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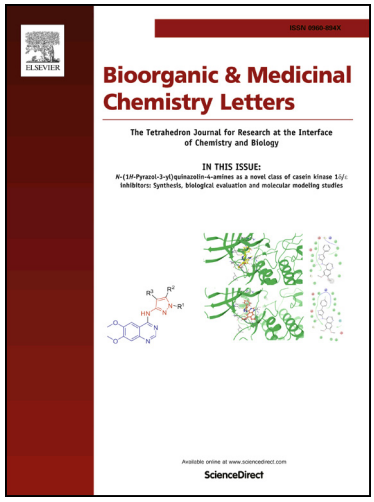

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Synthesis and antibacterial activity of 5-methylphenanthridium derivatives as FtsZ inhibitors

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Running title: 5-Methylphenanthridium derivatives

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Abstract

5-Methylphenanthridium derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity and cell division inhibitory activity against various Gram-positive and -negative bacteria. Among them, compounds **5A2**, **5B1**, **5B2**, **5B3**, **5C1** and **5C2** displayed the best on-target antibacterial activity with an MIC value of 4 μg/mL against *B. subtilis* ATCC9372 and *S. pyogenes* PS, showing over 2-fold better activity than sanguinarine. The SARs showed that the 5-methylphenanthridium derivatives with the alkyl side chains at the 2-postion, especially the straight alkyl side chains exerted better on-target antibacterial activity.

Keywords: Antimicrobials; Biological evaluation; FtsZ; Structure-activity relationships; 5-Methylphenanthridium derivatives.

Although the great development of antimicrobials in the past century has gave rise to the tremendous advances for human beings, making the rate of mortality from bacterial infections decrease remarkably and presenting a perception of the final triumph to the public in the battle against bacteria.[1] However, bacterial infections remain to be one of the exceedingly serious threats to the human society, impacting the lives of people all over world and the global economy significantly.[2] On the one hand, the profligate use of antibacterial agents has accelerated the evolution of bacterial resistance and even the epidemiological landscape of the Gram-positive and -negative pathogens.[3] On the other hand, the past almost three-decade-long drug discovery in the field of antibacterial agents has avoided any new classes of antimicrobial drugs.[4] In the current status, except for the restrictive use of antibacterial agents which provides a solution to the rapidly increasing bacterial resistance, it is becoming ever more urgent to discover new antimicrobial agents with novel or so-far-underexplored targets to address the recent antibiotic crisis.

Filamentous temperature-sensitive protein Z (FtsZ), as a crucial bacterial cell division protein, has been identified as an appealing target for the development of novel antimicrobials in the recent years, because of its high conservatism among bacterial pathogens and absence in mammalian cells.[5, 6] It plays a pivotal role in the bacteria cell division. When the bacterial division starts, FtsZ protein self-polymerizes into protofilaments in a GTP-dependent fashion, and then forms the so-called Z-ring at the center of the cell.[7, 8] The resulting Z-ring serves as a scaffold to recruit other cell division proteins.[9, 10] After the recruitment, Z-ring contracts, enabling the septum formation and the final cell separation. Therefore, any factor disturbing the function of FtsZ protein will eventually result in the failure of cell division and then the bacterial death.

Sanguinarine (Fig. 1), a kind of benzophenanthridine alkaloid, has been identified as a small molecule inhibiting FtsZ with antibacterial activity.[11] It can strongly induce bacterial filaments and alter Z-ring formation. Some *in vitro* experiments showed that sanguinarine inhibits FtsZ induced polymerization and hinders lateral interaction between the raw silks. Therefore, the number of FtsZ bundles is reduced, thereby inhibiting the formation of Z rings and reducing the frequency of their occurrence. However, it can also inhibit tubulin polymerization into microtubules, producing toxic side effects on mammalian cells.[12] Chelerythrine (Fig. 1), similar to sanguinarine in the structure, showed similar activity against *S. aureus* and *B. subtilis*. By modifying their structures and reducing their toxic effects on the human body, it is possible to develop new antibacterial drugs.

