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Syntheses and structure-activity relationships in antibacterial activity against *Clostridium difficile* and XBP1 activation property of 13-(*N*-alkyl)-aminomethyl-8-oxodihydrocoptisines

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13-(*N*-alkyl)-aminomethyl-8-oxo-dihydrocotisines were synthesized to evaluate antibacterial activity against *Clostridium difficile* and activating x-box-binding protein 1 activity, biological properties both associated with ulcerative colitis. Improving structural stability and ameliorating biological activity were major concerns. Different substituents on the structural modification site were involved to explore the influence of diverse structures on the bioactivities. The target compounds exhibited the desired activities with definite structure-activity relationship. In the 13-(*N*-*n*-alkyl)-aminomethyl-8-oxo-dihydrocotisines series, the length of *n*-alkyls has a definite effect on the bioactivity, elongation of the length increasing the antibacterial activity. The synthesized compounds were determined to display strong or weak XBP1-activating activity in vitro. The preliminary results of this study warrant further medicinal chemistry studies on these synthesized compounds.

Keywords: Quaternary coptisine chloride, Structure modification, Antibacteria, XBP1-activating, Structure-activity relationships

Introduction

Quaternary coptisine chloride (QCC), which has the structure of 6,7-dihydro-[1,3]dioxolo[4',5':7,8]isoquinolino[3,2-*a*][1,3]dioxolo[4,5-*g*]isoquinolin-5-ium chloride (**1**, Figure 1), was a kind of natural quaternary protoberberine alkaloids (QPAs). QCC and one of its reduction products, tertiary tetrahydrocoptisine, exist in many well-known medicinal plants in the natural world, such as the former in *Coptis chinensis* Franch of Ranunculaceae and the latter in *Corydalis yanhusuo* W. T. Wang ex Z. Y. Su et C. Y. Wu of Papaveraceae.[1] The natural resource of QCC is very rich. The synthetic preparation of QCC has been developed.[2,3] In biology, QCC was found to have a wide range of pharmacological activities, such as activities of antibacteria,[4,5] anti-tumors,[6,7] anti-viruses,[8] antidiabetes,[9] myocardial protection,[10,11] gastric mucosa protection,[12] type-A monoamine oxidase inhibition,[13] and anti-fungi,[14] and the like. But, according to structural analysis and pharmacokinetic studies, including those conducted by our group, it is known that the bioavailability of QCC is very limited, which has drastically limited its drug research.[15] In recent years, it was found in our laboratory that another reduction product of QCC, tertiary dihydrocoptisine (DHC), can ameliorate intestinal mucosal barrier damage and intestinal inflammation in animal model of ulcerative colitis (UC) by activating a key downstream transcription factor (TF) related to uncontrollable endoplasmic reticulum stress in intestinal epithelial cells (IECs), x-box-binding protein 1 (XBP1).[16-18] Nevertheless, the chemical instability of DHC, which is easy to aromatize, hinders the study of its drug-forming properties. In order to improve the physical and chemical properties and pharmacological activities of QCC and DHC as lead structures, our continuing study designed and synthesized 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs of structure stability with QCC as substrate. In addition, it is noticed in the process of our study that microbe *Clostridium difficile* infection (CDI) is very common in inflammatory bowel disease (IBD) patients, with a higher incidence of CDI in IBD patients being demonstrated compared to non-IBD patients.[19] *C. difficile* is a gram-positive spore-forming anaerobic pathogenic organism associated with the development of a spectrum of clinical illnesses.[19,20] In particular, CDI is more common in UC than in Crohn's disease (CD). Thus, UC is an independent risk factor for CDI.[19-21] In view of the association between UC and CDI, the antibacterial activity and XBP1 activation property were screened in the pharmacological studies of this work to evaluate the biological importance of the synthesized compounds for UC therapeutic study and to analyze the structure-activity relationship (SAR). This article reports on the design and syntheses of the target compounds, the evaluation on the in vitro activities of antibacterium against *C. difficile* and XBP1 activation, and SAR analysis.

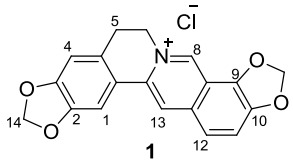

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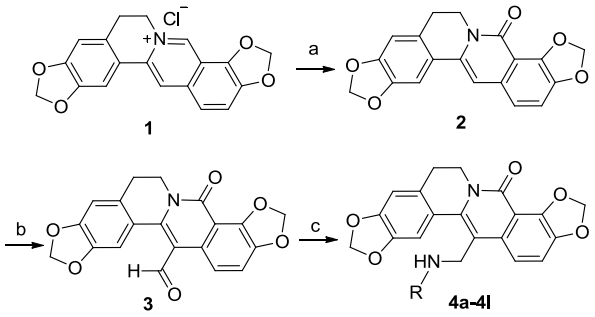
Figure 1. Structure of QCC (**1**).

