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Synthesis and biological evaluation of bufalin-3-yl nitrogen-containing-carbamate derivatives as anticancer agents

Min Lei [a,b,*], Zhiyong Xiao [a], Biao Ma [a], Yijia Chen [a], Miao Liu [a], Junhua Liu [a,b], De'an Guo [a], Xuan Liu [a,*], Lihong Hu [a,*]

[a] Shanghai Research Center for the Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

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ABSTRACT

A series of bufalin-3-yl nitrogen-containing-carbamate derivatives **3** were designed, synthesized, and evaluated for their proliferation inhibition activities against human cervical epithelial adenocarcinoma (HeLa) cell line. The structure–activity relationships (SARs) of this new series are described in this paper. Cytotoxicity data revealed that the C3 moiety had an important influence on cytotoxic activity. Compound **3i-HCl** exhibited significant *in vitro* antiproliferative activity against the ten tested tumor cell lines, with IC_{50} values ranging from 0.30 to 1.09 nM. Furthermore, **3i-HCl** can significantly inhibit tumor growth by 100% at the dose 2 mg/kg by iv, or 4 mg/kg by ig.

1. Introduction

Cardiac glycosides are a class of natural compounds with prominent cardiotonic effects. In clinical research, compounds such as digoxin, digitoxin, lanatosaide C, strophanthin K, and divarcosise, are used widely as drugs for treatment of heart disease. In 1960s, Shiratori [1] and Hartwell et al. [2] reported that cardiac glycosides exhibited significant antitumor activities *in vitro*, respectively. From then on, many papers have been published on studies of cardiac glycosides as antitumor therapeutic agents [3–8]. In recent years, several cardiac glycosides, such as Anvirzel [9], PBI-05204 [10], and UNBS-1450 [11], are being tested in phase I and II clinical trials as new anti-cancer agents. Cardiac glycosides as novel drugs for treatment of tumor might be a promising research area.

Chansu is a Traditional Chinese Medicine made from the dried skin and parotid venom glands of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider. Huachansu injection, the water-soluble extract of the dried skin of *Bufo bufo gargarizans* Cantor, has been successfully used in the clinic for treatment of various cancers in China. Bufalin (Fig. 1), a cardiac glycoside, is one of the major active components of Chansu. As is known in the literature, bufalin exhibits remarkable cytotoxic activity with IC_{50} values of 10^{-8} to 10^{-9} mol/L for various tumor cells including human prostate carcinoma cells (PC3, DU145) [12], human leukemia cells (U937, HL60) [13,14], human cervical carcinoma cells (HeLa) [15] and non-small-cell lung cancer cells (A549) [16]. Although the antitumor mechanism of bufalin has not been reported, bufalin, as with other cardiac glycosides, could bind to Na^+/K^+-ATPase and inhibit the Na^+/K^+ pump [17–20].

Through years of research, the antitumor activity of cardiac glycosides *in vivo* and *in vitro* has been widely recognized. However, these compounds exhibited high toxicity which severely limits their clinical application. Hence, reducing toxicity, enhancing activity and improving drug-like properties of cardiac glycosides might be an important strategy for the structural modification. Bufalin, just as other cardiac glycosides, has the toxicity problems. In our previous study, we found that bufalin-3-yl piperidin-4-carboxylate (IC_{50} value on HeLa 0.76 nM) displayed significant cytotoxic potency compared to the parent bufalin (IC_{50} value on HeLa 26.3 nM) [16]. These results suggest that the hydroxyl on C3 of bufalin is a suitable site for the structural modification.

Although bufalin-3-yl piperidin-4-carboxylate showed significant cytotoxic potency *in vitro*, this compound was inactive *in vivo*. We speculated that the ester was prone to metabolism *in vivo* because ester groups are susceptible to hydrolysis by

* Corresponding authors at: Shanghai Research Center for the Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China (M. Lei).
E-mail addresses: mlei@simm.ac.cn (M. Lei), xuanliu@simm.ac.cn (X. Liu), lhhu@simm.ac.cn (L. Hu).

