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Syntheses and structure-activity relationships on antibacterial and anti-ulcerative colitis properties of quaternary 13-substituted palmatines and 8-oxo-13-substituted dihydropalmatines

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ABSTRACT

In this study, quaternary palmatine is used as a lead compound to design and synthesize derivatives to evaluate bioactivities, with twenty-seven compounds of four series being obtained. Antibacterial activity was examined by determining the minimal inhibitory concentration (MIC) values on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, three series of derivatives being found to exhibit activity in vitro with significant structure-activity relationship (SAR). Elongating the carbon chain led to the antibacterial activity increased, with quaternary 13-hexanoylpalmatine chloride, quaternary 13-(ω-ethoxycarbonyl) heptylpalmatine chloride, and 8-oxo-13-(*N*-*n*-nonyl)aminomethyldihydropalmatine, all of which possess the longest aliphatic carbon chain in the corresponding series of derivatives, showing the MIC values of 62.5, 7.81, and 15.63 μg/ml against *S. aureus*, respectively. The property of anti-ulcerative colitis (anti-UC) was assessed at the levels of both in vitro and in vivo, with X-box-binding protein 1 (XBP1) being targeted in vitro. Seven compounds were found not only to be hypocytotoxic toward intestinal epithelial cells, but also to exhibit activity of activating the transcription of XBP1 in vitro. Five compounds were found to possess significant dose-effect relationship with EC_{50} values at a level of 10^{-7} μM in vitro. 8-Oxo-13-formyldihydropalmatine as an intermediate was found to display significant curative effect on UC in vivo based on the biomarkers of body weight change, colon length change, and calculated values of disease activity index and colon macroscopic damage index of the experimental animals, as well as the examination into the pathological changes of the colon tissue of the modeled animals.

1. Introduction

Quaternary palmatine, a kind of quaternary protoberberine alkaloid (QPA), is one of the chemical constituents of a famous traditional Chinese herbal medicine, *Coptis chinensis* Franch from the Ranunculaceae family.[1] QPAs were reported to exhibit pharmacological properties, including anti-microbes,[2] hypoglycemia,[3] anti-arrhythmia,[4] and anti-oxidation,[5] among others. Efforts to improve bioactivities of natural QPAs were conducted by medicinal chemists using approaches of structural modifications. Based on the principles in organic chemistry, C-8 and 13 positions, as well as several other aromatic sites, of QPA are perceived to possess the most active chemical properties for structural modification. Nucleophilic reagents, such as cyano,[6,7] methoxy,[8] pyrrolyl,[9,10] trichloromethyl,[8,10] alkyl, and phenyl,[11] and so on, were introduced into C-8 position of QPA. The syntheses of 13-substituted derivatives of QPA, such as 13-alkyl and 13-benzyl derivatives, were achieved largely.[12–19] To improve the antimicrobial activities of quaternary palmatine, introduction of substituents at C-8 and C-13 positions were conducted, and the structure-activity relationship (SAR) was evaluated.[20,21] In addition, in view of the anti-ulcerative colitis (anti-UC) activity of QPA derivatives revealed in

Abbreviations: QPA, quaternary protoberberine alkaloid; TF, transcriptional factor; UC, ulcerative colitis; IEC-6, intestinal epithelial cell-6; XBP1, X-box-binding protein 1; MIC, minimal inhibitory concentration; SAR, structure-activity relationship; SASP, Sulfasalazine; CC, column chromatography; DSS, dextran sodium sulfate; DAI, disease activity index; CMDI, colon macroscopic damage index; MHB, Mueller-Hinton Broth; SDB, Sabouraud Dextrose Broth; DIEA, *N*,*N*-diisopropylethylamine; HATU, 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

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our laboratory,[22–24] curative effect of palmatine derivatives on inflammations also attracted our attention. In the present paper, four series of palmatine derivatives, including quaternary 13-alkacylpalmatine chlorides, quaternary 13-(ω-ethoxycarbonyl)alkylpalmatine chlorides, tertiary amide-type 8-oxo-13-(*N*-alkyl) aminomethyldihydropalmatines, and tertiary amide-type 8-oxodihydropalmatine-13-(*N*-alkyl/benzyl)formamides, were designed and synthesized to create synthetic methods, obtain target compounds, and assess their bioactivities. Significant or moderate antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* and anti-UC activity targeting the transcriptional factor (TF) x-box-binding protein 1 (XBP1) were corroborated on several target compounds. This is the first findings so far for palmatine derivatives to exhibit anti-UC activity.

2. Results and discussion

2.1. Chemistry

The basic skeleton of QPA belongs to the 3,4-dihydrodibenzo[*a*, *g*]quinolizine-5-ium system on the systematic nomenclature. The sensitivity of the polar 7,8-imine salt functional group to nucleophilic addition and reduction reactions, followed by the formation of the 7,8-reduced 8-substitution product on the special numbering system of QPA, characterized one of the chemical reactivities of this skeleton. Based on this chemical reactivity, many QPA derivatives were obtained with (±)-8-acetonyldihydroprotoberberines or dihydroprotoberberines as key intermediates.[12–19] The oxidation reaction of the 7,8-imine salt functional group to form the 8-oxodihydroberberines is another feature of chemical property of QPA. Just like (±)-8-acetonyldihydroprotoberberines or dihydroprotoberberines, the bipolar 13a,7-enamine structure of 8-oxodihydroprotoberberines significantly enhances the chemical reactivity of QPA. In the present study, in order to study the medicinal chemistry of natural quaternary palmatine (**1**) extensively, all the three intermediates, dihydropalmatine, (±)-8-acetonyldihydropalmatine, and 8-oxodihydropalmatine, were first prepared to design and synthesize series of palmatine derivatives to evaluate the bioactivities. The syntheses of all the target compounds were depicted as follows.

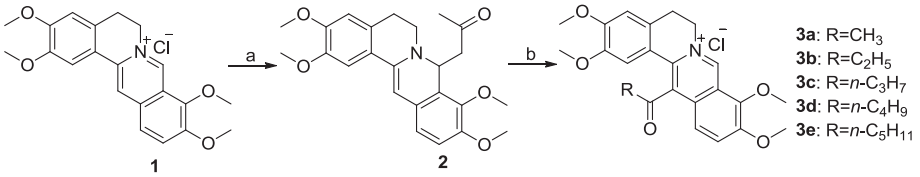
In Scheme 1, the intermediate tertiary amine (±)-8-acetonyldihydropalmatine (**2**) was prepared by utilizing nucleophilic addition of nucleophilic acetonyl anion over the 7,8-imine salt functional group with sodium hydroxide in acetone-water solution being used as catalyst. The target compounds of quaternary 13-alkacylpalmatine chlorides (**3a**–**e**) were obtained by reacting **2** with acyl chlorides undergoing an acylation of enamine dipole structures in yields of between 7.8% and 11.7% from **2**. The low yields were probably due to the instability of tertiary amine (±)-8-acetonyldihydropalmatine in acidic conditions of the reaction mixture which caused the return of **2** to the starting material **1** undergoing an aromatization process. For a systematic goal to screen the target activities and to evaluate the SAR, the acyl groups of acetyl, propionyl, butanoyl, pentanoyl, and hexanoyl were involved and explored.

As depicted in Scheme 2, the 7,8-imine salt function group of **1** was reduced using sodium borohydride ($NaBH_4$) in the presence of potassium carbonate (K_2CO_3) to obtain dihydropalmatine (**4**), a 81.1% yield being achieved. Quaternary 13-(ω-ethoxycarbonyl) alkylpalmatine chlorides **5a**–**g** as the second series of target compounds were produced *via* the enamine alkylation reaction of dihydropalmatine with ethyl ω-halogenated aliphatic acid ester under reflux conditions. Just as the tertiary amine (±)-8-acetonyldihydropalmatine, the stability of tertiary amine dihydropalmatine in acidic conditions of the reaction mixture was also poor, leading to the return of **4** to the starting material **1** undergoing an aromatization process, a cause of the lower yields of all this second series of target compounds. Ethoxycarbonyl and other (ω-ethoxycarbonyl)alkyl groups, including ethoxycarbonylmethyl, ethoxycarbonylpropyl, ethoxycarbonylbutyl, ethoxycarbonylpentyl, ethoxycarbonylhexyl, and ethoxycarbonylheptyl, were involved and explored for a systematical consideration. But, the synthesis of quaternary 13-ethoxycarbonylethylpalmatine chloride failed somehow.

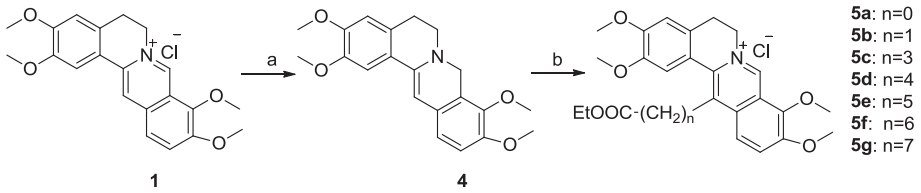
8-Oxodihydropalmatine (**6**) was synthesized mostly modeled after a previously reported method with somewhat a little alteration for the reaction time and post-processing method of extraction.[25] Then, compound **6** was reacted with Vilsmeier reagent, which was freshly prepared *via* reacting $POCl_3$ with DMF at 0 °C for 1 h, at 110 °C for 6 h to yield intermediate 8-oxo-13-formyldihydropalmatine (**8**) in a 41.4% yield from compound **6**. In this process, 9-*O*-demethyl derivative (**7**) of 8-oxo-13-formyldihydropalmatine as a by-product was generated and obtained due to the demethylation reaction. But, compound **7** were conveniently converted into compound **8** by methylation reaction in the presence of Cs_2CO_3 and CH_3I. The third series of target compounds, 8-oxo-13-(*N*-alkyl)aminomethyldihydropalmatines **9a**–**g**, were produced *via* reduction amination reaction, i.e., treating compound **8** with aliphatic amine in MeOH solution of glacial acetic acid under reflux conditions, and then reducing the imine intermediate with $NaBH_4$. The yields for synthesizing the third series of target compounds ranged from 19.3% to 53.8% from compound **8** after separation and purification on silica gel column chromatography (CC) and recrystallization (Scheme 3). The systematically considered (*N*-alkyl)aminomethyl groups uninterruptedly included those from (*N*-*n*-propyl)aminomethyl to (*N*-*n*-nonyl)aminomethyl. On the other hand, compound **8** was conveniently converted into 8-oxodihydropalmatine-13-formic acid (**10**) using the Pinnick oxidation reaction in the presence of $NaClO_2$ and $NaH_2PO_4{\cdot}2H_2O$ in a yield of 52.2%. And the fourth series of compounds, 8-oxo-dihydropalmatine-13-(*N*-alkyl/benzyl)formamides **11a**–**e**, were synthesized by reacting compound **10** with primary amines undergoing a condensation reaction in yields of between 38.2% and 84.3% from **10**. The explored alkyl/benzyl groups included *n*-propyl, *n*-butyl, *n*-pentyl, cyclopropyl, and benzyl (Scheme 3).

2.2. Biological evaluation

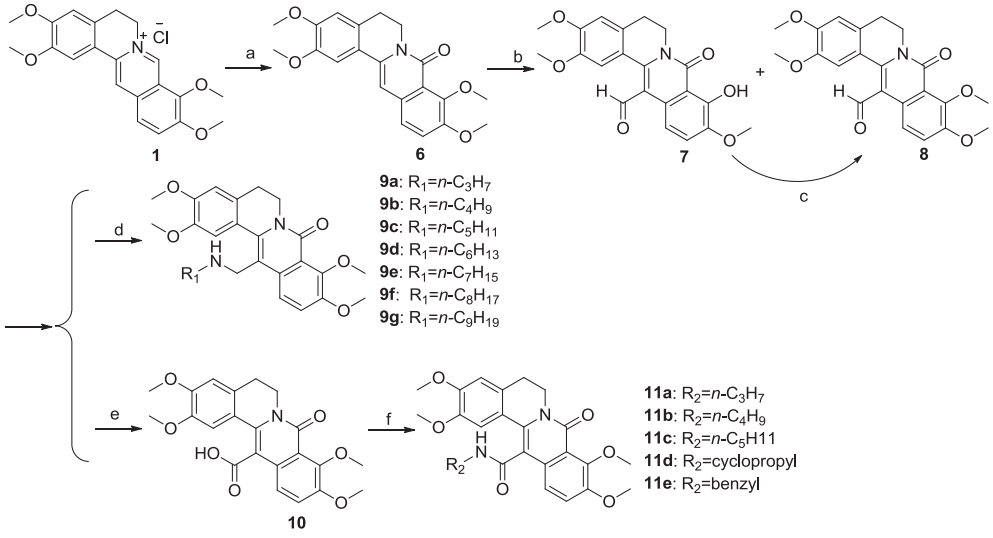
With the four series of target compounds in hand in enough amount, the next consideration of this work was to evaluate the bioactivities. Based on the clinical and folk applications of the



Scheme 1. Preparation of compounds **2** and **3a**–**e**. Reagents and conditions: (a) 5 N NaOH, CH_3COCH_3, rt; (b) NaI, RCOCl, reflux.



