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Synthesis and biological evaluation of pyridinium-functionalized carbazole derivatives as promising antibacterial agents

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ABSTRACT

Various pyridinium-functionalized carbazole derivatives were constructed by coupling the key fragments of carbazole skeleton and pyridinium nucleus in a single molecular architecture. Antibacterial bioassays revealed that some of the title compounds displayed impressive bioactivities against plant pathogens such as *Xanthomonas oryzae pv. oryzae*, *Ralstonia solanacearum*, and *Xanthomonas axonopodis pv. citri* with minimal EC_{50} values of up to 0.4, 0.3, and 0.3 mg/L, respectively. These bioactivities were achieved by systematically tuning and optimizing bridging linker, alkyl length of the tailor, and substituents on the carbazole scaffold. Compared with the bioactivity of the lead compound (**AP-10**), antibacterial efficacy dramatically increased by approximately 13-, 104- and 21-fold. This finding suggested that these compounds can serve as new lead compounds in research on antibacterial chemotherapy.

Disease-causing bacteria are gaining considerable attention over the last decade due to the significant threats they impose on agricultural products and their remarkable ability in acquiring additional resistance mechanisms.[1–3] *Xanthomonas oryzae pv. oryzae* (*Xoo*), *Ralstonia solanacearum* (*R. solanacearum*) and *Xanthomonas axonopodis pv. citri* (*Xac*) are three widely distributed Gram-negative opportunistic pathogens that can infect an array of individual species including rice, tomato, potato, tobacco and citrus.[4–6] Infection by these bacteria can present necrotic lesions on leaves, stems and/or fruits, which consequently result in a serious loss in agricultural output.[7,8] In addition, the emergence and worldwide spread of multidrug resistant pathogens has exacerbated the management of these persistent plant bacterial diseases. Although a few of the commercial drugs have been used to combat these diseases such as bismerthiazol (**BT**) and thiodiazole copper (**TC**), they failed to effectively treat the infected plants under field conditions considering their poor efficiency, high phytotoxicity and/or bacterial resistance.[9,10] Therefore, exploring and developing new anti-bacterial drugs is imperative with novel chemical motifs preferably owning unique modes of action rather than analogues of the existing ones.

Carbazole skeleton, owning impressive electronic and charge-transport properties, is a crucial type of nitrogen-containing aromatic heterocyclic scaffold, present in many naturally occurring products and biologically active substances.[11,12] In addition, this privileged building block can be easily tuned and modified with various functional groups, which endow carbazole-based derivatives and analogues with an admirable array of pharmacological activities such as anticancer, anti-inflammatory, antiviral, antifungal and antioxidant activities.[13–16] In particular, the antimicrobial activity has been widely investigated for their extensively potential applications in the pharmaceutical industry.[17,18] For example, Bremner and co-workers reported several carbazole-linked cyclic and acyclic peptoids as growth inhibitors of *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 15 μg/mL.[19] Gu and co-workers had synthesized and evaluated the antimicrobial activity of a series of new carbazole derivatives of ursolic acid and found that some compounds exhibited significant antibacterial activities against both Gram-positive and Gram-negative bacteria with MIC values ranging from 3.9 μg/mL to 15.6 μg/mL.[20] Thus, the fusion of a carbazole motif with the target molecule might probably lead to improved biological activity owing to the synergistic effect of these valuable moieties.

As an important functional fragment, pyridinium nucleus exists extensively in various kinds of pharmaceutical agents.[21,22] Normally, compounds featuring this scaffold always acquire various physicochemical properties and subsequently resulted in reformed

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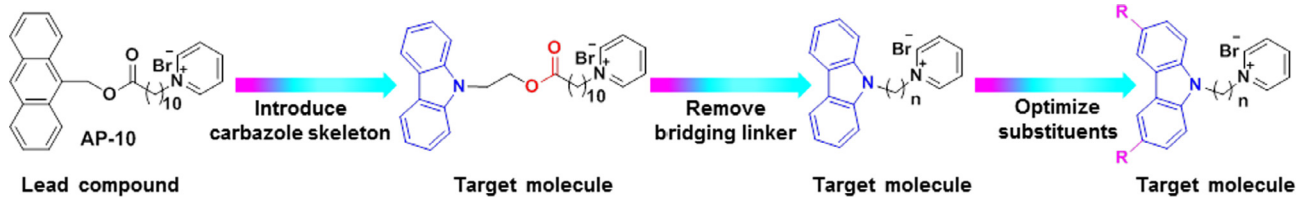
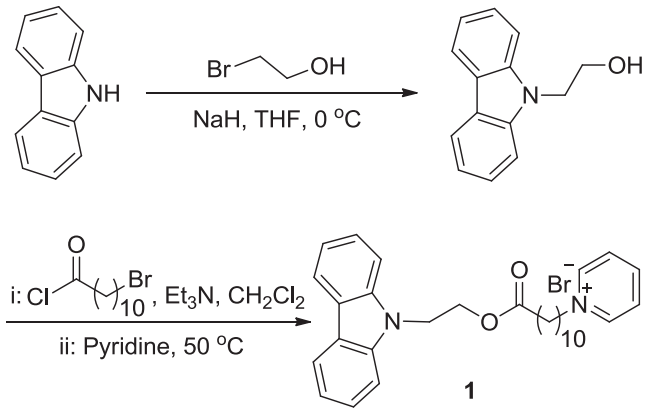