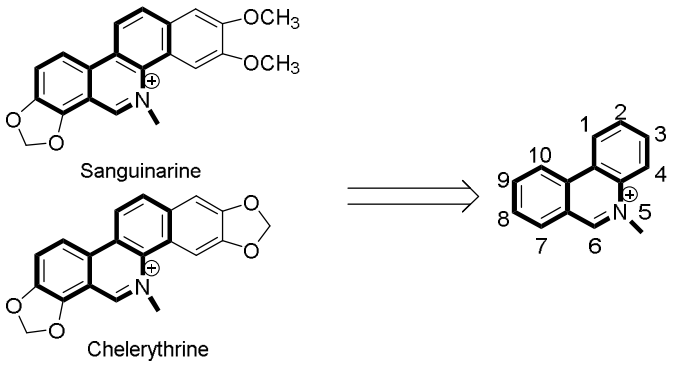
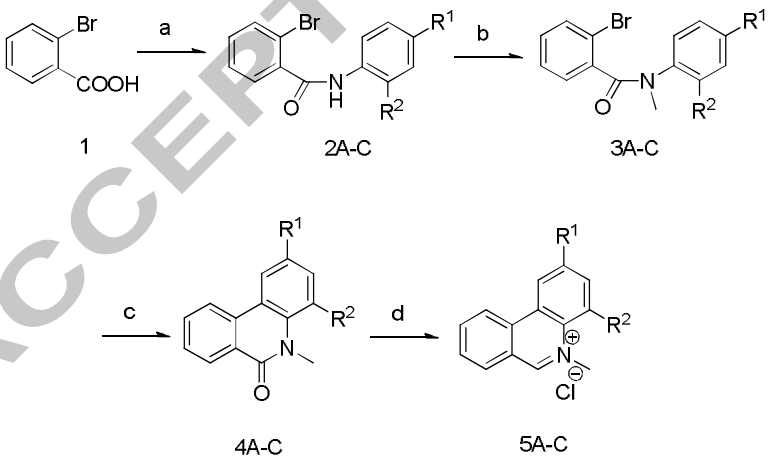


Fig. 1. The structures of sanguinarine, chelerythrine and 5-methylphenanthridium.

The general synthetic methodology employed for the synthesis of 5-methylphenanthridium derivatives is shown in Scheme 1. *O*-Bromobenzoic acid **1** was converted to the acid chloride in situ using $SOCl_2$, and this acid chloride was then reacted with the anilines to obtain the benzamides **2A-C** in high yields. After that, by treating the benzamides with NaH and CH_3I in DMF to give the corresponding N-methylbenzamides **3A-C**. The N-methylbenzamides were then cyclized to the phenanthridinones **4A-C** via an intramolecular Heck reaction using catalytic $Pd(OAc)_2$, PPh_3 and Na_3CO_3 in good yields. The phenanthridinones were reduced by DIBAL as the reducing agent and subsequent treatment with dilute HCl produced the phenanthridinium salts **5A-C** in high yields. The structures of the target compounds were characterized by MS, ^{1}H-NMR, and all the spectral data were in agreement with the proposed structures.



5A1	R^1=H	R^2=H
5A2	R^1=CH_3	R^2=H
5A3	R^1=t-butyl	R^2=H
5A4	R^1=n-butyl	R^2=H
5A5	R^1=CF_3	R^2=H
5A6	R^1=OCH_3	R^2=H
5A7	R^1=OCF_3	R^2=H
5A8	R^1=SCH_3	R^2=H
5A9	R^1=F	R^2=H
5A10	R^1=Cl	R^2=H
5B1	R^1=H	R^2=CH_3
5B2	R^1=H	R^2=isopropyl
5B3	R^1=H	R^2=OCF_3
5B4	R^1=H	R^2=OCH_3
5B5	R^1=H	R^2=SCH_3
5C1	R^1=CH_3	R^2=CH_3
5C2	R^1=OCH_3	R^2=OCH_3

Scheme 1. Reagents and conditions: (a) i) $SOCl_2$, reflux, 2 h; ii) phenyl anilines, TEA, DCM, rt, 3 h 78.6~89.3%; (b) NaH, DMF, 0 °C~rt, 70.1~75.6%; (c) $Pd(OAc)_2$, PPh_3, Na_2CO_3, dry DMF, reflux, 5 h, 76.8~83.2%; (d) i) DIBAL, -10 °C~rt; ii) 2 M HCl, 20 min, 15.2~29.0%.

