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PII: S0223-5234(18)30970-X

DOI: https://doi.org/10.1016/j.ejmech.2018.11.016

Reference: EJMECH 10873

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 11 December 2017

Revised Date: 25 October 2018

Accepted Date: 6 November 2018

Please cite this article as: L. Su, J. Li, Z. Zhou, D. Huang, Y. Zhang, H. Pei, W. Guo, H. Wu, X. Wang, M. Liu, C.-G. Yang, Y. Chen, Design, synthesis and evaluation of hybrid of tetrahydrocarbazole with 2,4-diaminopyrimidine scaffold as antibacterial agents, *European Journal of Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.ejmech.2018.11.016.

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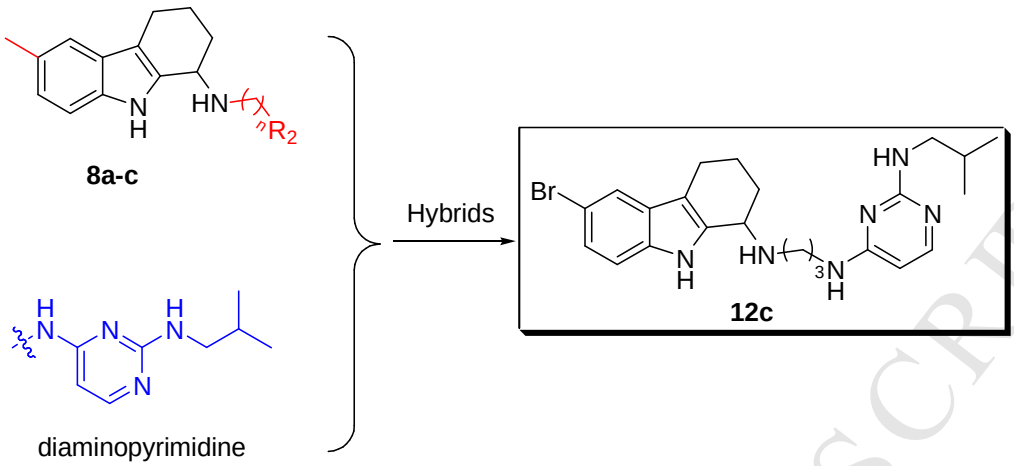
Graphical abstract



SAR analysis resulted in the discovery of hybrids of tetrahydrocarbazole with 2,4-diaminopyrimidine scaffold with potent antibacterial activities not only against *S. aureus* strain Newman and *Escherichia coli* AB1157 strain but also multidrug-resistant *S. aureus* strains.

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Design, synthesis and evaluation of hybrid of tetrahydrocarbazole with 2,4-diaminopyrimidine scaffold as antibacterial agents

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Abstract: Several 6-substituted tetrahydrocarbazole derivatives were designed, synthesized and evaluated for the antibacterial activities against *Staphylococcus aureus* Newman strain. Subsequently, 2,4-diaminopyrimidine scaffold was merged

with the tetrahydrocarbazole unit to generate a series of novel hybrid derivatives and the antibacterial activities were also investigated. Among these novel hybrids, compound **12c** showed the most potent activity with a MIC of 0.39-0.78 μg/mL against *S. aureus* Newman and *Escherichia coli* AB1157 strain. In addition, compound **12c** exhibited low MIC values against a panel of multidrug-resistant strains of *S. aureus*.

1. Introduction

The discovery of antibiotics has been vital to human survival, and has added an average of 20 years to the human lifespan [1, 2]. Unfortunately, the overuse of antibiotics has put pressure on bacteria to evolve resistance. Nowadays, bacteria are resistant to almost all antibiotics [3, 4]. To make matters worse, the drug-discovery pipeline of new antibacterial drugs is almost empty [3]. According to the World Health Organization, infections caused by resistant bacteria have become increasingly widespread, weakening the efficacy of antibiotics which have been regarded as the most successful discovery in medical history [5]. This poses a significant future risk as common infections become untreatable. Therefore, discovery of novel classes of antibiotics that are effective against a broad range of bacteria, including those which have developed resistance to known drugs, has become an urgent challenge [6].

Tetrahydrocarbazole is a well-known privileged skeleton, which possesses many different biological functions such as anti-tumor [5, 7, 8], antibiotics [6, 9], analgesia [10], anti-depression [11, 12], and anti-virus [13, 14] (**2**, Fig. 1). Beyond that,

compound **1** (GSK983), as a novel tetrahydrocarbazole (Fig. 1), has shown broad spectrum antiviral activity [14], and recently was reported to take effect through inhibiting the pyrimidine biosynthesis enzyme dihydroorotate dehydrogenase (DHODH) [15]. Interestingly, the 2,4-diaminopyrimidine scaffold was generally existed in bioactive small-molecule agents, including the antibiotic trimethoprim (TMP, **3**), and *Bacillus anthracis* inhibitor **4** (Fig. 1) [16, 17]. As for its antibiotic effect, the diaminopyrimidine can interfere with the folic acid metabolism by binding dihydrofolate reductase, which eventually leads to the prevention of bacterial reproduction [16-18].

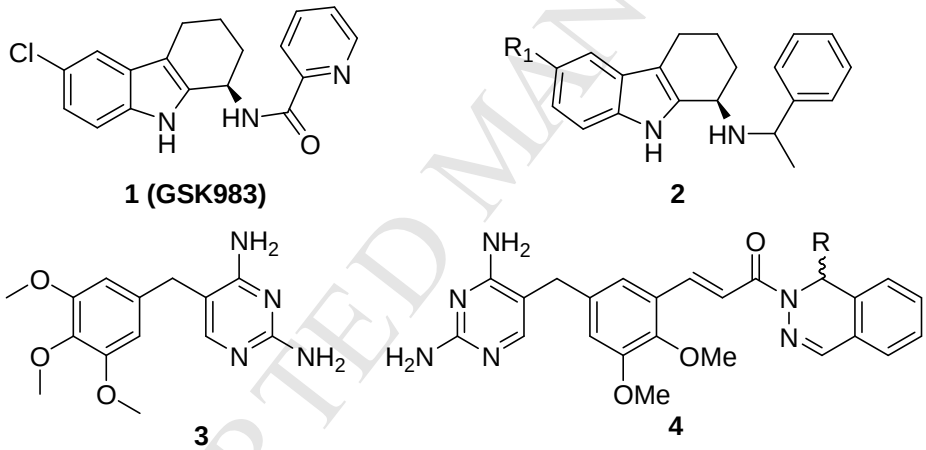


Fig. 1. Several molecules of tetrahydrocarbazoles and diaminopyrimidines with antibacterial and antiviral activities.

In order to identify novel antibacterial drug candidates, we synthesized a series of tetrahydrocarbazole derivatives and evaluated their activities. Compound **5** was identified as a potential hit with the antibacterial activity against *S. aureus* Newman with MIC values of 5.0 - 6.6 μg/mL (Fig. 2, Table 1). In an extension of our previous study, several novel tetrahydrocarbazole derivatives (**8a-c,** Table 2) of compound **5** were designed and optimized with a goal of exploring the active site, necessary

groups and suitable length of linker motifs. The *in vitro* antibacterial activities against *S. aureus* Newman were evaluated. Subsequently, since many substituted 2,4-diaminopyrimidine derivatives have also shown to have potent antibacterial efficacy, the structural combination of tetrahydrocarbazole and diaminopyrimidine scaffolds might lead to several novel antibacterial compounds (Fig. 3, **12a-p,** Table 2, 3). These hybrids not only could retain the excellent antibacterial activities as observed in our previous work, but also might overcome the deficiency of trimethoprim antibiotics which are susceptible to resistance development [19]. Five compounds with the most potent *in vitro* antibacterial activities against *S. aureus* Newman and *E. coli* AB1157 were evaluated against a panel of multidrug-resistant *S. aureus*, respectively.

