Bioorganic & Medicinal Chemistry xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

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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ARTICLE INFO

Article history:
Received 4 March 2018
Accepted 10 April 2018
Available online xxxx

Keywords:
Quaternary palmatine
Structure modification
Antibacteria
Anti-ulcerative colitis
Structure-activity relationships

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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https://doi.org/10.1016/j.bmc.2018.04.025
0968-0896/

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.

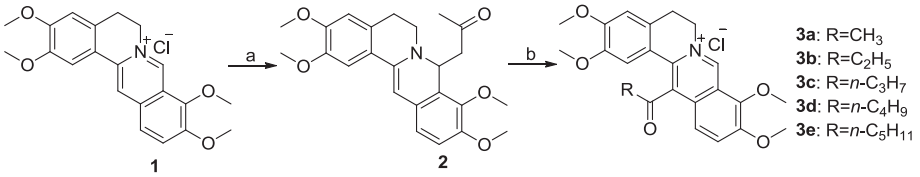
In Scheme 1, the intermediate tertiary amine (±)-8-acetonyldihydropalmatine (**2**) was prepared by utilizing nucleophilic addition of nucleophilic acetonyl anion over the 7,8-imine salt functional group with sodium hydroxide in acetone-water solution being used as catalyst. The target compounds of quaternary 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.