Results and Discussion

Synthesis

In previous studies, we performed the oxidation of QCC using potassium ferricyanide solution in alkaline condition. The reaction conditions were optimized and 8-oxo-DHC (**2**) was obtained.[16] The *N*-7 tertiary amine structure of **2** possesses obvious electron-donating effect to C-13 double bond carbon to make it easy to cause electrophilic reactions. Thus, based on the synthesis of **2**, the current study applied the Vilsmeier-Haack reaction to introduce a formyl group at C(13) of **2** under the condition of DMF as a formylation reagent and $POCl_3$ as the catalyst. The key intermediate 13-formyl-8-oxo-DHC (**3**) was successfully obtained in yield of 53.0%. Then, compound **3** was reacted with amines under reductive amination of $NaBH_4$ as reducing agent and a mixed solvent of CH_2Cl_2/MeOH (v/v = 1:1) as reaction solvent. The target compounds 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs (**4a-l**) were afforded in yields of 49.7%~85.8% (Scheme 1 and Table 1). The structures of all the synthesized compounds were established by the NMR and HRESI-MS methods, and others. In the ^{1}H NMR spectra of all the synthesized compounds, the 13-aminomethyl-8-oxo-DHC core was identified by the specific resonance peaks. The resonance peaks of the two relatively isolated aromatic protons of H(1) and H(4) of the 13-aminomethyl-8-oxo-DHC core are shown both as single peak of one proton; the two vicinal aromatic protons of H(11) and H(12) as either an overlapped broad peak of two protons together or two doublets of one proton each with coupling constant of around 8.0~8.8 Hz; the two vicinal aliphatic methylenes of CH_2(5) and CH_2(6) both as a non-resolved broad signal of two protons; and the two methylenedioxys both as a single peak of two protons. The *N*-substituted aminomethyls at C(13) of the 8-oxo-DHC core were mainly showed as relatively flat peaks due to the influence of nitrogen atom of electric quadrupole moment with very uneven substitutions. Several compounds are not easy to identify the *N*-substituted aminomethyls due to the overlapping of signals, but according to the integral values, the structures can be determined explicitly. The compounds **4i** of 13-(*N-cyclo*-propyl)-aminomethyl-8-oxo-DHC and **4l** of 13-(*N*-homopiperonyl)-aminomethyl-8-oxo-DHC are somewhat out of the common, with the former showing a very broad singlet and the latter nearly no signal for the *N*-substituted aminomethyls. The ^{13}C NMR spectrum of compound **4l** displayed all the twenty-nine carbon signals with exactly the right type, confirming the structure definitely. On top of the above described signature signals of the 13-(*N*-alkyl)-aminomethyl-8-oxo-DHC core, all other signals in the ^{1}H NMR spectra, as well as the data of the ^{13}C NMR, IR, and positive HRESIMS, were consistent with the substituents substituted on the nitrogen atom of C(13) aminomethyl, confirming the above right analyses for the structures of all the synthesized compounds (See Experimental section and Figures S1-52).



Scheme 1. Reagents and conditions: (a) $K_3[Fe(CN)_6]$, 5N NaOH, reflux; (b) DMF, $POCl_3$, Ar, 110°C; (c) RNH_2, $NaBH_4$, CH_2Cl_2/MeOH (v/v = 1:1), reflux.

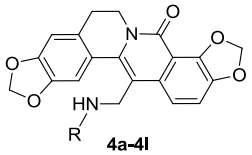
Biological activities

One thing that has been learned from literatures, including the findings by our group, in the field of medicinal chemistry about QPA is that QCC and some other QPAs have a certain antibacterial activities, but the antibacterial activities are generally very weak.[4,5] In addition, because of the quaternary ammonium structure, the bioavailability of QCC is very limited, which seriously limits the drug development and research.[15] There is also the problem of structural instability for DHC. In order to solve these problems, the structural modification of the current study aimed to improve the liposolubility of the quaternary ammonium salt structure and the chemical stability of the DHC structure, with the 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs being designed and synthesized successfully.

Antibacterial activity of 13-formyl-8-oxo-DHC (**3**) and all the synthesized 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs (**4a-l**) against gram-positive anaerobic bacillus *C. difficile* was examined with determining the minimal inhibitory concentration (MIC) of these compounds using the twofold

microdilution broth method. Levofloxacin, a widely used synthetic antibacterial agent in clinic, and QCC, were used as positive controls and reference compounds. In order to explore the antibacterial activities against other microorganisms not associated with UC to determine the specificity of the active compounds, antibacterial activities against gram-positive bacterium *Staphylococcus aureus and* gram-negative bacteria *Escherichia coli* and *Salmonella enterica* were also explored in the pharmacological experiments. Experimental results showed that all the tested synthesized compounds exhibited nearly no antimicrobial activities against *S. aureus, E. coli,* and *S. enterica*. The MIC values were equal to or greater than 250 μg/mL. But, the 13-(*N*-*n*-alkyl)-aminomethyl-8-oxo-DHCs showed definite antimicrobial activity against *C. difficile*, albeit some being poor and some significant. In particular, the MIC values of antibacterial activity of compounds **4g** and **4h** bearing the *n*-nonyl and *n*-decyl, respectively, as the *N*-*n*-alkyl substituents of the C-13 aminomethyl were both 7.8 μg/mL, equivalent to that of levofloxacin and sixteen times that of QCC against *C. difficile.* When the *N*-*n*-alkyl of the C(13) aminomethyl was replaced by other substituents, such as *cyclo*-propyl (**4i**), *cyclo*-pentyl (**4j**), *cyclo*-hexyl (**4k**), and homopiperonyl (**4l**), the target compounds showed very poor or no antibacterial activity against *C. difficile*, with the MIC values being 125 μg/mL or greater than 125 μg/mL. The antibacterial activity of 13-formyl-8-oxo-DHC (**3**) against *C. difficile* was also very poor. In addition, experimental results of this study showed that the active 13-(*N*-*n*-alkyl)-aminomethyl-8-oxo-DHCs series exhibited a clear SAR in the antibacterial activity against *C. difficile.* The carbon chain length of the *N*-*n*-alkyl substituents of the C(13) aminomethyl was found to be an important factor to affect the activity of the end-products. In the range of three to seven carbon atoms of the *N*-*n*-alkyl substituents, the synthesized compounds **4a-e** was found to inhibit *C. difficile* growth in a MIC value of 125 μg/mL, albeit very poor. The MIC values of compounds **4f-h** with the *n*-alkyl substituents of *n*-octyl, *n*-nonyl, and *n*-decyl were 62.5 μg/mL, 7.8 μg/mL, and 7.8 μg/mL, respectively. Thus, an obvious trend was shown that the activity increases with the elongation of *n*-alkyl carbon chain of *N*-*n*-alkyl substituents of the C(13) aminomethyl. Under this demonstrated SAR, when the carbon chain elongated to *n*-nonyl group, the activity began to increase obviously, the MIC value is equivalent to that of levofloxacin, i.e., 7.8 μg/mL (Table 1). In addition, specific selectivity of antibacterial activity is also a meaningful goal in the field of medicinal chemistry.

Table 1. The structures of **3** and **4a-4l** and their MIC values of against *S. aureus*, *E. coli, S. enterica*, and *C. difficile*.