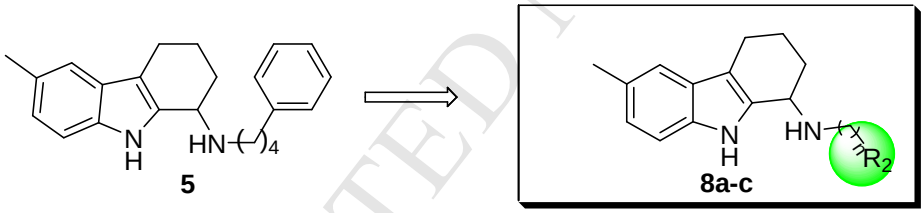


Fig. 2. Simple modification of based on tetrahydrocarbazole **5.**

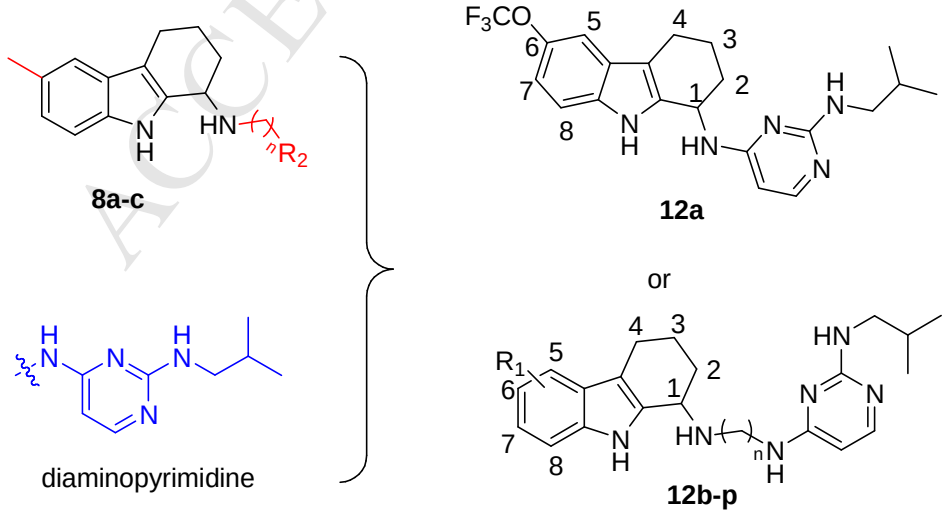


Fig. 3. Hybridation and optimization based on the tetrahydrocarbazole and

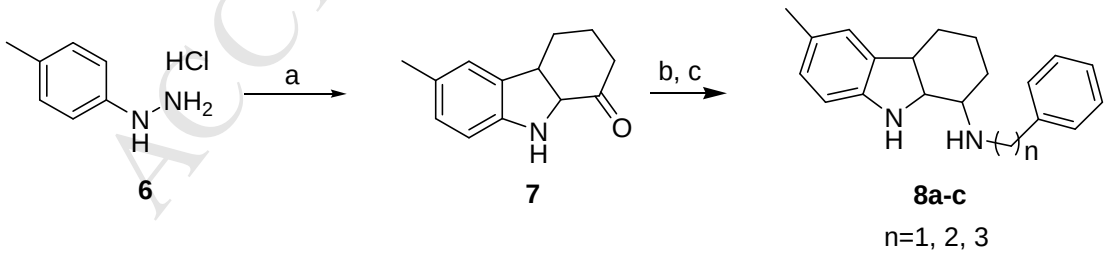
diaminopyrimidine scaffolds.

Table 1 MICs of compound **5** against Newman and multidrug-resistant *S. aureus*.

Compound	MIC (μg/mL)					
	Newman	**NRS-1**	**NRS-70**	**NRS-100**	**NRS-108**	**NRS-271**
5	5.0-6.6	2.9-4.2	2.9-4.2	2.9-4.2	4.2-5.8	4.2-5.8
Kanamycin	< 18.70	>75	> 75	< 9.38	> 75	< 9.38
Vancomycin	< 3.75	1.25-2.5	< 1.25	< 1.25	< 1.25	< 1.25
Tetracycline	< 2.50	> 12.5	< 1.56	< 1.56	< 1.56	< 1.56

2. Chemistry

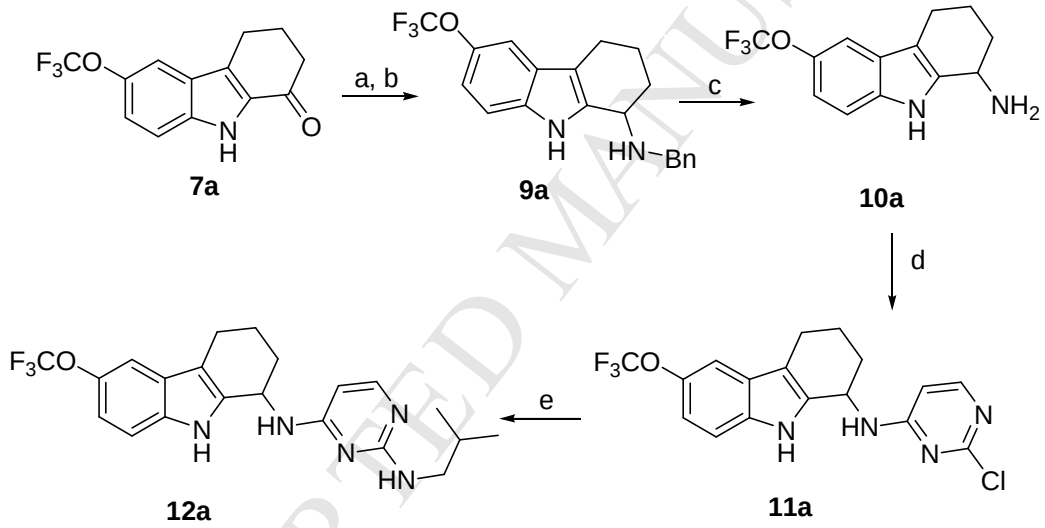
With the purpose of discovering novel antibacterial agents with higher activities, we first synthesized tetrahydrocarbazole derivatives **8a-c** as demonstrated in Scheme 1. Intermediate **7** was constructed from *p*-tolylhydrazine hydrochloride and 1,2-cyclohexadione in the presence of hydrochloric acid and acetic acid using the Fischer indole synthesis [20]. Subsequently, treatment of **7** with different primary amines generated compounds **8a-c** [21].



Scheme 1. Synthesis of compounds **8a-c**. Reagents and conditions: (a) 1,2-cyclohexanedione, conc. HCl, AcOH, MeOH, 60 ^{o}C; (b) *p*-TsOH, amines, toluene,

140 °C, 16 h; (c) $NaBH_4$, MeOH, 80 °C, 4 h.

