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新型 γ-咔啉衍生物的合成及其抑菌活性研究

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摘要 以哌啶酮和 4-甲氧基苯肼为起始原料, 通过 Fischer 吲哚合成得到中间体 **9**, 经过氧化、酰化以及烷基化构建出新型衍生物 45 个. 所有的目标产物都进行了抗金黄色葡萄球菌、大肠埃希菌、铜绿假单胞杆菌、茄科雷尔氏菌的抑菌活性研究. 结果显示: 5-(4'-甲氧基苯甲酰基)-8-甲氧基-γ-咔啉(**11g**)、5-(2'-呋喃甲酰基)-8-甲氧基-γ-咔啉(**11n**)、5-(2'-溴苄基)-8-甲氧基-γ-咔啉(**12f**)、5-(3'-溴苄基)-8-甲氧基-γ-咔啉(**12g**)、5-(4'-三氟甲氧基苄基)-8-甲氧基-γ-咔啉(**12h**)、溴化 2-(4'-氯苄基)-8-甲氧基-γ-咔啉(**13e**)具有良好的抑菌活性, 特别是化合物 **12f** 和 **12h** 展现出了与阳性对照氨苄西林钠相当的活性, 其最小抑菌浓度为 8 μg/mL. 探索了化合物 **12h** 对铜绿假单胞菌的作用机制, 确定了其抑菌机制是通过破坏细菌的细胞壁发挥抑菌作用. 进一步对该系列衍生物的构效关系进行了讨论, 并总结出若干影响该类化合物生物活性的关键因素, 为开发新的抗生素提供参考.

关键词 8-甲氧基-N^2 & N^5 取代 γ-咔啉; 抑菌活性; 构效关系; 抑菌机制

Novel γ-Carboline Derivatives as Antibacterial Agents: Synthesis and Antibacterial Evaluation

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Abstract Forty-five novel 8-methoxyl-γ-carboline derivatives were designed and effectively synthesized with commercially available starting materials, 4-methoxyphenylhydrazine hydrochloride **7** and piperidone **8**, *via* Fischer indole synthesis process to prepare key intermediate **9**, which underwent oxidation, acylation or alkylation to yield the target derivatives. The antibacterial activities of all compounds were evaluated against *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Ralstonia solanacearum* (*R. solanacearum*). The results indicated that (8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(4-methoxyphenyl)methanone (**11g**), furan-2-yl(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)methanone (**11n**), 5-(2-bromobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12f**), 5-(3-bromobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12g**), 8-methoxy-5-(4-(trifluoromethoxy)benzyl)-5*H*-pyrido[4,3-*b*]indole (**12h**) and 2-(4-chlorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13e**) showed a broad spectrum of antibacterial activity. Especially compounds **12f** and **12h** exhibited comparable antibacterial activity with the positive control (ampicillin sodium) with minimum inhibitory concentration (MIC) value of 8 μg/mL. Moreover, membrane-active mechanism was studied further *via* fluorescent microscopy, which suggested that derivative **12h** exerted its antibacterial effect by damaging the membranes of the bacteria. Some structure-activity relationships among the forty-five derivatives are discussed and several important determinants for the activity of these compounds are identified. This work offers useful instructions for the development of new antibiotics.

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Keywords 8-methoxyl-N^2 & N^5-substituted γ-carbolines; antibacterial activity; structure-activity relationships; mechanism

1 Introduction

Infectious diseases including various bacterial infection and viral infection are serious causes of morbidity and mortality worldwide. The epidemic of drug resistant microorganisms (methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa*) and lethal virus poses an enormous threat to public health. Moreover, bacterial wilt, caused by *R. solanacearum*, causes serious economic losses to crops such as tomato and potato *etc*.[1-3] Antibiotics, which can be classified into different categories based on their chemical structures, action mechanisms, action spectra and the route of administrations, are the products of rapid innovations in the health sector and the availability of antibiotics for treating infectious diseases improving the health as well as life expectancy of humans and animals significantly.[4-7] Although significant progresses have been made in the understanding of microbiology and the control of microorganisms, the antimicrobial resistance is still a looming public health crisis. The spread of antibiotic-infections caused by resistant bacteria as well as the emergence of multi-resistant gram-positive and gram-negative pathogens is tough threat to the health-care community.[8-11] What's worse, the frequency of the emergence of resistant bacteria is accelerated for the use and abuse of antibiotics. Thus, it is highly desirable to discover new and more effective reagents to settle these problems.

Carbolines, containing α-, β-, γ- and δ-carbolines, are the main skeletons of many natural products and bioactive compounds, and β-carboline alkaloids are the most popular agents in nature.[12-13] In the contrast, the γ-carboline core structure is less abundant in natural products (ingenine B **2** and isocanthine **3**) and less explored due to its unavailability (Figure 1). In recent years, the metal-catalyzed cyclization reactions offer powerful strategies to build polycyclic molecules having potent bioactivities,[14-15] and some disclosures reported classical methods to synthesize γ-carboline derivatives.[16-21] On the basis of these findings, the bioactivities of γ-carboline derivatives have been investigated, and these alkaloids showed a variety of pharmaceutical activities such as antifungal activity (**4**),[22] neurotrophic activity,[23-24] antiviral activity,[25] enzyme modulators or inhibitors[26] (**5**), cytotoxity,[27] and antimicrobial activity (**6**)[12] (Figure 1). Therefore, the development of a facile and efficient way to synthesize some novel γ-carbolines with unique scaffolds is of great importance.

In current study, a series of novel 8-methoxyl-N^3&N^5-substituted γ-carboline analogues were designed and synthesized according to the derivatization methods of reported bioactive β-carbolines.[28] All derivatives were assayed for their antibacterial activity *in vitro* against a gram-positive pathogen *S. aureus*, and three gram-negative pathogens *E. coli*, *P. aeruginosa* and *R. solanacearum*. They showed promising antibacterial activity, and some extend structure-activity relationships of γ-carboline derivatives were discussed to discover potent inhibitors and meet the urgent need of the development of new antibiotics.

2 Results and discussion

The chemical synthesis of 8-methoxyl-N^3 & N^5-substituted γ-carboline derivatives is depicted in Scheme 1. Intermediate **9** was synthesized from commercial available 4-methoxyphenylhydrazine hydrochloride **7** and piperidone derivative **8** *via* Fischer indole synthesis process according to the reported methods in a high yield.[29-31] The oxidation of compound **9** with Pd/C gave γ-carboline derivative **10** in an excellent yield,[32] which reacted with different chlorides and alkyl halides in the presence of corresponding bases to deliver the desired products **11a**～**11o** and **12a**～**12k** in moderate to high yields, respectively (Table 1). Moreover, stirring the key synthon **10** with var-

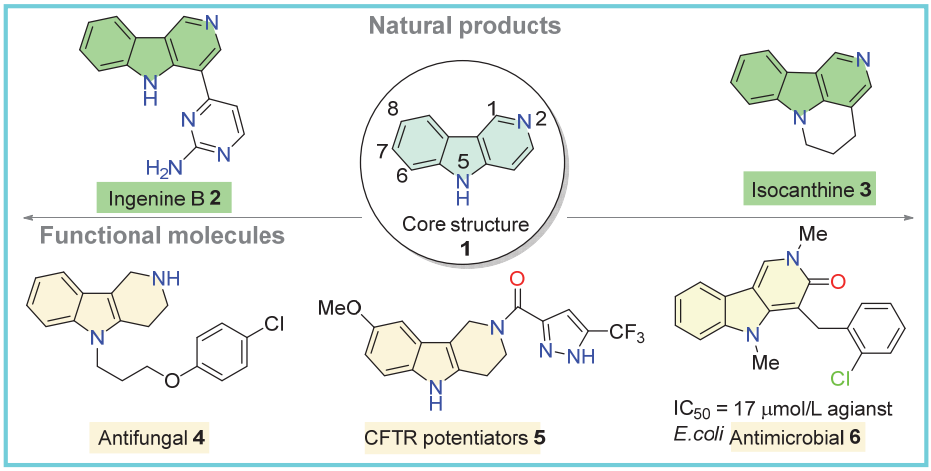
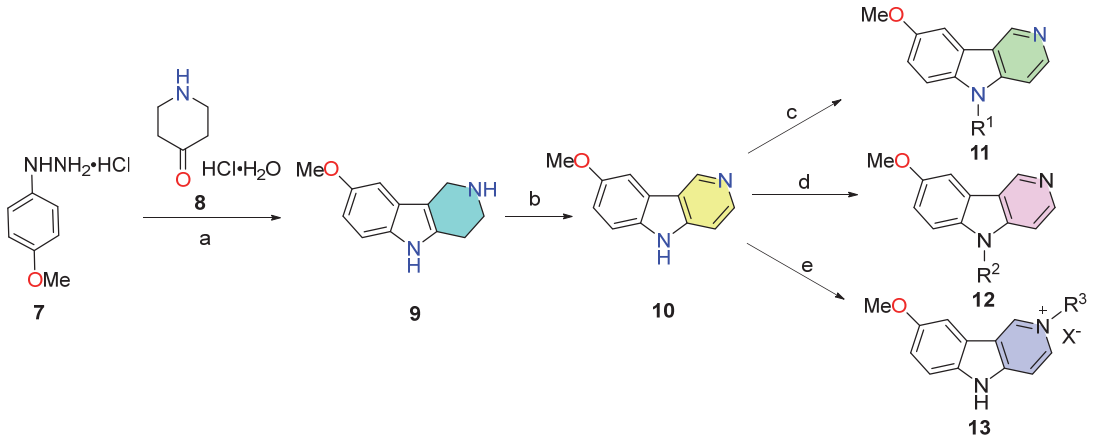