4a-4l

Compounds	MIC (μg/mL)			
	S. aureus	*E. coli*	*S. enterica*	*C. difficile*
QCC	>250	>250	250	125
levofloxacin	0.1	2.0	1.0	7.8
3	>250	>250	250	250
4a (R = *n*-C_3H_7)	>250	>250	>250	125
4b (R = *n*-C_4H_9)	>250	>250	>250	125
4c (R = *n*-C_5H_{11})	>250	>250	>250	125
4d (R = *n*-C_6H_{13})	>250	>250	>250	125
4e (R = *n*-C_7H_{15})	>250	>250	>250	125
4f (R = *n*-C_8H_{17})	>250	>250	>250	62.5
4g (R = *n*-C_9H_{19})	>250	>250	>250	7.8
4h (R = *n*-$C_{10}H_{21}$)	>250	>250	>250	7.8
4i (R = cyclopropyl)	>250	>250	>250	>250
4j (R = cyclopentyl)	>250	>250	>250	125
4k (R = cyclohexyl)	>250	>250	>250	125
4l (R = homopiperonyl)	>250	>250	>250	250

Our previous studies demonstrated that DHC as a reduced product of QCC had significant bioactivities both in activating XBP1 in vitro and in treating UC in animal experiment, which significantly ameliorated the intestinal mucosal barrier damage and intestinal inflammation in vivo.[17] But, as indicated above, DHC has an obvious defect, that is, it is chemically very easy to aromatize in the environment of digestive tract because of the chemical instability. One of the aims of the current study is to inhibit the aromatization instability of DHC through the structural modification by 8-oxo of DHC to obtain active compounds for the treatment of UC. Our own research showed that 8-oxo-DHC as another derivative of QCC also had the XBP1-activating property. But, the activation effect of 8-oxo-DHC on XBP1 is weaker than that of DHC.[16,17] These considerations warrant further structural modification over 8-oxo-DHC

with structural stability and study on the bioactivity of the target compounds. After the establishment for antibacterial activity of the target compounds against *C. difficile*, the examination for the XBP1-activating property was conducted to confirm the desired bioactivity. The experiment was largely modeled after our previously reported method[16-18] and the experimental results for the active compounds were presented in Figure 2, in which con 1 was the pGL3-basic vector control. The XBP1 agonist DHC was used as a positive control which gave a relative activation rate of 1.2 times as compared with con 1 in the current experiment. As shown in Figure 2, all the synthesized compounds, including 13-formyl-8-oxo-DHC (**3**) and 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs (**4a-l**), were determined to display XBP1-activating property in vitro with the relative activation rates arranging from 0.9 to 1.3 times as compared with con 1. Of course, in our laboratory, based on a benchmark relative to con 1, compounds **3**, **4a-g**, and **4j-l** were considered to have XBP1-activating activity, which showed the relative activation rates greater than 1.0 times as compared with con 1. Among these active compounds, **4d** with *n*-hexyl for the *N*-alkyl substituent of the C(13) aminomethyl showed the strongest activity by 1.3 times that of con 1, a better activity than DHC, and **4a** also has strong activity with 1.2 times that of con 1, equivalent to that of DHC. In addition, according to the experimental results, the skeleton of DHC or 8-oxo-DHC was still determined to be the key pharmacophore for XBP1-activating activity as a whole, as is mentioned above that all the synthesized compounds with 8-oxo-DHC core displayed XBP1-activating property. And it can be seen that the structure of the *N*-*n*-alkyl substituents of the C(13) aminomethyl has a rough effect on the XBP1 activation activity of target compounds. Although there are some deviation in the accurate experimental data, on the whole, when the carbon chain of the *N*-*n*-alkyl substituents of the C(13) aminomethyl is long, for example, when there are more than nine carbon atoms, the XBP1-activating activity of the compounds would gradually and definitely weaken. The target compounds with *cyclo*-alkyl substituents on the nitrogen atom of the C(13) aminomethyl, **4i-k**, also activate XBP1, but with relatively weaker activity than the active compounds **4a-e** with relatively shorter carbon chain for the *N*-*n*-alkyl substituents of the C(13) aminomethyl. The activation rate of compound **4l** with a homopiperonyl substituent on the nitrogen atom of the C(13) aminomethyl is 1.0 times that of con 1, a relatively weaker value than those of **4a-e**. Although the trends of the antibacterial activity against *C. difficile* and XBP1-activating property were not exactly matched in between for the active compounds series, this is a real experimental result, and all the activities are definite.

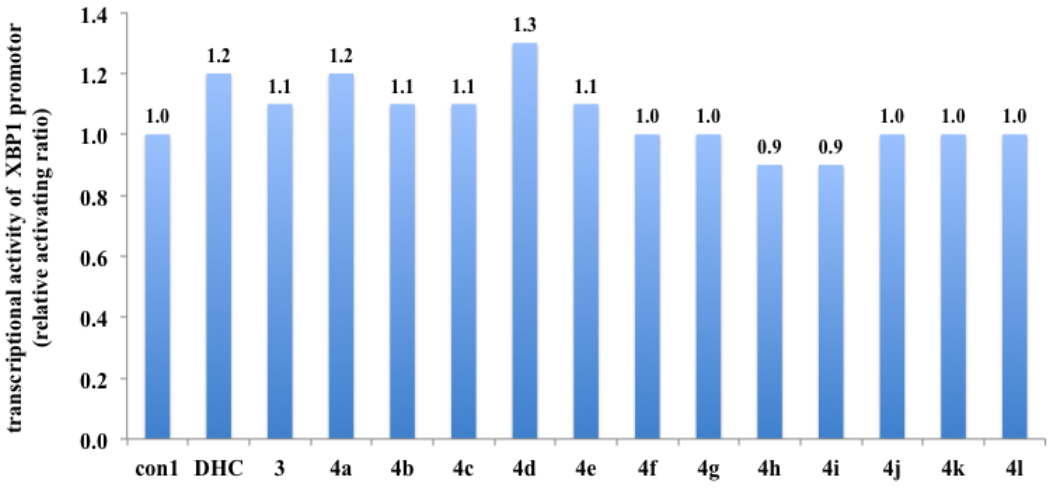


Figure 2. Effects of **3** and **4a-l** on activating XBP1 activity.