Scheme 2. Preparation of compounds **4** and **5a–g**. Reagents and conditions: (a) K_2CO_3, $NaBH_4$, rt; (b) ClCOOEt or $Br(CH_2)_nCOOEt$ (n > 0), NaI, reflux.



Scheme 3. Preparation of compounds **6**, **7**, **8**, **9a–g**, **10**, and **11a–e**. Reagents and conditions: (a) KOH, air, 80 °C; (b) DMF, $POCl_3$, 0 °C, then 110 °C; (c) CH_3I, Cs_2CO_3; (d) primary amine, HOAc, reflux, then $NaBH_4$; (e) $NaClO_2$, $NaH_2PO_4{\cdot}2H_2O$, 2-methyl-2-butene; (f) DIEA, HATU, primary amine.

crude drugs containing QPA, especially the quaternary palmatine, from traditional Chinese medicine (TCM) and our previous investigations on the biological properties of QPA derivatives, several categories of in vitro bioactivity were first tested with all the synthesized compounds. Bioactivities of antimicrobe and XBP1-activating involved in the treatment of UC were demonstrated.

2.2.1. Antimicrobial activity evaluation

Antimicrobial activities of all the synthesized palmatine derivatives against Gram-positive bacterium *S. aureus*, Gram-negative bacterium *E. coli*, and fungus *C. albicans* were examined on determining the minimal inhibitory concentration (MIC) of these compounds using the twofold microdilution broth method.[26] Levofloxacin, a widely used antibacterial agent, and quaternary palmatine were used as positive controls and reference compounds. Three series of compounds, quaternary 13-alkylacylpalmatine chlorides (**3a–e**), quaternary 13-(ω-ethoxycarbonyl) alkylpalmatine chlorides (**5a–g**), and tertiary amide-type 8-oxo-13-(*N*-alkyl)aminomethyldihydropalmatines (**9a–g**), showed antimicrobial activities against *S. aureus*. The MIC values were between 62.5 μg/ml and 250 μg/ml, 7.81 μg/ml and 250 μg/ml, and 15.63 μg/ml and 250 μg/ml for the three series of active compounds, respectively (Table S1, Supplementary data). Typical structure-dependent manner was observed for all the three series of compounds. Activities of **3a–e** against *S. aureus* increased as the length of the carbon chain of the acyls increased, with quaternary 13-hexanoylpalmatine chloride **3e**, which possesses the longest aliphatic carbon chain in this series of compounds, displaying the most significant activity among the synthesized quaternary 13-alkylacylpalmatine chlorides by its MIC value of 62.5 μg/ml (Fig. 1), four times that of quaternary palmatine. The quaternary 13-(ω-ethoxycarbonyl)alkylpalmatine chlorides and 8-oxo-13-(*N*-alkyl)aminomethyldihydropalmatines displayed the similar SAR to the quaternary 13-alkylacylpalmatine chlorides (Figs. 2 and 3). Compounds **5g** and **9g**, which possess, respectively, the longest carbon chain in their respective series, showed the strongest antibacterial activities against *S. aureus* by MIC values of 7.81 and 15.63 μg/ml, respectively. By comparison, the antibacterial activities of all the synthesized compounds against Gram-negative bacterium *E. coli* and fungus *C. albicans* were relatively very poor, but also clearly in a structure-dependent manner and with a similar experimental results to the previously reported findings for other palmatine derivatives.[20,21] The MIC values of the synthesized end compounds against *E. coli* and *C. albicans* were mostly more than 250 μg/ml, except for compounds **3e**, **5g**, and **9g**, all of these three compounds possessing the longest aliphatic carbon chains in their respective series of compounds (The MIC values of these

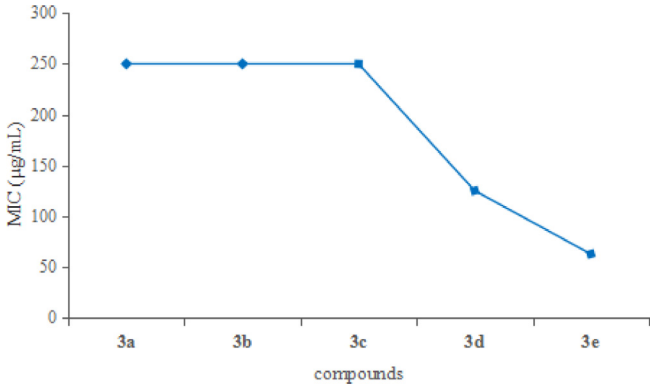


Fig. 1. MIC of **3a–e** against *S. aureus*.

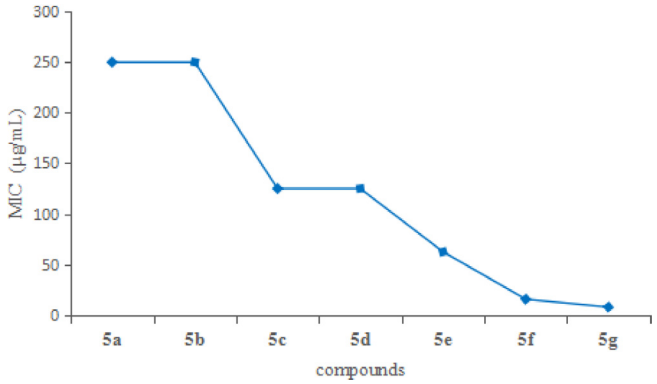


Fig. 2. MIC of **5a**–**g** against *S. aureus*.

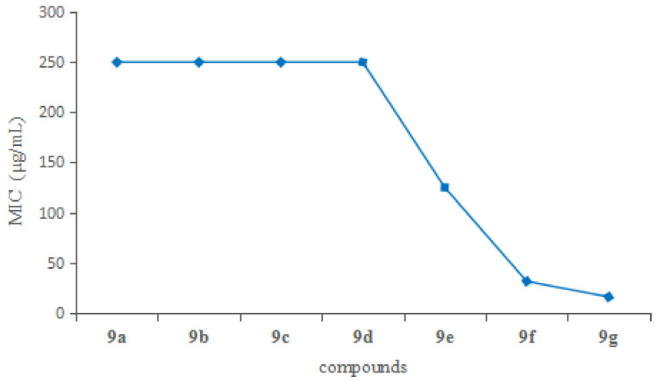


Fig. 3. MIC of **9a**–**g** against *S. aureus*.

three compounds were 250, 250, and >250 μg/ml, respectively, against *E. coli*, and >250, 62.5, and 62.5 μg/ml, respectively, against *C. albicans*). These findings were somewhat valuable in the antibacterial medicinal chemistry of palmatine for giving out the information of SAR. Based on these findings about the SAR, plus other reported results, it was predicted that chemical modifications of natural quaternary palmatine to introduce certain aliphatic carbon chains to increase the liposolubility of end compounds can improve or remarkably improve the anti-bacterial activity against certain bacteria, especially Gram-positive bacteria. But, in the current study, the syntheses of more target compounds containing longer carbon chains encountered difficulties. Compounds **11a**–**e** displayed no obvious antibacterial activity in this study.

2.2.2. In vitro XBP1-activating assay

It is well-known that compounds of hypocytotoxicity may be very interesting in exploring other potential bioactivities, such as, in the present case of quaternary palmatine derivatives, the activity of anti-UC. Thus, to assess the activity of anti-UC, all of the synthesized compounds were first evaluated for their cytotoxicity towards the normal intestinal epithelial cell-6 (IEC-6) cell line with blank control group using the MTT reduction assay procedure that was similar to our previous publication,[23] at a constant concentration of 10 μM. After coincubated with IEC-6 cells for 72 h, different levels of toxicity or safety for all the synthesized compounds were observed based on the final survival rate (SR) of IEC-6 cells. Noncytotoxic or hypocytotoxic compounds were defined as those with SRs ranged from 70% to 100% (not all data being shown, Fig. 4). This experiment gave out a conclusion similar to that of our previous publication[27] that, for the quaternary 13-alkacylpalmatine chlorides (**3a**–**e**), elongation of the aliphatic chain of the *n*-alkanoyls made the cytotoxicity towards the normal IEC-6 cell decreased in the situation of the aliphatic chain to be shorter than five carbons, but as soon as the aliphatic chain of the *n*-alkanoyls was elongated beyond five carbon atoms, i.e., the *n*-alkanoyls possessed six carbons or more, the corresponding compounds displayed apparently increased cytotoxicity on normal IEC-6 cell. No very clear correlation between the cytotoxicity and structures can be deduced from this cytotoxicity examination result for the other three series of tested compounds.

Only the noncytotoxic or hypocytotoxic compounds were tested in vitro for their bioactivity in activating the transcription of XBP1, which is associated with the occurrence, exacerbation, and potential treatment of UC. The examination for the XBP1-activating abilities was largely modeled after our previously reported method[22–24] and the results for the active compounds were presented in Fig. 5, in which con 1 was the pGL3-basic vector control. The XBP1 agonist dihydrocoptisine (DHC) was used as a positive control which gave a relative activation rate of 1.87 times as compared with con 1. As shown in Fig. 5, based on the activation times relative to con 1, seven compounds were determined to display XBP1-activating activity in vitro with the relative activation rates ranged from 1.01 to 1.59 times as compared with con 1. The active compounds included quaternary 13-propionylpalmatine chloride (**3b**), quaternary 13-butanoylpalmatine chloride (**3c**), quaternary 13-pentanoylpalmatine chloride (**3d**), 8-oxo-13-formyldihydropalmatine (**8**), 8-oxo-dihydropalmatine-13-(*N*-*n*-pentyl)formamide (**11c**), 8-oxo-dihydropalmatine-13-(*N*-cyclopropyl)formamide (**11d**), and 8-oxo-dihydropalmatine-13-(*N*-benzyl)formamide (**11e**). The relative activation rates of these compounds were listed in Fig. 5.

To confirm the XBP1-activating abilities at molecular level, all of the seven active compounds were also evaluated by determining

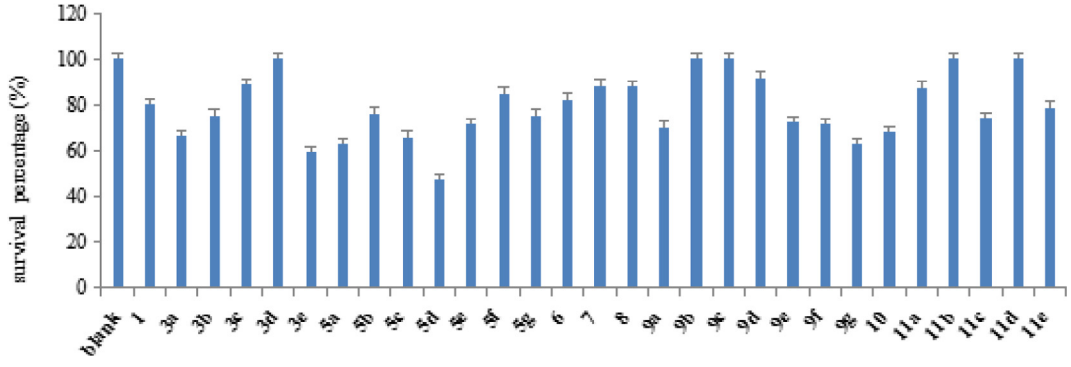


Fig. 4. Toxicity test results of synthesized compounds on IEC-6 cells determined by MTT assay.

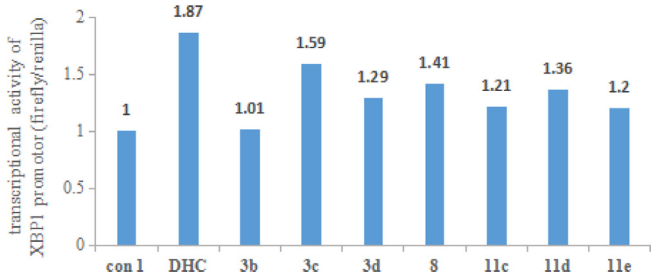


Fig. 5. Effects of compounds **3b–d**, **8**, and **11c–e** on activating XBP1 transcriptional activity.

the in vitro dose-effect relationship. The three quaternary 13-acylpalmatine derivatives **3b–d** showed significant dose-effect relationship by EC_{50} values all at 10^{-7} μM level (Figs. 6–8). The intermediate **8** displayed an EC_{50} value at the same 10^{-7} μM level as **3b–d**, but a little better than all the other compounds as considering the numerical value alone (Fig. 9). The 8-oxo-dihydropalmatine-13-(*N*-alkyl/benzyl)formamides exhibited relatively lower XBP1-activating activity than compounds **3b–d**, with the EC_{50} values of **11c** and **11d** being at 10^{-6} μM level, and **11e** 10^{-7} μM level (Fig. 10) but a bit poorer than **3b–d** as considering the numerical values alone.

