfloxacin, oxacillin and penicillin were used. Test compounds were prepared in dimethylsulfoxide (DMSO), the final concentration of which did not exceed 0.05%. The MIC was defined as the concentration of a test compound that completely inhibited bacteria growth during 24 h incubation at 37 ℃. Bacteria growth was determined by measuring the absorption at 630 nm using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were carried out three times.

4.4 Evaluation of bacterial resistance development

In order to evaluate the propensity for developing bacterial resistance, two of the compounds with high antibacterial activity (**1d** and **2d**) were used. The initial MIC values of **1d**, **2d**, and antibiotic control norfloxacin against *Staphylococcus aureus* CMCC 25923 and *Escherichia coli* CMCC 44568 were obtained in the above assay (Table 1). Subsequently, serial passaging was initiated by transferring bacterial suspension grown at the sub-MIC of the compounds/antibiotic (MIC/2) to a new plate and subjecting it to another assay to determine the (new) MIC. After 22 h incubation, cells grown at the sub-MIC of the test compounds/antibiotic were once again transferred and the MIC was determined. The process was repeated for 21 passages. The MICs for compounds **1d**, **2d**, and norfloxacin were plotted as a function of time in days (number of passages) to determine the propensity of bacterial resistance development.[24]

4.5 Time-kill assay

Multi-drug resistant *S. aureus* ATCC 43300 grown in MHB was used to determine time-kill kinetics. Bacterial suspensions (10^5 CFU/mL) containing test compounds (norflocaxin, compound **2d**) at final concentrations of 1/2× MIC, 1×MIC and 2×MIC were incubated at 37 ℃ with shaking. Aliquots (10 μL) were removed from the cultures after 0, 0.5, 1, 2, 3, 4, 6, 8 and 12 h, serially diluted 1000-fold in nutrient solution, and plated onto sterile Mueller-Hinton agar medium. Plates were then incubated for 24 h at 37 ℃, the number of CFU was counted, and the total bacterial was determined.

4.6 Evaluation of cytotoxicity activity *in vitro*

Human embryonic kidney 293T cells (HEK 293T cells) were used to test the cytotoxic activity of the target compounds according to operation methods in Ref. [18].

4.7 Docking studies

Molecular docking of compound **1d** with *E. coli* LpxC (PDB code: 3P3G) and *P. aeruginosa* LpxC (PDB code: 3P3E), was carried out using Discovery Studio software (version 2.0). The structures of 3P3G and 3P3E were downloaded from the Protein Data Bank. The three-dimensional (3D) structure of compound **1d** was constructed using Chem3D Ultra 12.0 software and energetically minimised in the DS. Before the docking, hydrogen atoms of the protein were added and water/impurities were removed. Types of docking interactions of the protein with compound **1d** were analysed and ranked, and those with maximum binding energy were selected to analyse the interaction patterns.

Supporting Information ^{1}H NMR, ^{13}C NMR and HRMS spectra of target compounds **1a**～**1f** and **2a**～**2e**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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