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Synthesis, in vitro antibacterial activities of a series of 3-*N*-substituted canthin-6-ones

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ABSTRACT

An improved synthetic route of canthin-6-one was accomplished. To further enhance the antibacterial potency and improve water solubility, a series of 3-*N*-alkylated and 3-*N*-benzylated canthin-6-ones were designed and synthesized, and their in vitro antibacterial activities were evaluated. A clear structure–activity relationship with peak minimal inhibitory concentration (MIC) values of 0.98 ($\mu g \cdot mL^{-1}$) was investigated. Particularly, compounds **6i–r** and **6t** were found to be the most potent compounds with minimal inhibitory concentration (MIC) values lower than 1.95 ($\mu g \cdot mL^{-1}$) against *Staphylococcus aureus*.

The canthin-6-one is a subclass of β-carboline alkaloids with an additional D-ring, which have been isolated from various sources, principally including Rutaceae, Simaroubaceae families and the fungi.[1,2] Simultaneously, canthin-6-one alkaloids are pharmacologically active natural products, which have shown to possess a wide-range biological activities consisting of cytotoxic, antiviral, antimicrobial, anti-inflammatory, anti-parasitic, anticancer, and enzyme inhibitory.[3–9] Based on its potentiality in drug development, a growing number of scientists have focused on it. Specially, its total synthesis continues to the present day.[10–12] Gollner et al. reported a 'non-classical' high yield (95%) route which relies on a concomitant Pd-catalyzed Suzuki–Miyaura C–C coupling followed by a Cu-catalyzed C–N coupling in 2010.[13] Their route is the most easy and low-cost strategy to date. However, most of these routes are tedious and harsh, which are not suitable for the preparation of canthin-6-one. In order to obtain more substrate for drug development under mild conditions, our group has explored a relatively simple method through improved the previous approach.[14]

Staphylococcus aureus is one of the leading causes of bacterial infections in humans with symptoms ranging from simple skin infections to severe necrotizing fasciitis and pneumonia.[15] *Bacillus cereus* could cause food poisoning.[16] *Ralstonia solanacearum* and *Pseudomonas solanacearum* are major components of plant pathogens.[17] We could see that all of these diseases caused by bacteria constitute a major threat to humans' life and property. Although penicillin is currently the first choice of antibiotics to treat bacterial infections, the overuse of penicillin has resulted in a situation that higher dose is required to successfully treat the bacterial infection.[18] Many studies have showed that compounds with large planar surface areas could function at the DNA level via intercalation between base pairs.[19] Moreover, it has been proved that DNA intercalation of compound could disrupt the function of DNA topoisomerases and lead to cell death ultimately.[20] First described in 2014 by Dejos et al., canthin-6-one could decrease the synthesis of DNA and possess the properties of antiproliferative.[21] In 2007, O'Donnell et al. reported the antibacterial activity of canthin-6-one alkaloids which displayed minimum inhibitory concentrations (MICs) in the range of 8–82 $\mu g \cdot mL^{-1}$ against a panel of fast-growing *Mycobacterium* species and 8–64 $\mu g \cdot mL^{-1}$ against multidrug-resistant (MDR) and methicillin-resistant (MRSA) strains of *Staphylococcus aureus*.[22] At the same time, Ostrov et al. used structure-based molecular docking to identify novel drug-like small molecules. They found that canthin-6-one could not effectively accumulate inside of the *Escherichia coli* might be due to drug instability or metabolic inactivation by bacterial enzymes, while it could inhibit the DNA supercoiling activity of purified *E. coli* DNA gyrase.[8] Based on the above studies, canthin-6-one which has a highly conjugated large

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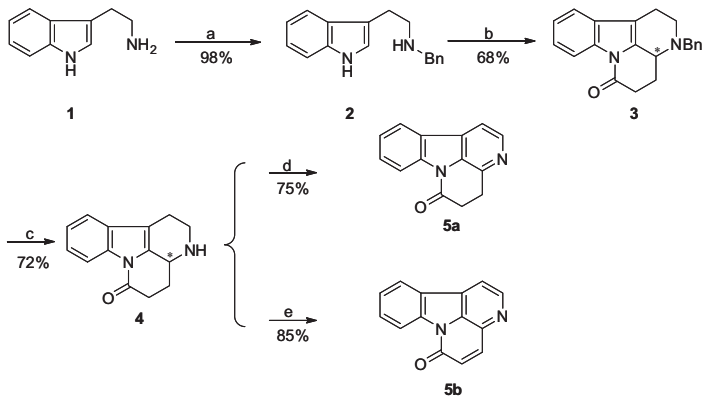
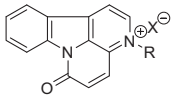


Scheme 1. Synthesis of canthin-6-one **5b**. Reagents and conditions: (a) (i) benzaldehyde, dry CH_3OH, rt, 36 h; (ii) $NaBH_4$, rt, 1 h; (b) α-ketoglutaric acid, dry toluene/dioxane (6:4, v/v), *p*-TSA, DST, reflux, 48 h; (c) $HCOONH_4$, Pd/C, dry CH_3OH, dry toluene, reflux, 5 h; (d) xylene, Pd/C, reflux, 4 h; (e) xylene, Pd/C, reflux, 48 h.