Fig. 1. Design strategy for the target compounds.



Scheme 1. Synthesis of compound **1**.

or enhanced biological activities, because the positive charge can strengthen their specificity for the target species.[23] Moreover, pyridinium-tailored amphiphiles are considered one of the powerful molecular templates to create biologically active compounds, especially in the field of antimicrobial drug discovery.[24,25] For example, Eren et al. investigated the antibacterial activity of some pyridinium functionalized polynorbornenes and found that compounds bearing octyl tails on the pyridine rings showed potent inhibitory effects against *Escherichia coli* and *Bacillus subtilis*.[26] Apparently, these repeatedly numerous studies on this functional scaffold opened a new avenue for the discovery and development of novel high-efficient bioactive molecules.

In our previous work, 1-[11-(9-anthracenyl methoxy)-11-oxoundecyl] pyridinium bromide (**AP-10**) exhibited good antibacterial activities towards plant pathogens.[27] Encouraged by the aforementioned facts and in a continuing search for high-efficient antibacterial agents, we report herein the design and synthesis of a series of pyridinium-tailored carbazole derivatives by coupling key fragments of carbazole skeleton and pyridinium nucleus in a single molecular architecture (Fig. 1). The carbazole group, which has desirable electron characteristics, is probably responsible for better binding with the enzymes or the receptors of bacteria; pyridinium, which has a positive charge, is employed to possibly interact with anionic cell components and increase the water solubility as well as membrane permeability; bridging linker and alkyl chain length of the tailor are used to tune the balance of hydrophobicity/hydrophilicity of target compounds. Better bioactive structures would be fabricated by utilizing the synergistic effect of these privileged moieties. The antibacterial activities of all the title compounds were tested against plant pathogens such as *Xoo*, *R. solanacearum* and *Xac*.

To investigate the antibacterial effect after replacing the anthracene ring of **AP-10** into carbazole skeleton, compound **1** was firstly designed and synthesized. As indicated in Scheme 1, intermediate 2-(9*H*-carbazol-9-yl)ethanol was obtained by adding 2-bromoethanol into a mixture of carbazole and NaH in dry THF, followed by treating it by two-step consecutive reactions with 11-bromoundecanoyl chloride and pyridine to provide the title molecule **1**. The structure was confirmed by ^{1}H NMR, ^{13}C NMR and MS experiments (detailed information see Supplementary data). The antibacterial bioassays against *Xoo*, *R. solanacearum* and *Xac* were performed as previously described,[27,28] and the commercial antibacterial agents (**BT** and **TC**) were co-assayed as positive controls under similar conditions. The inhibitory effect of **1** was indeed improved by introducing carbazole moiety in the target molecule (Table 1). In particular, anti-*R. solanacearum* activity was significantly increased with EC_{50} values from 31.3 mg/L to 1.6 mg/L, approximately 19-fold enhancement in the antibacterial efficacy, which validated the author's assumption.

Further, we wonder if the removed ester group within the alkyl chain would reform the bioactive efficacy, because compound **1** exhibited good antimicrobial potentials. Thereby, compound **2** was primarily constructed by sequential substitution reactions of carbazole with 1,12-dibromododecane to produce bromide-tailored carbazole, which was then treated with pyridine at 50 °C (Scheme 2). The structure was also characterized by ^{1}H NMR, ^{13}C NMR and MS experiments (detailed information see Supplementary data). The bioassay result is shown in Table 1. A dramatically

Table 1
Antibacterial activities of target compounds **1**–**5** against plant pathogen *Xoo*, *R. solanacearum* and *Xac in vitro*.