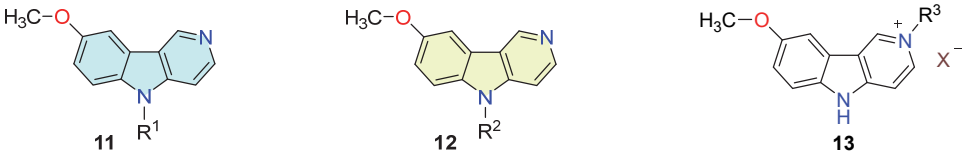
Figure 1 Representative bioactive molecules

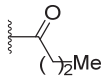
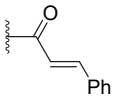
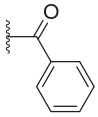
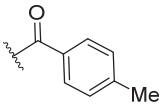
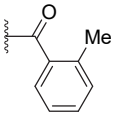
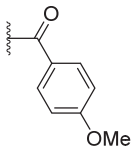
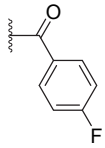
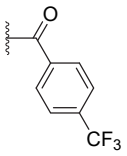
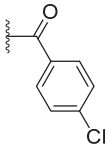
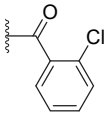
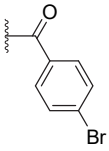
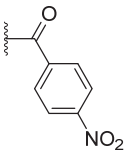
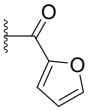


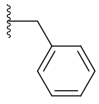
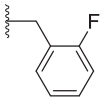
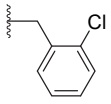
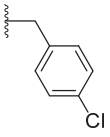
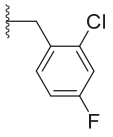
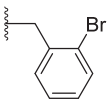
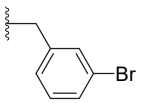
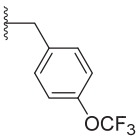
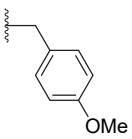
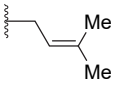
Reagents and conditions: (a) AcOH, EtOH, 70 °C, 12 h; (b) Pd/C, 150 °C, 4 h, 75% two steps; (c) RCOCl, Et_3N, DCM, 0 °C～r.t., 1 h; (d) $ArCH_2Br$, NaH, 0 °C～r.t., 1 h; (e) RX, CH_3CN, 70 °C.

Scheme 1 Synthetic route of γ-carboline derivatives

Table 1 Chemical structures of compounds **11a**～**11n**, **12a**～**12k** and **13a**～**13t**



Compd.	R^1	Compd.	R^1	Compd.	R^1	Compd.	R^1	Compd.	R^1
11a		**11b**		**11c**		**11d**		**11e**	
11f		**11g**		**11h**		**11i**		**11j**	
11k		**11l**		**11m**		**11n**			

Compd.	R^2	Compd.	R^2	Compd.	R^2	Compd.	R^2	Compd.	R^2
12a		**12b**		**12c**		**12d**		**12e**	
12f		**12g**		**12h**		**12i**		**12j**	
12k									

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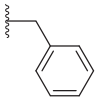
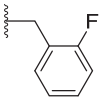
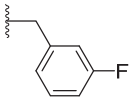
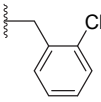
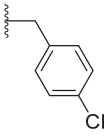
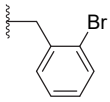
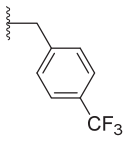
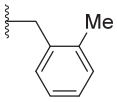
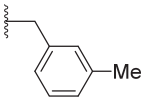
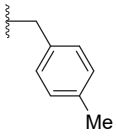
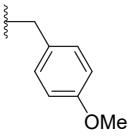
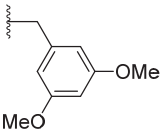
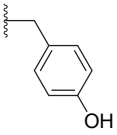
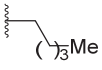
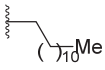
Compd.	R^3	Compd.	R^3	Compd.	R^3	Compd.	R^3	Compd.	R^3
13a		**13b**		**13c**		**13d**		**13e**	
13f		**13g**		**13h**		**13i**		**13j**	
13k[b]		**13l**		**13m**		**13n**[b]		**13o**	
13p		**13q**		**13r**		**13s**		**13t**	

[a] X=Cl; [b] X=I.

ious haloalkanes heating in acetonitrile afforded the ammonium salts **13a**～**13t** in moderate to high yields (Table 1).

The structures of all synthesized compounds were determined by ^{1}H NMR, ^{13}C NMR and high resolution mass spectra (HRMS) and were all in full agreement with the proposed structures. In the ^{1}H NMR, the successful oxidation of compound **9** to form compound **10** was confirmed by the signals ranging from δ 11.53 to 7.09. When 8-methoxyl-γ-carboline **10** reacted with corresponding reactants to afford the carboline derivatives, the missing of N—H signal and the appearance of signals of the different substitutions, aliphatics (**11a**～**11c** and **12k**～**12l**) or aromatics (**11d**～**11o** and **12a**～**12j**), in the aliphatic region or the aromatic region, respectively, confirmed the successful synthesis of the target derivatives. Moreover, the structure of compound **13k** was established by X-ray crystallographic analysis (CCDC numbers 2281688), and other ammonium salts were assigned by analogy (Figure 2).

All the new synthesized γ-carboline analogues were evaluated for *in vitro* antibacterial activity using ampicillin sodium as reference drug against four pathogens. The minimum inhibitory concentrations (MICs) for all compounds were listed in Table 2. In general, all the derivatives exhibited inhibitory activity against the tested pathogens, especially compounds **12f** and **12h**. As illustrated in Table 2, the amide derivatives **11g** with 4-methoxybenzoyl group and **11j** bearing methyl moiety showed promising antibacterial activity with MIC values of 16 μg/mL against *S. aureus* and *R. solanacearum*, which were more potent than the analogues with acetyl, butyryl and cinnamoyl group (**11a**～**11c**). When halogen atoms and nitryl were introduced to the 2- or 4-position of the benzoyl group, the derivatives **11d**～**11f** and **11h**～**11n** showed inferior activity to compound **11g**, which indicating that the electron-donating groups were beneficial for the improvement of the activity.

The γ-carboline derivatives **12a**～**12k** bearing alkyl side chains at the N(5)-atom exhibited superior antibacterial activity to the amide derivatives **11a**～**11n**. Analogues

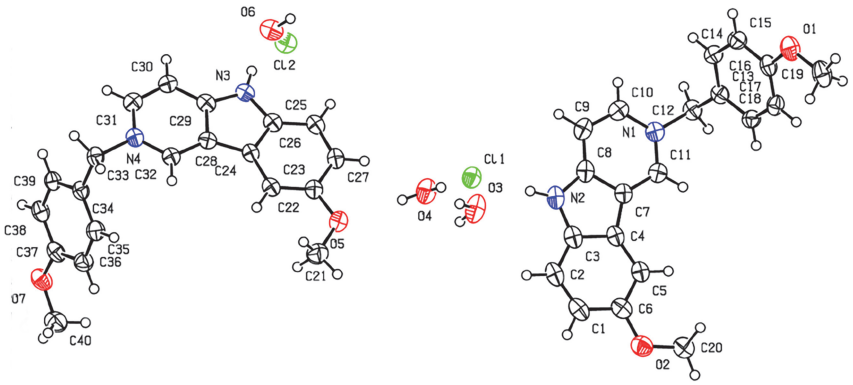


Figure 2 X-ray structure of compound **13k**

Table 2 MICs (μg/mL) of antibacterial activities of γ-carboline analogues[a]

Compound	*S.a*	*E.c*	*P.a*	*R.s*	Compound	*S.a*	*E.c*	*P.a*	*R.s*
11a	—	—	16	32	**12g**	64	—	—	32
11b	—	32	64	32	**12h**	16	—	**8**	64
11c	—	—	64	64	**12i**	16	—	16	16
11d	—	—	64	64	**12k**	16	64	64	16
11e	—	—	—	64	**13a**		—		64
11f	—	—	—	64	**13b**		—		64
11g	16	—	16	16	**13c**		64		16
11h	—	—	64	—	**13e**	16	—		8
11i	—	16	—	—	**13f**	16	—		32
11j	16	—	—	16	**13j**		64		
11k	—	—	—	16	**13k**	32	64		32
11m	—	—	—	16	**13o**	-	—		64
11n	—	64	32	—	**13p**	64	—		64
12a	—	—	64	32	**13q**	64	—		
12b	64	—	64	16	**13s**	—	64		
12c	—	—	32	—	**13t**	—	64		
12d	—	—	—	64	**13u**	—	64		
12f	**8**	—	64	**8**	A.S.[b]	8	4	8	2

[a] Compounds with all minimum inhibitory concentration (MIC) values ＞64 μg/mL against four strains were not listed in the table; *S.a* (*S. aureus*), *E.c* (*E. coli*), *P.a* (*P. aeruginosa*), *R.s* (*R. solanacearum*); [b] positive control, A.S.＝ampicillin sodium.