Table 1
Antibacterial activity of 3-*N*-substituted canthin-6-ones against two Gram-positive bacteria and two Gram-negative bacteria (MIC[a] values, μg·mL^{-1})



Compounds	R	X	Gram-positive bacteria		Gram-negative bacteria	
			S. aureus	*B. cereus*	*P. solanacearum*	*R. solanacearum*
3	—	—	>125	>125	>125	>125
4	—	—	>125	>125	>125	>125
5a	—	—	125	15.63	7.81	15.63
5b	—	—	31.25	7.81	3.91	7.81
6a	$-CH_3$	I	>125	>125	>125	>125
6b	$-CH_2CH_3$	I	>125	>125	>125	>125
6c	$-(CH_2)_2CH_3$	I	>125	>125	>125	>125
6d	$-CH(CH_3)_2$	I	>125	>125	>125	>125
6e	$-CH_2CH{=}CH_2$	Br	62.50	125	62.50	62.50
6f	$-(CH_2)_3CH_3$	I	62.50	125	62.50	62.50
6g	$-CH_2CH(CH_3)_2$	I	125	>125	62.50	>125
6h	$-(CH_2)_4CH_3$	I	31.25	62.50	15.63	31.25
6i	$-(CH_2)_7CH_3$	Br	0.98	62.50	7.81	31.25
6j	$-CH_2Ph$	Br	0.98	3.91	7.81	3.91
6k	$-CH_2Ph$	Cl	1.95	3.91	1.95	3.91
6l	$-CH_2Ph(o\text{-}CH_3)$	Cl	1.95	7.81	3.91	7.81
6m	$-CH_2Ph(m\text{-}CH_3)$	Cl	1.95	15.63	7.81	15.63
6n	$-CH_2Ph(p\text{-}CH_3)$	Cl	1.95	15.63	7.81	31.25
6o	$-CH_2Ph(o\text{-}F)$	Cl	1.95	7.81	3.91	15.63
6p	$-CH_2Ph(p\text{-}F)$	Cl	1.95	31.25	15.63	31.25
6q	$-CH_2Ph$(2,4-difluoro)	Cl	0.98	31.25	7.81	31.25
6r	$-CH_2Ph$(2,6-difluoro)	Cl	0.98	15.63	7.81	15.63
6s	$-CH_2Ph(p\text{-}Cl)$	Cl	7.81	15.63	15.63	15.63
6t	$-CH_2Ph(p\text{-}OCH_2Ph)$	Cl	0.98	15.63	3.91	7.81
6u	$-CH_2Ph(p\text{-}CF_3)$	Cl	7.81	62.50	62.50	15.63
6v	$-CH_2Ph(p\text{-}CH_2Br)$	Br	7.81	62.50	31.25	31.25
7	→O	—	15.63	15.63	7.81	15.63
Ampicillin sodium[b]	—	—	3.91	3.91	7.81	7.81
Fosfomycin sodium[b]	—	—	3.91	3.91	125	1.95

[a] Minimal inhibitory concentration.
[b] Two kinds of positive controls.

planar surface areas, have shown great potential in antibacterial drug. Herein series of 3-*N*-alkylated and 3-*N*-benzylated canthin-6-ones were designed and synthesized to further enhance the antibacterial potency and improve water solubility of canthin-6-one. And all compounds were successively screened of their inhibition activity against *Staphylococcus aureus*, *Bacillus cereus*, *Ralstonia solanacearum* and *Pseudomonas solanacearum*.

The initial synthetic aim of this study was to obtain a convenient route for the preparation of canthin-6-one alkaloids. So it was decided to begin our efforts from the preparation of the N_b-benzyltryptamine **2** which could direct the chemistry regioselectivity.[23] Subsequently, in the presence of a catalytic amount of *p*-TSA, the Pictet–Spengler and N-acylation reaction in aprotic media afforded N_b-benzylhexahydrocanthin-6-one **3**.[24] Then the debenzylated product **4** was provided under the conditions of the catalytic transfer hydrogenation. Excitingly, with the palladium catalyst, compound **5a** which has been proved by HMBC spectra was first generated (Fig. S1). And then the canthin-6-one **5b** was acquired (Scheme 1).[25] Although the overall yield (40.78%) is less than the previous synthesis strategy (48.22%), our present method

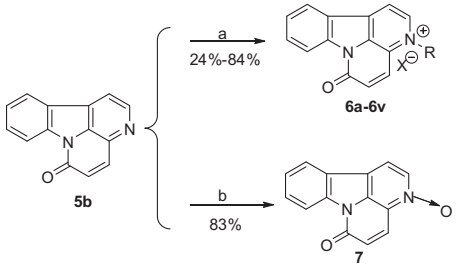
achieved the preparation of canthin-6-one under mild conditions, which shorten the reaction steps, reduced the cost of the materials and environmental pollution.