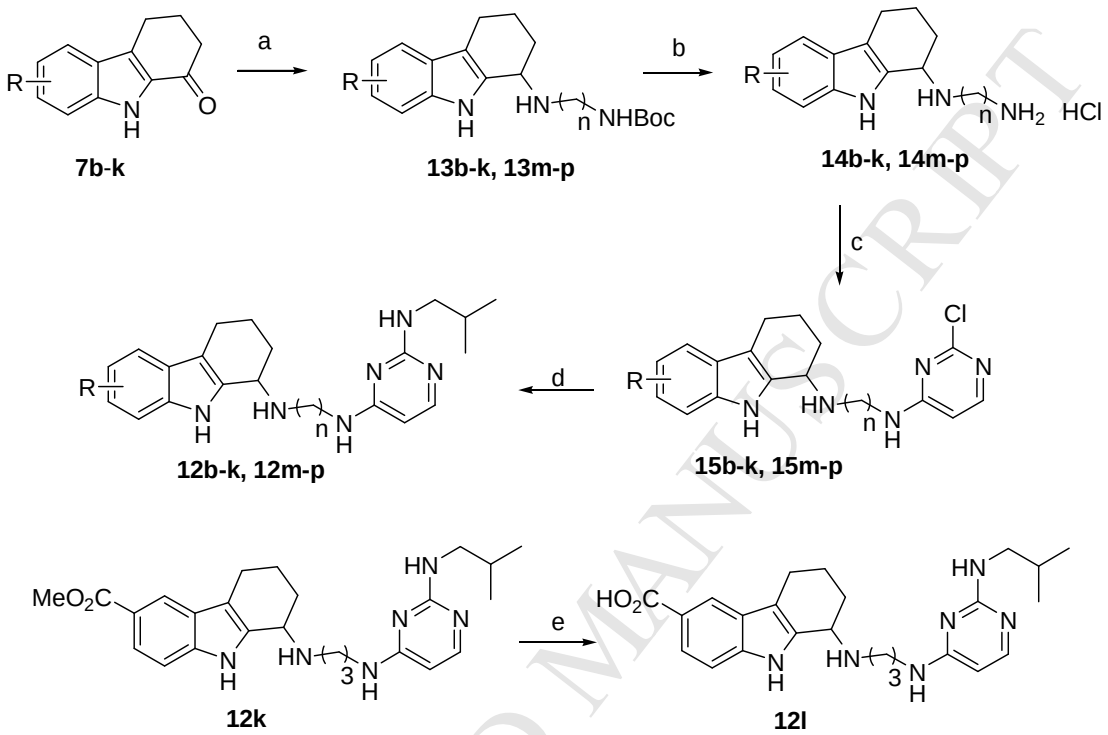
Compound **12a** was prepared as described in Scheme 2. The starting material **7a** was synthesized using procedures similar to those in Scheme 1. Compound **9a** was prepared from 6-trifluoromethoxy-2,3,4,9-tetrahydro-1*H*-1-carbazolone by a similar method. Debenzylation of **9a** with Pd/C under H_2 then afforded the key intermediate **10a**, which was subsequently coupled with 2,4-dichloropyrimidine to provide **11a**. Finally, the target compound **12a** was obtained by nucleophilic aromatic substitution of chloro by isobutylamine (Scheme 2).



Scheme 2. Synthesis of compound **12a**. Reagents and conditions: (a) *p*-TsOH, benzylamine, toluene, 140 °C, 16 h; (b) $NaBH_4$, MeOH, 80 °C, 4 h; (c) Pd/C (10%), H_2, MeOH, r.t.; (d) 2,4-dichloropyrimidine, DIEA, EtOH, r.t.; (e) isobutylamine, *n*-BuOH, 120 °C.

In the process of preparing the target compounds **12b-k** and **12m-p** (Scheme 3), there were just two steps different from the synthesis of compound **12a**. The free amino groups of starting compounds **7b-k** were reacted with various aliphatic diamines protected by a Boc group that was then readily cleaved under acidic conditions to give

compounds **14b-k** and **14m-p**. The remaining steps paralleled to those used to prepare **12a**. Compound **12l** was prepared by hydrolyzed **12k** with the sodium hydroxide solution.



Scheme 3. Synthesis of compounds **12b-p**. Reagents and conditions: (a) *p*-TsOH, corresponding amines, toluene, 140 ^{o}C, 16 h; $NaBH_4$, MeOH, 80 ^{o}C, 4 h; (b) anhydrous DCM, 1,4-dioxane, 0 ^{o}C to r.t.; (c) 2,4-dichloropyrimidine, DIEA, EtOH, r.t.; (d) isobutylamine, *n*-BuOH, 120 ^{o}C; (e) NaOH, EtOH.

3. Antibacterial studies

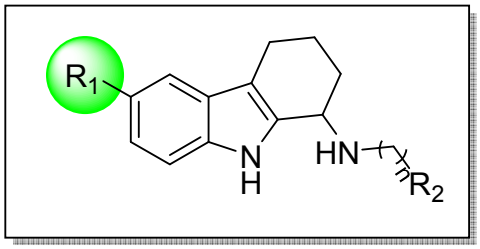
To evaluate the antibacterial activities of the synthesized tetrahydrocarbazole derivatives, we chose the lab-cultured *S. aureus* Newman and *Escherichia coli* AB1157 as the experimental strains, which are representatives of Gram-positive and Gram-negative bacteria, respectively. We also investigated the activities of the

synthetic compounds against a panel of multidrug-resistant strains of *S. aureus*, including NRS-1 (resistant to aminoglycosides and tetracycline), NRS-70 (resistant to erythromycin), NRS-100 (resistant to oxacillin and tetracycline), NRS-108 (resistant to gentamicin), and NRS-271 (resistant to linezolid) [22]. The MIC values of these compounds were in the range of 0.39 - 50 μg/mL.

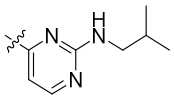
4. Results and discussion

In this study, novel antibiotics were designed and synthesized for the treatment of multiple *S. aureus* strains and *E. coli* AB1157. In order to demonstrate which groups or moieties were beneficial or essential for enhancing the antibacterial activities of the compounds, several modifications to the linker moiety of compound **5** were performed to afford compounds **8a-c** (Table 1). The results showed that the linker with three or four methylenes may be more suitable for maintaining antibacterial activities. In addition, the aromaticity of the group at the end of the linker is essential for the antibacterial activities of the compounds based on our previous results.

Table 2 MICs of compounds dating from explorative modification for compound **5** against *S. aureus* Newman.



Compound	R_1	n	R_2	MIC
				μg/mL

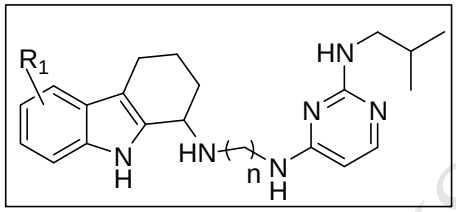
5	$-CH_3$	4	Ph	5.0-6.6
8a	$-CH_3$	3	Ph	4.0-8.0
8b	$-CH_3$	2	Ph	15.02-30.2
8c	$-CH_3$	1	Ph	14.5-20.9
12a	$-OCF_3$	0		0.78-1.56
Vancomycin	-	-	-	0.78-1.56
Kanamycin	-	-	-	1.95-3.90
Tetracycline	-	-	-	<0.20

By referring to the literature, we noticed that the 2,4-diaminopyrimidine scaffold is one of the dominant pharmacophores in antibacterial agents. Therefore, compound **12a** was designed innovatively by hybridizing tetrahydrocarbazole scaffold with the 2,4-diaminopyrimidine moiety directly. As we expected, the inclusion of these rings in **12a** showed an improved antibacterial activity against *S. aureus* strain Newman with a MIC of 0.78-1.56 µg/mL. A series of novel derivatives **12b-p** were further synthesized to investigate the influence of linker-length and tetrahydrocarbazole substituents on the antibacterial activities.