12f～**12k** showed significant improvements in antibacterial activity against all the tested bacteria in comparison with the amide derivatives. Detailedly, derivative **12f** containing *ortho*-bromo on the benzyl group was superior *in vitro* to the derivatives with *meta*-bromo and other halogens (F, Cl) on the benzyl group against *S. aureus* and *R. solanacearum* with MIC value of 8 μg/mL. Further, compound **12h** bearing trifluoromethoxyl group exhibited potent antibacterial activity against *P. aeruginosa* with MIC value of 8 μg/mL, which was comparable to the reference drug. The introduction of propargyl and isopentenyl groups resulted in a dramatic decrease in activity against all tested bacteria. Overall, several compounds exhibited moderate to significant antibacterial activity against gram-positive and gram-negative pathogens, and the alkyl derivatives were more efficient than the amide derivatives.

To further test the effects of N(2)-substituents on biological activity, various ammonium salts (**13a**～**13t**) were prepared. Derivative **13e** with *para*-chloro and **13f** having *ortho*-bromo on the benzyl group showed promising antibacterial activity against *S. aureus* with MIC values of 8 and 16 μg/mL, respectively. Compounds **13c** and **13k** also showed antibacterial activity against *R. solanacearum* and *S. aureus*, respectively. In the contrast, Hybrids **13a**～**13b**, **13d**, **13g**～**13j** and **13l**～**13t** with different functional groups substituted benzyl groups and alkyl chains did not show potent antibacterial activity against the tested bacterial strains. Obviously, antibacterial activity of these ammonium derivatives was relevant to their hydrophobicity, which could be estimated by the log *P* values. The *c*log *P* values of compounds **13a**～**13t** decreased, accompanied by a decrease in antibacterial activity, which indicating that the cationic moieties would affect the hydrophobicity of the γ-carboline derivatives, resulting in a loss in antibacterial activity.

According to the above antibacterial activity results of all the derivatives, the structure-activity relationships can be summarized in Figure 3. In general, the introduction of alkane substituents at N(5) position is more favorable than N(5)-amides and N(2)-ammonium derivatives for improving the antibacterial activity. (I) For the effects of substituents of N(5)-benzyl for the antibacterial activity: 2-Br＞4-OCF_3＞4-OCH_3＞2-F＞alkyl chains. (II) For the effects of substituents of N(5)-benzoyl for the antibacterial activity: 4-OCH_3＞4-Cl＞4-OCF_3＞alkyl acyl. (III) For the effects of substituents of N(2)- benzyl for the antibacterial activity: 4-Cl＞2-Br＞3-F＞4-OCH_3＞alkyl chains.

In order to investigate additional mode of action of these compounds, their impacts on bacterial membranes were achieved by fluorescent microscopic observation. The most potent derivative **12h** was selected to examine its ability to compromise bacterial membranes of *P. aeruginosa* using Hoechst 33342 and propidium iodide (PI) as two dyes, which could be used to differentiate the bacterial cells either with an intact or a damaged membrane. As shown in Figure 4, in the Hoechst channel, the *P. aeruginosa* exhibited blue fluorescence and no strains showed fluorescence in the PI channel without treated with compound **12h** indicating that the membranes of the pathogen were intact (Figure 4, A1, A2). After the cells treated with derivative **12h**, the bacteria were stained by both Hoechst and PI, suggesting that the membranes of *P. aeruginosa* were damaged (Figure 4, B1, B2).

3 Conclusions

In summary, three series of novel 8-methoxyl-N^5 and

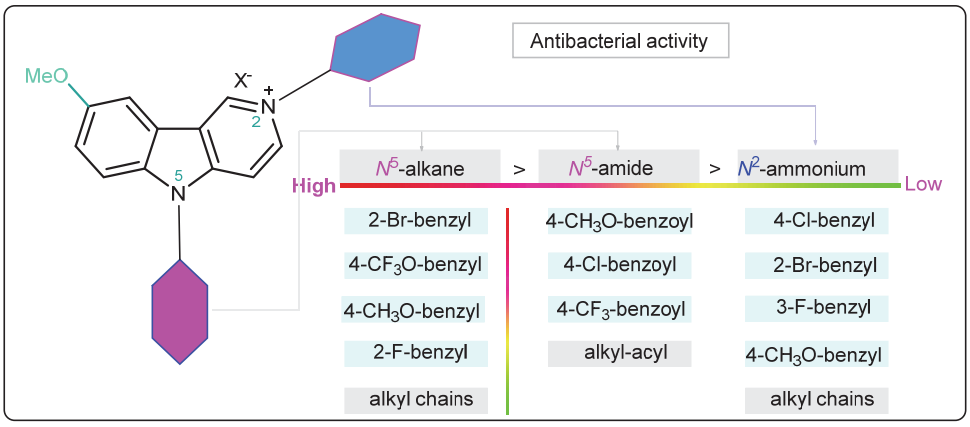


Figure 3 Structure-activity relationship of *γ*-carboline derivatives

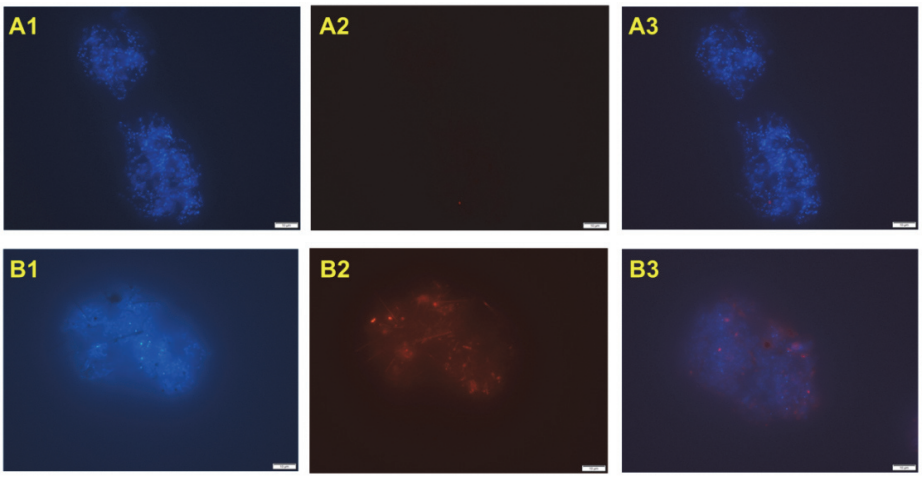


Figure 4 Florescence micrographs of *P. aeruginosa* treated or not treated with compound **12h**

(A1) No treatment, Hoechst stained; (A2) no treatment, PI stained; (A3) no treatment, merge graph; (B1) treatment with **12h**, Hoechst stained; (B2) treatment with **12h**, PI stained; (B3) treatment with **12h**, merge graph. Scale bars: 10 μm.

N^2-substituted *γ*-carboline derivatives were synthesized and evaluated for their antibacterial activity, and most compounds exhibited moderate to significant activity against four pathogens. Among them, compound **12f** showed promising antibacterial activity with MIC value of 8 μg/mL against *S. aureus* and *R. solanacearum*. In addition, derivative **12h** also exhibited potent antibacterial activity against *P. aeruginosa* with MIC value of 8 μg/mL, which was comparable to the reference drug ampicillin sodium. Study of the mechanism of action of these hybrids indicating that the *γ*-carboline derivatives reported here could compromise bacterial membranes and kill bacterial rapidly. These *γ*-carboline derivatives illustrated their potential to serve as a platform to develop more potent candidates to solve the current antibiotic crisis.

4 Experimental section

4.1 General information

Materials obtained from commercial suppliers were used directly without further purification unless otherwise noted. Dry tetrahydrofuran (THF) was distilled over sodium under N(2) prior to use. Dry dichloromethane (DCM) was distilled over calcium hydride at least for 2 h prior to use. All reactions sensitive to air or moisture were conducted under nitrogen atmosphere in dry solvents.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance spectrometer at 500 and 125 MHz in $CDCl_3$, CD_3OD or DMSO-*d* with reference to residual solvents signals [^{1}H NMR $CDCl_3$ (*δ* 7.26), CD_3OD (*δ* 3.31); ^{13}C NMR $CDCl_3$ (*δ* 77.16), CD_3OD (*δ* 49.00)]. Proton multiplicity is reported as a singlet (s), a broad singlet (br), a doublet (d), a triplet (t), a triplet double (td) or a multiplet (m). High resolution mass spectrometry (HRMS) was performed with a Thermo Scientific LTQ Orbitrap XL unless otherwise noted. The bacterial: *Staphylococcus aureus* (*S. aureus*, CGMCC 1.8721), *Escherichia coli* (*E. coli*, CGMCC 1.1574), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 9027) and *Ralstonia solanacearum* (*R. solanacearum*, CGMCC 1.12711).

