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Synthesis and tumor cell growth inhibitory activity of biotinylated annonaceous acetogenins

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ABSTRACT

Nineteen biotinylated squamocin/bullatacin derivatives have been synthesized for targeted delivery to biotin receptor overexpressed tumor cells. Most biotinylated squamocin and bullatacin derivatives show similar *in vitro* cytotoxicity against the biotin receptor non-overexpressed L1210 cells as squamocin and bullatacin, respectively, while against biotin receptor overexpressed 4T1 and P815 tumor cells, several derivatives show significantly higher potency and better selectivity. Among all the synthesized compounds, 15,28-di-*O*-(6-biotinylamidohexanoyl)squamocin (**16**) is the most potent, which is 10 and 26 times more active than squamocin against 4T1 and P815 cells, respectively. Compound **16** also appears to be six and fifteen times more selective than squamocin towards 4T1 and P815 cells, respectively, against L1210 cells. The structure activity relationship analysis has revealed that the preferred site for biotinylation is different for squamocin and bullatacin, and it also depends on whether a linking spacer is present.

1. Introduction

Annonaceous acetogenins (ACGs) are secondary metabolites occurring only in some plants of Annonaceae family. They are derivatives of long-chain fatty acid (C_{32} or C_{34}) bearing a terminal γ-lactone ring and have been reported to potently inhibit the activity of NADH-ubiquinone oxidoreductase (respiratory complex I) [1–4]. These long-chain fatty acid derivatives display impressive cytotoxicity against various tumor cell lines with an IC_{50} value ranging from 10^{-6} M to 10^{-14} M [5,6], as well as *in vivo* antitumor effect [7]. Squamocin (**1**) and bullatacin (**2**) (Fig. 1) are two best known examples of annonaceous acetogenins belonging to adjacent bis-THF (tetrahydrofuran) type of acetogenins [8]. They both possess a terminal α,β-unsaturated γ-lactone and a central polar part consisting of two THF rings and three secondary alcohol functional groups. Squamocin (**1**) has good activity with clear inhibitory effect on the growth of HepS tumor in mice (with an inhibition rate of 78% at a dose of 30 μg/kg) [9]. It has been shown that squamocin is more potent than piericidin-A, previously considered to be the most potent complex I inhibitor known [10]. Bullatacin (**2**), one of the most potent acetogenins, is effective in treating P388 [11] and L1210 [12] murine leukemias in normal mice. It also displays high cytotoxicity against A2780 [12] human ovarian carcinoma xenografts in athymic mice at a dose of only 50 μg/kg per day which is 300 times more potent than the *in vivo* activity of taxol [12,13].

Both bullatacin and squamocin have significant cytotoxicity on cancer cells and their antitumor effect has been demonstrated in several tumor models; however, their lack of tumor specificity and high toxicity toward normal cells prevents their use in clinic for cancer treatment [14,15]. In order to enhance the therapeutic potential and reduce systemic toxicity, selective targeting to cancer cells is important. Targeted cancer therapies are currently being developed for more effective treatments of cancer with fewer side effects [16–18]. These therapies are thought to be less harmful to normal cells by focusing on molecular and cellular changes that are specific to cancer cells.

Most tumor-specific delivery systems are based on carrier-mediated delivery to selective tissues and organs through ligands recognized by receptors or other address molecules which are overexpressed on target cancer cells [19]. Several ligands such as antibodies [20–22], peptides [23–25], vitamin [26–29], and integrin [30,31] could be used as suitable targeting moieties in targeted drug delivery systems [32,33].

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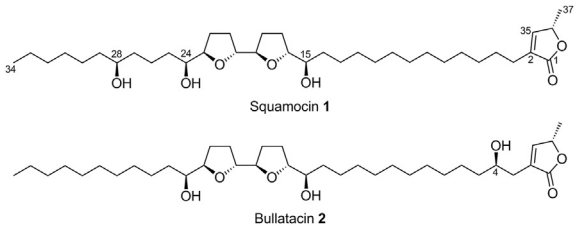


Fig. 1. Structures of squamocin and bullatacin.

Biotin (vitamin B_7, vitamin H) belongs to a category of essential micronutrients required for normal cellular functions [32,34]. It is a growth promoter at cellular level and its content in tumors is substantially higher than in normal tissues [35] due to the rapidly growing nature of cancer cells. Consequently, the receptors involved in the uptake of biotin are overexpressed in many cancer cells, such as leukemia (L1210FR), ovarian (OV 2008, ID8), Colon (Colo-26), mastocytoma (P815), lung (M109), renal (RENCA, RD0995), and breast (4T1, JC, MMT06056) cancer cell lines [32,36]. Recently, biotin receptors have been explored for targeted delivery of cytotoxic drugs to cancer cells by covalently linking the drug molecule to biotin [34,37–40]. For example, Ojima and coworkers demonstrated that biotin–taxoid conjugates exhibited much higher potency against L1210FR cells that overexpress biotin receptors on cell surfaces than other two cell lines (L1210 and W138) which do not overexpress biotin receptors on cell surfaces [32,41].

In order to improve the therapeutic potential of squamocin and bullatacin for cancer therapy, we decide to covalently link biotin to these molecules to generate biotin–acetogenin conjugates such as conjugate I (Fig. 2). Selective delivery of such conjugates to biotin overexpressed cancer cells and subsequent uptake into cytosome are anticipated to be mediated by biotin receptors. The targeting component biotin and/or the linking spacer in conjugate I will be readily cleaved to release the cytotoxic drug squamocin. Reported here are the chemical synthesis of a series of biotinylated squamocin and biotinylated bullatacin conjugates and their growth inhibitory activity against cancer cells.