Conclusions

To sum up, in this article, 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs (**4a-l**) were designed and synthesized based on QCC as a lead compound and 8-oxo-DHC (**2**) and 13-formyl-8-oxo-DHC (**3**) as two key intermediates. The biological importance was targeted at antibacterial activity against gram-positive anaerobic bacillus *C. difficile* and XBP1-activating activity, both associated with the disease of UC. Especially, the antibacterial activity against *C. difficile* presents a newly considered aspect for the exploration of active compounds against UC. In order to explore the influence of diverse structures on the target bioactivities, the *n*-alkyls, *cyclo*-alkyls, and substituent containing aromatic group were taken into consideration for the *N*-alkyl substituents of the C(13) aminomethyl. Apparently, on top of being chemically stable, the target compounds have rendered their liposolubility improved compared to the substrates. Experimental results did show that some of the synthesized compounds exhibited significant biological activities in anti-microorganism against *C. difficile* and in XBP1-activating. In addition, significant SAR was observed. In the series of 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs with *N*-*n*-alkyl as the *N*-substituents of the C-13 aminomethyl (**4a-h**), the length of the *n*-alkyl carbon chain has a significant influence on the bacterial activity against *C. difficile*. In the tested carbon chain length of three to ten carbons, with the elongation of carbon chain, the antibacterial activity against *C. difficile* remained or gradually increased. Compounds **4a-e** with the three to seven carbons for the carbon chains showed definite antibacterial activity against *C.*

difficile all by the MIC value of 125 μg/mL, which is equivalent to QCC. Change occurred in compound **4f** with eight carbons for the carbon chain, which showed the antibacterial activity against *C. difficile* by the MIC value of 62.5 μg/mL. When the carbon chain extended to *n*-nonyl group, the activity began to increase obviously, with compounds **4g** and **4h** both showed antibacterial activity against *C. difficile* by the MIC value of 7.8 μg/mL, equivalent to that of positive control, levofloxacin, and sixteen times that of substrate QCC. About the XBP1-activation, all the synthesized target compounds, 13-(*N*-alkyl)-aminomethyl-8-oxo-DHCs (**4a-l**), as well as intermediate 13-formyl-8-oxo-DHC (**3**), were determined to display activity in vitro. Compound **4d** with *n*-hexyl for the *N*-alkyl substituent of the C(13) aminomethyl showed the strongest activity by 1.3 times that of con 1, and **4a** with *n*-propyl for the *N*-alkyl substituent of the C(13) aminomethyl also has strong activity with 1.2 times that of con 1, equivalent to that of DHC. About the SAR, a rough tendency can be seen that when the carbon chain of the *N*-*n*-alkyl substituents of the C(13) aminomethyl elongated, the XBP1-activating activity of the compounds gradually and definitely weaken, albeit there are some deviation in the accurate experimental data on the whole. Although the trends of the antibacterial activity against *C. difficile* and XBP1-activating property were not exactly matched in between for the active compounds series, all the activities are definite. Thus, these preliminary results warrant further medicinal chemistry studies with these synthesized QCC derivatives.

Experimental Section

General

The IR spectra were recorded on a Nicolet 5700 FT-IR Microscope spectrometer (Thermo Electron Corporation, Madison, WI, USA). Nuclear magnetic resonance (NMR) spectra were recorded on either a Varian Mercury-400 (Varian, Inc., Palo Alto, CA, USA) or a Bruker Avance III 500 NMR spectrometer (Bruker Instruments Inc., Fällanden, Switzerland) and reported with tetramethylsilane (TMS) as an internal standard and chloroform-*d* ($CDCl_3$) (D, 99.8% + 0.05% v/v TMS) or dimethyl sulfoxide-d_6 (DMSO-d_6) (D, 99.9% + 0.05% v/v TMS) (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) as solvents. Chemical shifts (δ values) and coupling constants (*J* values) are given in ppm and Hz, respectively. HRESIMS$^+$ were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer (Agilent, Santa Clara, CA, USA). QCC was isolated from natural resource in our laboratory. The purity of QCC was determined to be over 98% by high-pressure liquid chromatography (HPLC). The structure of QCC was confirmed on the basis of chemical and spectroscopic data (data not shown). Column chromatography (CC) was carried out using silica gel (200-300 mesh size; Qingdao Haiyang Chemical, Qingdao, China) as an adsorbent. All the reagents and solvents were reagent grade of analytically pure or were purified by standard methods before using. The reaction progress was monitored by thin-layer chromatography (TLC) on a high-efficiency TLC plate with precoated silica gel GF_{254} produced by Qingdao Haiyang Chemical (Qingdao, China). The spots were visualized by I_2 steam or under UV light (254 nm and 365 nm simultaneously). The concentration of solution after reactions involved the use of a rotary evaporator operated at a reduced pressure of ca. 9.0 mbar.

Preparation of compounds

8-oxo-dihydrocoptisine (2). Potassium ferricyanide (1.10 g, 3.35 mmol) was weighed and placed into the reaction bottle. Aqueous 5N sodium hydroxide solution (6.5 mL) was added in at rt and the mixed solution was heated to 45°C under stirring. After dissolution of the solid oxidant, QCC (1 g, 2.81 mmol) was added batchwise and the reaction was performed at 80 °C for 8 h under stirring and refluxing. After the reaction was completed and the reaction solution was cooled to rt, the reaction mixture was filtered and the filter cake was washed using water, until the filtrate was neutral. The filter cake was purified using silica gel CC eluted using a mixed solvent of $CHCl_3$/MeOH (v/v = 120:1) to yield **2** (600 mg, 63.5%) as a yellow solid. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 7.46 (s, 1H, ArH), 7.33 (d, *J* = 8.4 Hz, 1H, ArH), 7.14 (d, *J* = 8.4 Hz, 1H, ArH), 7.11 (s, 1H, ArH), 6.91 (s, 1H, ArH), 6.18 (s, 2H, OCH_2O), 6.06 (s, 2H, OCH_2O), 4.08 (t, *J* = 6.0 Hz, 2H, NCH_2CH_2Ar), 2.85 (t, *J* = 6.0 Hz, 2H, NCH_2CH_2Ar); HRESI-MS (*m/z*): 336.0859 $[M+H]^+$ (calcd for $C_{19}H_{14}NO_5$, 336.0867). [16]

