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Communication

Synthesis of quaternary 8-(1-acylethene-1-yl)-13-methylcoptisine chlorides and their selective growth inhibitory activity between human cancer cell lines and normal intestinal epithelial cell-6

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ABSTRACT

In this paper, quaternary 8-(1-acylethene-1-yl)-13-methylcoptisine chlorides targeting thioredoxin reductases (TrxRs) were designed to test the growth inhibitory activity against human cancer cell lines and the effect on viability of the normal intestinal epithelial cell-6 (IEC-6) *in vitro* and to evaluate structure-activity relationship (SAR). The introduced α, β-unsaturated ketone groups at C-8 consisting of *n*-alkanoyls possessing five to ten carbons or aroyls or cyclohexylcarbonyl increased the tested activity against the target cancer cell lines. By and large, this type of improvement was increasingly graced by the elongation of the aliphatic chain of the *n*-alkanoyls in the range of less than ten carbon atoms. The relatively more polar 1-acylethene-1-yls displayed no effect on improving the activity. All the explored aroyls showed significant effect on improving the activity of the target compounds against the tested cancer cell lines with no SAR being observed. The findings of this study suggested that oil/water partition coefficient of the test compounds was one of the key factors impacting the target activity against the tested cancer cell lines. At the concentration of 10 μmol/L, except for the compounds with *n*-alkanoyls possessing seven or more carbons or with α-naphthoyl, none of the other compounds displayed obvious cytotoxicity on normal IEC-6 cell when co-incubated. The survival rate of IEC-6 cell ranged from 75% to 100% for the noncytotoxic compounds.

Quaternary coptisine (**1**, Fig. 1) is a typical natural quaternary protoberberine alkaloid (QPA). It was obtained previously from several *Coptis* species of the Ranunculaceae family and *Corydalis* species of the Papaveraceae family [1,2]. The existence of two aromatic methylenedioxy groups at C-2/3 and C-9/10 positions, respectively, on the numbering system of QPA is its distinctive structural features compared with other natural QPAs. The 7,8-imine salt functional group is one of its most active sites for chemical reaction. Quaternary coptisine was reported to possess certain biological and pharmacological activities worthy of inquiring for drug development, such as the properties of attenuating obesity-related inflammation [3], anti-alzheimer's disease [4], anti-hypercholesterolaema [5], anti-osteosarcoma [6], anti-fungus [7], cardioprotection [8,9], and anti-proliferation of vascular smooth muscle cells [10,11], among others [12–14]. But, by and large, extensive investigation into the medicinal chemistry of quaternary coptisine is relative scarce to date [15–17], especially compared with another well-known natural QPA, quaternary berberine. In view of this situation, certain structural modifications on quaternary coptisine and pharmacological investigations with the synthesized compounds were conducted recently in our laboratory. Several coptisine analogues were proved to be potential candidates for developing new drugs, such as anti-Ulcerative colitis (anti-UC) new drug [15–17].

Over the past several years, the selenoprotein thioredoxin reductases (TrxRs) as a potential target for cancer treatment attracted the attention of pharmacologists and medicinal chemists. The Michael acceptor of α, β-unsaturated carbonyl as a key structural feature was reported to be capable of improving the antitumor property of explored substrates and cancer chemotherapeutic drugs *via* a mechanism of targeting TrxRs inhibitively [18–26]. In our ongoing studies into the medicinal chemistry of

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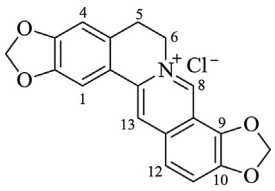


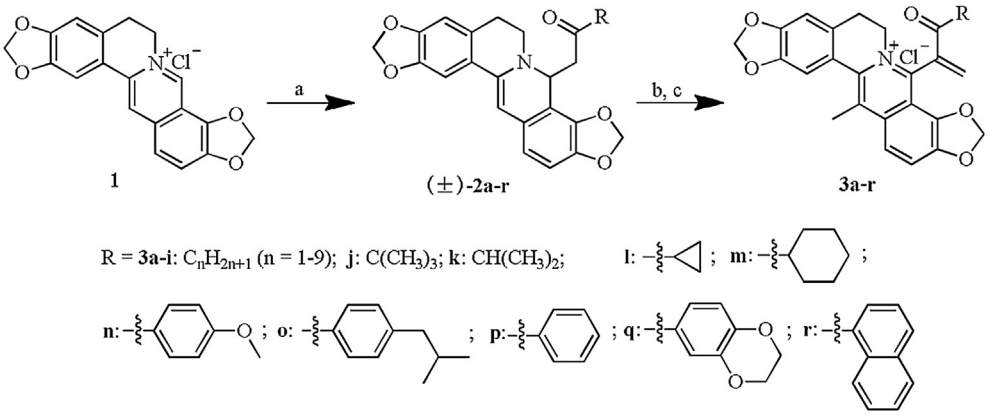
1

Fig. 1. Structure of **1**.

quaternary coptisine, the advantage of 7,8-imine salt functional group as chemically more active site than other structural moieties was again taken to carry out the structural modification to introduce an α, β-unsaturated ketone moiety, *i.e.*, 1-acylethene-1-yl, at C-8 position. Eighteen target quaternary ammoniums were synthesized and different kinds of acyls were involved. In the course of synthesizing the target compounds, a methyl was inevitably introduced to C-13 when the Michael acceptor of α, β-unsaturated ketone moiety was built. Thus, the synthesized target compounds were actually quaternary 8-(1-acylethene-1-yl)-13-methylcoptisine chlorides. This paper describes the design, synthesis, and structural identification of target compounds. It also describes the pharmacological studies on the synthesized compounds, including the screening of the growth inhibitory activity against three human cancer cell lines, the effect on the viability of normal intestinal epithelial cell-6 (IEC-6) *in vitro*, the evaluation of efficacy by IC_{50} values as an indicator, and the structure-activity relationship (SAR) analysis of the growth inhibitory activity.