4.2 Experimental procedures

4.2.1 Synthesis of compound **9**

To a solution of 4-methoxyphenylhydrazine hydrochlo-

ride (10 mmol, 1.74 g, 1 equiv.) in 10 mL of AcOH and 30 mL of EtOH was added 4,4-piperidinediol hydrochloride (11 mmol, 1.68 g, 1.1 equiv.) and the resulting mixture was heated at 70 ℃ for 12 h. Upon completion, the reaction mixture was evaporated to dry, and the crude product was used to the next step without further purification.

4.2.2 Synthesis of compound **10**

To a solution of **9** (20 mmol,) in 30 mL of dry xylene was added Pd/C (0.1 equiv.) and the reaction was stirred at 150 ℃ for 4 h. Upon completion, the reaction was filtered through a celite plug, concentrated to give the crude product, which was purified by chromatography (eluted with DCM/MeOH, *V*∶*V*=20∶1～10∶1) to get light yellow solid 8-methoxy-5*H*-pyrido[4,3-*b*]indole (**10**) in 75% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.53 (s, 1H), 9.32 (s, 1H), 8.38 (d, *J*=5.7 Hz, 1H), 7.81 (d, *J*=2.5 Hz, 1H), 7.47 (d, *J*=8.7 Hz, 1H), 7.42 (d, *J*=5.7 Hz, 1H), 7.10 (dd, *J*=8.8, 2.5 Hz, 1H), 3.86 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 153.9, 144.2, 143.9, 142.9, 134.2, 121.3, 119.5, 115.7, 112.2, 106.4, 103.3, 55.6. HRMS (ESI) calcd for $C_{12}H_{11}N_2O$ $[M+H]^+$ 199.0866, found 199.0870.

4.2.3 Syntheses of compounds **11a**～**11n**

To a solution of compound **10** (20 mg, 0.1 mmol) in 3 mL of dry DCM was added Et_3N (20 mg, 0.2 mmol), and various acyl chlorides (0.12 mmol) in 1 mL of dry DCM was added dropwise at 0 ℃. The resulting mixture was stirred at room temperature for 1 h, upon completion, the reaction was quenched with 10 mL of H_2O, extracted with DCM (30 mL×3). The organic layers were combined, dried over Na_2SO_4, evaporated to dry to get crude product, which was purified by chromatography (eluted with DCM/MeOH, *V*∶*V*=50∶1～30∶1) to get compounds **11** in moderate to high yields.

1-(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)ethan-1-one (**11a**): 91 yield%. Light yellow solid, m.p. 108～109 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.17 (s, 1H), 8.60 (d, *J*=6.0 Hz, 1H), 8.08 (d, *J*=9.1 Hz, 1H), 7.97 (d, *J*=5.8 Hz, 1H), 7.44 (d, *J*=2.3 Hz, 1H), 7.07 (dd, *J*=9.2, 2.7 Hz, 1H), 3.92 (s, 3H), 2.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.7, 157.0, 147.5, 143.8, 142.7, 132.9, 125.3, 122.5, 117.3, 116.2, 110.9, 103.4, 55.9, 27.5. HRMS (ESI) calcd for $C_{14}H_{13}N_2O_2$ $[M+H]^+$ 241.0972, found 241.0971.

1-(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)butan-1-one (**11b**): 85 yield%. Light yellow wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.19 (s, 1H), 8.61 (d, *J*=5.9 Hz, 1H), 8.11 (d, *J*=9.1 Hz, 1H), 8.00 (d, *J*=5.8 Hz, 1H), 7.45 (d, *J*=2.6 Hz, 1H), 7.08 (dd, *J*=9.1, 2.6 Hz, 1H), 3.92 (s, 3H), 3.04 (t, *J*=7.2 Hz, 2H), 1.98～1.91 (m, 2H) 1.12 (t, *J*=7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 172.9, 156.9, 147.4, 143.7, 142.6, 132.8, 125.2, 122.5, 117.5, 116.2, 111.2, 103.3, 55.9, 41.0, 18.0, 13.9. HRMS (ESI) calcd for $C_{16}H_{17}N_2O_2^+$ $[M+H]^+$ 269.1285, found 269.1287.

(*E*)-1-(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)-3-phenylprop-2-en-1-one (**11c**): 76 yield%. Light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.22 (s, 1H), 8.61 (s, 1H), 7.98～7.93 (m, 3H), 7.66～7.64 (m, 2H), 7.48～7.46 (m, 4H), 7.29 (s, 1H), 7.09 (dd, *J*=9.0, 2.6 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 165.8, 157.0, 147.1, 146.6, 143.9, 142.8, 134.3, 132.8, 131.2, 129.3, 128.6, 125.0, 120.0, 116.6, 116.2, 110.4, 103.6, 55.9. HRMS (ESI) calcd for $C_{21}H_{17}N_2O_2$ $[M+H]^+$ 329.1285, found 329.1283.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(phenyl)methanone (**11d**): 93 yield%. Light yellow solid, m.p. 106～107 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.23 (s, 1H), 8.43 (d, *J*=5.8 Hz, 1H), 7.69～7.65 (m, 3H), 7.55～7.49 (m, 4H), 7.17 (d, *J*=5.8 Hz, 1H), 6.97 (dd, *J*=9.1, 2.5 Hz, 1H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.1, 157.0, 146.6, 144.4, 142.8, 135.0, 133.5, 132.9, 129.1, 129.1, 124.9, 122.2, 116.9, 116.0, 110.4, 103.2, 55.9. HRMS (ESI) calcd for $C_{19}H_{15}N_2O_2$ $[M+H]^+$ 303.1128, found 303.1128.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(*p*-tolyl)methanone (**11e**): 92 yield%. Light yellow wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.24 (s, 1H), 8.44 (d, *J*=5.7 Hz, 1H), 7.60 (d, *J*=8.1 Hz, 2H), 7.54 (d, *J*=9.1 Hz, 1H), 7.50 (d, *J*=2.5 Hz, 1H), 7.32 (d, *J*=7.9 Hz, 2H), 7.22 (d, *J*=5.8 Hz, 1H), 6.98 (dd, *J*=9.1, 2.6 Hz, 1H), 3.91 (s, 3H), 2.48 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.2, 156.8, 146.5, 144.5, 143.9, 142.8, 133.6, 132.0, 129.8, 129.4, 124.8, 122.1, 116.9, 115.9, 110.4, 103.2, 55.9, 21.9. HRMS (ESI) calcd for $C_{20}H_{17}N_2O_2$ $[M+H]^+$ 317.1285, found 317.1281.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(*o*-tolyl)methanone (**11f**): 88 yield%. Light yellow solid, m.p. 115～116 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.24 (s, 1H), 8.42 (s, 1H), 7.57～7.50 (m, 3H), 7.40～7.34 (m, 3H), 7.00～6.97 (m, 2H), 3.91 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 169.5, 157.2, 147.1, 144.1, 142.7, 135.8, 135.8, 133.1, 131.5, 131.4, 127.4, 126.8, 125.2, 122.6, 117.2, 116.1, 110.4, 103.3, 55.9, 19.1. HRMS (ESI) calcd for $C_{20}H_{17}N_2O_2$ $[M+H]^+$ 317.1285, found 317.1283.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(4-methoxyphenyl)methanone (**11g**): 80 yield%. Light yellow solid, m.p. 121～122 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.25 (s, 1H), 8.45 (s, 1H), 7.68 (d, *J*=8.8 Hz, 2H), 7.57～7.41 (m, 2H), 7.27 (d, *J*=5.7 Hz, 1H), 7.07～6.79 (m, 3H), 3.91 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.5, 163.7, 156.7, 146.3, 144.6, 142.7, 133.7, 131.9, 126.6, 124.6, 116.6, 116.0, 114.3, 113.5, 110.3, 103.1, 55.9, 55.7. HRMS (ESI) calcd for $C_{20}H_{17}N_2O_3$ $[M+H]^+$ 333.1234, found 333.1236.