13-Formyl-8-oxo-dihydrocoptisine (3). DMF was measured and placed into a reaction bottle. After stirring in an ice bath condition for 5 min, $POCl_3$ (0.42 mL, 4.47 mmol) was added dropwise and the mixture was stirred for another 1 h. Then, compound **2** (500 mg, 1.49 mmol) was added batchwise and the reaction mixture was heated to 110 °C under the protection of argon gas and stirred for 20 h until the reaction was completed according to TLC monitoring. The reaction solution was concentrated under reduced pressure to remove the solvent. Water (10 mL) was added into the residue. The solution was extracted using a mixed solvent of CH_2Cl_2/MeOH (v/v = 10:1) three times (20 mL/time) in a separatory funnel. The organic layer was integrated, dried using anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to remove the solvent. The residue was purified using silica gel CC eluted using a mixed solvent of CH_2Cl_2/MeOH (v/v = 15:1) to yield compound **3** (287 mg, 53.0%) as a yellow amorphous solid. IR(KBr): ν_{max} 3298, 3077, 2926, 2856, 1672, 1653, 1611, 1539, 1503, 1381, 882 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.82 (s, 1H, CHO), 8.78 (d, *J* = 8.8 Hz, 1H, ArH), 7.27 (d, *J* = 8.8 Hz, 1H, ArH), 6.90 (s, 1H, ArH), 6.86 (s, 1H, ArH), 6.24 (s, 2H, OCH_2O), 6.08 (s, 2H, OCH_2O), 4.22 (t, *J* = 6.4 Hz, 2H, NCH_2CH_2Ar), 2.94 (t, *J* = 6.4 Hz, 2H, NCH_2CH_2Ar); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 190.7, 159.4, 150.7, 149.6, 147.0, 147.0, 146.6, 134.6, 127.6, 120.5, 118.7, 114.5, 113.5, 112.3, 109.8, 108.1, 102.7, 102.1, 39.7, 29.0; (+)HRESI-MS (*m/z*): 364.0817 $[M+H]^+$ (calcd for $C_{20}H_{14}NO_6$, 364.0816).

13-(*N*-*n*-propyl)-aminomethyl-8-oxo-dihydrocoptisine (4a). Compound **3** (100 mg, 0.28 mmol) was weighed and placed into a reaction bottle. A mixed solvent of CH_2Cl_2/MeOH (v/v = 1:1, 16 mL), as well as *n*-propylamine (115 μL, 1.38 mmol), were also added into the reaction bottle in turn. Then, the

reaction mixture was reacted for 12 h under refluxing and stirring. After the reaction mixture was cooled to rt, $NaBH_4$ (31 mg, 0.83 mmol) was added batchwise. The reaction mixture was reacted for another 2 h under refluxing and stirring until it was completed according to TLC monitoring. The reaction solution was concentrated under reduced pressure to remove the solvents. Water (10 mL) was added into the residue. The solution was extracted using CH_2Cl_2 three times (20 mL/time) in a separatory funnel. The organic layer was integrated, dried using anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to remove the solvent. The residue was purified using silica gel CC eluted using a mixed solvent of CH_2Cl_2/MeOH (v/v = 100:1) to yield compound **4a** (96 mg, 85.8%) as a light yellow amorphous solid. IR(KBr): ν_{max} 3025, 2958, 2900, 1649, 1620,1589, 1571, 1500, 1377, 809 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H, ArH), 7.24 (d, J = 8.8 Hz, 1H, ArH), 7.22 (d, J = 8.8 Hz, 1H, ArH), 6.73 (s, 1H, ArH), 6.23 (s, 2H, OCH_2O), 6.02 (s, 2H, OCH_2O), 3.82-3.88 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.77-2.80 (m, 4H, N$\underline{CH_2}CH_2CH_3$, $NCH_2\underline{CH_2}Ar$), 1.63 (sestet, J = 7.2 Hz, 2H, $CH_2\underline{CH_2}CH_3$), 1.50-1.60 (br, 2H, N$\underline{CH_2}$C-13), 1.03 (t, J = 7.2 Hz, 3H, $CH_2CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.0, 132.1, 124.0, 115.9, 113.7, 111.5, 111.0, 110.4, 107.3, 102.7, 101.4, 51.6, 48.5, 40.9, 29.5, 23.0, 12.1; (+)HRESI-MS (*m/z*): 429.1407 $[M+Na]^+$ (calcd for $C_{23}H_{22}N_2O_5Na$, 429.1421).

13-(*N*-*n*-butyl)-aminomethyl-8-oxo-dihydrocoptisine (4b). Target compound **4b** was obtained (86 mg, 49.7%) as a light yellow amorphous solid from compound **3** (150 mg, 0.41 mmol), *n*-butylamine (206 μL, 2.06 mmol), and $NaBH_4$ (47 mg, 1.23 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3307, 3013, 2928, 2900, 1633, 1585, 1506, 1371, 823 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.05 (brs, 1H, ArH), 7.20-7.26 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.23 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.81-3.91 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.75-2.86 (m, 4H, $NCH_2\underline{CH_2}Ar$, N$\underline{CH_2}CH_2CH_2CH_3$), 1.49-1.61 (m, 6H, N$\underline{CH_2}$C-13, $NCH_2\underline{CH_2}\underline{CH_2}CH_3$), 0.97 (t, J = 7.2 Hz, 3H, $NCH_2CH_2CH_2\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.1, 132.0, 124.0, 115.9, 113.7, 111.4, 111.0, 110.4, 107.3, 102.7, 101.4, 49.4, 48.5, 41.0, 31.9, 29.5, 20.6, 14.0; (+)HRESI-MS (*m/z*): 443.1566 $[M+Na]^+$ (calcd for $C_{24}H_{24}N_2O_5Na$, 443.1577).

