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Short communication

Syntheses and structure–activity relationships in cytotoxicities of 13-substituted quaternary coptisine derivatives

Zhi-Hui Zhang [a], An-Jun Deng [a], Lian-Qiu Wu [a], Lian-Hua Fang [a], Jin-Qian Yu [b], Zhi-Hong Li [a], Tian-Yi Yuan [a], Wen-Jie Wang [a], Guan-Hua Du [a], Hai-Lin Qin [a, *]

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China
[b] Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, PR China

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ABSTRACT

Twenty five 13-substituted quaternary coptisine derivatives were synthesized to test their cytotoxicities against several cancer cell-lines and on intestinal epithelial cell-6 (IEC-6) *in vitro* to evaluate structure–activity relationship (SAR). Introduction of the alkyl groups into the C-13 position of quaternary coptisine (**1**) led to significant increase of the cytotoxic activity, while the substitution of arylmethyl groups and others at the same position showed no effect on improving cytotoxicities against the same cancer cell-lines. The cytotoxicities of quaternary 13-alkylcoptisines was significantly reinforced as the length of the aliphatic chain increased, with quaternary 13-*n*-undecylcoptisine (**4l**) showing 7, 23, 12, and 9 times, respectively, more active than quaternary coptisine (**1**) against HCT, A549, Bel7402, and C33A, and being 4, 11, 2, and 3 times, respectively, more active than the positive control, fluorouracil (5-FU), against the same cell-lines, by IC_{50} values. In comparison to quaternary 13-*n*-undecylcoptisine (**4l**) and the above references, quaternary 13-*n*-dodecylcoptisine (**4m**) almost showed the same cytotoxicities. In contrast with the *n*-alkyl chains, the arylmethyl substituents at C-13 displayed low cytotoxicity, except for naphthyl rings or phenyl rings with CF_3 or methyl substituents. However, their low cytotoxicity could make them useful as drug candidates for other diseases (bowel, etc).

1. Introduction

Alkaloids are well known to be a group of important natural products in medicinal chemistry due to their extensive biological activities. The isoquinoline derivatives, which are widely distributed in several botanical families as natural products, are a category of especially noteworthy alkaloids, with many therapeutic applications having been reported [1].

The basic skeleton of quaternary protoberberine alkaloids (QPAs), which are also classified as benzylisoquinoline alkaloids, is 5, 6-dihydrodibenzo[*a*, *g*]quinolizinium. Quaternary coptisine (**1**) (Fig. 1), which is a typical natural compound of 2, 3, 9, 10-tetrasubstituted QPA and was isolated from some *Coptis* and *Corydalis* species belonging to the plant families of Ranunculaceae and Papaveraceae, respectively, have been demonstrated to exert wide ranges of biological and pharmacological activities, including cytotoxicities and some others [2–12]. From the viewpoint of medicinal chemistry, it is very important to investigate the chemical reactivities of natural active organic compounds and clarify the key structure characteristics involving certain biological and pharmacological activities within their analogs. However, the investigation on the structural modification of coptisine is very rare up to now, with, to the best of our knowledge, only one paper having reported the syntheses and antimicrobial activity of 8-alkylcoptisines, which didn't involve the cytotoxic activity of coptisine derivatives [13]. Herein, we would like to report the first syntheses and structure–activity relationship (SAR) investigation of cytotoxic and non-cytotoxic quaternary 13-substituted coptisine derivatives.

Abbreviations: FU, fluorouracil; QPA, quaternary protoberberine alkaloid; SAR, structure–activity relationship; HCT, human colon cancer cell; A549, human lung cancer cell; Bel7402, human hepatoma cell; C33A, human cervical cancer cell; IEC, intestinal epithelial cell.