Compounds **12b-g** were obtained by modifying the length of the linker between tetrahydrocarbazole skeleton and the terminal pyrimidine ring to identify the optimum linker length. As shown in Table 3, the antibacterial effect of compounds **12b-d** and **12e-g** indicated a parabolic trend for two-carbon, three-carbon and four-carbon linkers, with **12c**, having a three-carbon linker exhibiting the best activity against *S. aureus* Newman and *E. coli* AB1157 among the investigated compounds. The above studies

illustrated that the antibacterial activity was enhanced by maintaining three methylene group in the linker moiety of the hybrid. Therefore, in the subsequent modifications, the linker containing three methylene groups was retained.

Table 3 MICs of synthetic hybrid derivatives against *S. aureus* Newman and *E. coli* AB1157



Compound	R_1	n	MIC (μg/mL)	
			Newman	**AB1157**
12b	6-Br	2	1.56-3.13	1.56-3.13
12c	6-Br	3	0.39-0.78	0.39-0.78
12d	6-Br	4	0.78-1.56	1.56-3.13
12e	6-OCF_3	2	3.13-6.25	3.13-6.25
12f	6-OCF_3	3	1.56-3.13	1.56-3.13
12g	6-OCF_3	4	1.56-3.13	3.13-6.25
12h	6-F	3	12.5-25.0	12.5-25.0
12i	6-Cl	3	1.56-3.13	1.56-3.13
12j	6-CF_3	3	1.56-3.13	1.56-3.13
12k	6-CO_2Me	3	25.0-50.0	> 50.0
12l	6-COOH	3	> 50.0	> 50.0
12m	6-OMe	3	25.0-50.0	> 50.0
12n	6-H	3	12.5-25.0	12.5-25.0
12o	8-Br	3	3.13-6.25	6.25-12.5
12p	8-OMe	3	12.5-25.0	12.5-25.0
Vancomycin	-	-	0.78-1.56	> 50.0
Kanamycin	-	-	3.13-6.25	6.25-12.5

Compounds **12h-p** were prepared by altering the substituents on the tetrahydrocarbazole ring of the hybrid as presented in Table 3. The phenyl rings with electron-withdrawing substituents, such as chlorine (**12i**), bromine (**12c** and **12o**), trifluoromethyl (**12j**) and trifluoromethoxy (**12f**) exhibited more potent activity with MICs of 0.39-12.5 µg/mL, while compounds with electron-donating substituents such as methoxy (**12m** and **12p**) and unsubstituted compound **12n** with MICs of 12.5-25.0 µg/mL. However, the fluorine substituent (**12h**) was an exception, showing an MIC of 12.5-25.0 µg/mL. Surprisingly, analogues with carboxyl (**12l**) or methyl ester (**12k**) substituents showed little or no inhibition (>50 µg/mL). In addition, it seems that tetrahydrocarbazole substitution at the 6-position with electron-withdrawing substituent increased the inhibitory effects obviously compared to the corresponding compounds substituted at C8 (see **12c** and **12o**). The SARs of these compounds revealed that the antibacterial activity was enhanced by the introduction of small electron-withdrawing substituents into the tetrahydrocarbazole ring at the 6-position.

The five most potent compounds against *S. aureus* Newman and *E. coli* AB1157 were further evaluated against multidrug-resistant strains of *S. aureus* NRS-1, NRS-70, NRS-100, NRS-108 and NRS-271 (Table 4). The derivatives incorporating tetrahydro-carbazole and diaminopyrimidine moieties exhibited good antibacterial activity *in vitro*, with MICs ranging from 0.39-6.25 µg/mL. Among them, compounds **12c, 12f, 12i** and **12j** were more effective with MICs of 0.39 -3.13 µg/mL, because all compounds have electron-withdrawing substituents at C6, compound **12c** was the

most potent one against Newman strain and multidrug-resistant strains of *S. aureus*, and its effectiveness was comparable to vancomycin. The mechanism and target of compound **12c** is unclear, however. It appears likely that the compound binds to the bacterial DNA clamp in the previously described "Subsite I".[9] We also suspect that the 2,4-diaminopyrimidine moieties may be able to reach around to "Subsite II".

Table 4 MICs of the hybrid derivatives against five multidrug-resistant strains of *S. aureus*: **NRS-1**, **NRS-70**, **NRS-100**, **NRS-108**, **NRS-271**.

Compound	**MIC** (μg/mL)				
	NRS-1	**NRS-70**	**NRS-100**	**NRS-108**	**NRS-271**
12c	0.39-0.78	0.78-1.56	0.78-1.56	0.78-1.56	0.78-1.56
12f	1.56-3.13	1.56-3.13	1.56-3.13	1.56-3.13	1.56-3.13
12i	1.56-3.13	3.13-6.25	3.13-6.25	1.56-3.13	1.56-3.13
12j	1.56-3.13	1.56-3.13	1.56-3.13	1.56-3.13	1.56-3.13
12o	3.13-6.25	6.25-12.5	6.25-12.5	6.25-12.5	6.25-12.5
Vancomycin	1.56-3.13	0.78-1.56	0.78-1.56	0.78-1.56	0.78-1.56
Kanamycin	> 50.0	> 50.0	0.39-0.78	> 50.0	6.25-12.5

Preliminary pharmacokinetic studies of compound 12c *in vivo*

In order to further evaluate the potential of this series of compounds as an antibacterial lead, compound **12c** was selected to explore the preclinical pharmacokinetic properties with oral administration, the plasma concentration in SD rats after a single oral gavage administration at a dose of 10 mg/kg was measured, and

pharmacokinetic parameters were calculated, as shown in Table 5. The concentration of compound **12c** reached the peak value (*Cmax* = 86.32 ± 25.57 ng/mL) at 3.60 ± 0.89 hr (T_{max}), and the area under the curve (AUC_{0-t}) was 1196.68 ± 238.10. The relatively low concentration in rats may be due to the poor dissolution and absorption rate of compound **12c**, which indicate more hydrophilic groups should be introduced to the scaffold. In addition, it possessed of a long half-life in plasma (*t½* = 10.80 ± 0.89 hr). Also, the plasma clearance (*CL*) was 6.60 ± 1.20 L/min/kg. Which means that the compound was not easily metabolized in the body, reminding us to pay attention to dosing intervals in subsequent multiple dosing trials. The mean values of the apparent volume of distribution (*Vd*) were quite high for oral administration, indicating that compound **12c** was widely distributed in tissues.

Table 5. Pharmacokinetic parameters of **12c** after oral administration in rats (n = 5, mean ± SD)

Dose (mg/kg)	10
C_{max} *(ng/mL)*	86.32 ± 25.57
T_{max} *(hr)*	3.60 ± 0.89
t½ (hr)	10.80 ± 0.57
AUC_{0-t} *(hr*ng/mL)*	1196.68 ± 238.10
$AUC_{0-\infty}$ *(hr*ng/mL)*	1557.25 ± 298.17
Vd (L/kg)	103.15 ± 21.46
CL (L/min/kg)	6.60 ± 1.20
MRT (min)	16.19 ± 0.82

Conclusion

In the initial screening of a small molecule library, a tetrahydrocarbazole compound **5** displayed antibacterial activity and classified as a hit compound. A series of new analogues and hybrids bearing the 2,4-diaminopyridimine scaffold were designed and synthesized based on hit compound **5** and investigated on the antibacterial activity *in vitro* against *S. aureus* Newman and *E. coli* AB1157. Several potent compounds were further evaluated against five multidrug-resistant *S. aureus* strains (NRS-1, NRS-70, NRS-100, NRS-108 and NRS-271). The structure-activity relationship analysis indicated that the linker and the electric properties of substituents on the terminal aromatic ring had a significant influence on the antibacterial activities. Compound **12c** exhibited excellent activity. For Gram-positive bacterium *S. aureus* Newman and Gram-negative bacterium *E. coli* AB1157, **12c** showed highest activity with a MIC of 0.39-0.78 μg/mL; for multidrug-resistant bacteria of *S. aureus*, **12c** displayed the most potent activity with a MIC of 0.39-1.56 μg/mL. Here we offered a series of promising novel hybrid of tetrahydrocarbazole and diaminopyrimidine derivatives for the discovery of new chemical entities against multidrug-resistant bacteria. Further in-depth SAR studies of the active scaffolds and the mechanism (of action) are also required.