The 3-*N*-alkylated and 3-*N*-benzylated canthin-6-ones **6a**–**v** (Table 1) were prepared from canthin-6-one by reacting with halogenated hydrocarbon in heated acetonitrile (Scheme 2).[26,27] And the canthin-6-one *N*-oxide was prepared from canthin-6-one in the presence of 3-chloroperbenzoic acid.

All compounds were evaluated for their in vitro antibacterial activity against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative bacteria (*Ralstonia solanacearum* and *Pseudomonas solanacearum*) with ampicillin sodium and fosfomycin sodium as the positive controls. The MIC values were also listed in Table 1.

A clear structure–activity relationship with peak MIC values of 0.98 ($\mu g \cdot mL^{-1}$) was explored. Comparing the MIC values of compound **5b** with **4** and **5a**, we find that the unsaturated bonds could improve their antibacterial activity. For example, **5b** has MIC values that are about triple of **5a** against *Staphylococcus aureus*. Against *Bacillus cereus*, *Ralstonia solanacearum* and *Pseudomonas solanacearum*, the MIC values of **5b** are about double compared to **5a**. So we summarize that conjugated unsaturated bonds are beneficial. Meanwhile, we could also see that the antibacterial activity gradually increases with the growth of the 3-*N*-alkyl chain length for all tested bacteria. Unfortunately, the side alkyl chain group and short alkyl chain are not conducive to improve the antibacterial activity. In general, 3-*N*-alkylated quaternarization is not effective.

Inspiringly, 3-*N*-benzylated canthin-6-ones generally show good antibacterial activity. Specially, compared to the positive controls, compounds **6j**, **6k**, **6l**, **6m**, **6n**, **6o**, **6p**, **6q**, **6r** and **6t** are found to be the most potent compounds with MIC values lower than 1.95 ($\mu g \cdot mL^{-1}$) against *Staphylococcus aureus*. And compounds **6j**, **6k**, **6l**, **6m**, **6n**, **6o**, **6q**, **6r** and **6t** are also found to be the most potent compounds against *Pseudomonas solanacearum* with MIC values lower than 7.81 ($\mu g \cdot mL^{-1}$). The MIC values against *Bacillus cereus* of compounds **6j**, **6k**, **6l** and **6o** are equal or superior than canthin-6-one which MIC values is 7.81 ($\mu g \cdot mL^{-1}$). Similarly, the MIC values against *Ralstonia solanacearum* of compounds **6j**, **6k**, **6l** and **6t** are lower than 7.81 ($\mu g \cdot mL^{-1}$), which are equal to ampicillin sodium. The structure–activity relationship of the antibacterial activity evaluation against *Staphylococcus aureus* is depicted in Figure 1. For all tested bacteria other than *Staphylococcus aureus*, we find that 3-*N*-alkylated quaternarization is also not effective and the unsaturated bonds are beneficial. However, the structure–activity relationship is ambiguous on 3-*N*-benzylated quaternarization. The data revealed that not all the activities of 3-*N*-benzylated derivatives are better than canthin-6-one. Additionally, the halogen of these compounds has some effect on the in vitro antibacterial activity via analyzing the activity data of compounds **6j** and **6k**. A clear relationship has not been investigated due to the limitation of the number of samples.



Scheme 2. Synthesis of canthin-6-one analogs. Reagents and conditions: (a) CH_3CN, RX, 50 °C; (b) CH_2Cl_2, *m*-CPBA, 0 °C, 2 h.

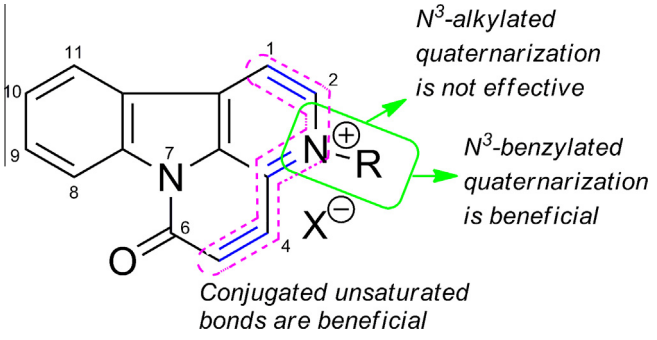


Figure 1. Structure–activity relationship of antibacterial activity evaluation against *Staphylococcus aureus*.

In conclusion, we have synthesized the parent natural product canthin-6-one via an easier synthetic route. Moreover, a collection of hydrophilic analogs of canthin-6-one were synthesized and their in vitro antibacterial activities were tested. The data clearly indicate that some of these compounds such as **6k** and **6l**, are more potent than canthin-6-one and may become lead compounds for further diseases-relevant studies. It is worthy to mention that the mechanism of these compounds' antibacterial activity is still ongoing to explore.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.11.070. These data include MOL files and InChiKeys of the most important compounds described in this article.

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