2.2.3. In vivo anti-UC efficacy assay

As being among those exhibiting more significant in vitro XBP1-activating abilities with better dose-effect relationship, compounds **3c** and **8** were selected to conduct animal experiments to assess the in vivo curative effect on UC. The acute UC animal models induced by dextran sodium sulfate (DSS) with C57BL/6J mice were built using the reported method.[23,24] Typical symptoms of UC were observed and confirmed from the differences of the body weight and colon length before and after the experiments, and the calculated values of disease activity index (DAI)[28] and colon macroscopic damage index (CMDI) scores[29] of the experimental animals compared with those of the normal control animals, and from the examination into the pathological changes of the colon tissue of the modeled animals (see Tables 1–3 and Fig. 11A & B),

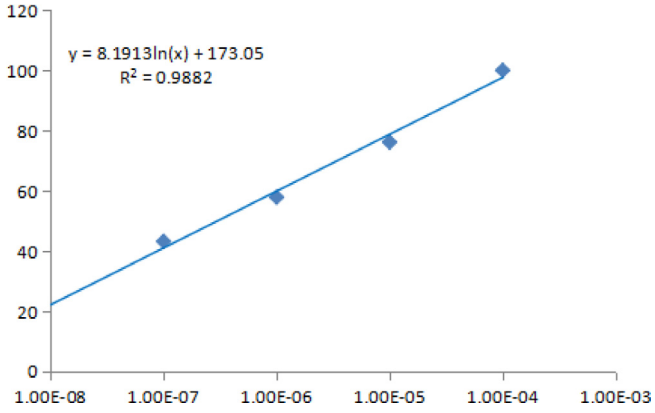


Fig. 6. EC_{50} value of **3b** (EC_{50}: 0.301 μM).

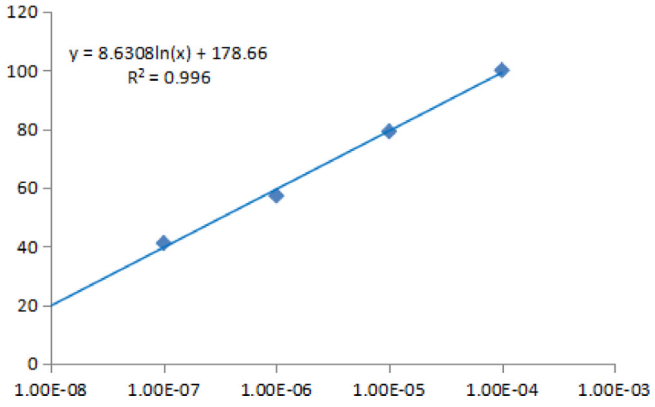


Fig. 7. EC_{50} value of **3c** (EC_{50}: 0.338 μM).

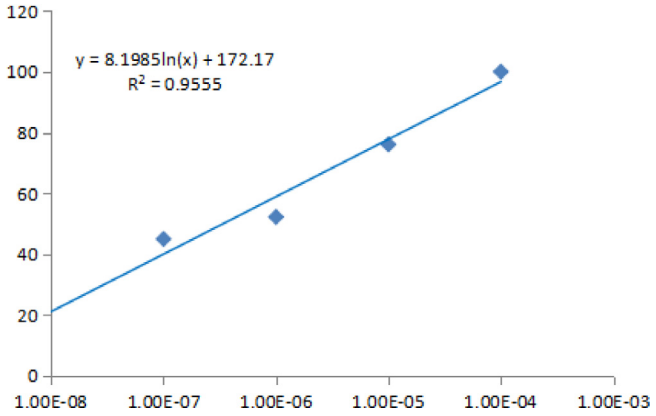


Fig. 8. EC_{50} value of **3d** (EC_{50}: 0.34 μM).

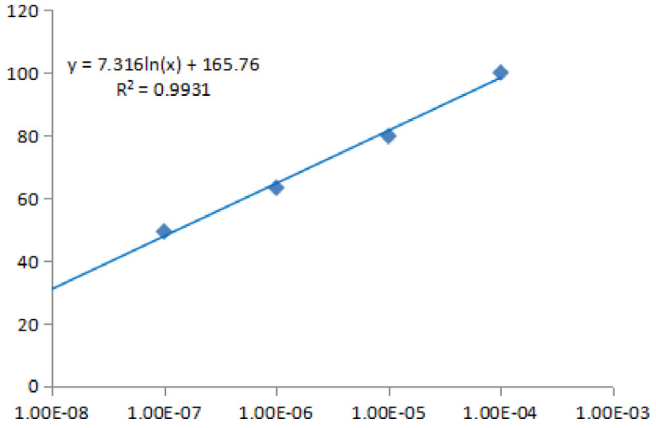


Fig. 9. EC_{50} value of **8** (EC_{50}: 0.135 μM).

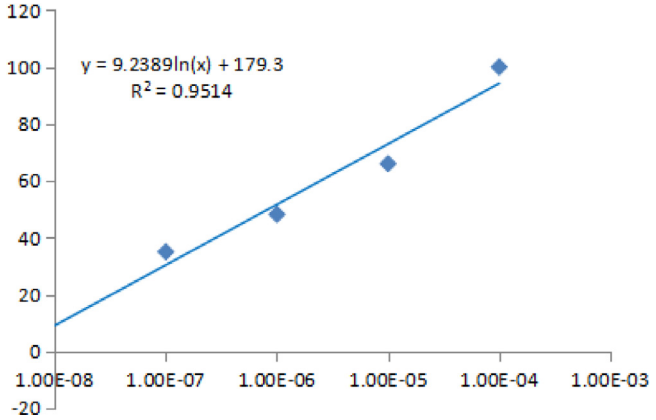


Fig. 10. EC_{50} value of **11e** (EC_{50}: 0.834 μM).

Table 1
Therapeutic effects of **3c** and **8** in vivo evaluated by the body weight change on C57BL/6J mice with DSS-induced acute UC.

Groups	n (start/end)	Body weight (g) x ± SD		Changes of body weight (%)
		Start	End	
Normal control group	8/8	18.13 ± 0.70	19.56 ± 0.51	+7.34
Model group (DSS-induced)	8/8	18.01 ± 0.37	14.59 ± 1.02	−23.96***
SASP group (500 mg/kg)	8/8	17.98 ± 0.44	16.98 ± 0.78	−6.01###
3c (150 mg/kg)	8/8	18.22 ± 0.78	15.67 ± 1.85	−17.28
8 (150 mg/kg)	8/8	17.95 ± 0.71	16.98 ± 1.09	−5.92###

*** $p < 0.001$ when compared with the normal control group.
$p < 0.001$ when compared with the model group.

Table 2
Effects of **3c** and **8** on improving the colon contracture of C57BL/6J mice with DSS-induced acute UC evaluated by contracture percentage (%).

Groups	n (start/end)	Colon length (cm)	Percentage (%)
Normal control group	8/8	8.15 ± 0.24	0
Model group (DSS-induced)	8/8	4.74 ± 0.64	41.87***
SASP group (500 mg/kg)	8/8	5.85 ± 0.63	28.22##
3c (150 mg/kg)	8/8	5.13 ± 0.90	37.01
8 (150 mg/kg)	8/8	5.77 ± 0.97	29.24#

*** $p < 0.001$ when compared with the normal control group.
# $p < 0.05$.
$p < 0.01$ when compared with the model group.

Table 3
Effects of **3c** and **8** on treatment of UC evaluated by DAI and CMDI scores of colon tissue in C57BL/6J mice with DSS-induced acute UC.

Groups	n (start/end)	DAI (IR, %)	CMDI
Normal control group	8/8	0.00 ± 0.00	0.00 ± 0.00
Model group (DSS-induced)	8/8	3.63 ± 0.38***	4.13 ± 0.78**
SASP group (500 mg/kg)	8/8	1.63 ± 0.93 (55.17%)###	3.13 ± 0.60#
3c (150 mg/kg)	8/8	2.50 ± 0.51 (31.03%)###	3.25 ± 0.43#
8 (150 mg/kg)	8/8	1.33 ± 0.82 (63.22%)###	2.25 ± 0.43##

IR: inhibition ratio.
** $p < 0.01$.
*** $p < 0.001$ when compared with the normal control group.
# $p < 0.05$.
$p < 0.01$.
$p < 0.001$ when compared with the model group.

confirming the success for the building of animal models. The experiments were carried out according to the published procedure.[23,24] Compounds **3c** and **8** were administered orally at a dose of 150 mg/kg each day in the form of suspension prepared with 0.5% carboxymethylcellulose sodium and the anti-UC agent Sulfasalazine (SASP) was used as a positive control and was administered at a dose of 500 mg/kg each day.

First, at the end of experiments, the change of animal body weight was examined (Table 1). The animal body weight of normal control group increased on average by 7.34% compared with the initial value, whereas all the other groups exhibited body weight loss to different extents. But, as compared with the model group which displayed a body weight loss by 23.96%, the administration groups of SASP and compound **8** exhibited significantly meliorated effects on the body weight loss with similar body weight loss rates of 6.01% and 5.92%, respectively. The administration group of **3c** showed animal body weight loss by 17.28% compared with the initial value. These data was compatible with a positive efficacy.

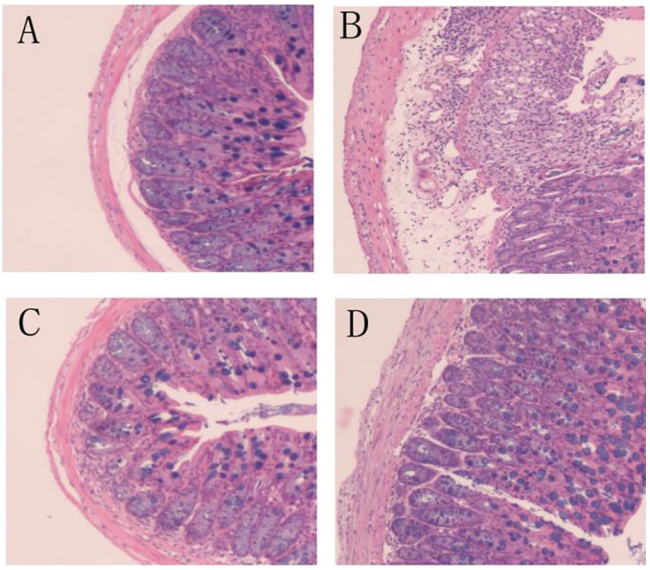


Fig. 11. Effect of **8** on pathological change of colon tissue of C57BL/6J mice with acute UC induced by DSS (HE, ×100). (A) Normal control group; (B) DSS model group; (C) positive SASP group (500 mg/kg); (D) **8** group (150 mg/kg).

Next, as another biomarker to evaluate the curative effect on UC, the colon contractures of the experimental animals were examined by measuring the colon length of each experimental animal after sacrificing them at the end of the trial. The experimental result was shown in Table 2. The colon length of the normal control group was 8.15 cm. In contrast, the colon length of the model group was shortened sharply to 4.74 cm, the colon contracture being up to 41.87% compared with the normal control group. By contrast, the administration groups of SASP, **3c**, and **8** all showed alleviating effects on colon contracture of C57BL/6J mice with DSS-induced acute UC, significant curative effect of SASP and **8** being indicated by their decreased colon contracture of 28.22% and 29.24% compared with the normal control group, respectively, and **3c** showing a weak improving effect on UC with colon contracture of 37.01%.

The calculated DAI and CMDI scores were used to evaluate the anti-UC efficacy of the target compounds. The DAI scores were achieved on the basis of testing for body weight loss, stool features, and hematochezia, whereas the CMDI scores were computed on the basis of testing the hyperemia of the intestinal mucosa, the edema of the bowel wall, and the ulcer size. Lower DAI and CMDI scores in the experimental animals meant closer to the physiological status of the normal control group. As shown in Table 3, the model group possessed the highest scores of DAI and CMDI by the values of 3.63 ± 0.38 and 4.13 ± 0.78, respectively. The positive control SASP exhibited significant curative effect on DSS-induced UC, with the scores of DAI and CMDI decreased to 1.63 ± 0.93

and 3.13 ± 0.60, respectively. Compared with the positive control, compound **8** displayed better curative effects on UC with lower scores of 1.33 ± 0.82 and 2.25 ± 0.43, respectively. Similar to the foregoing other biomarkers, the DAI score of 2.50 ± 0.51 and CMDI score of 3.25 ± 0.43 for **3c** implied that it showed a certain curative effect on DSS-induced UC, but the effect was relatively weaker than that of **8**.