6. Experiment section

6.1 General methods for chemistry

All reagents and chemicals used in experiments were purchased from Adamas-beta and Aladdin Reagents Inc., Sigma-Aldrich Inc., Bide Pharmatech Ltd., and J&K Scientific Ltd.. Unless otherwise indicated, all reagents were used as received. All

reactions were carried out with standard techniques under an inert atmosphere (Ar or N_2). Column chromatography was performed on silica gel (Qingdao, 200-300 mesh) using the indicated eluents. Preparative thin layer chromatography was carried out on 200 mm × 200 mm plate coated with 0.4-0.5 mm of silica gel. NMR spectra were collected on a Bruker 300 or 500 MHz instrument using $CDCl_3$ or DMSO-d_6; High-resolution mass data were obtained on an Agilent 6550 iFunnel Q-TOF LC/MS System. HPLC was employed for purity detection with an Agilent Technologies 1200 Series, using the following method: Eclipse XDB C18 column, 5 μm, 4.6 mm × 150 mm, column temperature 40 °C; solvent A: ultrapure water (with 0.1% triethylamine), solvent B: MeOH (with 0.1% trimethylamine); flow rate = 1.5 mL/min. The biological activities of compounds were tested on the assumption that compound purity was more than 95%.

6.2. Synthesis of 6-methyl-1-(4-phenylbutylamino)-1*H*-2,3,4,9-tetrahydrocarbazole (**5)**

To a solution of 1,2-cyclohexanedione (2243 mg, 20 mmol) and concentrated hydrochloric acid (13 mL) in acetic acid (40 mL), *p*-tolylhydrazine hydrochloride (1586 mg, 10 mmol) in methanol (25 mL) was added dropwise slowly over 10 mins. After the addition, the resulting mixture was heated to 60 °C, and stirred overnight. The solvent was evaporated, and the residue was pH adjusted to weak alkaline with saturated $NaHCO_3$. The mixture was extracted with AcOEt (3 × 20 mL). The combined organic extract was washed with brine, dried over anhydrous Na_2SO_4, and

concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 12/1 v/v) to give intermediate **7** (1235 mg, 62%) as a brown powder.

The mixture of intermediate **7** (102 mg, 0.5 mmol), 4-phenylbutylamine (0.12 mL, 0.8 mmol) and catalytic *p*-TsOH in toluene (10 mL) was refluxed at 140 ^{o}C for 16 h with a Dean-Stark trap in place. The solvent was evaporated and the residue was dissolved in methanol. $NaBH_4$ (177 mg) was then added at 0 ^{o}C. The solution was heated to 80 ^{o}C until TLC indicated the reaction was complete. The reaction was quenched with water and concentrated. Subsequently, the mixture was extracted with AcOEt twice and the combined organic layers were dried over anhydrous Na_2SO_4. After evaporation of the solvent, the resulting residue was purified by column chromatography on silica gel (petroleum ether/AcOEt, 4/1 v/v) to give compound **5** (123 mg, Yield: 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.33 – 7.27 (m, 2H), 7.26 – 7.23 (m, 1H), 7.23 – 7.11 (m, 4H), 6.99 – 6.92 (m, 1H), 3.97 – 3.85 (m, 1H), 2.89 – 2.77 (m, 1H), 2.71 – 2.63 (m, 4H), 2.63 – 2.59 (m, 1H), 2.44 (s, 3H), 2.28 – 2.14 (m, 1H), 2.08 – 1.95 (m, 1H), 1.83 –1.65 (m, 4H), 1.63–1.48 (m, 4H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.28, 136.74, 134.21, 128.29, 128.18, 127.10, 126.27, 125.57, 121.96, 117.38, 110.68, 108.76, 51.61, 46.07, 35.22, 29.72, 29.34, 28.92, 21.27, 20.89, 20.76. HR MS (ESI): calcd for $C_{23}H_{29}N_2$ $[M + H]^+$; 333.2331; found 333.2328.

6.3. Syntheses of compounds **8a-c**

6.3.1. 6-Methyl-1-(3-phenylpropylamino)-1*H*-2,3,4,9-tetrahydrocarbazole (**8a**)

The title compound **8a** was obtained according to the procedure for compound **5** except utilizing 3-phenylpropylamine instead of 4-phenylbutylamine. Yield: 71%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.38 – 7.27 (m, 2H), 7.25 – 7.10 (m, 4H), 6.97 (d, *J* = 8.2 Hz, 1H), 3.96 – 3.87 (m, 1H), 2.92 – 2.78 (m, 1H), 2.76 – 2.57 (m, 5H), 2.45 (s, 3H), 2.29 – 1.95 (m, 2H), 1.92 – 1.68 (m, 3H), 1.66 – 1.57 (m, 1H), 1.27 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 142.33, 136.89, 134.20, 128.31, 128.19, 127.11, 126.25, 125.55, 121.94, 117.37, 110.67, 108.69, 51.57, 45.67, 33.06, 32.04, 29.42, 21.27, 20.89, 20.71. HR MS (ESI): calcd for $C_{22}H_{27}N_2$ $[M + H]^+$; 319.2174; found 319.2172.

6.3.2. 6-Methyl-1-(2-phenylethylamino)-1*H*-2,3,4,9-tetrahydrocarbazole (**8b**)

The title compound **8b** was obtained according to the procedure for compound **5** except utilizing 2-phenylethylamine instead of 4-phenylbutylamine. Yield: 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.38 – 7.19 (m, 6H), 7.14 (d, *J* = 8.2 Hz, 1H), 6.95 (dd, *J* = 8.6, 0.8 Hz, 1H), 4.01 – 3.90 (m, 1H), 3.06 – 2.91 (m, 2H), 2.68 – 2.56 (m, 2H), 2.43 (s, 3H), 2.17 – 2.07 (m, 1H), 2.06 – 1.91 (m, 1H), 1.82 – 1.64 (m, 2H), 1.63 – 1.57 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 140.41, 136.52, 134.19, 128.57, 128.25, 127.03, 126.30, 125.85, 122.03, 117.40, 110.71, 108.80, 51.36, 48.20, 36.50, 29.37, 21.25, 20.81, 20.50. HR MS (ESI): calcd for $C_{21}H_{25}N_2$ $[M + H]^+$; 305.2018; found 305.2011.

6.3.3. 6-Methyl-1-benzylamino-1*H*-2,3,4,9-tetrahydrocarbazole (**8c**)

The title compound **8c** was obtained according to the procedure for compound **5** except utilizing benzylamine instead of 4-phenylbutylamine. Yield: 50%. ^{1}H NMR

(300 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.45 – 7.13 (m, 7H), 6.95 (d, *J* = 8.2 Hz, 1H), 4.00 (d, *J* = 12.9 Hz,1H), 3.98 (s, 1H), 3.86 (d, *J* = 12.9 Hz, 1H), 2.73 – 2.59 (m, 2H), 2.43 (s, 3H), 2.30 (dd, *J* = 12.0, 5.1 Hz, 1H), 2.14 – 1.98 (m, 1H), 1.86 – 1.60 (m, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.29, 136.82, 134.29, 128.10, 128.02, 127.17, 126.52, 126.39, 122.06, 117.44, 110.77, 108.97, 51.28, 49.95, 29.41, 21.31, 20.97, 20.92. HR MS (ESI): calcd for $C_{20}H_{23}N_2$ $[M + H]^+$; 291.1861; found 291.1856.