No.	*Xoo*			*R. solanacearum*			*Xac*		
	Regression equation[a]	r	EC_{50} (mg/L)	Regression equation	r	EC_{50} (mg/L)	Regression equation	r	EC_{50} (mg/L)
AP-10	y = 3.29x + 2.60	0.93	5.3 ± 0.7	y = 1.01x + 3.50	0.97	31.3 ± 8.2	y = 2.74x + 2.80	0.96	6.3 ± 0.5
1	y = 3.54x + 3.36	0.98	2.9 ± 0.3	y = 12.73x + 2.39	0.98	1.6 ± 0.2	y = 12.87x − 2.71	0.94	4.0 ± 0.2
2	y = 16.27x + 10.85	0.98	0.4 ± 0.1	y = 16.91x + 15.26	0.96	0.3 ± 0.1	y = 3.59x + 6.60	0.95	0.3 ± 0.1
3	y = 9.67x + 5.07	0.98	1.0 ± 0.1	y = 9.55x + 8.89	1.00	0.4 ± 0.1	y = 2.03x + 4.57	1.00	1.6 ± 0.4
4	y = 14.16x − 2.52	0.96	3.4 ± 0.1	y = 4.73x + 4.89	0.98	1.1 ± 0.1	y = 5.18x + 4.38	0.95	1.3 ± 0.1
5	y = 3.91x + 0.26	0.95	16.4 ± 2.4	y = 3.85x + 2.07	0.99	5.8 ± 0.5	y = 2.12x + 4.24	1.00	2.3 ± 0.2
BT	y = 1.50x + 2.05	0.98	92.6 + 2.2	/	/	/	/	/	/
TC	/	/	/	y = 1.03x + 2.94	0.99	99.1 ± 5.1	y = 2.15x + 0.94	0.98	77.0 ± 2.0

[a] Five different concentrations (such as 80, 40, 20, 10, 5 mg/L, depending on the bioactivity of different compounds, the concentrations were chosen in two times decline trend to make sure the EC_{50} values are inside the concentration ranges tested) of the test compounds and positive control were selected to test the corresponding inhibition rates. By using the SPSS 17.0 software and the obtained inhibition rates at different concentrations, a related regression equation was provided to calculate the related EC_{50} values.[10,27,28]

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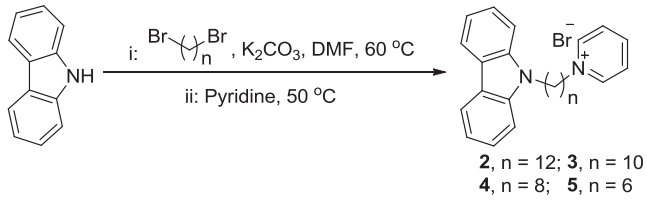
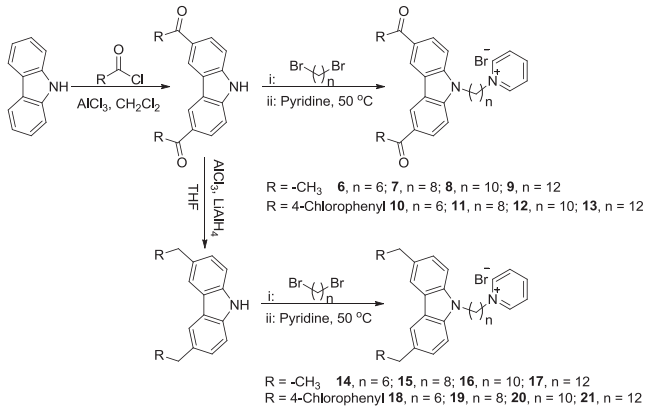
improved antibacterial potency was observed against *Xoo*, *R. solanacearum* and *Xac*, and their EC_{50} values varied from 2.9 mg/L to 0.4 mg/L, 1.6 mg/L to 0.3 mg/L and 4.0 mg/L to 0.3 mg/L, respectively, suggesting that the ester group might serve as a disadvantageous factor to block any further interactions between compound **1** and bacterial receptors.

Comparing the bioactivity of the lead compound (**AP-10**), the antibacterial efficacy was strongly enhanced by approximately 13-, 104- and 21-fold, respectively, indicating that a better bioactive structure was achieved. To tune the balance of hydrophobicity/hydrophilicity of target molecules, pyridinium-tailored carbazoles (**3**, **4**, **5**) bearing alkyls of different lengths were fabricated following the synthetic protocols of compound **2**. EC_{50} values



Scheme 2. Synthesis of compounds **2**, **3**, **4**, and **5**.