In the end, the animals of all the experimental groups were inspected to look at the pathological changes of the colon tissue using a microscopic examination. The results were shown in Fig. 11. The normal control group exhibited a typical colon mucosal histological structure with the IEC cells being arranged regularly (Fig. 11A). Compared with the normal control group, it was observed that the colonic mucosa of the animal in the DSS model group showed obvious exfoliation, the mucous membrane and mucoderm were touched by inflammation, the recesses were destroyed. A large body of inflammatory cells was observed to infiltrate into the inflammatory lesion region, with the inflammatory cells largely being lymphocytes and neutrophils. Inflammatory ulceration was found to exist in some regions of serious inflammation and, under the ulcers, the hyperblastosis of inflammatory fibers was apparent (Fig. 11B). These findings indicated that the construction of the animal model was successful. In contrast, the positive control SASP and the target compound **8** both improved the pathological status of the colons of the experimental UC animals for the better. SASP exerted a definite curative effect on UC, partial disappearance of inflammatory edema being observed (Fig. 11C). Compound **8** exhibited more significant curative effect, with regular arrangement of IEC cells being observed. And, the polar arrangement of IEC cells even recovered to the normal physiological status (Fig. 11D).

3. Conclusion

In this article, four series of palmatine derivatives, quaternary 13-alkacylpalmatine chlorides, quaternary 13-(ω-ethoxycarbonyl) alkylpalmatine chlorides, 8-oxo-13-(*N*-alkyl)aminomethyldihydropalmatines, and 8-oxo-dihydropalmatine-13-(*N*-alkyl/benzyl)-formamides, were designed and synthesized. Biological/pharmacological activities of the synthesized compounds were screened, with in vitro antimicrobial activities against *S. aureus*, *E. coil*, and *C. albicans* and XBP1-activiting activity and in vivo anti-UC efficacy of some compounds being found and evaluated. The series of quaternary 13-alkacylpalmatine chlorides (**3a**–**e**), 13-(ω-ethoxycarbonyl)alkylpalmatine chlorides (**5a**–**g**), and 8-oxo-13-(*N*-alkyl)aminomethyldihydropalmatines (**9a**–**g**) all showed antibacterial activity against *S. aureus* with significant SAR of enhancing activity as the carbon chain involved in the substitutes increased in carbon numbers. In the experiment for cytotoxicity assay on normal IEC-6 cell, it was found that some compounds were hypocytotoxic or noncytotoxic. An SAR was confirmed for the quaternary 13-alkacylpalmatine chlorides. Elongation of the aliphatic chain of the *n*-alkanoyls made the cytotoxicity decreasing as the aliphatic chain was shorter than five carbons. But as soon as the aliphatic chain of the *n*-alkanoyls possessed six carbons or more, the corresponding compounds displayed increased cytotoxicity. No clear correlation between the cytotoxicity and structures was deduced from the cytotoxicity examination result for the other three series of tested compounds. Seven hypocytotoxic compounds displayed XBP1-activating activity in vitro, the relative activation rates ranging from 1.01 to 1.59 times the pGL3-basic vector control (con 1). Compounds **3b**–**d**, **8**, and **11e** exhibited EC_{50} values at the level of 10^{-7} μM. In the end, compound **8** was confirmed to show the better in vivo anti-UC efficacy in animal experiments by the selected biomarkers involved in the alterations of the body weight and colon length, the calculated values of DAI and CMDI scores, and the examination into the pathological changes of the colon tissue, and thus, it was a promising candidate compound for developing innovative drug treating UC.

4. Experimental

4.1. Chemistry

4.1.1. General experimental procedures

Quaternary palmatine chloride was purchased from market and purified *via* recrystallization method. The purity was determined to be over 98% using high-pressure liquid chromatography (HPLC). The structure was confirmed on the basis of chemical and spectroscopic data (data not shown). All the reagents and solvents were reagent grade or were purified by standard methods before using. The reaction progress was monitored using thin-layer chromatography (TLC) on glass plates with precoated silica gel GF_{254} (Qindao Haiyang Chemical, Qingdao, China). The spots were visualized under UV light. CC was carried out with silica gel (200–300 mesh size; Qingdao Haiyang Chemical, Qingdao, China). The concentrating of solution after reactions involved the use of a rotary evaporator operated at a reduced pressure of ca. 9.0 mbar.

The ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Mercury-400 NMR spectrometer or a Bruker AV-III-500 NMR spectrometer and reported with tetramethylsilane (TMS) as an internal standard and chloroform-*d* ($CDCl_3$) (D, 99.8% + 0.05% v/v TMS) or pyridine d_5 (C_5D_5N) (D, 99.5% + 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.

4.1.2. Procedure for the synthesis of (±)-8-acetonyldihydropalmatine (**2**)

To a stirred solution of **1** (2 g, 5.16 mmol) in aqueous 5 N NaOH (12 ml) was added acetone (4.58 ml) dropwise. After stirred for 3 h, the reaction mixture was filtered, and the obtained precipitate was washed with water until the filtrate was neutral, then recrystallized from acetone/water (4:1) to provide **2** (1.16 g, 54.9% yield) as yellow crystal. ^{1}H NMR (500 MHz, DMSO d_6) δ: 2.03 (s, 3H, $CHCH_2COC\underline{H}_3$), 2.31 (dd, J = 14.5 and 2.5 Hz, 1H, $CHC\underline{H}_2COCH_3$), 2.71–2.81 (m, 2H, $NCH_2C\underline{H}_2$), 2.94 (dd, J = 14.5 and 6.0 Hz, 1H, $CHC\underline{H}_2COCH_3$), 3.21 (t, J = 10.5 Hz, 1H, $NC\underline{H}_2CH_2$), 3.28–3.29 (m, 1H, $NC\underline{H}_2CH_2$), 3.76 (s, 9H, $ArOCH_3\times3$), 3.81 (s, 3H, $ArOCH_3$), 5.21 (m, 1H, $C\underline{H}CH_2COCH_3$), 6.05 (s, 1H, ArCH=C), 6.74 (d, J = 8.0 Hz, 1H, ArH), 6.76 (s, 1H, ArH), 6.86 (d, J = 8.0 Hz, 1H, ArH), 7.20 (s, 1H, ArH).

4.1.3. General procedure for the syntheses of compounds ***3a–e***

To a stirred solution of **2** (2.44 mmol) in acetonitrile (20 ml) was added aliphatic acyl chlorides (24.4 mmol) and NaI (3.0 mmol). The reaction mixture was refluxed for 4 h. The solvent was removed from the mixture *via* evaporation under reduced pressure, and the residue was acidified using aqueous 2 N HCl, stirred at rt. for 1 h. The mixture was extracted three times with $CHCl_3$ in a separatory funnel. The organic layers were combined together and washed successively with, at first, saturated aqueous $NaHCO_3$ solution, next saturated aqueous NaCl solution, and then water. Finally, the solution was dried with anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give a crude product, which was purified using silica CC, eluted using a

150:1 → 100:1 (v/v) gradient elution of $CHCl_3$/MeOH, then the residue of the eluate was recrystallized using acetone or a 3:1 (v/v) solvent of acetone/ethyl acetate as crystallizing solvent to yield **3a**–**e**.

*4.1.3.1. Quaternary 13-acetylpalmatine chloride (**3a**).* Yellow amorphous powder from acetone; yield, 8.7%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.36 (s, 3H, $COCH_3$), 3.50 (br, 2H, $NCH_2C\underline{H}_2$), 3.87 (s, 3H, $ArOCH_3$), 4.01 (s, 3H, $ArOCH_3$), 4.08 (s, 3H, $ArOCH_3$), 4.44 (s, 3H, $ArOCH_3$), 5.19 (br, 2H, $NC\underline{H}_2CH_2$), 6.96 (s, 1H, ArH), 7.10 (s, 1H, ArH), 7.62 (d, J = 9.5 Hz, 1H, ArH), 7.84 (d, J = 9.0 Hz, 1H, ArH), 10.46 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 27.4, 32.8, 56.3, 56.4, 56.8, 57.0, 63.3, 111.2, 111.3, 117.8, 119.6, 122.1, 126.4, 129.3, 131.3, 132.8, 134.3, 146.5, 147.9, 149.0, 151.0, 152.5, 203.1; positive-ion mode HRESIMS m/z 394.16489 $[M-Cl]^+$ (calcd for $C_{23}H_{24}NO_5$, 394.16490).

*4.1.3.2. Quaternary 13-propionylpalmatine chloride (**3b**).* Yellow amorphous powder from acetone/ethyl acetate (3:1); yield: 11.3%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.13 (t, J = 5.6 Hz, 3H, $COCH_2C\underline{H}_3$), 2.52 (q, J = 5.6 Hz, 2H, $COC\underline{H}_2CH_3$), 3.29 (br, 1H, $NCH_2C\underline{H}_2$), 3.86 (s, 4H, $ArOCH_3$ and $NCH_2C\underline{H}_2$), 4.01 (s, 3H, $ArOCH_3$), 4.08 (s, 3H, $ArOCH_3$), 4.43 (br, 4H, $ArOCH_3$ and $NC\underline{H}_2CH_2$), 5.89 (br, 1H, $NC\underline{H}_2CH_2$), 6.97 (s, 1H, ArH), 7.05 (s, 1H, ArH), 7.55 (d, J = 9.5 Hz, 1H, ArH), 7.83 (d, J = 9.0 Hz, 1H, ArH), 10.46 (s, 1H, ArCH = N); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 8.0, 27.4, 38.6, 56.2, 56.4, 56.8, 57.0, 63.3, 110.9, 111.3, 118.0, 119.6, 122.0, 126.4, 129.6, 131.2, 133.0, 134.3, 146.5, 147.8, 149.0, 151.0, 152.3, 206.3; positive-ion mode HRESIMS m/z 408.18030 $[M-Cl]^+$ (calcd for $C_{24}H_{26}NO_5$, 408.18055).

*4.1.3.3. Quaternary 13-butanoylpalmatine chloride (**3c**).* Yellow amorphous powder from acetone/ethyl acetate (3:1); yield: 11.7%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.89 (t, J = 7.0 Hz, 3H, $COCH_2CH_2C\underline{H}_3$), 1.62 (br, 2H, $COCH_2C\underline{H}_2CH_3$), 2.46 (t, J = 7.5 Hz, 2H, $COC\underline{H}_2CH_2CH_3$), 3.20 (br, 1H, $NCH_2C\underline{H}_2$), 3.85 (br, 4H, $ArOCH_3$ and $NCH_2C\underline{H}_2$), 4.01 (s, 3H, $ArOCH_3$), 4.08 (s, 3H, $ArOCH_3$), 4.43 (br, 4H, $ArOCH_3$ and $NC\underline{H}_2CH_2$), 5.93 (br, 1H, $NC\underline{H}_2CH_2$), 6.96 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.56 (d, J = 9.0 Hz, 1H, ArH), 7.83 (d, J = 9.0 Hz, 1H, ArH), 10.47 (s, 1H, ArCH = N); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.6, 17.3, 27.4, 47.0, 56.2, 56.5, 56.9, 57.0, 63.3, 111.2, 111.3, 118.0, 119.6, 122.0, 126.4, 129.6, 131.2, 132.9, 134.3, 146.4, 147.7, 148.9, 150.9, 152.3, 205.7; positive-ion mode HRESIMS m/z 422.19580 $[M-Cl]^+$ (calcd for $C_{25}H_{28}NO_5$, 422.19620).

*4.1.3.4. Quaternary 13-pentanoylpalmatine chloride (**3d**).* Yellow amorphous powder from acetone/ethyl acetate (3:1); yield: 9.4%; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.83 (t, J = 7.6 Hz, 3H, $CO(CH_2)_3C\underline{H}_3$), 1.21–1.31 (m, 2H, $CO(CH_2)_2C\underline{H}_2CH_3$), 1.57 (br, 2H, $COCH_2C\underline{H}_2CH_2CH_3$), 2.48 (t, J = 7.2 Hz, 2H, $COC\underline{H}_2(CH_2)_2CH_3$), 3.19 (br, 1H, $NCH_2C\underline{H}_2$), 3.86 (br, 4H, $ArOCH_3$ and $NCH_2C\underline{H}_2$), 4.01 (s, 3H, $ArOCH_3$), 4.08 (s, 3H, $ArOCH_3$), 4.44 (br, 4H, $ArOCH_3$ and $NC\underline{H}_2CH_2$), 5.96 (br, 1H, $NC\underline{H}_2CH_2$), 6.94 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.56 (d, J = 9.2 Hz, 1H, ArH), 7.82 (d, J = 9.2 Hz, 1H, ArH), 10.49 (s, 1H, ArCH = N); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.7, 21.9, 25.8, 27.4, 44.8, 56.2, 56.4, 56.8, 57.0, 63.3, 111.1, 111.2, 118.0, 119.6, 122.0, 126.4, 129.6, 131.1, 133.0, 134.3, 146.5, 147.8, 148.9, 151.0, 152.3, 205.8; positive-ion mode HRESIMS m/z 436.21158 $[M-Cl]^+$ (calcd for $C_{26}H_{30}NO_5$, 436.21185).