6.4. Synthesis of compounds **12a-p**

6.4.1.

6-Trifluoromethoxy-1-((2-isobutylaminopyrimidine)-4-amino)-1*H*-2,3,4,9-tetrahydro carbazole (**12a)**

To a solution of intermediate **10a** (132 mg, 0.5 mmol) in EtOH (5 mL) at r.t. was added 2,4-dichloropyrimidine (112 mg, 0.75 mmol) and DIEA (0.25 mL) and the reaction was stirred for 8 h. Upon completion, the mixture was quenched with water, concentrated, and extracted with AcOEt (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to give crude intermediate **11a** (48 mg). A solution of intermediate **11a** (48 mg, 0.13 mmol), isobutylamine (0.13 mL), DIEA (67 μL) in *n*-butanol (3 mL) was heated at 120 ^oC overnight. Upon completion, the title compound **12a** was obtained by the post-processing steps which were the same as above. Yield: 67%. ^1H NMR (500 MHz, DMSO-d_6) δ 12.26 (s, 1H), 7.77 (d, *J* = 6.0 Hz, 1H), 7.47 – 7.32 (m, 2H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.09 (d, *J* = 6.0 Hz, 1H), 5.43 (d, *J* = 5.5 Hz, 1H), 3.30 – 3.10 (m, 2H),

3.10 – 3.01 (m, 1H), 2.78 – 2.59 (m, 2H), 2.09 (s, 1H), 1.94 (s, 2H), 1.90 – 1.78 (m, 2H), 1.25 – 1.14 (m, 1H), 0.90 (d, J = 5.5 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.62, 154.15, 141.46, 135.13, 134.57, 126.51, 121.49, 119.47, 114.81, 112.15, 111.54, 110.42, 97.18, 47.64, 45.30, 44.47, 29.36, 27.76, 20.34, 19.95. HR MS (ESI): calcd for $C_{21}H_{25}F_3N_5O$ $[M + H]^+$; 420.2011; found 420.2004.

6.4.2.

6-Bromo-1-((2-isobutylaminopyrimidine)-4-aminoethyl)amino-1*H*-2,3,4,9-tetrahydro carbazole (**12b**)

Intermediate **7a** was synthesized according to the procedure for compound **5** except utilizing 4-bromophenylhydrazine instead of *p*-tolylhydrazine, *N*-Boc-ethylene-diamine instead of 4-phenylbutylamine. Compound **14b** was synthesized after removal of Boc group with 4.6 N hydrogen chloride dissolved in 1,4-dioxane. The remaining steps are essentially the same as for the conversion of **12a** to **12b**. Yield: 11%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.87 (s, 1H), 7.61 (s, 1H), 7.51 (d, J = 1.5 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H), 7.11 (dd, J = 8.5, 1.8 Hz, 1H), 6.78 (s, 1H), 6.35 (s, 1H), 5.70 (d, J = 5.7 Hz, 1H), 3.89 (s, 1H), 3.45 – 3.35 (m, 2H), 3.00 (t, J = 6.4 Hz, 2H), 2.85 – 2.71 (m, 2H), 2.55 (m, 2H), 2.07 – 1.92 (m, 2H), 1.85 – 1.74 (m, 1H), 1.73 – 1.64 (m, 2H), 1.25 (m, 1H), 0.83 (d, J = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.61, 162.16, 154.46, 138.49, 134.47, 128.70, 122.84, 119.98, 112.89, 110.62, 109.23, 95.08, 62.80, 51.21, 48.28, 45.45, 29.33, 27.89, 21.32, 20.56, 20.29. HR MS (ESI): calcd for $C_{22}H_{30}BrN_6$ $[M + H]^+$; 457.1715; found 457.1759.

6.4.3.

6-Bromo-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydr ocarbazole (**12c**)

The title compound **12c** was synthesized according to the procedure for compound **12b** except utilizing *N*-Boc-propylene-1,3-diamine instead of *N*-Boc-ethylenediamine. Yield: 47%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.60 (s, 1H), 7.52 (d, *J* = 1.5 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 1H), 7.12 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.85 (s, 1H), 6.35 (s, 1H), 5.68 (d, *J* = 5.5 Hz, 1H), 3.91 (s, 1H), 2.98 (t, *J* = 6.5 Hz, 2H), 2.79 – 2.62 (m, 2H), 2.57 (s, 2H), 2.04 – 1.89 (m, 3H), 1.82 – 1.63 (m, 5H), 1.24 (s, 2H), 0.83 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.36, 149.93, 134.59, 130.42, 130.26, 127.87, 124.57, 120.83, 113.41, 112.93, 111.33, 96.48, 54.95, 50.75, 47.97, 42.58, 27.78, 25.89, 25.65, 20.33, 20.11, 19.66. HR MS (ESI): calcd for $C_{23}H_{32}BrN_6$ $[M + H]^+$; 471.1872; found 471.1867.

6.4.4.

6-Bromo-1-((2-isobutylaminopyrimidine)-4-aminobutyl)amino-1*H*-2,3,4,9-tetrahydro carbazole (**12d**)

The title compound **12d** was synthesized according to the procedure for compound **12b** except utilizing *N*-Boc-butylene-1,4-diamine instead of *N*-Boc-ethylenediamine. Yield: 14%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.37 (s, 1H), 7.63 (m, 2H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.21 (dd, *J* = 8.6, 1.8 Hz, 1H), 5.77 (d, *J* = 6.0 Hz, 1H), 4.39 (s, 1H), 3.39 (s, 1H), 3.28 (s, 2H), 3.12 – 2.88 (m, 4H), 2.73 – 2.56 (m, 3H), 2.16 – 2.06 (m, 1H), 2.04 – 1.95 (m, 2H), 1.85 – 1.74 (m, 2H), 1.67 (m, 2H), 1.59 (d, *J* = 6.4 Hz, 2H),

1.27 – 1.21 (m, 1H), 0.85 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.45, 152.79, 134.56, 131.65, 128.01, 124.32, 120.72, 115.72, 113.35, 112.30, 111.23, 95.90, 62.81, 50.81, 48.19, 44.73, 27.85, 26.28, 26.19, 24.30, 20.25, 20.17, 19.67. HR MS (ESI): calcd for $C_{24}H_{34}BrN_6$ $[M + H]^+$; 485.2028; found 485.2023.

6.4.5.

6-Trifluoromethoxy-1-((2-isobutylaminopyrimidine)-4-aminoethyl)amino-1*H*-2,3,4,9-tetra-hydrocarbazole (**12e**)

The title compound **12e** was synthesized according to the procedure for compound **12b**. Yield: 23%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 7.64 (s, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.34 (s, 1H), 7.01 (d, *J* = 8.6 Hz, 1H), 5.80 (d, *J* = 5.9 Hz, 1H), 4.09 (s, 1H), 3.50 – 3.35 (m, 2H), 3.02 (t, *J* = 5.8 Hz, 2H), 2.95 – 2.79 (m, 2H), 2.61 (s, 2H), 2.03 (m, 1H), 1.91 (s, 1H), 1.82 – 1.69 (m, 3H), 1.37 – 1.19 (m, 3H), 0.84 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.57, 154.23, 141.65, 134.36, 130.34, 126.10, 121.45, 119.42, 115.88, 114.17, 112.50, 110.99, 97.11, 50.78, 47.46, 45.62, 42.68, 27.82, 25.42, 20.06, 19.86 ,19.75. HR MS (ESI): calcd for $C_{23}H_{30}F_3N_6O$ $[M + H]^+$; 463.2433; found 463.2423.