2. Results and discussion

2.1. Synthesis and structure characterization

Generally, a tumor targeting drug delivery system consists of a tumor recognition moiety and a cytotoxic warhead connected directly or through a suitable linker to form a conjugate [42]. There are three secondary alcohol functional groups in squamocin and bullatacin, which play less important role in their activity [43]. In this study we have designed two groups of squamocin/bullatacin derivatives by attaching the biotin residue either (a) directly or (b) through an ε-aminocaproic acid linking spacer to one of the free

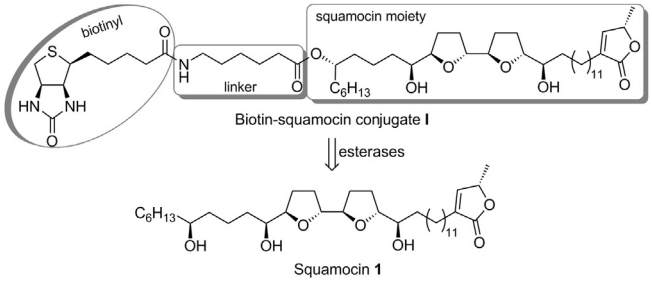
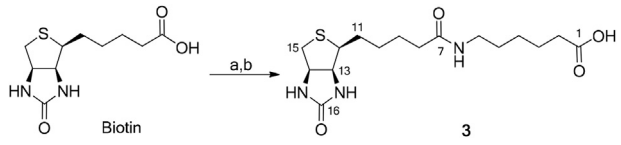


Fig. 2. General structure of biotin–squamocin conjugate **I** and the presumed cleavage mechanism to release the active drug.

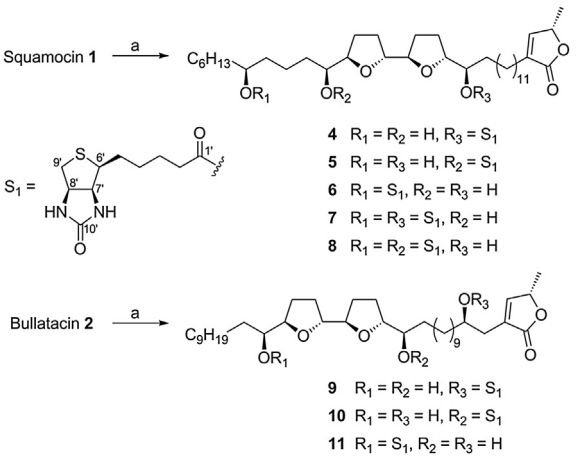


Scheme 1. Reagents and conditions: (a) DCC, DMF, rt, N_2, 16 h. (b) ε-aminocaproic acid, $NaHCO_3$, H_2O-DMF, rt, 16 h, 50%.

hydroxyl groups. The biotinylating agent containing the spacer (**3**, Scheme 1) can be readily prepared from biotin and ε-aminocaproic acid as reported [44].

The coupling between biotin/**3** and squamocin/bullatacin is mediated by dicyclohexylcarbodiimide (DCC) and *N*,*N*-dimethyl-4-aminopyridien (DMAP). This coupling condition is mild and does not affect the labile terminal α,β-unsaturated γ-lactone functionality. Thus, a series of biotinylated squamocin and bullatacin derivatives **4**–**22** were synthesized when biotin and biotin derivative **3** were treated with squamocin and bullatacin, respectively (Schemes 2 and 3). Although some selectivity was observed among the secondary hydroxyl groups in both squamocin and bullatacin toward acylation under this reaction condition, in most cases the selectivity was low (Table 1). The reaction typically resulted in a mixture of mono- and di-biotinylated derivatives when 1.1–1.5 equivalents of the acylating acid was used. After extensive purification of the crude products by silica gel chromatography and preparative HPLC, all products were purified and structurally confirmed.

Structure characterization was established by the analysis of 1D (^{1}H, ^{13}C and DEPT) and 2D (COSY, HSQC and HMBC) NMR spectra, and HR-ESIMS data. The ^{1}H and ^{13}C chemical shifts of the biotinylated derivatives were compared with those of squamocin or bullatacin. When the secondary hydroxyl group was esterified, the chemical shifts of the carbon (C-4, C-15, C-24, and C-28) bearing that hydroxyl group moved downfield (Table 2). Up to 3.6 ppm downfield shift was observed for C-24 in compound **11** while the shift for C-15 was relatively small (<1 ppm) in all acylated derivatives. On the other hand, the chemical shifts of the neighboring carbons (C-5, C-14, C-16, C-23, C-25, C-27, and C-29) moved upfield (in a magnitude of 1.5–3.7 ppm) upon esterification of the specific hydroxyl group. In the case of ^{1}H NMR data, a significant downfield shift (>1.1 ppm) was observed for the proton directly attached to the carbon bearing the hydroxyl group that is acylated (Table 3). The ^{1}H NMR chemical shifts of the protons observed here are



Scheme 2. Synthesis of biotinylsquamocins **4**–**8** and biotinylbullatacins **9**–**11**. Reagents and conditions: (a) Biotin, DCC, DMAP, DMF, CH_2Cl_2, rt, N_2, 16 h.