13-(*N*-*n*-pentyl)-aminomethyl-8-oxo-dihydrocoptisine (4c). Target compound **4c** was obtained (68 mg, 57.1%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-pentylamine (161 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3040, 3020, 2933, 2899, 1644, 1617, 1564, 1503, 1375, 808 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$): 8.05 (brs, 1H, ArH), 7.20-7.25 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.23 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.81-3.91 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.73-2.85 (m, 4H, N$\underline{CH_2}(CH_2)_3CH_3$, $NCH_2\underline{CH_2}Ar$), 1.40-1.61 (m, 8H, N$\underline{CH_2}$C-13, $NCH_2\underline{(CH_2)_3}CH_3$), 0.93 (t, J = 5.6 Hz, 3H, $NCH_2(CH_2)_3\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.1, 132.1, 124.0, 115.9, 113.7, 111.4, 111.0, 110.4, 107.3, 102.7, 101.4, 49.7, 48.5, 41.0, 29.6, 29.5, 29.4, 22.6, 14.1; (+)HRESI-MS (*m/z*): 457.1720 $[M+Na]^+$ (calcd for $C_{25}H_{26}N_2O_5Na$, 457.1734).

13-(*N*-*n*-hexyl)-aminomethyl-8-oxo-dihydrocoptisine (4d). Target compound **4d** was obtained (104 mg, 84.2%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-hexylamine (184 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3050, 3023, 2956, 2927, 1644, 1618, 1568, 1373, 807 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.06 (brs, 1H, ArH), 7.21-7.26 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.24 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.78-3.92 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.73-2.85 (m, 4H, N$\underline{CH_2}(CH_2)_4CH_3$, $NCH_2\underline{CH_2}Ar$), 1.33-1.60 (m, 10H, N$\underline{CH_2}$C-13, $NCH_2\underline{(CH_2)_4}CH_3$), 0.91 (t, J = 6.8 Hz, 3H, $NCH_2(CH_2)_4\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.1, 132.0, 124.0, 115.9, 113.7, 111.4, 111.0, 110.4, 107.3, 102.7, 101.4, 49.7, 48.5, 41.0, 31.8, 29.7, 29.5, 27.2, 22.7, 14.1; (+)HRESI-MS (*m/z*): 471.1877 $[M+Na]^+$ (calcd for $C_{26}H_{28}N_2O_5Na$, 471.1890).

13-(*N*-*n*-heptyl)-aminomethyl-8-oxo-dihydrocoptisine (4e). Target compound **4e** was obtained (85 mg, 66.9%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-peptylamine (208 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0,83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3045, 3021, 2923, 1643, 1620, 1585, 1502, 1373, 809 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.05 (brs, 1H, ArH), 7.42 (d, J = 8.4 Hz, 1H, ArH), 7.38 (d, J = 8.4 Hz, 1H, ArH), 6.97 (s, 1H, ArH), 6.21 (s, 2H, OCH_2O), 6.08 (s, 2H, OCH_2O), 3.64-3.74 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.78, 2.69 (2 × br, 2 × 2H, N$\underline{CH_2}(CH_2)_5CH_3$, $NCH_2C\underline{H_2}Ar$), 1.28-1.57 (m, 12H, N$\underline{CH_2}$C-13, $NCH_2\underline{(CH_2)_5}CH_3$), 0.87 (t, J = 6.8 Hz, 3H, $NCH_2(CH_2)_5\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.2, 147.2, 146.4, 146.4, 135.6, 133.1, 131.9, 123.9, 116.0, 113.8, 111.4, 110.9, 110.4, 107.4, 102.8, 101.4, 49.6, 48.3, 41.0, 31.8, 29.7, 29.5, 29.2, 27.4, 22.7, 14.1; (+)HRESI-MS (*m/z*): 485.2034 $[M+Na]^+$ (calcd for $C_{27}H_{30}N_2O_5Na$, 485.2047).

13-(*N*-*n*-octyl)-aminomethyl-8-oxo-dihydrocoptisine (4f). Target compound **4f** was obtained (85 mg, 64.8%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-octylamine (230 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3047, 3020, 2956, 2919, 1643, 1620, 1586, 1503, 1373, 810 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.05 (brs, 1H, ArH), 7.20-7.26 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.23 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.80-3.93 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.71-2.88 (br, 4H, N$\underline{CH_2}(CH_2)_6CH_3$, $NCH_2\underline{CH_2}Ar$), 1.31-1.62 (m, 14H, N$\underline{CH_2}$C-13, $NCH_2\underline{(CH_2)_6}CH_3$), 0.84-0.95 (br, 3H, $NCH_2(CH_2)_6\underline{CH_3}$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.6, 133.1, 131.9, 123.9, 116.0, 113.8, 111.3, 111.0, 110.4, 107.4, 102.8, 101.4, 49.6, 48.4, 41.0, 31.9, 29.7, 29.5, 29.5, 29.3, 27.5, 22.7, 14.1; (+)HRESI-MS (*m/z*): 499.2187 $[M+Na]^+$ (calcd for $C_{28}H_{32}N_2O_5Na$, 499.2203).

13-(*N*-*n*-nonyl)-aminomethyl-8-oxo-dihydrocoptisine (4g). Target compound **4g** was obtained (93 mg, 68.9%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-nonylamine (254 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3028, 3007, 2951, 2921, 1643, 1618, 1589, 1572, 1376, 808 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.04 (brs, 1H, ArH), 7.20-7.26 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.24 (s, 2H, OCH_2O), 6.03 (s, 2H, OCH_2O), 3.77-3.97 (br, 2H, N$\underline{CH_2}CH_2Ar$), 2.68-2.93 (br, 4H, N$\underline{CH_2}(CH_2)_7CH_3$, $NCH_2\underline{CH_2}Ar$), 1.29-

1.59 (m, 16H, NCH$_2$C-13, NCH$_2$(CH$_2$)$_7$CH$_3$), 0.80-0.97 (br, 3H, NCH$_2$(CH$_2$)$_7$CH$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.2, 148.2, 147.2, 146.4, 146.4, 135.7, 133.2, 131.9, 123.8, 116.1, 113.8, 110.9, 110.4, 110.0, 107.4, 102.8, 101.4, 49.5, 48.3, 41.0, 31.9, 29.7, 29.6, 29.5(2C), 29.3, 27.4, 22.7, 14.1; (+)HRESI-MS (*m/z*): 513.2347 [M+Na]$^+$ (calcd for $C_{29}H_{34}N_2O_5Na$, 513.2360).