6.4.6.

6-Trifluoromethoxy-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12f**)

The title compound **12f** was synthesized according to the procedure for compound **12c** except utilizing 4-trifluoromethoxyphenylhydrazine instead of 4-bromophenyl-hydrazine. Yield: 49%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (s,

1H), 7.61 (s, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.33 (s, 1H), 7.00 (d, *J* = 9.0 Hz, 1H), 5.70 (d, *J* = 5.5 Hz, 1H), 4.03 (s, 2H), 2.98 (t, *J* = 6.5 Hz, 2H), 2.76 (m, 2H), 2.65 – 2.56 (m, 3H), 1.91 (s, 3H), 1.83 – 1.64 (m, 4H), 1.25 (m, 4H),0.82 (d, *J* = 6.5 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.40, 154.61, 141.64, 134.35, 130.46, 126.08, 121.44, 119.42, 115.84, 114.03, 112.52, 110.95, 96.88, 54.92, 50.68, 47.69, 42.40, 27.72, 26.36, 25.41, 20.09, 19.99, 19.58. HR MS (ESI): calcd for $C_{24}H_{32}F_3N_6O$ [M + H]$^+$; 477.2590; found 477.2586.

6.4.7.

6-Trifluoromethoxy-1-((2-isobutylaminopyrimidine)-4-aminobutyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12g**)

The title compound **12g** was synthesized according to the procedure for compound **12d** except utilizing 4-trifluoromethoxyphenylhydrazine instead of 4-bromophenyl-hydrazine. Yield: 19%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.74 (s, 1H), 9.03 (s, 1H), 8.24 (s, 1H), 7.68 (s, 1H), 7.47 (d, *J* = 8.8 Hz, 2H), 7.11 (d, *J* = 9.1 Hz, 1H), 6.08 (d, *J* = 6.3 Hz, 1H), 4.61 (t, *J* = 5.3 Hz, 1H), 3.39 (s, 2H), 3.17 (s, 2H), 3.09 – 3.00 (m, 2H), 2.74 – 2.61 (m, 2H), 2.23 – 2.00 (m, 3H), 1.89 – 1.75 (m, 4H), 1.65 (s, 2H), 1.33 – 1.21 (m, 1H), 0.90 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.19, 154.21, 141.66, 134.34, 130.41, 126.10, 121.46, 119.44, 115.86, 114.07, 112.53, 110.97, 96.94, 62.82, 50.64, 47.63, 44.00, 27.75, 25.45, 25.31, 23.26, 20.09, 19.99, 19.59. HR MS (ESI): calcd for $C_{25}H_{34}F_3N_6O$ [M + H]$^+$; 491.2746; found 491.2734.

6.4.8.

6-Fluoro-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydr ocarbazole (**12h**)

The title compound **12h** was synthesized according to the procedure for compound **12c** except utilizing 4-fluorophenylhydrazine instead of 4-bromophenylhydrazine. Yield: 37%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.44 (s, 1H), 7.75 – 7.54 (m, 2H), 7.38 (dd, *J* = 8.7, 4.3 Hz, 1H), 7.28 – 7.22 (m, 1H), 7.02 – 6.94 (m, 1H), 5.98 (s, 1H), 4.22 (t, *J* = 6.5 Hz, 1H), 3.14 – 2.96 (m, 4H), 2.71 – 2.56 (m, 2H), 2.23 – 2.10 (m, 2H), 2.05 – 1.92 (m, 4H), 1.87 – 1.72 (m, 3H), 1.67 – 1.60 (m, 1H), 1.41 – 1.33 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ δ 162.30, 157.74, 155.89, 154.62, 132.60, 130.05, 126.22, 113.69, 112.48, 110.44, 103.36, 96.90, 61.83, 50.80, 47.75, 42.45, 29.05, 27.77, 25.46, 20.23, 20.05, 19.63. HR MS (ESI): calcd for $C_{23}H_{32}FN_6$ $[M + H]^+$; 411.2672; found 411.2661.

6.4.9.

6-Chloro-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydr ocarbazole (**12i)**

The title compound **12i** was synthesized according to the procedure for compound **12c** except utilizing 4-chlorophenylhydrazine instead of 4-bromophenylhydrazine**.** Yield: 53%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.85 (s, 1H), 7.59 (s, 1H), 7.37 (d, *J* = 1.7 Hz, 1H), 7.30 (d, *J* = 8.5 Hz, 1H), 7.00 (dd, *J* = 8.5, 1.9 Hz, 1H), 6.86 (s, 1H), 6.37 (s, 1H), 5.67 (s, 1H), 3.86 (s, 1H), 2.97 (t, *J* = 6.2 Hz, 2H), 2.79 – 2.59 (m, 3H), 2.56 (s, 2H), 2.05 – 1.88 (m, 3H), 1.81 – 1.73 (m, 1H), 1.73 – 1.61 (m, 4H), 1.23 (s, 1H), 0.81 (d, *J* = 6.2 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.59, 162.24, 154.89,

138.61, 134.25, 127.96, 122.65, 120.26, 116.92, 112.41, 109.27, 99.50, 59.74, 51.45, 48.30, 43.87, 29.93, 29.09, 27.85, 20.59, 20.51, 20.26. HR MS (ESI): calcd for $C_{23}H_{32}ClN_6$ $[M + H]^+$; 427.2377; found 427.2364.

6.4.10.

6-Trifluoromethyl-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12j**)

The title compound **12j** was synthesized according to the procedure for compound **12c** except utilizing 4-trifluoromethylphenylhydrazine instead of 4-bromophenylhydrazine. Yield: 44%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.76 (s, 1H), 8.53 (s, 1H), 7.88 (s, 1H), 7.66 (s, 1H), 7.58 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 8.6 Hz, 1H), 5.94 (s, 1H), 4.56 (s, 1H), 3.41 (s, 2H), 3.07 (s, 4H), 2.72 (m, 2H), 2.20 – 2.02 (m, 3H), 1.99 (m, 2H), 1.87 – 1.75 (m, 2H), 1.24 (s, 2H), 0.86 (d, J = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.39, 154.13, 137.38, 126.63, 125.42, 124.47, 119.74, 119.50, 118.42, 116.16, 114.15, 112.11, 96.37, 54.92, 50.71, 45.62, 42.64, 27.77, 26.37, 25.80, 20.10, 19.75, 19.65. HR MS (ESI): calcd for $C_{24}H_{32}F_3N_6$ $[M + H]^+$; 461.2641; found 461.2621.

6.4.11.

6-Methoxylcarbonyl-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12k**)

The title compound **12k** was synthesized according to the procedure for compound **12c** except utilizing methyl 4-hydrazinylbenzoate instead of 4-bromophenylhydrazine. Yield: 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.10 – 8.07 (m, 1H),

7.84 (s, 1H), 7.73 – 7.63 (m, 1H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.45 – 7.35 (m, 1H), 5.70 (s, 1H), 4.04 (s, 1H), 3.89 – 3.78 (m, 3H), 2.97 (s, 2H), 2.83 (s, 2H), 2.64 (s, 2H), 1.98 – 1.84 (m, 4H), 1.81 – 1.66 (m, 4H), 1.32 – 1.18 (m, 3H), 0.81 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 167.08, 166.60, 162.23, 138.46, 129.93, 125.62, 123.20, 121.06, 120.62, 120.32, 114.93, 111.41, 96.89, 60.16, 57.65, 51.69, 50.65, 42.52, 28.96, 27.72, 25.31, 19.98, 19.60, 19.56. HR MS (ESI): calcd for $C_{25}H_{35}N_6O_2$ $[M + H]^+$; 451.2821; found 451.2824.