*4.1.3.5. Quaternary 13-hexanoylpalmatine chloride (**3e**).* Yellow amorphous powder from acetone/ethyl acetate (3:1); yield: 7.8%; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.84 (t, J = 5.0 Hz, 3H, $CO(CH_2)_4C\underline{H}_3$), 1.21 (br, 4H, $CO(CH_2)_2C\underline{H}_2C\underline{H}_2CH_3$), 1.58 (br, 2H, $COCH_2C\underline{H}_2(CH_2)_2CH_3$), 2.47 (t, J = 7.5 Hz, 2H, $COC\underline{H}_2(CH_2)_3CH_3$), 3.15 (br, 1H, $NCH_2C\underline{H}_2$), 3.86 (br, 4H, $ArOCH_3$ and $NCH_2C\underline{H}_2$), 4.01 (s, 3H, $ArOCH_3$), 4.08 (s, 3H, $ArOCH_3$), 4.44 (br, 4H, $ArOCH_3$ and $NC\underline{H}_2CH_2$), 5.98 (br, 1H, $NC\underline{H}_2CH_2$), 6.94 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.56 (d, J = 9.0 Hz, 1H, ArH), 7.82 (d, J = 9.0 Hz, 1H, ArH), 10.48 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.8, 22.3, 23.4, 27.4, 30.9, 45.1, 56.2, 56.4, 56.8, 57.0, 63.3, 111.1, 111.3, 118.0, 119.6, 122.0, 126.4, 129.6, 131.1, 133.0, 134.3, 146.5, 147.7, 148.9, 150.9, 152.3, 205.8; positive-ion mode HRESIMS m/z 450.22714 $[M-Cl]^+$ (calcd for $C_{27}H_{32}NO_5$, 450.22750).

*4.1.4. Procedure for the synthesis of dihydropalmatine (**4**)*

To a stirred methanol solution (250 ml) containing **1** (10 g, 25.78 mmol) and K_2CO_3 (10.69 g, 41.25 mmol) was added aqueous 5% NaOH solution (15 ml) containing $NaBH_4$ (1.56 g, 41.25 mmol) dropwise. The reaction mixture was stirred at rt. for 3 h and then filtered to obtain the precipitated product. This product was washed sequentially with water (100 ml) and then 80% ethanol (100 ml), and dried under high vacuum to yield **4** (7.38 g, 81.1%) as yellow powder. ^{1}H NMR (500 MHz, C_5D_5N) δ: 2.86 (t, J = 5.5 Hz, 2H, $NCH_2C\underline{H}_2$), 3.09 (t, J = 5.5 Hz, 2H, $NC\underline{H}_2CH_2$), 3.74 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.85 (s, 3H, $ArOCH_3$), 3.87 (s, 3H, $ArOCH_3$), 4.52 (br, 2H, $ArCH_2N$), 6.48 (s, 1H, ArCH=C), 6.74 (s, 1H, ArH), 6.86 (d, J = 8.5 Hz, 1H, ArH), 6.93 (d, J = 8.5 Hz, 1H, ArH), 7.56 (s, 1H, ArH).

*4.1.5. General procedure for the syntheses of compounds **5a**–**g***

To a stirred solution of **4** (1.7 mmol) in acetonitrile (15 ml) was added ethyl ω-halogenated aliphatic acid ester (ClCOOEt or $Br(CH_2)_nCOOEt$ ($n > 0$)) (6.8 mmol) and NaI (6.8 mmol). The reaction mixture was refluxed overnight and filtered to obtain the filtrate. The solvent of the filtrate was removed under evaporation. The residue was acidified using aqueous 2 N HCl (10 ml). The solution was stirred at rt. for 1 h, extracted with CH_2Cl_2 (3 × 20 ml). The organic layers were combined together and dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure to give a crude product, which was purified *via* a silica gel CC, eluted using a 50:1 (v/v) isocratic elution of CH_2Cl_2/MeOH, then the residue of the eluate was recrystallized using a 2:1 (v/v) solvent of CH_2Cl_2/EtOAc as crystallizing solvent to yield **5a**–**g**.

*4.1.5.1. Quaternary 13-ethoxycarbonylpalmatine chloride (**5a**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 11.9%; ^{1}H NMR (500 MHz, DMSO d_6) δ: 1.28 (t, J = 7.0 Hz, 3H, $COOCH_2C\underline{H}_3$), 3.21 (t, J = 5.5 Hz, 2H, $NCH_2C\underline{H}_2$), 3.79 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.09 (s, 3H, $ArOCH_3$), 4.13 (s, 3H, $ArOCH_3$), 4.54 (q, J = 7.0 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.90 (t, J = 5.5 Hz, 2H, $NC\underline{H}_2CH_2$), 7.20 (s, 2H, ArH × 2), 7.84 (d, J = 9.0 Hz, 1H, ArH), 8.25 (d, J = 9.5 Hz, 1H, ArH), 10.11 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.7, 26.2, 55.7, 55.9, 56.2, 57.0, 62.1, 63.3, 109.7, 111.5, 117.7, 120.1, 120.9, 124.7, 127.5, 129.0, 131.5, 134.7, 144.5, 147.9, 148.0, 150.7, 151.7, 165.9; positive-ion mode HRESIMS m/z 424.17508 $[M-Cl]^+$ (calcd for $C_{24}H_{26}NO_6$, 424.17546).

*4.1.5.2. Quaternary 13-ethoxycarbonylmethylpalmatine chloride (**5b**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 20.6%; ^{1}H NMR (400 MHz, DMSO d_6) δ: 1.25 (t, J = 7.2 Hz, 3H, $COOCH_2C\underline{H}_3$), 3.13 (t, J = 5.2 Hz, 2H, $NCH_2C\underline{H}_2$), 3.77 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.09 (s, 3H, $ArOCH_3$), 4.12 (s, 3H, $ArOCH_3$), 4.23 (q, J = 7.2 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.50 (s, 2H, $=CC\underline{H}_2COOCH_2CH_3$), 4.86 (br, 2H, $NC\underline{H}_2CH_2$), 7.21 (s, 1H, ArH), 7.22 (s, 1H, ArH), 8.02 (d, J = 9.2 Hz, 1H, ArH), 8.24 (d, J = 9.2 Hz, 1H, ArH), 9.99 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, DMSO d_6) δ:

13.9, 26.7, 36.5, 55.4, 55.8, 56.9 (2 × C), 61.5, 62.0, 111.2, 112.1, 118.5, 120.8 (2 × C), 125.6, 126.3, 132.0, 132.8, 137.2, 144.1, 145.7, 147.4, 150.2, 150.8, 170.7; positive-ion mode HRESIMS *m/z* 438.19077 $[M-Cl]^+$ (calcd for $C_{25}H_{28}NO_6$, 438.19111).

*4.1.5.3. Quaternary 13-(ω-ethoxycarbonyl)propylpalmatine chloride (**5c**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 7.6%; ^{1}H NMR (500 MHz, DMSO d_6) δ: 1.16 (t, J = 7.0 Hz, 3H, $COOCH_2C\underline{H}_3$), 2.02–2.09 (m, 2H, $=CCH_2C\underline{H}_2CH_2COOCH_2CH_3$), 2.53 (m, 2H, $=C(CH_2)_2C\underline{H}_2$ $COOCH_2CH_3$), 3.12 (t, J = 5.5 Hz, 2H, $NCH_2C\underline{H}_2$), 3.50 (t, J = 7.5 Hz, 2H, $=CC\underline{H}_2(CH_2)_2COOCH_2CH_3$), 3.86 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.03 (q, J = 7.0 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.10 (s, 3H, $ArOCH_3$), 4.11 (s, 3H, $ArOCH_3$), 4.82 (br, 2H, $NC\underline{H}_2CH_3$), 7.18 (s, 1H, ArH), 7.31 (s, 1H, ArH), 8.23 (d, J = 9.5 Hz, 1H, ArH), 8.35 (d, J = 9.5 Hz, 1H, ArH), 9.91 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, DMSO d_6) δ: 14.0, 25.8, 26.8, 28.5, 32.7, 55.6, 55.7, 56.9, 57.1, 59.9, 61.9, 111.0, 112.5, 118.8, 121.1 (2 × C), 125.9, 132.0, 132.4, 132.9, 136.1, 144.1, 144.5, 147.3, 150.0, 150.5, 172.5; positive-ion mode HRESIMS *m/z* 466.22208 $[M-Cl]^+$ (calcd for $C_{27}H_{32}NO_6$, 466.22241).

*4.1.5.4. Quaternary 13-(ω-ethoxycarbonyl)butylpalmatine chloride (**5d**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 9.3%; ^{1}H NMR (400 MHz, DMSO d_6) δ: 1.15 (t, J = 7.2 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.69–1.76 (m, 2H, $=CCH_2C\underline{H}_2(CH_2)_2COOCH_2CH_3$), 1.81–1.89 (m, 2H, $=C(CH_2)_2C\underline{H}_2CH_2COOCH_2CH_3$), 2.35 (t, J = 7.2 Hz, 2H, $=C(CH_2)_3C\underline{H}_2COOCH_2CH_3$), 3.11 (t, J = 5.2 Hz, 2H, $NCH_2C\underline{H}_2$), 3.39 (t, J = 7.6 Hz, 2H, $=CC\underline{H}_2(CH_2)_3COOCH_2CH_3$), 3.87 (s, 3H, $ArOCH_3$), 3.89 (s, 3H, $ArOCH_3$), 4.03 (q, J = 7.2 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.096 (s, 3H, $ArOCH_3$), 4.102 (s, 3H, $ArOCH_3$), 4.82 (m, 2H, $NC\underline{H}_2CH_2$), 7.19 (s, 1H, ArH), 7.29 (s, 1H, ArH), 8.18 (d, J = 9.6 Hz, 1H, ArH), 8.21 (d, J = 9.6 Hz, 1H, ArH), 9.90 (s, 1H, ArCH = N); ^{13}C NMR (100 MHz, DMSO d_6) δ: 14.0, 24.3, 26.8, 29.0, 30.1, 33.0, 55.8 (2 × C), 56.9, 57.1, 59.7, 61.9, 111.1, 112.5, 118.9, 121.1, 121.2, 125.8, 132.1, 132.2, 133.3, 135.9, 144.1, 144.5, 147.3, 150.0, 150.6, 172.6; positive-ion mode HRESIMS *m/z* 480.23773 $[M-Cl]^+$ (calcd for $C_{28}H_{34}NO_6$, 480.23806).

*4.1.5.5. Quaternary 13-(ω-ethoxycarbonyl)pentylpalmatine chloride (**5e**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 10.1%; ^{1}H NMR (500 MHz, DMSO d_6) δ: 1.17 (t, J = 7.0 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.41–1.52 (m, 2H, $=C(CH_2)_2C\underline{H}_2(CH_2)_2COOCH_2CH_3$), 1.54–1.59 (m, 2H, $=CCH_2C\underline{H}_2(CH_2)_3COOCH_2$ CH_3), 1.84(m, 2H, $=C(CH_2)_3$ $C\underline{H}_2CH_2COOCH_2CH_3$), 2.28 (t, J = 6.5 Hz, 2H, $=C(CH_2)_4C\underline{H}_2COOCH_2CH_3$), 3.11 (t, J = 5.5 Hz, 2H, $NCH_2C\underline{H}_2$), 3.37 (t, J = 7.0 Hz, 2H, $=CC\underline{H}_2(CH_2)_4COOCH_2CH_3$), 3.86 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.04 (q, J = 7.0 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.10 (s, 3H, $ArOCH_3$), 4.11 (s, 3H, $ArOCH_3$), 4.82 (br, 2H, $NC\underline{H}_2CH_2$), 7.19 (s, 1H, ArH), 7.30 (s, 1H, ArH), 8.19 (d, J = 9.5 Hz, 1H, ArH), 8.21 (d, J = 9.5 Hz, 1H, ArH), 9.90 (s, 1H, ArCH = N); ^{13}C NMR (100 MHz, DMSO d_6) δ: 14.0, 24.1, 26.8, 28.5, 29.2, 30.5, 33.3, 55.7, 55.8, 56.9, 57.1, 59.6, 61.9, 111.1, 112.5, 118.9, 121.1, 121.2, 125.9, 132.1, 132.2, 133.4, 135.9, 144.1, 144.4, 147.2, 150.0, 150.6, 172.6; positive-ion mode HRESIMS *m/z* 494.25317 $[M-Cl]^+$ (calcd for $C_{29}H_{36}NO_6$, 494.25371).