Under the conditions of building the α, β-unsaturated ketone moiety at C-8 *via* reactions of nucleophilic addition, aldol condensation, and dehydration of alcohol using methyl ketones and formaldehyde as reagents, a methyl was inevitably introduced at C-13 of the substrate. The synthesis of the desired quaternary 8-(1-acylethene-1-yl)-13-methylcoptisine chlorides as target compounds were modeled after our earlier study *via* the same three-step sequences as that indicated by Scheme 1. For a systematic goal to screen the growth inhibitory activity against the target cancer cell lines and to evaluate the SAR, the considered acyl groups involved *n*-alkanoyls, branched chain alkanoyls, cycloalkylcarbonyls, and aroyls. The *n*-alkanoyls involved those containing two to ten carbon atoms (**a**-**i**); the branched chain alkanoyls involved *tert*-butylcarbonyl (**j**) and *iso*-propylcarbonyl (**k**); the cycloalkylcarbonyls involved cyclopropylcarbonyl (**l**) and cyclohexylcarbonyl (**m**); and the aroyls involved 4-methoxybenzoyl (**n**), 4-isobutylbenzoyl (**o**), benzoyl (**p**), 2,3-dihydrobenzo[*b*][1,4]dioxin-6-ylcarbonyl (**q**), and naphthalen-1-yl-carbonyl (**r**). As pointed out in our previous study, in addition to the purification of (±)-8-acetonyldihydrocoptisine [(±)-**2a**] as one of the intermediates, which was achieved by recrystallization from acetone solvent, all the other key intermediates of (±)-8-acylmethyl-substituted dihydrocoptisines [(±)-**2b-r**] were directly applied in the next reaction step to conduct the syntheses of target compounds without any processing endeavor because there were no facile approach to separate and purify those intermediates. The experimental results affirmed the feasibility of this strategy. And, in addition to the reported modest yields between 22.2% and 71.5% from **1** for the synthesis of compounds **3a**-**d**, **3j**-**l**, **3n**, and **3o**, the new synthesized congeners **3e**-**i**, **3m**, and **3p**-**r** were obtained at yields between 17.8% and 71.5% from **1**. The structures of all the synthesized compounds were identified by the combination of the ^{1}H NMR and ^{13}C NMR data and ESIMS data (see Supporting information). In addition, some other chemical modifications of **1** were conducted in this study, including the reported inactive quaternary 13-methylcoptisine chloride (**4a**), which was considered as a pseudosubstrate for the synthesized compounds according to its structural features, and active quaternary 13-*n*-undecylcoptisine chloride (**4l**) and quaternary 13-*n*-dodecylcoptisine chloride (**4m**) [17], both of which were used as reference compounds and positive control to assess the effect of the α, β-unsaturated ketone moiety introduced into quaternary coptisine on the growth inhibitory activity against human cancer cell lines.

In order to evaluate the effect of the introduced α, β-unsaturated ketone moieties on the growth inhibitory activity of the target compounds against human cancer cell lines and to clarify the SAR, all the synthesized compounds **3a**-**r** were investigated for their activity *in vitro* using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. Quaternary coptisine (**1**), quaternary 13-methylcoptisine chloride (**4a**), quaternary 13-*n*-undecylcoptisine chloride (**4l**), and quaternary 13-*n*-dodecylcoptisine chloride (**4m**) were screened in the same batches of experiments. The explored human cancer cell lines included human lung adenocarcinoma (A-549), human hepatoma (Bel7402), and human colorectal cancer (HCT-8) cell lines. On evaluation of these compounds, all target cancer cells were treated successively using every tested compound for 96 h, respectively. The MTT reduction assay procedure was modeled after our earlier study [17]. Results of means of three replicates presented in Table 1 were expressed as the concentration for samples to inhibit the cell growth by 50%, *i.e.*, the IC_{50} values, when the growth inhibition rate (GIR) of tested compounds was more than the value of 50%. Among the tested compounds, the active quaternary 8-(1-acylethene-1-yl)-13-methylcoptisine chlorides were defined by the IC_{50} values in the range of 1.23–9.31 μmol/L. Several compounds were found to show the IC_{50} values at the same level as those of the reference compounds, **4l** and **4m**, in the present study. On this benchmark,



Scheme 1. Reagents and conditions for syntheses of (±)-**2a**-**r** and **3a**-**r**. (a) Methyl ketone, 5 mol/L NaOH, 60 °C; (b) HCHO, THF, CH_3COOH, reflux; (c) 2 mol/L HCl, CH_3OH, r.t.

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