(4-Fluorophenyl)(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)methanone (**11h**): 77 yield%. Light yellow wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.22 (s, 1H), 8.50～8.32 (m, 1H), 7.80～7.66 (m, 2H), 7.53～7.38 (m, 2H), 7.31～7.12 (m, 3H), 7.02～6.95 (m, 1H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.9, 165.6 (J_{CF}=253.75 Hz), 157.0, 146.7, 144.4, 142.9, 133.4, 131.9 (J_{CF}=11.25 Hz), 131.0 (J_{CF}=3.75 Hz), 124.9, 122.2, 116.7, 116.5 (J_{CF}=22.5 Hz), 116.0, 110.3, 103.3, 55.9. HRMS (ESI) calcd for $C_{19}H_{14}FN_2O_3$ $[M+H]^+$ 321.1034, found 321.1035.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(4-(trifluoromethyl)phenyl)methanone (**11i**): 70 yield%. Light yellow solid, m.p. 160～161 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.25 (s, 1H), 8.48 (d, J=5.7 Hz, 1H), 7.82 (s, 4H), 7.50 (d, J=2.3 Hz, 1H), 7.45 (d, J=9.1 Hz, 1H), 7.21 (d, J=5.7 Hz, 1H), 6.98 (dd, J=9.1, 2.4 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.7, 157.3, 146.9, 144.3, 142.8, 138.3, 134.5 (J_{CF}=33.75 Hz), 133.1, 129.4, 127.9 (J_{CF}=270.0 Hz), 126.3 (J_{CF}=3.75 Hz), 125.1, 122.4, 116.9, 116.1, 110.4, 103.5, 56.0. HRMS (ESI) calcd for $C_{20}H_{14}F_3N_2O_2$ $[M+H]^+$ 371.1002, found 371.1000.

(4-Chlorophenyl)(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)methanone (**11j**): 88 yield%. Light yellow solid, m.p. 132～133 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.26 (s, 1H), 8.48 (d, J=5.6 Hz, 1H), 7.66 (d, J=8.4 Hz, 2H), 7.56～7.42 (m, 4H), 7.27 (s, 1H), 7.01 (dd, J=9.1, 2.6 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.1, 157.2, 146.9, 144.4, 143.0, 139.6, 133.4, 133.3, 130.7, 129.6, 125.0, 122.3, 116.9, 116.1, 110.4, 103.5, 56.0. HRMS (ESI) calcd for $C_{19}H_{14}ClN_2O_2$ $[M+H]^+$ 337.0738, found 337.0735.

(3-Chlorophenyl)(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)methanone (**11k**): 77 yield%. Light yellow wax. ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.22 (s, 1H), 8.35 (d, J=5.9 Hz, 1H), 7.72～7.54 (m, 5H), 7.43 (s, 1H), 7.15 (s, 1H), 6.98 (dd, J=9.2, 2.7 Hz, 1H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 167.5, 158.7, 147.2, 145.1, 143.1, 136.4, 133.7, 133.7, 131.9, 131.4, 129.9, 129.1, 126.1, 124.1, 117.5, 117.4, 111.3, 104.5, 56.3. HRMS (ESI) calcd for $C_{19}H_{14}ClN_2O_2$ $[M+H]^+$ 337.0738, found 337.0737.

(4-Bromophenyl)(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)methanone (**11l**): 73 yield%. Light yellow solid, m.p. 149～150 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.26 (s, 1H), 8.49 (d, J=5.1 Hz, 1H), 7.69 (d, J=8.4 Hz, 2H), 7.59 (d, J=8.4 Hz, 2H), 7.51 (d, J=8.8 Hz, 2H), 7.32～7.17 (m, 1H), 7.00 (dd, J=9.1, 2.6 Hz, 1H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 168.2, 157.1, 146.9, 144.3, 143.0, 133.7, 133.3, 132.6, 130.8, 128.0, 125.0, 122.3, 116.9, 116.1, 110.4, 103.4, 56.0. HRMS (ESI) calcd for $C_{19}H_{14}BrN_2O_2$ $[M+H]^+$ 381.0233, found 381.0230.

(8-Methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl)(4-nitrophenyl)-methanone (**11m**): 79 yield%. Yellow solid, m.p. 165～166 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.27 (s, 1H), 8.50 (d, J=5.6 Hz, 1H), 8.42 (d, J=8.6 Hz, 2H), 7.89 (d, J=8.6 Hz, 2H), 7.53 (d, J=2.4 Hz, 1H), 7.43 (d, J=9.1 Hz, 1H), 7.24 (d, J=5.7 Hz, 1H), 6.99 (dd, J=9.1, 2.6 Hz, 1H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 167.0, 157.5, 150.2, 147.2, 144.1, 143.1, 140.6, 132.9, 130.1, 125.3, 124.5, 122.5, 116.9, 116.2, 110.4, 103.7, 56.0. HRMS (ESI) calcd for $C_{19}H_{14}N_3O_4$ $[M+H]^+$ 348.0979, found 348.0974.

Furan-2-yl(8-methoxy-5*H*-pyrido[4,3-*b*]indol-5-yl) methanone (**11n**): 86 yield%. Light yellow wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 8.56 (d, J=5.9 Hz, 1H), 7.66 (s, 1H), 7.57～7.48 (m, 2H), 7.37 (d, J=3.5 Hz, 1H), 7.34 (d, J=5.9 Hz, 1H), 7.04 (dd, J=9.1, 2.6 Hz, 1H), 6.69 (dd, J=3.6, 1.7 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 157.6, 157.1, 146.9, 146.6, 146.0, 144.4, 142.7, 133.2, 124.3, 122.3, 120.7, 116.7, 116.1, 113.1, 110.0, 103.2, 56.0. HRMS (ESI) calcd for $C_{17}H_{13}N_2O_3$ $[M+H]^+$ 293.0921, found 293.0924.

4.2.4 Syntheses of compounds **12a**～**12k**

To a solution of compound **10** (20 mg, 0.1 mmol) in 3 mL of dry THF was added NaH (20 mg, 60% dispersion in mineral oil, 0.5 mmol), and various benzyl bromides (0.12 mmol) was added dropwise at 0 ℃. The resulting mixture was stirred at room temperature for 1 h, upon completion, the reaction was quenched with 10 mL of H_2O, extracted with DCM (30 mL×3). The organic layers were combined, dried over Na_2SO_4, evaporated to dry to get crude product, which was purified by chromatography (eluted with DCM/MeOH, V∶V=50∶1～30∶1) to get compound **12** in moderate to high yields.

5-Benzyl-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12a**): 73 yield%. Light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.21 (s, 1H), 8.36 (d, J=4.6 Hz, 1H), 7.69 (s, 1H), 7.41 (d, J=5.8 Hz, 1H), 7.36 (d, J=8.9 Hz, 1H), 7.23～7.22 (m, 3H), 7.14 (d, J=8.9 Hz, 1H), 7.09 (d, J=6.9 Hz, 2H), 5.52 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 155.9, 146.1, 143.1, 141.5, 136.5, 136.3, 129.4, 128.4, 127.0, 122.4, 117.3, 111.4, 105.6, 104.3, 100.6, 56.4, 47.3. HRMS (ESI) calcd for $C_{19}H_{17}N_2O$ $[M+H]^+$ 289.1335, found 289.1334.

5-(2-Fluorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12b**): 79 yield%. Light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.37 (s, 1H), 8.55 (s, 1H), 7.65 (s, 1H), 7.34 (t, J=7.9 Hz, 2H), 7.29～7.22 (m, 1H), 7.18～7.07 (m, 2H), 6.95 (t, J=7.8 Hz, 1H), 6.77 (t, J=7.8 Hz, 1H), 5.52 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 160.5 (J_{CF}=245.0 Hz), 155.4, 145.3, 142.5, 135.4, 129.9 (J_{CF}=8.75 Hz), 128.3 (J_{CF}=3.75 Hz), 124.7 (J_{CF}=3.75 Hz), 123.1 (J_{CF}=15.0 Hz), 122.2, 116.8, 115.8 (J_{CF}=21.25 Hz), 110.5, 104.8, 103.7, 56.2, 40.8 (J_{CF}=5.0 Hz). HRMS (ESI) calcd for $C_{19}H_{16}FN_2O$ $[M+H]^+$ 307.1241, found 307.1244.

5-(3-Chlorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12c**): 81 yield%. Light yellow wax; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.33 (s, 1H), 8.50 (d, J=5.7 Hz, 1H), 7.67 (d, J=2.5 Hz, 1H), 7.45 (dd, J=8.0, 1.2 Hz, 1H), 7.25～7.18 (m, 3H), 7.13～7.09 (m, 1H), 7.01 (td, J=7.6, 1.2 Hz, 1H), 6.46 (dd, J=7.8, 1.5 Hz, 1H), 5.55 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.2, 145.3, 145.1, 142.9, 135.4, 133.6, 132.6, 129.9, 129.1, 127.4, 127.2, 122.3, 120.0, 116.4, 110.4, 104.5, 103.8, 56.2, 44.5. HRMS (ESI) calcd for $C_{19}H_{16}ClN_2O$ $[M+H]^+$ 323.0946, found 323.0946.

5-(4-Chlorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12d**): 90 yield%. Light yellow wax; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 8.51 (d, J=5.0 Hz, 1H), 7.64 (s, 1H), 7.25～7.23 (m, 4H), 7.13 (d, J=8.8 Hz, 1H), 7.02 (d, J=7.8 Hz, 2H), 5.44 (s, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.4, 145.3, 144.2, 142.3, 135.3, 134.5,

133.9, 129.3, 127.8, 122.2, 120.0, 116.8, 110.5, 104.6, 103.8, 56.2, 46.4. HRMS (ESI) calcd for $C_{19}H_{16}ClN_2O$ $[M+H]^+$ 323.0946, found 323.0949.