Scheme 3. Synthesis of compounds **6**–**21**.

provided an enhancive tendency with the decrease in the alkyl chain length, demonstrating that the decrease in molecular lipophilicity was unfavourable to the bioactivity.

To study the effect of the substitution on the carbazole ring towards bioactivity, title compounds **6**–**21** possessing different substitutional units were designed and synthesized from Scheme 3. In general, the intermediates containing diacetyl or di-(p-chlorlbenzoyl) groups at the 3, 6-positions of the carbazole skeleton were provided through the Friedel Crafts reaction with acetyl chloride or 4-chlorobenzoyl chloride,[29,30] which subsequently reacted with lithium aluminum hydride to produce the other two crucial intermediates bearing diethyl or di-(p-chlorobenzyl) groups. Finally, a series of target compounds simultaneously owning different alkyl chain lengths (n = 6, 8, 10, 12) were obtained through the synthetic protocols of compound **2**.

Preliminary antibacterial bioassays revealed that these compounds exhibited poor to good activities against the three tested strains. As noted in Table 2, the substituents had a considerable influence towards bioactivity. Compounds **6**–**9** and **14**–**17** containing diacetyl or diethyl groups can effectively inhibit the growth of *Xoo* at 80 mg/L, even lowered the concentration to 40 mg/L. By contrast, the anti-*Xoo* capacity was significantly decreased after placing the sterically hindered groups (p-chlorlbenzoyl or p-chlorobenzyl) at the 3, 6-positions of the carbazole ring. A phenomenon for EC_{50} values of compounds **6**–**9** against *Xoo* was firstly decreased and then increased with improvement in the length of alkyl tailors (Table 3). Thereby, compound **8** produced the minimal EC_{50} value of up to 1.4 mg/L, suggesting that even slight changes in the ratio of hydrophobicity/hydrophilicity can affect their bioactivities. However, the EC_{50} values of compounds **14**–**17** followed the order of **14** (0.9 mg/L) < **15** (1.1 mg/L) < **16** (10.3 mg/L) < **17** (10.9 mg/L), revealing that the enhancement of molecular lipophilicity might result in negative effects to the anti-*Xoo* activity. The electronic effect of substituents on the carbazole scaffold also had a significant effect on the anti-*Xoo* activity, illuminated by comparing the EC_{50} values of **6** (15.4 mg/L) and **14** (0.9 mg/L), **7** (5.3 mg/L) and **15** (1.1 mg/L), **8** (1.4 mg/L) and **16** (10.3 mg/L), **9** (3.3 mg/L) and **17** (10.9 mg/L) with the former bearing electron-withdrawing groups (CH_3CO-) and the latter containing electron-donating groups (CH_3CH_2–). The antibacterial efficacy towards *R. solanacearum* was dramatically reduced after introducing small or large sterically hindered fragments, though compound **14** showed an inhibition rate of 92.7% at a dosage of 80 mg/L. For

Table 2
Inhibitory effect of target compounds **6**–**21** against plant pathogen *Xoo*, *R. solanacearum* and *Xac in vitro*.

No.	Inhibition (%)					
	Xoo		*R. solanacearum*		*Xac*	
	80 mg/L	40 mg/L	80 mg/L	40 mg/L	80 mg/L	40 mg/L
6	100	85.6 ± 4.8	22.5 ± 9.4	11.6 ± 2.3	96.5 ± 2.6	62.6 ± 3.7
7	100	98.4 ± 0.3	41.8 ± 4.3	17.9 ± 0.7	100	100
8	100	100	34.3 ± 4.0	27.5 ± 6.9	100	100
9	100	100	5.9 ± 3.1	4.5 ± 3.8	100	100
10	61.4 ± 1.5	58.3 ± 3.7	0	0	36.8 ± 5.4	28.4 ± 1.1
11	66.4 ± 2.0	58.3 ± 8.0	0	0	21.2 ± 2.1	17.1 ± 1.4
12	33.5 ± 9.5	18.6 ± 8.7	0	0	22.7 ± 1.7	18.3 ± 9.9
13	55.8 ± 7.4	25.1 ± 4.6	0	0	24.6 ± 6.3	16.5 ± 0.7
14	100	100	92.7 ± 3.2	66.7 ± 6.0	100	100
15	100	100	20.4 ± 7.4	0	100	100
16	100	100	0	0	100	100
17	100	95.5 ± 2.8	0	0	49.4 ± 6.9	27.7 ± 8.9
18	53.3 ± 5.1	26.7 ± 2.6	0	0	40.8 ± 7.0	30.2 ± 5.4
19	38.8 ± 2.1	18.8 ± 3.6	0	0	10.0 ± 3.5	8.9 ± 2.3
20	26.9 ± 9.4	0	0	0	17.3 ± 4.6	11.0 ± 2.5
21	4.1 ± 5.8	0	0	0	18.9 ± 2.7	13.8 ± 4.6
BT	45.1 ± 5.2	35.3 ± 3.4	/	/	/	/
TC	/	/	43.2 ± 1.6	12.1 ± 2.3	51.2 ± 3.2	32.4 ± 2.8