The 5-methylphenanthridium derivatives (**5A1**-**5C1**) were determined for their *in vitro* antibacterial activity and cell division inhibitory activity, respectively. The *in vitro* antibacterial activity was tested using tube dilution method recommended by NCCLS and the cell division inhibitory activity was measured by assessing cell morphology using phase-contrast light microscopy. The tested strains included *S. aureus* ATCC25923, *S. aureus* ATCC29213 (MRSA), *S. epidermidis*, *S. pyogenes* PS, *E. Coli* ATCC25922, *B. subtilis* ATCC9372 and *P. Aeruginosa* ATCC27853. *S. aureus* ATCC25923, *B. subtilis* ATCC9372, *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 were used to determine the cell division inhibitory activity of the tartget compounds. The results in the unit of μg/mL are shown in Table1, Table 2 and Fig. 2.

The 5-methylphenanthridium derivatives universally exhibited better antibacterial activity against *B. subtilis* ATCC9372, *S. aureus* PR, *S. pyogenes* PS and *S. pyogenes* PR than the other tested strains. Among them, many derivatives also showed superior or comparable activity against *B. subtilis* ATCC9372 and *S. pyogenes* PS to sanguinarine and ciprofloxacin. Particularly, compounds **5A2**, **5B1**, **5B2**, **5B3**, **5C1** and **5C2** displayed the best antibacterial activity with an MIC value of 4 μg/mL against *B. subtilis* ATCC9372, showing over 8, 2, 2, and 2-fold better activity than curcumin, sanguinarine, ciprofloxaxin and oxacillin sodium, respectively. In the inhibition of *S. pyogenes* PS, the most active compounds**5A2**, **5B1**, **5B2**, **5B3**, **5C1** and **5C2** exhibited the same MIC value of 4 μg/mL, being 2, 16, and 4-fold better than sanguinarine and oxacillin sodium, respectively. Furthermore, compound **5B5** also showed the strongest activity with an MIC value of 16 μg/mL against *S. aureus* ATCC29213 in all of the tested compounds, being 8-fold and 8-fold more potent than curcumin, and oxacillin sodium, but 2- and weaker 4-fold weaker than sanguinarine and ciprofloxacin. Compounds **5A2**, **5B1**, **5C1** and **5C2** exhibited the same activity against *S. aureus* PR to sanguinarine. Besides, all of the compounds showed basically the same activity against *S. pyogenes* PS and *S. pyogenes* PR.

Table 1. Antibacterial activity of 5-methylphenanthridium derivatives (μg/mL)

Compounds	*B. subtilis* ATCC9372 [a)]	*E. coli* ATCC25922 [b)]	*P. aeruginosa* ATCC27853 [c)]	*S. aureus* ATCC25923 [d)]	*S. aureus* ATCC29213 [e)]	*S. aureus* PR [f)]	*S. epidermidis* [g)]	*S. pyogenes* PS [h)]	*S. pyogenes* PR [i)]
5A1	16	>128	128	>128	>128	32	>128	32	32
5A2	4	>128	>128	64	128	4	128	4	8
5A3	8	>128	>128	64	>128	16	128	16	16
5A4	4	>128	>128	64	>128	8	>128	8	8
5A5	128	>128	>128	128	>128	128	128	128	128
5A6	8	>128	>128	>128	128	8	64	8	16
5A7	128	>128	>128	128	>128	128	128	128	128
5A8	8	>128	>128	128	>128	32	>128	32	32
5A9	16	>128	128	>128	>128	32	>128	32	32
5A10	16	128	>128	>128	>128	32	>128	32	32
5B1	4	>128	>128	128	>128	4	128	4	8
5B2	4	>128	>128	8	64	16	64	4	8
5B3	4	>128	>128	16	64	8	32	4	8
5B4	8	>128	>128	>128	>128	16	128	16	16
5B5	8	>128	>128	16	16	8	>128	8	8
5C1	4	>128	>128	64	32	4	128	4	8
5C2	4	>128	>128	128	32	4	64	4	8
Sang [j)]	8	>128	>128	8	8	4	64	8	8
Cipro [k)]	8	8	8	32	4	16	>128	16	>128
OS [l)]	8	128	128	8	128	>128	8	1	2