5-(2-Chloro-4-fluorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12e**): 68 yield%. Light yellow wax. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.32 (s, 1H), 8.50 (d, J=5.6 Hz, 1H), 7.66 (d, J=2.3 Hz, 1H), 7.24～7.17 (m, 3H), 7.12 (dd, J=8.9, 2.4 Hz, 1H), 6.73 (td, J=8.3, 2.6 Hz, 1H), 6.44 (dd, J=8.7, 5.9 Hz, 1H), 5.49 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 162.0 (J_{CF}=248.75 Hz), 155.3, 145.2, 145.2, 143.0, 135.2, 133.1 (J_{CF}=10.0 Hz), 129.5 (J_{CF}=3.75 Hz), 128.4 (J_{CF}=8.75 Hz), 122.3, 120.0, 117.4 (J_{CF}=25.0 Hz), 116.4, 114.7 (J_{CF}=21.25 Hz), 110.2, 104.4, 103.9, 56.2, 44.0. HRMS (ESI) calcd for $C_{19}H_{15}ClFN_2O$ $[M+H]^+$ 341.0851, found 341.0847.

5-(2-Bromobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12f**): 67 yield%. Light yellow solid, m.p. 130～131 ℃; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.33 (s, 1H), 8.50 (s, 1H), 7.67 (d, J=2.3 Hz, 1H), 7.64 (d, J=8.0 Hz, 1H), 7.22 (d, J=8.9 Hz, 1H), 7.19 (d, J=5.7 Hz, 1H), 7.15～7.09 (m, 2H), 7.04 (t, J=7.5 Hz, 1H), 6.40 (d, J=7.6 Hz, 1H), 5.50 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.2, 145.2, 145.2, 143.0, 135.3, 135.1, 133.1, 129.4, 128.0, 127.2, 122.3, 122.3, 116.3, 110.4, 104.5, 103.8, 56.2, 47.1. HRMS (ESI) calcd for $C_{19}H_{16}BrN_2O$ $[M+H]^+$ 367.0441, found 367.0444.

5-(3-Bromobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**12g**): 73 yield%. Light yellow solid, m.p. 125～126 ℃; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.34 (s, 1H), 8.53 (s, 1H), 7.63 (s, 1H), 7.38 (d, J=7.8 Hz, 1H), 7.30～7.20 (m, 3H), 7.16～7.08 (m, 2H), 6.96 (d, J=7.5 Hz, 1H), 5.42 (s, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.4, 145.2, 144.6, 142.6, 138.4, 135.3, 131.3, 130.7, 129.5, 125.0, 123.2, 122.2, 120.1, 116.8, 110.4, 104.6, 103.8, 56.2, 46.4. HRMS (ESI) calcd for $C_{19}H_{16}BrN_2O$ $[M+H]^+$ 367.0441, found 367.0440.

8-Methoxy-5-(4-(trifluoromethoxy)benzyl)-5*H*-pyrido-[4,3-*b*]indole (**12h**): 65 yield%. Light yellow wax. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.33 (s, 1H), 8.51 (s, 1H), 7.64 (s, 1H), 7.24 (t, J=8.2 Hz, 2H), 7.13～7.11 (m, 5H), 5.46 (s, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.3, 148.9, 145.2, 144.7, 142.7, 135.2, 134.9, 127.9, 122.2, 121.6, 120.5 (J_{CF}=256.25 Hz), 120.0, 116.5, 110.3, 104.4, 103.8, 56.2, 46.2. HRMS (ESI) calcd for $C_{20}H_{16}F_3N_2O_2$ $[M+H]^+$ 373.1158, found 373.1155.

8-Methoxy-5-(4-methoxybenzyl)-5*H*-pyrido[4,3-*b*]-indole (**12i**): 89 yield%. Light yellow wax. ^1H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.23 (s, 1H), 8.38 (s, 1H), 7.66 (s, 1H), 7.42 (d, J=6.1 Hz, 2H), 7.37 (d, J=8.9 Hz, 1H), 7.14 (d, J=8.7 Hz, 1H), 7.04 (d, J=8.2 Hz, 2H), 6.77 (d, J=8.2 Hz, 1H), 5.44 (s, 2H), 3.90 (s, 3H), 3.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 159.8, 155.9, 146.0, 142.7, 141.2, 136.3, 128.4, 128.4, 122.3, 117.5, 114.8, 111.5, 105.8, 104.2, 56.4, 55.5, 46.9. HRMS (ESI) calcd for $C_{20}H_{19}N_2O_2$ $[M+H]^+$ 319.1441, found 319.1442.

8-Methoxy-5-(prop-2-yn-1-yl)-5*H*-pyrido[4,3-*b*]indole (**12j**): 63 yield%. Light yellow oil. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.27 (s, 1H), 8.56 (d, J=5.8 Hz, 1H), 7.63 (s, 1H), 7.45 (d, J=8.9 Hz, 1H), 7.36 (dd, J=5.8, 1.0 Hz, 1H), 7.19 (dd, J=8.9, 2.5 Hz, 1H), 5.00 (d, J=2.6 Hz, 2H), 3.94 (s, 3H), 2.31 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.3, 145.1, 144.5, 143.0, 134.6, 122.4, 120.1, 116.2, 110.2, 104.4, 103.8, 73.2, 77.0, 56.2, 32.7. HRMS (ESI) calcd for $C_{15}H_{13}N_2O$ $[M+H]^+$ 237.1022, found 237.1019.

8-Methoxy-5-(3-methylbut-2-en-1-yl)-5*H*-pyrido[4,3-*b*]indole (**12k**): 66 yield%. Light yellow oil. ^1H NMR (500 MHz, $CDCl_3$) δ: 9.29 (s, 1H), 7.82 (dd, J=6.9, 1.5 Hz, 1H), 7.72～7.41 (m, 3H), 7.03 (dd, J=8.9, 2.5 Hz, 1H), 5.53～5.36 (m, 1H), 5.19 (d, J=7.5 Hz, 2H), 3.84 (s, 3H), 1.88 (d, J=1.4 Hz, 3H), 1.79 (d, J=1.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 155.9, 144.9, 142.7, 136.5, 136.1, 135.1, 120.6, 120.2, 119.1, 117.2, 114.3, 108.9, 103.5, 100.1, 57.9, 56.1, 26.0, 18.7. HRMS (ESI) calcd for $C_{20}H_{19}N_2O_2$ $[M+H]^+$ 267.1492, found 267.1490.

4.2.5 Syntheses of compounds **13a**～**13t**

To a solution of compound **10** (20 mg, 0.1 mmol) in 3 mL of dry CH_3CN was added different haloalkanes R—X (0.3 mmol) at room temperature. The resulting mixture was stirred at 70 ℃ for 6 h, upon completion, the reaction was evaporated to dry to get crude product, which was purified by chromatography (eluted with DCM/MeOH, V∶V=50∶1～10∶1) to get compounds **13** in moderate to high yields.

2-Benzyl-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13a**): 86 yield%. White solid, m.p. 133～134 ℃; ^1H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.77 (s, 1H), 8.58～8.44 (m, 1H), 7.91～7.77 (m, 2H), 7.60～7.46 (m, 3H), 7.43～7.34 (m, 3H), 7.18 (dd, J=8.9, 2.4 Hz, 1H), 5.78 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.0, 146.1, 138.0, 137.5, 136.8, 134.9, 130.1, 130.0, 129.0, 122.0, 121.7, 120.3, 114.3, 109.5, 104.5, 63.7, 56.3. HRMS (ESI) calcd for $C_{19}H_{17}N_2O$ $[M-Br]^+$ 289.1335, found 289.1331.

2-(2-Fluorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13b**): 90 yield%. White solid, m.p. 129～130 ℃; ^1H NMR (500 MHz, $CDCl_3$) δ: 12.96 (s, 1H), 9.64 (s, 1H), 8.13 (d, J=7.0 Hz, 1H), 7.86 (d, J=7.5 Hz, 1H), 7.64 (d, J=6.9 Hz, 1H), 7.55 (d, J=8.9 Hz, 1H), 7.52 (d, J=2.2 Hz, 1H), 7.32 (q, J=6.9 Hz, 1H), 7.16 (t, J=7.5 Hz, 1H), 7.06～6.95 (m, 2H), 5.94 (s, 2H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.1 (J_{CF}=247.5 Hz), 156.1, 144.8, 137.2, 136.0, 135.8, 132.1 (J_{CF}=7.5 Hz), 131.9 (J_{CF}=2.5 Hz), 125.4 (J_{CF}=3.75 Hz), 121.4 (J_{CF}=15.0 Hz), 120.5 (J_{CF}=21.25 Hz), 119.4, 116.1 (J_{CF}=21.25 Hz), 114.2, 108.6, 103.8, 57.6, 56.1. HRMS (ESI) calcd for $C_{19}H_{16}FN_2O$ $[M-Br]^+$ 307.1241, found 307.1237.