Table 3
Antibacterial activities of target compounds (**6**–**11**, **13**–**18**) against pathogen *Xoo*, *R. solanacearum* and *Xac in vitro*.

No.	*Xoo*			*R. solanacearum*			*Xac*		
	Regression equation	r	EC_{50} (mg/L)	Regression equation	r	EC_{50} (mg/L)	Regression equation	r	EC_{50} (mg/L)
6	y = 1.29x + 3.47	0.94	15.4 ± 1.2	/	/	>80	y = 2.49x + 1.67	0.91	21.8 ± 0.3
7	y = 2.73x + 3.02	0.97	5.3 ± 0.4	/	/	>80	y = 2.18x + 3.15	0.94	7.1 ± 1.4
8	y = 2.83x + 4.57	0.94	1.4 ± 0.1	/	/	>80	y = 2.73x + 3.40	0.99	3.8 ± 0.5
9	y = 3.25x + 3.32	0.93	3.3 ± 0.1	/	/	>80	y = 0.83x + 5.22	0.91	0.5 ± 0.1
10	y = 0.77x + 3.85	0.95	31.2 ± 0.5	/	/	>80	/	/	>80
11	y = 0.99x + 3.56	0.99	28.1 ± 5.4	/	/	>80	/	/	>80
13	y = 1.92x + 1.41	0.97	73.9 ± 3.0	/	/	>80	/	/	>80
14	y = 2.94x + 5.16	0.99	0.9 ± 0.1	y = 1.95x + 2.51	0.92	19.1 ± 4.4	y = 4.45x + 1.83	1.00	5.1 ± 0.7
15	y = 1.11x + 4.95	0.94	1.1 ± 0.1	/	/	>80	y = 2.25x + 3.12	1.00	6.8 ± 0.2
16	y = 1.35x + 3.63	0.96	10.3 ± 1.3	/	/	>80	y = 1.18x + 3.48	0.98	19.4 ± 5.3
17	y = 2.07x + 2.85	0.96	10.9 ± 0.7	/	/	>80	/	/	>80
18	y = 1.74x + 1.71	0.98	77.6 ± 5.2	/	/	>80	/	/	>80
BT	y = 1.50x + 2.05	0.98	92.6 + 2.2	/	/	/	/	/	/
TC	/	/	/	y = 1.03x + 2.94	0.99	99.1 ± 5.1	y = 2.15x + 0.94	0.98	77.0 ± 2.0

anti-*Xac* bioassays, compounds **7**–**9** and **14**–**16** exerted potent growth suppression effects, even at 40 mg/L, whereas compounds **10**–**13** and **18**–**21** did not produce significant activity even at a high concentration of 80 mg/L, further demonstrating that large groups on the carbazole ring would block the bioactivity of title compounds. Compounds **6**–**9** displayed enhanced anti-*Xac* activity with increasing alkyl chain length, and their EC_{50} values reached a minimum of 0.5 mg/L. This fact might be ascribed to the enhanced molecular lipophilicity. In comparison, compounds **14**–**17** provided reduced anti-*Xac* ability with the increase in the alkyl tailors. In view of the above study findings, the antibacterial activity can be affected by a variety of factors including alkyl chain length of the tailor, bridging linker, electronic properties and steric hindrance of substituents on the carbazole scaffold, which reminded us to carefully optimize the molecular structures in the exploration of novel, high-efficient bioactive substances.

In conclusion, a series of pyridinium-tailored carbazole compounds were designed and synthesized by rationally tuning and optimizing bridging linker, alkyl length of the tailor, and substituents on the carbazole skeleton. Antibacterial evaluation results revealed that some of the target compounds exhibited promising bioactivities against plant pathogen *Xoo*, *R. solanacearum* and *Xac*, with minimal EC_{50} values of 0.4, 0.3 and 0.3 mg/L, respectively. Considering their simple structures and conveniently synthetic protocols, these kinds of compounds can be further studied as new lead compounds in the field of antibacterial chemotherapy.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.08.040.

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