[a)] *B. subtilis* ATCC9372: penicillin-susceptible strain; [b)] *E. coli* ATCC25922: penicillin-susceptible strain; [c)] *P. aeruginosa* ATCC27853: penicillin-susceptible strain; [d)] *S. aureus* ATCC25923: penicillin-susceptible strain; [e)] *S. aureus* ATCC29213: methicillin-resistant strain; [f)] *S. aureus* PR: penicillin-resistant strain isolated clinically, not characterized; [g)] *S. epidermidis*: penicillin-resistant strain isolated clinically, not characterized; [h)] *S. pyogenes* PS: penicillin-susceptible strain; [i)] *S. pyogenes* PR: penicillin-resistant strain; [j)] Sang: Sanguinarine [k)] Cipro: ciprofloxacin; [l)] OS: oxacillin sodium.

All the above results indicated that 5-methylphenanthridium and its derivatives were the effective simplified forms of sanguinarine and chelerythrine. The study of SARs showed that the introduction of the alkyl side chains at the 2-position of the 5-methylphenanthridium (such as compounds **5A2**, **5A3** and **5A4**) could improve the antibacterial activity. Furthermore, the straight alkyl side chain was found to be better than the branched alkyl side chain in the antibacterial activity. By comparison, introducing halogen group into the 2-position (such as compounds **5A9** and **5A10**) slightly decrease the antibacterial activity while introducing methoxyl and methylthio groups at the 2-position retained the antibacterial activity. However, the substituent group at the 4-position was less rigorous and regular. Nonetheless, introduction of the alkyl side chain (such as compounds **5B1**, **5B2** and **5B3**) into the 4-position could enhance the antibacterial activity as well. Additionally, introduction of the side chains at the 2- and 4-positions simultaneously (such as compounds **5C1** and **5C2**) could increase the antibacterial activity.

As seen from Table 2 and Fig. 3, basically the cell division inhibitory activity of all the target compounds fitted their *in vitro* antibacterial activity against *S. aureus* ATCC25923, *B. subtilis* ATCC9372, *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853. Therefore, the above results indicated that the 5-methylphenanthridium derivatives were promising FtsZ inhibitors with on-target antibacterial activity.

Table 2. Cell division inhibitory activity of 5-methylphenanthridium derivatives (μg/mL)

Compounds	*B. subtilis* ATCC9372 [a)]	*E. coli* ATCC25922 [b)]	*P. aeruginosa* ATCC27853 [c)]	*S. aureus* ATCC25923 [d)]
5A1	16	>128	128	>128
5A2	4	>128	>128	32
5A3	8	128	>128	64
5A4	4	128	>128	64
5A5	64	>128	>128	128
5A6	8	>128	>128	>128
5A7	64	>128	>128	128
5A8	8	128	>128	128
5A9	16	>128	128	>128
5A10	16	128	>128	>128
5B1	4	>128	>128	128
5B2	4	>128	>128	8
5B3	4	>128	>128	16
5B4	8	>128	>128	>128
5B5	8	128	>128	16
5C1	2	>128	128	64
5C2	2	>128	128	128
Sang [e)]	8	>128	>128	8

[a)] *B. subtilis* ATCC9372: penicillin-susceptible strain; [b)] *E. coli* ATCC25922: penicillin-susceptible strain; [c)] *P. aeruginosa* ATCC27853: penicillin-susceptible strain; [d)] *S. aureus* ATCC25923: penicillin-susceptible strain; [e)] Sang:Sanguinarine