2-(3-Fluorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13c**): 87 yield%. White solid, m.p. 130～131 ℃; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.84 (d, J=1.6 Hz, 1H), 8.59 (dd, J=7.1, 1.5 Hz, 1H), 7.89～7.79 (m, 2H), 7.55 (d, J=8.9 Hz, 1H), 7.44～7.37 (m, 1H), 7.33 (d, J=

7.8 Hz, 1H), 7.28 (d, J=9.3 Hz, 1H), 7.22 (dd, J=8.9, 2.4 Hz, 1H), 7.12～7.05 (m, 1H), 5.83 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 163.9 (J_{CF}=245.0 Hz), 157.4, 146.4, 138.5, 138.0, 137.8 (J_{CF}=7.5 Hz), 137.1, 132.0 (J_{CF}=8.75 Hz), 125.0 (J_{CF}=2.5 Hz), 122.3, 122.1, 120.5, 117.1 (J_{CF}=21.25 Hz), 116.2 (J_{CF}=22.5 Hz), 114.8, 109.7, 104.7, 63.0, 56.4. HRMS (ESI) calcd for $C_{19}H_{16}FN_2O$ [M−Br]$^+$ 307.1241, found 307.1245.

2-(2-Chlorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13d**): 75 yield%. White solid, m.p. 141～142 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD, one proton of NH couldn't be detected due to its exchange with the solvent) δ: 9.28 (s, 1H), 8.07 (d, J=7.1 Hz, 1H), 7.53～7.38 (m, 2H), 7.19 (d, J=9.0 Hz, 2H), 7.12～7.06 (m, 1H), 7.06～7.00 (m, 2H), 6.87 (dd, J=8.9, 2.5 Hz, 1H), 5.52 (s, 2H), 3.53 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 156.1, 145.2, 137.2, 136.7, 135.8, 133.8, 131.2, 131.1, 130.9, 129.9, 127.5, 120.8, 120.7, 119.2, 113.2, 108.2, 103.3, 60.2, 55.1. HRMS (ESI) calcd for $C_{19}H_{16}ClN_2O$ [M−Br]$^+$ 323.0946, found 323.0949.

2-(4-Chlorobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13e**): 85 yield%. Light yellow solid, m.p. 145～146 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.80 (s, 1H), 8.49 (d, J=6.2 Hz, 1H), 7.79 (d, J=8.5 Hz, 2H), 7.57～7.42 (m, 3H), 7.33 (d, J=7.6 Hz, 2H), 7.18 (d, J=8.1 Hz, 1H), 5.78 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 156.4, 145.4, 137.3, 136.7, 136.1, 135.5, 132.9, 130.0, 129.5, 121.4, 121.0, 119.7, 113.7, 108.9, 103.9, 62.2, 55.8. HRMS (ESI) calcd for $C_{19}H_{16}ClN_2O$ [M−Br]$^+$ 323.0946, found 323.0949.

2-(2-Bromobenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13f**): 90 yield%. White solid, m.p. 136～137 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 13.64 (s, 1H), 9.29 (s, 1H), 7.92 (d, J=6.9 Hz, 1H), 7.86 (d, J=7.5 Hz, 1H), 7.64 (d, J=8.9 Hz, 1H), 7.57 (d, J=8.0 Hz, 1H), 7.51 (d, J=7.0 Hz, 1H), 7.47～7.38 (m, 2H), 7.27 (t, J=10.3 Hz, 1H), 7.11 (dd, J=8.9, 2.3 Hz, 1H), 5.99 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 156.2, 145.0, 136.9, 136.3, 135.9, 133.8, 133.2, 132.9, 131.7, 128.9, 124.8, 120.7, 120.2, 119.4, 114.5, 108.5, 103.5, 63.1, 56.1. HRMS (ESI) calcd for $C_{19}H_{16}BrN_2O$ [M−Br]$^+$ 367.0441, found 367.0444.

2-(4-(Trifluoromethyl)-8-methoxy-5*H*-pyrido[4,3-*b*]-indol-2-ium bromide (**13g**): 89 yield%. Light yellow solid, m.p. 146～147 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.83 (d, J=1.5 Hz, 1H), 8.55 (dd, J=7.1, 1.5 Hz, 1H), 7.89～7.74 (m, 2H), 7.61 (d, J=8.8 Hz, 2H), 7.54 (d, J=8.8 Hz, 1H), 7.32～7.13 (m, 3H), 5.83 (s, 2H), 3.91 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 156.4, 149.8, 145.5, 137.4, 136.9, 136.2, 133.2, 130.3, 121.7, 121.3 (J_{CF}=43.75 Hz), 120.3 (J_{CF}=256.25 Hz), 119.3, 113.7, 109.0, 103.9, 62.0, 55.7. HRMS (ESI) calcd for $C_{20}H_{16}F_3N_2O$ [M−Br]$^+$ 357.1209, found 357.1203.

2-(2-Methylbenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13h**): 86 yield%. White solid, m.p. 124～125 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.41 (s, 1H), 7.97 (d, J=6.9 Hz, 1H), 7.65 (d, J=7.0 Hz, 1H), 7.60 (d, J=8.9 Hz, 1H), 7.50 (d, J=2.2 Hz, 1H), 7.27～7.21 (m, 1H), 7.20～7.12 (m, 3H), 7.06 (dd, J=8.9, 2.3 Hz, 1H), 5.88 (s, 2H), 3.81 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 156.1, 145.1, 137.1, 136.9, 136.2, 135.9, 132.0, 131.4, 129.8, 129.7, 127.1, 120.6, 120.4, 119.4, 114.4, 108.8, 103.7, 61.6, 56.1, 19.5. HRMS (ESI) calcd for $C_{20}H_{19}N_2O$ [M−Br]$^+$ 303.1492, found 303.1496.

2-(3-Methylbenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13i**): 90 yield%. Light yellow solid, m.p. 132～133 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.54 (s, 1H), 8.07 (d, J=7.0 Hz, 1H), 7.58～7.47 (m, 2H), 7.23～7.14 (m, 3H), 7.09 (d, J=6.7 Hz, 1H), 7.01 (dd, J=8.9, 2.3 Hz, 1H), 5.82 (s, 2H), 3.80 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 156.0, 145.0, 139.5, 137.0, 136.1, 135.9, 134.0, 130.3, 129.4, 129.2, 125.7, 120.6, 120.5, 119.3, 114.3, 108.8, 103.8, 77.4, 77.2, 76.9, 63.4, 56.1, 21.4. HRMS (ESI) calcd for $C_{20}H_{19}N_2O$ [M − Br]$^+$ 303.1492, found 303.1490.

8-Methoxy-2-(4-methylbenzyl)-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13j**): 77 yield%. Light yellow solid, m.p. 127～128 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.69 (s, 1H), 8.59～8.35 (m, 1H), 7.89～7.70 (m, 2H), 7.52 (d, J=8.9 Hz, 1H), 7.41～7.31 (m, 2H), 7.24～7.10 (m, 3H), 5.71 (s, 2H), 3.90 (s, 3H), 2.29 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 156.3, 145.4, 139.6, 137.1, 136.7, 136.1, 131.3, 130.0, 128.4, 121.3, 121.0, 119.6, 113.7, 108.8, 103.8, 63.0, 55.7. HRMS (ESI) calcd for $C_{20}H_{19}N_2O$ [M−Br]$^+$ 303.1492, found 303.1493.

2-(4-Methoxybenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]-indol-2-ium choloride (**13k**): 90 yield%. Light yellow solid, m.p. 120～121 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.70 (s, 1H), 8.56 (d, J=7.1 Hz, 1H), 7.92～7.79 (m, 2H), 7.61 (d, J=8.9 Hz, 1H), 7.46 (d, J=8.2 Hz, 2H), 7.30 (dd, J=8.9, 2.5 Hz, 1H), 6.97 (d, J=8.3 Hz, 2H), 5.71 (s, 2H), 3.94 (s, 3H), 3.79 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 159.8, 155.9, 146.0, 142.7, 141.2, 136.3, 128.4, 128.4, 122.3, 117.5, 114.8, 111.5, 105.8, 105.8, 104.2, 56.4, 55.5, 46.9. HRMS (ESI) calcd for $C_{20}H_{19}N_2O_2$ [M − Cl]$^+$ 319.1441, found 319.1441.

2-(3,5-Dimethoxybenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13l**): 89 yield%. Light yellow solid, m.p. 138～139 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.64 (s, 1H), 8.48 (d, J=6.9 Hz, 1H), 7.83 (d, J=7.0 Hz, 1H), 7.78 (s, 1H), 7.57 (d, J=8.9 Hz, 1H), 7.25 (d, J=11.3 Hz, 1H), 6.57 (s, 2H), 6.44 (s, 1H), 5.67 (s, 2H), 3.92 (s, 3H), 3.76 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 162.2, 157.0, 146.2, 137.8, 137.5, 136.9, 136.8, 122.0, 121.7, 120.3, 114.4, 109.4, 107.1, 104.4, 101.2, 63.7, 56.3, 55.8. HRMS (ESI) calcd for $C_{21}H_{21}N_2O_3$ [M−Br]$^+$ 349.1547, found 349.1549.