*4.1.5.6. Quaternary 13-(ω-ethoxycarbonyl)hexylpalmatine chloride (**5f**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 9.2%; ^{1}H NMR (500 MHz, DMSO d_6) δ: 1.17 (t, J = 7.0 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.30–1.31 (m, 2H, $=C(CH_2)_3C\underline{H}_2(CH_2)_2COOCH_2CH_3$), 1.48–1.52 (m, 4H, $=CCH_2(C\underline{H}_2)_2(CH_2)_3COO$ CH_2CH_3), 1.83 (m, 2H, $=C(CH_2)_4C\underline{H}_2CH_2COOCH_2CH_3$), 2.26 (t, J = 7.0 Hz, 2H, $=C(CH_2)_5$-$C\underline{H}_2COOCH_2CH_3$), 3.11 (br, 2H, $NCH_2C\underline{H}_2$), 3.37 (t, J = 6.5 Hz, 2H, $=CC\underline{H}_2(CH_2)_5COOCH_2CH_3$), 3.86 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.04 (q, J = 7.0 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.09 (s, 3H, $ArOCH_3$), 4.10 (s, 3H, $ArOCH_3$), 4.81 (br, 2H, $NC\underline{H}_2CH_2$), 7.18 (s, 1H, ArH), 7.30 (s, 1H, ArH), 8.20 (br, 2H, ArH × 2), 9.89 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, DMSO d_6) δ:14.0, 24.3, 26.8, 28.1, 28.7, 29.2, 30.7, 33.3, 55.7 (2 × C), 56.9, 57.1, 59.6, 61.9, 111.1, 112.6, 118.9, 121.1 (2 × C), 125.9, 132.1, 132.2, 133.5, 135.9, 144.1, 144.4, 147.2, 150.0, 150.6, 172.7; positive-ion mode HRESIMS *m/z* 508.26898 $[M-Cl]^+$ (calcd for $C_{30}H_{38}NO_6$, 508.26936).

*4.1.5.7. Quaternary 13-(ω-ethoxycarbonyl)heptylpalmatine chloride (**5g**).* Yellow amorphous powder from CH_2Cl_2/EtOAc (2:1); yield: 9.4%; ^{1}H NMR (500 MHz, DMSO d_6) δ: 1.17 (t, J = 7.0 Hz, 3H, $COOCH_2C\underline{H}_3$), 1.24–1.29 (m, 4H, $=C(CH_2)_3(C\underline{H}_2)_2(CH_2)_2COOCH_2$-$CH_3$), 1.45–1.52 (m, 4H, $=CCH_2(C\underline{H}_2)_2(CH_2)_4$ $COOCH_2CH_3$), 1.82 (m, 2H, $=C(CH_2)_5C\underline{H}_2CH_2COOCH_2CH_3$), 2.26 (t, J = 7.5 Hz, 2H, $=C(CH_2)_6C\underline{H}_2COOCH_2CH_3$), 3.11 (t, J = 5.0 Hz, 2H, $NCH_2C\underline{H}_2$), 3.37(t, J = 8.0 Hz, 2H, $C=CC\underline{H}_2(CH_2)_6COOCH_2CH_3$), 3.86 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.04 (q, J = 7.5 Hz, 2H, $COOC\underline{H}_2CH_3$), 4.09 (s, 3H, $ArOCH_3$), 4.10 (s, 3H, $ArOCH_3$), 4.81 (br, 2H, $NC\underline{H}_2CH_2$), 7.19 (s, 1H, ArH), 7.31 (s, 1H, ArH), 8.20 (br, 2H, ArH × 2), 9.89 (s, 1H, ArCH=N); ^{13}C NMR (100 MHz, DMSO d_6) δ: 14.0, 24.3, 26.8, 28.3 (2 × C), 28.8, 29.2, 30.8, 33.3, 55.7, 55.8, 56.9, 57.1, 59.5, 61.9, 111.1, 112.6, 118.9, 121.1, 121.2, 125.9, 132.2 (2 × C), 133.6, 135.8, 144.1, 144.4, 147.2, 150.0, 150.6, 172.8; positive-ion mode HRESIMS *m/z* 522.28485 $[M-Cl]^+$ (calcd for $C_{31}H_{40}NO_6$, 522.28501).

*4.1.6. Procedure for the synthesis of 8-oxo-dihydropalmatine (**6**)*

To a stirred 80 °C water solvent (10 ml) was added **1** (1 g, 2.58 mmol) until the compound was completely dissolved. Then an aqueous alkali solution (5 ml) containing KOH (7.7 g) was added dropwise. The reaction mixture was kept at 80 °C for 0.5 h. While cooled to rt., the reaction mixture was extracted with CH_2Cl_2 three times. The combined organic solution was acidified using aqueous 2 N HCl solution and separated in a separatory funnel. The obtained organic layer was dried using anhydrous $MgSO_4$ and then filtered. The filtrate was concentrated under reduced pressure to give the crude product, which was purified using silica gel CC, eluted using a 150:1 (v/v) isocratic elution of $CHCl_3$/MeOH, to yield compound **6** (310 mg, 32.7%) as light yellow amorphous powder. ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.90 (t, J = 6.0 Hz, 2H, $NCH_2C\underline{H}_2$), 3.78 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $ArOCH_3$), 3.87 (s, 6H, $ArOCH_3$ × 2), 4.14 (t, J = 6.0 Hz, 2H, $NC\underline{H}_2CH_2$), 6.94 (s, 1H, ArCH=C), 7.16 (s, 1H, ArH), 7.42 (s, 1H, ArH), 7.44 (d, J = 8.8 Hz, 1H, ArH), 7.53 (d, J = 8.8 Hz, 1H, ArH).

*4.1.7. Procedure for the syntheses of 9-O-demethyl-8-oxo-13-formyldihydropalmatine (**7**) and 8-oxo-13-formyldihydropalmatine (**8**)*

To a stirred 0 °C DMF (5 ml) solvent was added $POCl_3$ (300 μl, 3.24 mmol) dropwise. The solution was kept at 0 °C for 1 h, then **6** (400 mg, 1.09 mmol) was added. The reaction mixture was heated to 110 °C and was kept for 6 h in an Ar atmosphere. After cooled to rt., the reaction mixture was concentrated under reduced pressure to remove most of the solvent. After ice water was added into the residue and then alkalized with aqueous 1 N NaOH, the alkaline solution was extracted with CH_2Cl_2 three times. The combined organic layer was dried using anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give the crude product, which was purified using silica gel CC, eluted first using a 1:0 → 0:1 (v/v) gradient elution of petroleum ether/CH_2Cl_2,

and then a 100:1 → 50:1 (v/v) gradient elution of CH_2Cl_2/EtOAc, to yield compound **7** (134 mg) as pale yellow amorphous powder and compound **8** (178 mg, 41.4%) as yellow amorphous powder. Compound **7**: ^{1}H NMR (500 MHz, DMSO d_6) δ: 3.02 (br, 2H, $NCH_2C\underline{H}_2$), 3.79 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 3.90 (s, 3H, $ArOCH_3$), 4.14 (br, 2H, $NC\underline{H}_2CH_2$), 6.97 (s, 1H, ArH), 7.19 (s, 1H, ArH), 7.56 (d, J = 9.0 Hz, 1H, ArH), 8.47 (d, J = 9.0 Hz, 1H, ArH), 9.77 (s, 1H, CHO), 12.99 (s, 1H, ArOH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 28.4, 39.9, 56.4, 56.5, 56.6, 110.1, 110.6, 113.2, 114.7, 116.7, 119.0, 119.2, 126.4, 132.4, 146.0, 147.7, 147.8, 150.2, 152.2, 165.7, 191.1; positive-ion mode HRESIMS m/z 382.12799 $[M+H]^+$ (calcd for $C_{21}H_{20}NO_6$, 382.12851). Compound **8**: ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.98 (t, J = 5.6 Hz, 2H, $NCH_2C\underline{H}_2$), 3.78 (s, 3H, $ArOCH_3$), 3.79 (s, 3H, $ArOCH_3$), 3.875 (s, 3H, $ArOCH_3$), 3.900 (s, 3H, $ArOCH_3$), 4.12 (br, 2H, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.17 (s, 1H, ArH), 7.63 (d, J = 9.2 Hz, 1H, ArH), 8.94 (d, J = 9.2 Hz, 1H, ArH), 9.74 (s, 1H, CHO); ^{13}C NMR (100 MHz, DMSO d_6) δ: 27.8, 40.0, 56.2, 56.4, 56.5, 61.1, 110.2, 111.3, 117.3, 118.2, 119.0, 119.4, 120.8, 128.2, 133.6, 147.0, 148.6, 150.4, 151.9, 152.1, 158.7, 190.5; positive-ion mode HRESIMS m/z 396.14423 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_6$, 396.14416).

*4.1.8. Conversion of **7** to **8***

To a stirred 0 °C solution of **7** (1.14 g, 3.0 mmol) in THF (15 ml) and DMF (5 ml) was added Cs_2CO_3 (1955 mg, 6.0 mmol) and CH_3I (1.5 ml, 24.0 mmol). The mixture was stirred overnight at rt. Then, the solution was added additional Cs_2CO_3 (978 mg, 3.0 mmol) and CH_3I (1.13 ml, 18.0 mmol) and heated to 40 °C and kept for another 4 h. The reaction mixture was concentrated under reduced pressure to remove most of the solvent. The residue was poured into ice water (100 ml). The water solution was filtered. The obtained precipitate was washed using water until the filtrate was neutral and then the crude product was dried under vacuum. The purification of this crude product was carried out *via* silica gel CC, eluted using, first, a 5:1 → 0:1 (v/v) gradient elution of petroleum ether/CH_2Cl_2, and then, a 1:0 → 10:1 (v/v) gradient elution of CH_2Cl_2/EtOAc, to yield compound **8** (924 mg) in a yield of 78.0% from **7**.

4.1.9. Syntheses of 8-oxo-13-(N-alkyl)aminomethyldihydropalmatines

*4.1.9.1. Procedure for the synthesis of 8-oxo-13-(N-n-propyl) aminomethyldihydropalmatine (**9a**).* To a stirred solution of compound **8** (150 mg, 0.38 mmol) in MeOH (4 ml), *n*-propylamine (158 μl, 1.9 mmol) and glacial acetic acid (2 drops) were added successively. After the reaction mixture was refluxed for 3.5 h, additional *n*-propylamine (158 μl, 1.9 mmol) was added, and stirred for another 1 h. Then, $NaBH_4$ (22 mg, 0.57 mmol) was added portionwise to the reaction mixture in an ice bath. After stirred for 1 h at rt., the reaction was quenched using aqueous 1 N NaOH, then extracted with CH_2Cl_2 three times. The combined organic layer was dried using anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give the crude product, which was purified using silica gel CC, eluted using a 150:1 (v/v) isocratic elution of CH_2Cl_2/MeOH. The residue of the eluate was recrystallized from EtOAc to yield **9a** (32 mg, 19.3%) as white amorphous powder. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.94 (t, J = 7.5 Hz, 3H, $CH_2CH_2C\underline{H}_3$), 1.52–1.59 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.02 (s, 1H, NH), 2.71 (br, 2H, $C\underline{H}_2CH_2CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (br, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.80 (s, 3H, $ArOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.60 (d, J = 9.0 Hz, 1H, ArH), 7.63 (d, J = 9.5 Hz, 1H, ArH), 8.10 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 11.9, 22.6, 28.1, 40.6, 48.5, 51.7, 55.4, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7 (2 × C), 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.3, 149.3, 150.8, 158.2; positive-ion mode HRESIMS m/z 439.22229 $[M+H]^+$ (calcd for $C_{25}H_{31}N_2O_5$, 439.22275).