6.4.12.

6-Carboxy-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12l**)

To a solution of compound **12k** (301 mg, 0.67 mmol) in 12 mL of ethanol was added the aqueous sodium hydroxide solution (134 mg in 4 mL of H_2O) and the reaction mixture was stirred overnight. The reaction was then acidized with 1.0 N of hydrochloric acid solution. The mixture was extracted with AcOEt twice and the combined organic layers were dried over anhydrous Na_2SO_4. After evaporation of the solvent, the resulting residue was purified by column chromatography on silica gel to give the title compound **12l** (64 mg, yield: 37%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.37 (s, 1H), 11.76 (s, 1H), 8.81 (s, 1H), 8.16 (s, 1H), 8.11 – 7.91 (m, 1H), 7.76 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.68 (d, *J* = 4.1 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 6.02 (d, *J* = 2.0 Hz, 1H), 4.59 (s, 1H), 3.59 – 3.41 (m, 2H), 3.23 – 2.95 (m, 4H), 2.81 – 2.61 (m, 2H), 2.26 – 2.08 (m, 2H), 2.07 – 1.95 (m, 2H), 1.89 – 1.69 (m, 2H), 1.25 – 1.21 (m, 2H), 0.88 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.06, 168.37,

162.34, 155.70, 138.35, 129.91, 125.62, 123.49, 121.59, 121.17, 114.75, 111.14, 96.69, 62.82, 50.73, 47.84, 42.51, 29.02, 27.78, 25.47, 21.16, 20.08, 19.66. HR MS (ESI): calcd for $C_{24}H_{33}N_6O_2$ [M + H]$^+$; 437.2665; found 437.2645.

6.4.13.

6-Methoxy-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12m**)

The title compound **12m** was synthesized according to the procedure for compound **12c** except utilizing 4-methoxyphenylhydrazine instead of 4-bromophenylhydrazine. Yield: 46%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.31 (s, 1H), 8.73 (s, 1H), 7.64 (s, 1H), 6.95 – 6.61 (m, 2H), 6.14 (s, 1H), 5.05 – 4.81 (m, 1H), 4.67 (s, 1H), 3.81 (s, 2H), 3.71 – 3.60 (m, 1H), 3.49 (s, 2H), 3.14 (s, 2H), 3.05 – 2.88 (m, 2H), 2.68 (s, 2H), 2.36 – 2.19 (m, 3H), 1.83 (m, 2H), 1.61 (m, 1H), 1.28 (s, 3H), 0.91 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.28, 153.24, 142.30, 131.01, 129.63, 128.47, 126.28, 113.18, 112.39, 112.11, 100.25, 96.74, 55.33, 54.93, 50.90, 47.74, 42.27, 27.74, 25.47, 22.09, 20.33, 20.03, 19.78. HR MS (ESI): calcd for $C_{24}H_{35}N_6O$ [M + H]$^+$; 423.2872; found 423.2862.

6.4.14.

1-((2-Isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydro-carbazole (**12n**)

The title compound **12n** was synthesized according to the procedure for compound **12c** except utilizing phenylhydrazine instead of 4-bromophenylhydrazine. Yield: 8%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.29 (s, 1H), 8.39 (s, 1H), 7.66 (s, 1H), 7.48 (d, *J*

= 7.8 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.05 – 6.99 (m, 1H), 5.91 (s, 1H), 4.56 (s, 1H), 3.41 (s, 2H), 3.14 – 3.01 (m, 4H), 2.75 – 2.58 (m, 2H), 2.22 – 1.93 (m, 5H), 1.87 – 1.74 (m, 2H), 1.27 – 1.21 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.41, 152.41, 145.77, 135.93, 128.29, 126.02, 122.23, 118.83, 118.49, 113.26, 111.38, 96.10, 57.49, 50.87, 47.99, 42.42, 27.79, 25.85, 25.63, 20.27, 20.14, 19.77. HR MS (ESI): calcd for $C_{23}H_{33}N_6$ $[M + H]^+$; 393.2767; found 393.2763.

6.4.15.

8-Bromo-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydr ocarbazole (**12o**)

The title compound **12o** was synthesized according to the procedure for compound **12c** except utilizing 2-bromophenylhydrazine instead of 4-bromophenylhydrazine. Yield: 25%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.67 (s, 1H), 8.74 – 8.54 (m, 1H), 7.77 – 7.60 (m, 1H), 7.35 (d, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 6.94 – 6.87 (m, 1H), 6.06 (br s, 1H), 4.63 (s, 1H), 3.56 – 3.40 (m, 2H), 3.23 – 3.08 (m, 2H), 3.07 – 2.94 (m, 2H), 2.75 – 2.61 (m, 2H), 2.33 – 2.21 (m, 3H), 2.15 – 2.08 (m, 2H), 2.03 – 1.96 (m, 1H), 1.91 – 1.71 (m, 3H), 1.65 – 1.56 (m, 1H), 0.91 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.32, 154.89, 134.60, 129.40, 127.56, 124.70, 120.38, 118.07, 114.94, 108.34, 104.17, 96.67, 54.93, 50.56, 47.74, 41.82, 27.75, 25.58, 24.92, 22.08, 20.35, 20.05. HR MS (ESI): calcd for $C_{23}H_{32}BrN_6$ $[M + H]^+$; 471.1872; found 471.1866.

6.4.16.

8-Methoxy-1-((2-isobutylaminopyrimidine)-4-aminopropyl)amino-1*H*-2,3,4,9-tetrahydrocarbazole (**12p**)

The title compound **12p** was synthesized according to the procedure for compound **12c** except utilizing 2-methoxyphenylhydrazine instead of 4-bromophenylhydrazine. Yield: 18%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.07 (s, 1H), 7.67 (m, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 6.95 (dd, *J* = 7.9, 7.7 Hz, 1H), 6.72 (d, *J* = 7.7 Hz, 1H), 6.03 (d, *J* = 6.3 Hz, 1H), 3.91 (s, 2H), 3.46 (m, 2H), 3.16 (m, 2H), 3.08 (m, 2H), 2.71 – 2.61 (m, 2H), 2.20 – 2.11 (m, 2H), 2.04 (m, 4H), 1.82 (m, 2H), 1.41 – 1.32 (m, 1H), 0.89 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.19, 153.91, 145.93, 140.61, 127.30, 126.14, 119.49, 114.04, 111.20, 102.82, 96.98, 55.24, 54.95, 50.71, 47.54, 41.80, 37.70, 27.68, 25.07, 20.39, 20.13, 19.94. HR MS (ESI): calcd for $C_{24}H_{35}N_6O$ $[M + H]^+$; 423.2872; found 423.2868.

Acknowledgments

This work was supported by the grants from the National Natural Science Foundation of China (81673304 to Y.C., and 81661138004 and 21725801 to C.Y.), the Innovation Program of Shanghai Municipal Education Commission (2017-01-07-00-05-E00011 to M.L.) and the Science and Technology Commission of Shanghai Municipality (15431902200 to Y.C. and 17XD1404400 to C.Y.).

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A novel series of tetrahydrocarbazoles with 2,4-diaminopyrimidines was designed and optimized.

The structure-activity relationship of the synthetic compounds was adequately understood.

Compound **12c** exhibited excellent antibacterial activity against multidrug resistant *S. aureus* and *E. coli* and showed good pharmacokinetic properties.

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