13-(*N*-n-*d*ecyl)-aminomethyl-8-oxo-dihydrocoptisine (4h). Target compound **4h** was obtained (90 mg, 64.8%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), *n*-decylamine (281 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3023, 3004, 2950, 2923, 1646, 1618, 1589, 1569, 1376, 807 cm^{-1}; ^{1}H-NMR (500 MHz, CDCl$_3$): δ 8.07 (s, 1H, ArH), 7.21-7.25 (br, 2H, ArH), 6.74 (s, 1H, ArH), 6.24 (s, 2H, OCH$_2$O), 6.03 (s, 2H, OCH$_2$O), 3.81-3.88 (br, 2H, NCH$_2$CH$_2$Ar), 2.76-2.85 (br, 4H, NCH$_2$(CH$_2$)$_8$CH$_3$, NCH$_2$CH$_2$Ar), 1.28-1.60 (m, 18H, NCH$_2$C-13, NCH$_2$(CH$_2$)$_8$CH$_3$), 0.88 (t, *J* = 7.0 Hz, 3H, NCH$_2$(CH$_2$)$_8$CH$_3$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.6, 133.10, 132.0, 123.9, 116.0, 113.7, 111.4, 110.9, 110.4, 107.3, 102.7, 101.4, 49.6, 48.4, 41.0, 31.9, 29.7, 29.6(3C), 29.5, 29.4, 27.5, 22.7, 14.1; (+)HRESI-MS (*m/z*): 527.2504 [M+Na]$^+$ (calcd for $C_{30}H_{36}N_2O_5Na$, 527.2516).

13-(*N*-cyclopropyl)-aminomethyl-8-oxo-dihydrocoptisine (4i). Target compound **4i** was obtained (95 mg, 85.3%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), cyclopropylamine (393 μL, 5.40 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3089, 3019, 2956, 2928, 1651, 1621, 1571, 1501, 1374, 811 cm^{-1}; ^{1}H-NMR (500 MHz, CDCl$_3$): δ 7.99 (s, 1H, ArH), 7.30 (d, *J* = 8.0 Hz, 1H, ArH), 7.24 (d, *J* = 8.0 Hz, 1H, ArH), 6.74 (s, 1H, ArH), 6.24 (s, 2H, OCH$_2$O), 6.04 (s, 2H, OCH$_2$O), 3.92-4.04 (br, 2H, NCH$_2$CH$_2$Ar), 2.73-2.84 (br, 2H, NCH$_2$CH$_2$Ar), 2.29-2.35 (m, 1H, NCH), 1.52-2.11 (br, 2H, NCH$_2$C-13), 0.57-0.64, 0.48-0.56 (2×br, 2×2H, CH$_2$CHCH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.1, 148.1, 147.2, 146.3(2C), 135.7, 133.0, 132.1, 124.0, 115.9, 113.7, 111.0, 110.9, 110.5, 107.3, 102.7, 101.4, 48.3, 40.9, 30.6, 29.5, 5.9(2C); (+)HRESI-MS (*m/z*): 427.1249 [M+Na]$^+$ (calcd for $C_{23}H_{20}N_2O_5Na$, 427.1264).

13-(*N*-cyclopentyl)-aminomethyl-8-oxo-dihydrocoptisine (4j). Target compound **4j** was obtained (95 mg, 79.8%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), cyclopentylamine (137 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3026, 2943, 2896, 1645, 1620, 1591, 1570, 1373, 810 cm^{-1}; ^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.21 (brs, 1H, ArH), 7.22-7.25 (br, 2H, ArH), 6.73 (s, 1H, ArH), 6.23 (s, 2H, OCH$_2$O), 6.02 (s, 2H, OCH$_2$O), 3.83-3.93 (br, 2H, NCH$_2$CH$_2$Ar), 3.32 (quintet, *J* = 5.6 Hz, 1H, NCH), 2.79 (t, *J* = 6.0 Hz, 2H, NCH$_2$CH$_2$Ar), 1.56-1.83 (m, 10H, NCH$_2$C-13, N(CH$_2$)$_4$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.0, 132.0, 124.0, 115.8, 113.8, 111.2, 111.0, 110.7, 107.3, 102.7, 101.4, 60.5, 47.4, 40.9, 32.7, 29.5(2C), 23.9(2C); (+)HRESI-MS (*m/z*): 455.1565 [M+Na]$^+$ (calcd for $C_{25}H_{24}N_2O_5Na$, 455.1577).

13-(*N*-cyclohexyl)-aminomethyl-8-oxo-dihydrocoptisine (4k). Target compound **4k** was obtained (83 mg, 67.5%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), cyclohexylamine (159 μL, 1.38 mmol), and $NaBH_4$ (31 mg, 0.83 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3058, 3028, 2922, 1642, 1589, 1570, 1504, 1373, 821 cm^{-1}; ^{1}H-NMR (400 MHz, CDCl$_3$): δ 8.20 (brs, 1H, ArH), 7.21-7.26 (br, 2H, ArH), 6.73 (s, 1H, ArH), 6.23 (s, 2H, OCH$_2$O), 6.03 (s, 2H, OCH$_2$O), 3.88-4.01 (br, 2H, NCH$_2$CH$_2$Ar), 2.79 (t, *J* = 5.6 Hz, 2H, NCH$_2$CH$_2$Ar), 2.65-2.73 (m, 1H, NCH), 1.31-1.97 (m, 12H, NCH$_2$C-13, N(CH$_2$)$_5$); ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.2, 148.1, 147.2, 146.4, 146.3, 135.5, 133.0, 132.1, 123.9, 115.9, 113.8, 111.2, 111.0, 110.7, 107.3, 102.7, 101.4, 57.6, 46.1, 41.0, 33.0, 29.5(2C), 26.1, 24.9(2C); (+)HRESI-MS (*m/z*): 469.1720 [M+Na]$^+$ (calcd for $C_{26}H_{26}N_2O_5Na$, 469.1734).