*4.1.9.2. Procedure for the synthesis of 8-oxo-13-(N-n-butyl) aminomethyldihydropalmatine (**9b**).* To a stirred solution of compound **8** (150 mg, 0.38 mmol) in MeOH (4 ml), *n*-butylamine (189 μl, 1.9 mmol) and glacial acetic acid (2 drops) were added successively. After the reaction mixture was refluxed for 3 h, $NaBH_4$ (22 mg, 0.57 mmol) was added portionwise to the reaction mixture in an ice bath. After stirred for 1 h at rt., the reaction was quenched using aqueous 1 N NaOH, then extracted using CH_2Cl_2 three times. The combined organic layer was dried using anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to give the crude product, which was purified using silica gel CC, eluted using a 150:1 (v/v) isocratic elution of CH_2Cl_2/MeOH. The residue of the eluate was recrystallized from EtOAc to yield **9b** (66 mg, 38.4%) as white amorphous powder. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.92 (t, J = 7.0 Hz, 3H, $(CH_2)_3C\underline{H}_3$), 1.34–1.41 (m, 2H, $CH_2CH_2C\underline{H}_2$ CH_3), 1.49–1.55 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 1.99 (s, 1H, NH), 2.74 (s, 2H, $C\underline{H}_2CH_2CH_2CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (s, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.80 (s, 3H, $ArOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.89 (s, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.60 (d, J = 9.0 Hz, 1H, ArH), 7.63 (d, J = 9.5 Hz, 1H, ArH), 8.10 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.9, 20.1, 28.1, 31.6, 40.6, 48.6, 49.5, 55.3, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7 (2 × C), 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.3, 149.3, 150.7, 158.2; positive-ion mode HRESIMS m/z 453.23776 $[M+H]^+$ (calcd for $C_{26}H_{33}N_2O_5$, 453.23840).

*4.1.9.3. Procedure for the synthesis of 8-oxo-13-(N-n-pentyl) aminomethyldihydropalmatine (**9c**).* Target compound **9c** was obtained (51 mg, 21.6% yield) as white amorphous powder from compound **8** (200 mg, 0.51 mmol), *n*-pentylamine (298 μl, 2.53 mmol), glacial acetic acid (2 drops), and $NaBH_4$ (30 mg, 0.79 mmol) using a similar procedure to that for **9b**. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.89 (t, J = 6.5 Hz, 3H, $(CH_2)_4C\underline{H}_3$), 1.32–1.33 (m, 4H, $(CH_2)_2(C\underline{H}_2)_2CH_3$), 1.52–1.54 (m, 2H, $CH_2C\underline{H}_2(CH_2)_2CH_3$), 1.99 (s, 1H, NH), 2.73 (br, 2H, $C\underline{H}_2(CH_2)_3CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (br, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.79 (s, 3H, $ArOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.60 (d, J = 9.0 Hz, 1H, ArH), 7.62 (d, J = 9.0 Hz, 1H, ArH), 8.10 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.9, 22.0, 28.1, 29.1, 29.2, 40.6, 48.5, 49.8, 55.3, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7 (2 × C), 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.2, 149.3, 150.7, 158.2; positive-ion mode HRESIMS m/z 467.25323 $[M+H]^+$ (calcd for $C_{27}H_{35}N_2O_5$, 467.25405).

*4.1.9.4. Procedure for the synthesis of 8-oxo-13-(N-n-hexyl) aminomethyldihydropalmatine (**9d**).* Target compound **9d** was obtained (130 mg, 53.5% yield) as white amorphous powder from compound **8** (200 mg, 0.51 mmol), *n*-hexylamine (298 μl, 2.53 mmol), glacial acetic acid (2 drops), and $NaBH_4$ (30 mg, 0.79 mmol) using a similar procedure to that for **9b**. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.88 (t, J = 6.0 Hz, 3H, $(CH_2)_5C\underline{H}_3$), 1.29 (br, 4H, $(CH_2)_2(C\underline{H}_2)_2CH_2CH_3$), 1.32–1.36 (m, 2H, $(CH_2)_4C\underline{H}_2CH_3$), 1.50–1.54 (m, 2H, $CH_2C\underline{H}_2$ $(CH_2)_3CH_3$), 1.98 (s, 1H, NH), 2.72 (br, 2H, $C\underline{H}_2(CH_2)_4CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (br, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.79 (s, 3H, $ArOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.59 (d, J = 9.0 Hz, 1H, ArH), 7.62 (d, J = 9.0 Hz, 1H, ArH), 8.09 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.9, 22.0, 26.7, 28.1, 29.4, 31.2, 40.6, 48.6, 49.9, 55.3, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7 (2 × C), 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.3, 149.3, 150.8, 158.2; positive-ion mode HRESIMS m/z 481.26913 $[M+H]^+$ (calcd for $C_{28}H_{37}N_2O_5$, 481.26970).

*4.1.9.5. Procedure for the synthesis of 8-oxo-13-(N-n-heptyl) aminomethyldihydropalmatine (**9e**).* Target compound **9e** was obtained (105 mg, 42.0% yield) as white amorphous powder from compound **8** (200 mg, 0.51 mmol), *n*-heptylamine (383 μl, 2.53 mmol), glacial acetic acid (2 drops), and $NaBH_4$ (30 mg, 0.79 mmol) using a similar procedure to that for **9b**. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.87 (br, 3H, $(CH_2)_6C\underline{H}_3$), 1.27 (br, 8H, $(CH_2)_2(C\underline{H}_2)_4$-$CH_3$), 1.52 (br, 2H, $CH_2C\underline{H}_2(CH_2)_4CH_3$), 1.99 (s, 1H, NH), 2.72 (br, 2H, $C\underline{H}_2(CH_2)_5\ CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (br, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.79 (s, 3H, $ArOCH_3$), 3.83 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.60 (br, 2H, ArH × 2), 8.09 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.9, 22.0, 27.0, 28.1, 28.6, 29.5, 31.2, 40.6, 48.5, 49.8, 55.3, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7 (2 × C), 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.3, 149.3, 150.7, 158.2; positive-ion mode HRESIMS *m*/*z* 495.28494 $[M+H]^+$ (calcd for $C_{29}H_{39}N_2O_5$, 495.28535).

*4.1.9.6. Procedure for the synthesis of 8-oxo-13-(N-n-octyl) aminomethyldihydropalmatine (**9f**).* Target compound **9f** was obtained (93 mg, 36.2% yield) as white amorphous powder from compound **8** (200 mg, 0.51 mmol), *n*-octylamine (428 μl, 2.53 mmol), glacial acetic acid (2 drops), and $NaBH_4$ (30 mg, 0.79 mmol) using a similar procedure to that for **9b**. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.86 (t, J = 6.0 Hz, 3H, $(CH_2)_7C\underline{H}_3$), 1.26 (s, 8H, $(CH_2)_2(C\underline{H}_2)_4CH_2CH_3$), 1.32–1.33 (m, 2H, $(CH_2)_6C\underline{H}_2CH_3$), 1.49–1.53 (m, 2H, $CH_2C\underline{H}_2(CH_2)_5CH_3$), 1.97(s, 1H, NH), 2.71 (br, 2H, $C\underline{H}_2(CH_2)_5CH_3$), 2.80 (br, 2H, $NCH_2C\underline{H}_2$), 3.76 (br, 2H, $=CC\underline{H}_2NH$), 3.77 (s, 3H, $ArOCH_3$), 3.79 (s, 3H, $ArOCH_3$), 3.83 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.98 (s, 1H, ArH), 7.58 (d, J = 9.0 Hz, 1H, ArH), 7.61 (d, J = 9.0 Hz, 1H, ArH), 8.09 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 13.9, 22.0, 27.0, 28.1, 28.7, 29.0, 29.5, 31.2, 40.6, 48.6, 49.9, 55.3, 55.5, 56.1, 60.7, 109.4, 110.3, 113.4, 118.7, 118.8, 119.6, 122.0, 131.4, 131.8, 135.1, 146.6, 148.3, 149.3, 150.8, 158.2; positive-ion mode HRESIMS *m*/*z* 509.30042 $[M+H]^+$ (calcd for $C_{30}H_{41}N_2O_5$, 509.30100).

*4.1.9.7. Procedure for the synthesis of 8-oxo-13-(N-n-nonyl) aminomethyldihydropalmatine (**9g**).* Target compound **9g** was obtained (142 mg, 53.8% yield) as white amorphous powder from compound **8** (200 mg, 0.51 mmol), *n*-nonylamine (473 μl, 2.53 mmol), glacial acetic acid (2 drops), and $NaBH_4$ (30 mg, 0.79 mmol) using a similar procedure to that for **9b**. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.86 (t, J = 6.5 Hz, 3H, $(CH_2)_8C\underline{H}_3$), 1.27 (br, 10H, $(CH_2)_2(C\underline{H}_2)_5CH_2CH_3$), 1.32–1.35 (m, 2H, $(CH_2)_7C\underline{H}_2CH_3$), 1.49–1.55 (m, 2H, $CH_2C\underline{H}_2(CH_2)_6CH_3$), 1.98 (s, 1H, NH), 2.72 (br, 2H, $C\underline{H}_2(CH_2)_7CH_3$), 2.81 (br, 2H, $NCH_2C\underline{H}_2$), 3.77 (br, 5H, $ArOCH_3$, $=CC\underline{H}_2NH$), 3.79 (s, 3H, $ArOCH_3$), 3.83 (s, 3H, $ArOCH_3$), 3.89 (br, 5H, $ArOCH_3$, $NC\underline{H}_2CH_2$), 6.99 (s, 1H, ArH), 7.59 (d, J = 9.0 Hz, 1H, ArH),7.62 (d, J = 9.0 Hz, 1H, ArH), 8.09 (s, 1H, ArH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 14.5, 22.6, 27.6, 28.8, 29.2, 29.6 (2 × C), 30.1, 31.9, 41.2, 49.2, 50.5, 55.9, 56.1, 56.7, 61.3, 110.0, 110.9, 114.0, 119.3, 119.4, 120.2, 122.6, 132.0, 132.5, 135.8, 147.2, 149.0, 149.9, 151.4, 158.8; positive-ion mode HRESIMS *m*/*z* 523.31622 $[M+H]^+$ (calcd for $C_{31}H_{43}N_2O_5$, 523.31665).

*4.1.10. Procedure for the synthesis of 8-oxo-13-carboxydihydropalmatine (**10**)*

After 2-methyl-2-butene (12.3 ml) was added into a stirred solution of compound **8** (1.1 g, 2.78 mmol) in *t*-BuOH (10 ml) and THF (10 ml) in ice bath, a water solution (10 ml) containing $NaH_2PO_4{\cdot}2H_2O$ (4.34 g, 27.8 mmol) and $NaClO_2$ (3.35 g, 27.8 mmol) was added dropwise. The reaction mixture was stirred for 5 h at rt., then additional 2-methyl-2-butene (6.15 ml) and a water solution (5 ml) containing $NaH_2PO_4{\cdot}2H_2O$ (2.17 g, 13.9 mmol) and $NaClO_2$ (1.68 g, 13.9 mmol) were added again. After the reaction mixture was stirred for another 2 h, $Na_2S_2O_3{\cdot}5H_2O$ (10.36 g, 41.7 mmol) was added and the stirring was carried out for another 1 h at rt. to exhaust excess oxidizing agent. The reaction was stopped and the mixture was concentrated under reduced pressure to remove the organic solvents, then aqueous layer was extracted using CH_2Cl_2 three times. The combined organic layer was extracted using aqueous 1 N NaOH twice. The alkaline solution was combined and acidified to PH = 2–3 with aqueous concentrated HCl solution. The precipitate was obtained *via* filtration of the acidic solution, which was washed using water and dried under vacuum conditions to yield compounds **10** (597 mg, 52.2% yield) as orange powder. ^{1}H NMR (400 MHz, DMSO d_6) δ: 2.87 (t, J = 5.6 Hz, 2H, $NCH_2C\underline{H}_2$), 3.76 (s, 3H, $ArOCH_3$), 3.79 (s, 3H, $ArOCH_3$), 3.84 (s, 3H, $ArOCH_3$), 3.89 (s, 3H, $ArOCH_3$), 4.09 (t, J = 5.6 Hz, 2H, $NC\underline{H}_2CH_2$), 7.02 (s, 1H, ArH), 7.36 (s, 1H, ArH), 7.39 (d, J = 9.2 Hz, 1H, ArH), 7.61 (d, J = 9.2 Hz, 1H, ArH), 13.50 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO d_6) δ: 27.7, 39.7, 55.4, 55.6, 56.2, 60.8, 109.4, 110.7, 110.8, 118.0, 119.0, 119.7, 120.8, 128.2, 131.0, 133.2, 147.0, 148.4, 149.9, 151.2, 157.9, 170.1; positive-ion mode HRESIMS *m*/*z* 412.13840 $[M+H]^+$ (calcd for $C_{22}H_{22}NO_7$, 412.13908).