* Corresponding author. Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, PR China.
E-mail addresses: zhangzhihui@imm.ac.cn (Z.-H. Zhang), denganjun@imm.ac.cn (A.-J. Deng), wlq@imm.ac.cn (L.-Q. Wu), fanglh@imm.ac.cn (L.-H. Fang), yujinqian87528@126.com (J.-Q. Yu), zhl@imm.ac.cn (Z.-H. Li), yuantianyi@imm.ac.cn (T.-Y. Yuan), wwenj@imm.ac.cn (W.-J. Wang), dugh@imm.ac.cn (G.-H. Du), qinhailin@imm.ac.cn (H.-L. Qin).

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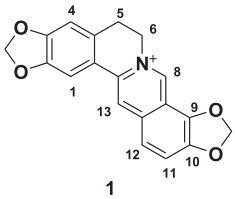

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Fig. 1. Structure of quaternary coptisine (**1**).

2. Results and discussion

2.1. Chemistry

In this study, the target compounds were synthesized by introduction of various aliphatic alkyl, arylmethyl, alkoxycarbonyl, and alkoxycarbonylmethyl groups into the C-13 position of lead compound, quaternary coptisine (**1**), to improve the cytotoxicities against some cancer cell-lines and on intestinal epithelial cell-6 (IEC-6).

The syntheses of the quaternary 13-substituted alkylcoptisine derivatives involved three steps, as shown in Scheme 1. Reduction of starting material quaternary coptisine (**1**), which was isolated and purified from natural resources of *Coptis chinensis*, with sodium borohydride ($NaBH_4$) and potassium carbonate (K_2CO_3) gave the key synthetic precursor dihydrocoptisine (**2**). Subsequently, in the presence of acetic acid, the enamine **2** was reacted with commercial aliphatic aldehydes under reflux. The condensation products underwent aromatization giving the acetates **3a**–**m**. For this step, according to our investigation, a solution in 80% ethanol was preferable to other solvents. Finally, the reaction of **3a**–**m** with 2N·HCl provided the target compounds **4a**–**m** in modest yield. The syntheses of these 13-alkylcoptisine derivatives are similar to those reported for the introduction of alkyl chains at C-13 of related protoberberine alkaloids [14–17]. For the synthesis of quaternary 13-substituted arylmethylcoptisines (**4n**–**w**), ethyl coptisine-13-formate (**4x**), and ethyl coptisine-13-acetate (**4y**), 8-acetonyldihydrocoptisine (**5**) was prepared as a key intermediate in the first place by condensation (nucleophilic addition) of coptisine with acetone under alkaline condition. The treatment of **5** with various substituted benzyl bromides or naphth-2-yl-methyl bromide or ethyl chloroformate or ethyl bromoacetate and sodium iodide (NaI) gave the corresponding 13-substituted coptisine halides with removal of acetone from C-8 and ring aromatization. The bromides were converted into chlorides (**4n**–**y**) of yellow-orange powder using 2N·HCl in MeOH (Scheme 2) [18–20]. The structures of all the intermediates and final quaternary substituted coptisines were elucidated by ^1H and ^{13}C NMR spectroscopic analyses and mass spectrometry. The target compounds showed disappearance of the H-13 signals (at δ 8.96 in quaternary coptisine). The high-field signals for the aliphatic chain moiety or the low-field signals for the aromatic parts in the linker-groups were particularly detected (see Experimental section).