2-(4-Hydroxybenzyl)-8-methoxy-5*H*-pyrido[4,3-*b*]-indol-2-ium bromide (**13m**): 80 yield%. Light yellow solid, m.p. 174～175 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.56 (s, 1H), 8.45 (d, J=6.3 Hz, 1H), 7.81 (d, J=6.3 Hz, 1H), 7.78 (s, 1H), 7.58 (d, J=8.9 Hz, 1H), 7.31 (d, J=7.9 Hz, 2H), 7.27 (d, J=8.9 Hz, 1H), 6.85 (d, J=7.7 Hz,

2H), 5.64 (s, 2H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 159.1, 157.2, 146.3, 137.4, 137.3, 137.0, 130.8, 125.2, 122.1, 121.8, 120.3, 116.9, 114.4, 109.5, 104.5, 63.8, 56.4. HRMS (ESI) calcd for $C_{19}H_{17}N_2O_2$ $[M-Br]^+$ 305.1285, found 305.1287.

8-Methoxy-2-methyl-5*H*-pyrido[4,3-*b*]indol-2-iumio-Dide (**13n**): 80 yield%. Light yellow solid, m.p. 251～252 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.62 (d, J=1.5 Hz, 1H), 8.41 (dd, J=7.0, 1.5 Hz, 1H), 7.91～7.73 (m, 2H), 7.56 (d, J=8.9 Hz, 1H), 7.25 (dd, J=8.9, 2.5 Hz, 1H), 4.41 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.3, 146.1, 139.0, 138.6, 136.9, 122.0, 121.9, 120.3, 114.4, 109.3, 104.7, 56.5, 47.4. HRMS (ESI) calcd for $C_{13}H_{13}N_2O$ $[M-I]^+$ 213.1022, found 213.1024.

2-Ethyl-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13o**): 70 yield%. Light yellow solid, m.p. 253～254 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.62 (d, J=1.5 Hz, 1H), 8.41 (dd, J=7.0, 1.5 Hz, 1H), 7.88～7.75 (m, 2H), 7.56 (d, J=8.9 Hz, 1H), 7.25 (dd, J=8.9, 2.5 Hz, 1H), 4.41 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.3, 146.4, 137.9, 137.6, 137.1, 122.2, 122.0, 120.3, 114.4, 109.6, 104.6, 56.4, 56.4, 17.3. HRMS (ESI) calcd for $C_{14}H_{15}N_2O$ $[M-Br]^+$ 227.1179, found 227.1176.

2-Butyl-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13p**): 88 yield%. Light yellow solid, m.p. 259～260 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.66 (d, J=1.5 Hz, 1H), 8.47 (dd, J=7.0, 1.5 Hz, 1H), 7.84 (dd, J=4.8, 2.2 Hz, 2H), 7.58 (d, J=8.9 Hz, 1H), 7.25 (dd, J=8.9, 2.5 Hz, 1H), 4.61 (t, J=7.4 Hz, 2H), 3.92 (s, 3H), 2.11～1.95 (m, 2H), 1.45～1.37 (m, 2H), 0.99 (t, J=7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.2, 146.3, 138.1, 137.8, 137.0, 122.1, 121.9, 120.3, 114.4, 109.5, 104.7, 60.9, 56.4, 34.4, 20.1, 13.8.. HRMS (ESI) calcd for $C_{16}H_{19}N_2O$ $[M-Br]^+$ 255.1492, found 255.1490.

8-Methoxy-2-pentyl-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13q**): 88 yield%. Light yellow solid, m.p. 244～245 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.66 (s, 1H), 8.47 (dd, J=7.0, 1.4 Hz, 1H), 7.93～7.79 (m, 2H), 7.58 (d, J=8.9 Hz, 1H), 7.25 (dd, J=8.9, 2.4 Hz, 1H), 4.60 (t, J=7.4 Hz, 2H), 3.93 (s, 3H), 2.19～1.96 (m, 2H), 1.56～1.28 (m, 4H), 0.90 (t, J=6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.3, 146.3, 138.1, 137.7, 137.0, 122.1, 121.9, 120.3, 114.4, 109.5, 104.7, 61.1, 56.4, 32.1, 28.9, 22.8, 14.1. HRMS (ESI) calcd for $C_{17}H_{21}N_2O$ $[M-Br]^+$ 269.1648, found 269.1647.

2-Dodecyl-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13r**): 91 yield%. Light yellow solid, m.p. 267～268 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.39 (s, 1H), 7.86 (d, J=6.9 Hz, 1H), 7.67 (d, J=6.9 Hz, 1H), 7.63～7.54 (m, 2H), 7.08 (dd, J=8.9, 2.4 Hz, 1H), 4.57 (t, J=7.4 Hz, 2H), 3.86 (s, 3H), 1.90 (q, J=7.3 Hz, 2H), 1.41～1.06 (m, 20H), 0.83 (t, J=6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 156.1, 144.9, 136.8, 136.2, 135.5, 120.6, 120.4, 119.3, 114.4, 109.0, 103.8, 60.8, 56.2, 32.0, 29.6, 29.5, 29.4, 29.4, 29.1, 26.3, 22.7, 14.2. HRMS (ESI) calcd for $C_{24}H_{35}N_2O$ $[M-Br]^+$ 367.2744, found 367.2745.

2-Allyl-8-methoxy-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13s**): 69 yield%. Light yellow solid, m.p. 257～258 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.60 (d, J=1.4 Hz, 1H), 8.43 (dd, J=7.0, 1.5 Hz, 1H), 7.87～7.84 (m, 1H), 7.81 (d, J=2.4 Hz, 1H), 7.60～7.55 (m, 1H), 7.24 (dd, J=8.9, 2.5 Hz, 1H), 6.26～6.11 (m, 1H), 5.53～5.40 (m, 2H), 5.24 (dt, J=6.3, 1.4 Hz, 2H), 3.92 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 156.4, 145.6, 137.3, 136.9, 136.2, 131.4, 121.4, 121.3, 121.1, 119.6, 113.7, 108.7, 103.7, 61.9, 55.6. HRMS (ESI) calcd for $C_{15}H_{15}N_2O$ $[M-Br]^+$ 239.1179, found 239.1176.

8-Methoxy-2-(prop-2-yn-1-yl)-5*H*-pyrido[4,3-*b*]indol-2-ium bromide (**13t**): 94 yield%. Light yellow solid, m.p. 253～254 ℃; ^{1}H NMR (500 MHz, $CDCl_3$ & CD_3OD) δ: 9.61 (s, 1H), 8.52 (dt, J=7.0, 1.6 Hz, 1H), 7.81 (dd, J=7.1, 1.4 Hz, 1H), 7.74 (d, J=2.2 Hz, 1H), 7.52 (dd, J=9.0, 1.4 Hz, 1H), 7.28～7.13 (m, 1H), 5.46 (t, J=2.0 Hz, 3H), 3.85 (d, J=1.5 Hz, 3H), 3.18 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$ & CD_3OD) δ: 157.4, 146.6, 137.8, 137.4, 137.1, 122.1, 122.0, 120.5, 114.6, 109.6, 104.6, 102.3, 79.8, 75.8, 56.4. HRMS (ESI) calcd for $C_{15}H_{13}N_2O$ $[M-Br]^+$ 237.1022, found 237.1024.

4.3 Antibacterial assay

4.3.1 Minimum inhibitory concentrations (MICs) test

MICs were determined by the procedures reported by the National Committee for Clinical Laboratory Standards. The concentrations of each bacterium were adjusted to 1×10^5 CFU/mL. All the tested compounds were dissolved in DMSO and the reference drug was dissolved in H_2O and diluted with the Luria-Bertani liquid medium (LB) to prepare the stock solution. The final required concentrations were 128, 64, 32, 16, 8, 4, 2 and 1 μg/mL, respectively, and the final content of DMSO was less than 0.5%. The MIC was defined as the minimum inhibitory concentration, each compound resulting in visible inhibition on bacteria growth (incubation at 37 ℃, 140 r/min, for 24 h).

4.3.2 Fluorescence microscopy assay

The dyes, PI and Hoechst, were used to determine the ability of compound **12h** to compromise the membranes of *P. aeruginosa*. The bacteria which incubated to the mid-logarithmic phase was diluted with LB to a concentration of 1×10^5 CFU/mL. 3 mL of bacteria suspension was collected by a 5 mL centrifugal tube, and then incubated in the presence of compound (0.0107 μmol/mL, 2×MIC) at 37 ℃ for 1 h. After incubation, the cells were concentrated for 15 min at 4000×*g* to remove the cultural supernatant and washed with 3 mL of phosphate-buffered saline (PBS) for three times. The collected cells were re-suspended with 3 mL of LB and incubated with PI and Hoechst (5 μg/mL) for 20 min in dark on ice, and then the mixture was concentrated, washed with LB for three times, and re-suspended with 1 mL of PBS. The suspension was applied on chamber slides and observed under LECIA DM6 B microscope using 100× oil-immersion objective.

Supporting Information ^{1}H NMR and ^{13}C NMR spectra of all compounds and X-ray diffraction data for **13k**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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