4.1.11. Syntheses of 8-oxo-dihydropalmatine-13-(N-alkyl/benzyl) formamides

*4.1.11.1. Procedure for the synthesis of 8-oxo-dihydropalmatine-13-(N-n-propyl) formamide (**11a**).* To a stirred solution of **10** (100 mg, 0.24 mmol) in DMF (3 ml) in ice bath was added DIEA (86 μl, 0.49 mmol) and HATU (140 mg, 0.36 mmol). After stirred for 1 h in ice bath, *n*-propylamine (31 μl, 0.36 mmol) was added, then stirred for 3 h at rt. The reaction mixture was poured into ice water (20 ml), and then filtered to get the precipitate, which was washed using water and dried under vacuum to obtain the crude product. The crude product was purified *via* silica gel CC, eluted using a 70:1 (v/v) isocratic elution of CH_2Cl_2/MeOH to yield compound **11a** (42 mg, 38.2% yield) as white amorphous powder. ^{1}H NMR (400 MHz, DMSO d_6) δ: 0.74 (t, J = 7.6 Hz, 3H, $(CH_2)_2C\underline{H}_3$), 1.34–1.43 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.88 (br, 2H, $NCH_2C\underline{H}_2$), 2.97 (br, 1H, $NC\underline{H}_2CH_2$), 3.33 (br, 2H, $CONHC\underline{H}_2CH_2CH_3$), 3.70 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 4.92 (br, 1H, $NC\underline{H}_2CH_2$), 6.98 (s, 1H, ArH), 7.34 (d, J = 9.2 Hz, 1H, ArH), 7.48 (s, 1H, ArH), 7.58 (d, J = 9.2 Hz, 1H, ArH), 8.34 (t, J = 5.6 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.3, 22.4, 28.8, 40.3, 42.0, 56.0, 56.1, 56.6, 61.6, 110.0, 111.2, 112.4, 118.7, 119.1, 120.4, 121.5, 129.6, 130.6, 133.3, 147.7, 149.3, 150.0, 151.8, 159.5, 169.1; positive-ion mode HRESIMS *m*/*z* 453.20135 $[M+H]^+$ (calcd for $C_{25}H_{29}N_2O_6$, 453.20201).

*4.1.11.2. Procedure for the synthesis of 8-oxo-dihydropalmatine-13-(N-n-butyl) formamide (**11b**).* Target compound **11b** was obtained (55 mg, 48.5% yield) as white amorphous powder from **10** (100 mg, 0.24 mmol), DMF (3 ml), DIEA (86 μl, 0.49 mmol), HATU (140 mg, 0.36 mmol), and *n*-butylamine (37 μl, 0.36 mmol) using a similar procedure to that for **11a**. ^{1}H NMR (400 MHz, DMSO d_6) δ: 0.80 (t, J = 7.2 Hz, 3H, $CONH(CH_2)_3C\underline{H}_3$), 1.08–1.18 (m, 2H, $(CH_2)_2C\underline{H}_2CH_3$), 1.30–1.37 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 2.86 (br, 2H, $NCH_2C\underline{H}_2$), 3.06 (br, 1H, $NC\underline{H}_2CH_2$), 3.32 (br, 2H, $C\underline{H}_2(CH_2)_2CH_3$), 3.70 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 4.93 (br, 1H, $NC\underline{H}_2CH_2$), 6.98 (s, 1H, ArH), 7.33 (d, J = 9.2 Hz, 1H, ArH), 7.47 (s, 1H, ArH), 7.58 (d, J = 8.8 Hz, 1H, ArH), 8.32 (t, J = 5.6 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.7, 20.0, 28.9, 31.1, 40.0, 40.3, 56.0, 56.1, 56.6, 61.6, 110.0, 111.2, 112.4, 118.7, 119.1, 120.4, 121.5, 129.6, 130.6, 133.4,

147.7, 149.4, 150.0, 151.8, 159.5, 169.1; positive-ion mode HRESIMS *m*/*z* 467.21732 $[M+H]^+$ (calcd for $C_{26}H_{31}N_2O_6$, 467.21766).

*4.1.11.3. Procedure for the synthesis of 8-oxo-dihydropalmatine-13-(N-n-pentyl) formamide (**11c**).* Target compound **11c** was obtained (81 mg, 81.6% yield) as white amorphous powder from **10** (85 mg, 0.21 mmol), DMF (2 ml), DIEA (73 μl, 0.41 mmol), HATU (119 mg, 0.31 mmol), and *n*-pentylamine (122 μl, 1.10 mmol) using a similar procedure to that for **11a**. ^{1}H NMR (400 MHz, DMSO d_6) δ: 0.80 (t, J = 6.8 Hz, 3H, $(CH_2)_4C\underline{H}_3$), 1.03–1.10 (m, 2H, $(CH_2)_3C\underline{H}_2CH_3$), 1.15–1.24 (m, 2H, $(CH_2)_2C\underline{H}_2CH_2CH_3$), 1.31–1.39 (m, 2H, $CH_2C\underline{H}_2(CH_2)_2$-$CH_3$), 2.87 (br, 2H, $NCH_2C\underline{H}_2$), 3.07 (br, 1H, $NC\underline{H}_2CH_2$), 3.20 (br, 2H, $C\underline{H}_2(CH_2)_3CH_3$), 3.70 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.82 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 4.93 (br s, 1H, $NC\underline{H}_2CH_2$), 6.98 (s, 1H, ArH), 7.33 (d, J = 8.8 Hz, 1H, ArH), 7.47 (s, 1H, ArH), 7.57 (d, J = 8.8 Hz, 1H, ArH), 8.31 (t, J = 5.6 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.9, 22.3, 28.7, 28.9, 29.0, 40.3(2 × C), 56.0, 56.1, 56.6, 61.6, 109.9, 111.2, 112.4, 118.7, 119.1, 120.4, 121.5, 129.6, 130.6, 133.4, 147.7, 149.4, 150.0, 151.8, 159.5, 169.1; positive-ion mode HRESIMS *m*/*z* 481.23300 $[M+H]^+$ (calcd for $C_{27}H_{33}N_2O_6$, 481.23331).

*4.1.11.4. Procedure for the synthesis of 8-oxo-dihydropalmatine-13-(N-cyclopropyl) formamide (**11d**).* To a stirred solution of **10** (120 mg, 0.29 mmol) in DMF (3 ml) in ice bath was added DIEA (103 μl, 0.58 mmol) and HATU (168 mg, 0.44 mmol). After stirring for 1 h in ice bath, cyclopropylamine (31 μl, 0.44 mmol) was added, then stirred for 3 h at rt. Additional cyclopropylamine (31 μl, 0.44 mmol) was added, and stirred for another 2 h. The reaction mixture was poured into ice water (20 ml), then filtered to get the precipitate, which was washed using water and dried under vacuum to obtain the crude product. The crude product was purified *via* silica gel CC, eluted using a 70:1 (v/v) isocratic elution of CH_2Cl_2/MeOH to yield compound **11d** (96 mg, 73.1% yield) as white amorphous powder. ^{1}H NMR (500 MHz, DMSO d_6) δ: 0.16 (s, 1H, $CONHCH(C\underline{H}_2)_2$), 0.36 (s, 1H, $CONHCH(C\underline{H}_2)_2$), 0.62 (s, 1H, $CONHCH(C\underline{H}_2)_2$), 0.67 (s, 1H, $CONHCH(C\underline{H}_2)_2$), 2.79–2.85 (m, 2H, $NCH_2C\underline{H}_2$), 2.89 (br, 1H, $NC\underline{H}_2CH_2$), 3.31 (br, 1H, $CONHC\underline{H}(CH_2)_2$), 3.75 (s, 3H, $ArOCH_3$), 3.77 (s, 3H, $ArOCH_3$), 3.83 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 4.90 (br, 1H, $NC\underline{H}_2CH_2$), 6.98 (s, 1H, ArH), 7.32 (d, J = 9.0 Hz, 1H, ArH), 7.50 (s, 1H, ArH), 7.58 (d, J = 9.0 Hz, ArH), 8.37 (d, J = 4.5 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.3 (2 × C), 23.6, 28.8, 40.3, 56.0, 56.2, 56.5, 61.5, 110.0, 111.3, 112.0, 118.6, 118.9, 120.3, 121.4, 129.5, 130.6, 133.4, 147.7, 149.2, 150.0, 151.7, 159.5, 170.5; positive-ion mode HRESIMS *m*/*z* 451.18607 $[M+H]^+$ (calcd for $C_{25}H_{27}N_2O_6$, 451.18636).

*4.1.11.5. Procedure for the synthesis of 8-oxo-dihydropalmatine-13-(N-benzyl) formamide (**11e**).* To a stirred solution of **10** (120 mg, 0.29 mmol) in DMF (3 ml) in ice bath was added DIEA (103 μl, 0.58 mmol) and HATU (168 mg, 0.44 mmol). After stirred for 1 h in ice bath, benzylamine (163 μl, 1.46 mmol) was added, then stirred for 3 h at rt. The reaction mixture was poured into ice water (20 ml), then filtered to get the precipitate, which was washed using water and dried under vacuum to obtain the crude product. The crude product was purified *via* silica CC, eluted using a 70:1 (v/v) isocratic elution of CH_2Cl_2/MeOH to yield compound **11e** (123 mg, 84.3% yield) as white amorphous powder. ^{1}H NMR (500 MHz, DMSO d_6) δ: 2.85–2.90 (m, 2H, $NCH_2C\underline{H}_2$), 3.23 (br, 1H, $NC\underline{H}_2CH_2$), 3.56 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 3.85 (s, 3H, $ArOCH_3$), 3.88 (s, 3H, $ArOCH_3$), 4.23 (br, 1H, $CONHC\underline{H}_2Ph$), 4.51 (br, 1H, $CONHC\underline{H}_2Ph$), 4.97 (br, 1H, $NC\underline{H}_2CH_2$), 7.01 (s, 1H, ArH), 7.11–7.12 (m, 2H, PhH × 2), 7.20–7.25 (m, 3H, PhH × 3), 7.31 (d, J = 9.0 Hz, 1H, ArH), 7.44 (s, 1H, ArH), 7.54 (d, J = 9.5 Hz, 1H, ArH), 8.86 (t, J = 6.0 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.8, 40.3, 44.3, 56.1 (2 × C), 56.6, 61.6, 110.0, 111.2, 112.0, 118.7, 119.1, 120.3, 121.5, 127.6 (2 × C), 127.7, 128.7 (2 × C), 129.5, 130.6, 133.7, 137.2, 147.8, 149.3, 150.1, 151.8, 159.5, 169.1; positive-ion mode HRESIMS *m*/*z* 501.20175 $[M+H]^+$ (calcd for $C_{29}H_{29}N_2O_6$, 501.20201).

4.2. Antibacterial activity assay

The microbes *S. aureus* and *E. coil* were obtained from clinical isolates and were cultivated in Mueller-Hinton Broth (MHB) at 37 °C. *C. albicans* CMCC(F) 98,001 was purchased from Shanghai Luwei Technology Co., Ltd. and was cultivated in Sabouraud Dextrose Broth (SDB) at 25 °C.

Antibacterial activity was examined by determining the minimal inhibitory concentration (MIC) using the twofold microdilution broth method.[26] The tested compounds and the positive control were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 20,000 μg/ml as reserve solutions. The *S. aureus* and *E. coli* microbes were diluted with MHB, and *C. albicans* with SDB, all to 10^6 colony forming units (CFUs) as bacterial suspensions. The determination of MIC was conducted as follows. First, MHB (SDB for experiment of *C. albicans*) was added to the experimental wells in a sterile 96 wells plate, with 180 μl in the first well and 100 μl in the next ten wells. Then, 200 μl of MHB was added to the twelfth well as a compound-free medium blank negative control. Next, 20 μl of reserve solution was added to the first well and mixed fully. Then, 100 μl was removed from the first well and transferred to the second well. After mixing it fully, 100 μl of the second mixture was removed and transferred to the third well. The same procedure was carried out step by step until the tenth well. The tenth mixture (100 μl) was removed from the tenth well and discarded to ensure the same volume conditions for this well as the aforementioned nine wells as well as the eleventh well, with the eleventh well being used as compound-free but bacterium-containing control. Finally, the bacterial suspension (100 μl) was added to each of the aforementioned first eleven wells and mixed fully. After incubating for 24 h in an ambient air incubator, the microbial growth was examined with the naked and unaided eye. The MICs 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.

4.3. In vitro cytotoxicity and XBP1-activating activity assay

An in vitro cytotoxicity assay with IEC-6 cells, a dual luciferase reporter assay, and an EC_{50} value assay were modeled after the procedures in our previous publications.[22–24]

4.4. Evaluation of curative effect of in vivo

The animal experiments were approved by the Institutional Ethical Committee For Animal Care and Use of the Chinese Academy of Medical Science (CAMS). The experimental procedure was modeled after previous work.[23,24]

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmc.2018.04.025.

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