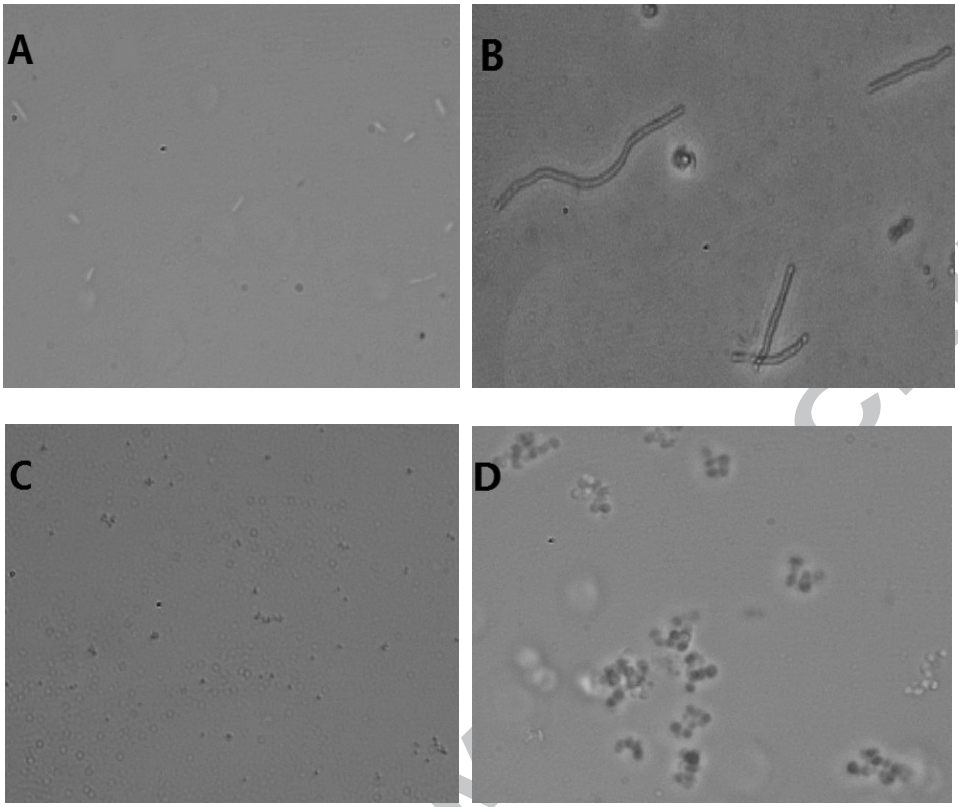


Fig. 2. *B. subtilis* ATCC9372 without (A) and with (B) compound 5B3 at 128 µg/mL; *S. aureus* ATCC25923 without (C) and with (D) compound 5B3 at 128 µg/mL.

Besides, the 5-methylphenanthridium derivatives were also determined using a commercial tubulin assay kit for their effect on the polymerisation of mammalian tubulin. The results showed that none of the compounds enhanced polymerisation of mammalian tubulin even though they were added at concentrations much higher than that of the positive control paclitaxel (Figure 3A on the page 6 in supplementary material). A marginal inhibitory effect was observed on tubulin polymerisation in the presence of compounds **5A5** and **5B2** (Figure 3B on the page 6 in supplementary material). These compounds were tested at relatively high concentrations though (32 µg/mL and 256 µg/ml for **5B2** and **5A7** respectively). The effect of compound **5A5** on mammalian tubulin polymerisation could not be determined as the compound interfered with the fluorescence of the reporter used in the assay kit. However, the MIC values of this compound were at least 128 µg/mL for all the bacterial strains tested. Hence, this derivative would not be selected for further optimisation anyway.

In conclusion, a series of 5-methylphenanthridium derivatives were designed, synthesized and evaluated for their *in vitro* antibacterial activity and cell division inhibitory activity against various Gram-positive and Gram-negative bacteria. Besides, the activity against polymerisation of mammalian tubulin was determined. Among them, compounds **5A2**, **5B1**, **5B2**, **5B3**, **5C1** and **5C2** displayed the best on-target antibacterial activity with an MIC value of 4 μg/mL against *B. subtilis* ATCC9372 and *S. pyogenes* PS, showing over 2-fold better activity than sanguinarine. The SARs showed that the introduction of the alkyl side chains at the 2-position of the 5-methylphenanthridium could improve its antibacterial activity, especially the straight alkyl side chain being better than the branched alkyl side chain in the antibacterial activity while introducing halogen group into the 2-position could cause slightly reduced antibacterial activity.

Acknowledgments

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Highlights

> 5-Methylphenanthridium derivatives were designed and synthesized. > They were tested for their antibacterial and cell division inhibitory activities. > Compounds **5A2**, **5B1**, **5B2**, **5B3**, **5C1** and **5C2** exerted the best on-target activity.