13-(*N*-homopiperonyl)-aminomethyl-8-oxo-dihydrocoptisine (4l). Target compound **4l** was obtained (109 mg, 77.3%) as a light yellow amorphous solid from compound **3** (100 mg, 0.28 mmol), homopiperonylamine (46 μL, 0.34 mmol), and $NaBH_4$ (16 mg, 0.42 mmol) using a reductive amination procedure similar to that for **4a**. IR(KBr): ν_{max} 3017, 2946, 2899, 1644, 1619, 1572, 1504, 1378, 801 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) : δ 7.93 (s, 1H, ArH), 7.34 (d, *J* = 8.8 Hz, 1H, ArH), 7.27 (d, *J* = 8.8 Hz, 1H, ArH), 6.97 (s, 1H, ArH), 6.86 (d, *J* = 0.8 Hz, 1H, ArH), 6.82 (d, *J* = 8.0 Hz, 1H, ArH), 6.73 (dd, *J* = 8.0, 0.8 Hz, 1H, ArH), 6.21 (s, 2H, OCH$_2$O), 6.09 (s, 2H, OCH$_2$O), 5.96 (s, 2H, OCH$_2$O), 3.68-3.76 (br, 2H, NCH$_2$CH$_2$Ar), 2.91 (t, *J* = 6.4 Hz, 2H, NCH$_2$CH$_2$Ar), 2.73-2.80 (m, 4H, HNCH$_2$CH$_2$Ar). In view of the fact that the resonance peak of amineomethyl protons did not appear, the ^{13}C-NMR spectrum of the compound was measured, which gave out the exact ^{13}C-NMR signals corresponding to the structure of *13-(N*-homopiperonyl*)-aminomethyl-8-oxo-DHC (**4l**)*. ^{13}C-NMR (100 MHz, CDCl$_3$): δ 159.1, 148.1, 147.7, 147.1, 146.4, 146.2, 146.0, 135.5, 133.6, 133.1, 132.0, 131.9, 123.9, 121.6, 115.9, 113.5, 110.9, 110.3, 109.1, 108.3, 107.3, 102.7, 101.4, 100.8, 50.8, 48.5, 40.9, 35.7, 29.5; (+)HRESI-MS (*m/z*): 535.1462 [M+Na]$^+$ (calcd for $C_{29}H_{24}N_2O_7Na$, 535.1476).

Antibacterial activities assay

The bacterial strains used in this research included *S. aureus*, *E. coli*, *S.enterica*, and *C. difficile*, all from American type culture collection (ATCC). The strains of *S. aureus* ATCC 25923 and *E. coil* ATCC 25922 was obtained from Beijing Institute of Radiation Medicine. *S. enterica* ATCC 14028 was purchased from Shanghai Luwei Technology Co. Ltd. and *C. difficile* ATCC 43593 from Beina Chuanglian Biotechnology (Beijing) Institute.

The MIC values of all the tested compounds were determined using twofold microdilution broth method. The tested compounds, the positive control levofloxacin, and QCC were dissolved in DMSO to a concentration of 2500 μg/mL as reserve solutions. The *S. aureus, E. coli,* and *S. enterica* strains were diluted using Nutrient Broth (NB), and *C. difficile* Fluid Thioglycollate Medium (FT), all to 5×10^4 colony forming units (CFUs) as bacterial suspensions.

The determination of MIC was conducted as follows. First, bacterial suspensions were added to the experimental wells in a sterile 96 wells plate, with 180 μl in the first wells of all the eight rows and 100 μl in the next eleven wells of all the eight rows. Next, 20 μl of NB in the experiments for *S. aureus, E.*

coli, and *S. enterica* (or FT for experiment of *C. difficile*) was added into the first well of the first row and 20 µl of DMSO to the first well of the second row, which were used as compound-free but bacterium-containing negative control group and compound-free but bacterium-containing solvent control group, respectively. The reserve solutions of every tested compounds, as well as levofloxacin, of 20 µl each were added into the remaining six wells in the first column of the 96 wells plate, respectively, which were experimental groups and positive control group, respectively. Then, after mixed up by inserting the wells with multichannel pipette and blowing evenly, 100 µL of the mixture was pipetted from the first well of each row and transferred to the second well. After mixed up, 100 µL of the second mixture was pipetted and transferred to the third well. The same procedure was carried out step by step until the twelfth well. And 100 µL of the mixture in the twelfth well was pipetted and discarded to ensure the same volume condition for this well as the aforementioned all wells. In this way, serial concentrations of 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0, 1.0, 0.5, 0.2, and 0.1 µg/mL were set. Finally, the 96 wells plates were put into incubator for culture. In the case of *S. aureus, E. coli,* and *S. enterica* strains, the incubating was carried out at 37°C for 24 h in an ambient air incubator. In the case of *C. difficile* strain, the 96 wells plates were first put into the anaerobic culture bag, and then put into the incubator for culture at 36°C for 24 h. The microbial growth was examined with the naked and unaided eye. The MIC values of the tested compounds were defined as the lowest concentration at which there was no visible bacterial growth. The experiment was performed with three replicates for all the tested compounds to ensure data accuracy.

In vitro XBP1-activating activity assay

A dual luciferase reporter assay was modeled after the procedure in our previous publications.[16-18]

Supplementary Material

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/MS-number.

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Author Contribution Statement

H. Q., *Y. X,* and *L. W* conceived and designed the experiments together; *J. L*. and *H. Z*. performed the experiments; *J. L*. and *A. D*. analyzed the data; *Z. L* and *A. D* contributed reagents/materials/analysis tools; *J. L*. and *H. Q*. wrote/corrected the paper.

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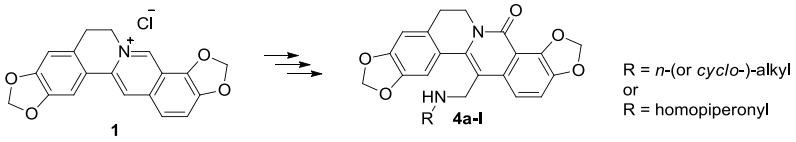
Entry for the Graphical Illustration

Syntheses and structure-activity relationships in antibacterial activity against *Clostridium difficile* and XBP1 activation property of 13-(*N*-alkyl)-aminomethyl-8-oxodihydrocoptisines

Jing Li, Hai-Jing Zhang, An-Jun Deng, Zhi-Hong Li, Ya-Ling Xing, Lian-Qiu Wu, Hai-Lin Qin



MIC values and XBP1 activation multiples of major active compounds are equivalent to those of positive controls.

Twitter Text

Coptisine derivatives were synthesized. Anti-*C. difficile* and XBP1-activating activities were tested. MIC values and XBP1 activation multiples of major compounds are equivalent to positive controls.