2.2. Biological evaluation

In order to establish SAR among the prepared quaternary 13-substituted coptisine analogs, all of the synthesized compounds were investigated preliminarily for their cytotoxicities *in vitro* against several human cancer cell-lines in the first place. The well-known antitumor drug, fluorouracil (5-FU), and the starting compound, quaternary coptisine (**1**), were used as positive control and reference compounds. On evaluation of these compounds, all cells were treated continuously with each sample for 96 h. Cell growth was measured using an MTT reduction assay procedure. Results of means of three replicates presented in Table 1 are expressed as the 50% inhibiting concentration for samples to inhibit cell growth (IC_{50}) when the growth inhibition rate (IR) of tested compound was more than the value of 50%. Among the tested compounds, the cytotoxic quaternary 13-substituted alkylcoptisine derivatives were determined to be in the range of 0.59–7.31 μmol of IC_{50} values for their activities against four cancer cell-lines, with the values of IR or IC_{50} of some compounds being significantly superior to that of positive 5-FU (Table 1). On the other hand, the quaternary 13-substituted arylmethyl-/alkoxycarbonyl-/alkoxycarbonylmethyl-coptisine derivatives showed no effect of increasing cytotoxic activity on the corresponding several cancer cell lines, i.e., these compounds showed the IR values of much less than 50% (see Table S1). Thus, from the viewpoint of SAR, some features can be explicitly pointed out, that is, the introduction of the alkyl groups into C-13 position of lead compound plays an important role in the tested cytotoxic activities against the four human cancer cell-lines. The cytotoxicities of quaternary 13-alkylcoptisines were significantly enhanced as the length of the aliphatic chain increased. This conclusion was very compatible with the similar investigation on related protoberberine alkaloids reported in previous works [16]. Among the synthesized quaternary 13-substituted alkylcoptisine analogs, quaternary 13-*n*-decylcoptisine (**4k**), 13-*n*-undecylcoptisine (**4l**), and 13-*n*-dodecylcoptisine (**4m**) showed the most significant activities, with their IC_{50} values ranging from 0.59 to 1.81 μmol. Quaternary 13-*n*-undecylcoptisine (**4l**) was 7, 23, 12, and 9 times, respectively, more active than quaternary coptisine (**1**) against HCT, A549, Bel7402, and C33A cancer cell-lines. The IC_{50} values of compound **1** against the four cancer cell-lines were 5.59, >14.05, >14.05, and >14.05 μmol/L, respectively. Besides, compound **4l** was 4, 11, 2, and 3 times, respectively, more active than the positive control, 5-FU, against the same four cancer cell-lines, by IC_{50} values. In comparison to quaternary 13-*n*-undecylcoptisine (**4l**), 13-*n*-dodecylcoptisine (**4m**) almost showed the same levels in active effects. The correlation of alkyl chain length with increased activities would suggest that lipophilicity, for the most part, contributes to the tested activities. Studies on cytotoxicities *in vivo* of the most potent compounds are in progress.

Another meaningful finding in the current SAR investigation was that, as mention above, the introduction of arylmethyl, alkoxycarbonyl, and alkoxycarbonylmethyl groups into C-13 position of lead compound showed no effect of increasing cytotoxic activity on the corresponding several cancer cell lines. Whereas this potential meaning, the cytotoxicity assay *in vitro* of the synthesized 13-arylmethyl-/alkoxycarbonyl-/alkoxycarbonylmethyl-substituted quaternary coptisine derivatives and others on IEC-6 was carried out using an MTT reduction assay procedure with blank control group. The results at 1×10^{-5} mol/L of each sample are displayed in Fig. 2. It is shown that only compounds **4q** (R = CH_2–C_6H_4-3–CF_3), **4r** (R = CH_2–C_6H_4-3,5-di–CF_3), **4v** (R = CH_2–C_6H_4-4-Me), and **4w** (R = CH_2-naphth-2-yl) displayed certain cytotoxicity on IEC-6 cell line and the others showed nearly no significant cytotoxicity when incubated with the tested cells for 24 h at this concentration. The result after 3 days of incubation was the same as that after 24 h (data not shown here) described above. Thus, a kind of possibility was proposed that aromaric methyl and its analogs cause cytotoxicity on IEC-6. It is well known that a compound with low cytotoxicity may be very interesting in exploring other biological activities, such as, in the case of non-cytotoxic 13-substituted quaternary coptisine derivatives, anti-ulcerative colitis, antifungal, and antimicrobial, and so forth, in order to develop other original new drugs. Further investigation of