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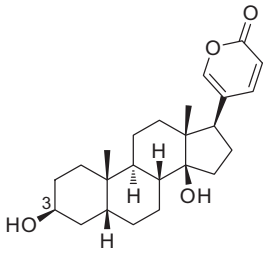
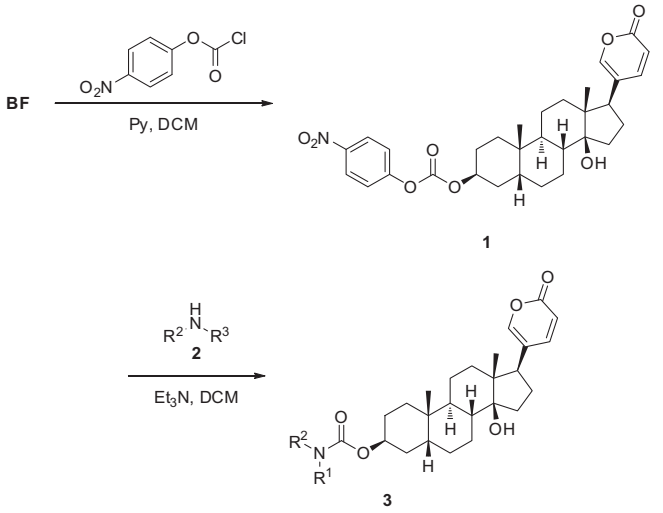


Fig. 1. The structure of bufalin (BF).

esterases in the blood. Therefore, we designed bufalin-3-yl piperazine-1-carbamate by bioisosteric replacement (Fig. 2), and the activity test indicated that this compound could maintain cytotoxic potency (IC_{50} (HeLa) = 0.77 nM). In order to find new compounds with better cytotoxic potency, a series of bufalin-3-yl nitrogen-containing-carbamate derivatives were designed, synthesized, and evaluated for their proliferation inhibition activities.



Scheme 1. The synthesis of bufalin-3-yl carbamate derivatives **3**.

2. Experimental

A two-step reaction route was designed for the synthesis of bufalin-3-yl carbamate derivatives as shown in Scheme 1. In the first step, bufalin was reacted with 4-nitrophenyl chloroformate in DCM using pyridine as the base to obtain the intermediate **1**. Then, **1** was reacted with amine **2** in the presence of Et_3N to obtain the desired products **3**.

All reactions were performed under a nitrogen atmosphere with dry solvents in oven-baked or flame-dried glassware, unless otherwise noted. All reagents were commercially available, and were used without further purification unless otherwise specified. All solvents were redistilled under argon atmosphere. The progress of reactions were monitored by thin layer chromatography (TLC) plates (silica gel 60GF, Yantai jiangyou company) visualized with 254-nm UV light and/or by staining with vanillin solution (2.7 g vanillin + 100 mL H_2O + 35 mL concentrated H_2SO_4 diluted to 300 mL with 95% ethanol). Melting points were measured by a WRS-1B micromelting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury-VX300 Fourier transform spectrometer or a Bruker AM-400 spectrometer, and chemical shifts (δ) were reported in ppm; the hydrogenated residues of deuterated solvent were used as internal standard $CDCl_3$: 7.26 ppm for ^1H NMR and 77.00 ppm for ^{13}C NMR. ESIMS was run on a Bruker Esquire 3000 plus spectrometer in MeOH. HRESIMS were determined on a Micromass Q-TOF Global mass spectrometer.

2.1. General synthesis of bufalin-3-yl carbamate derivatives

To a stirred solution of bufalin (100 mg, 0.28 mmol) and 4-nitrophenyl chloroformate (129 mg, 0.64 mmol, 2.3 eq) in anhydrous DCM (2 mL) at room temperature under nitrogen gas was added pyridine (50 mg, 0.64 mmol, 2.3 eq), and stirring continued for 2 h. After completion (by TLC), DCM (10 mL), and H_2O (10 mL) were added. The organic phase was separated, washed with aq. Na_2CO_3, H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo to afford yellow residue 2. To a solution of the residue in DCM (2.5 mL) was added amine (3 eq) and Et_3N (3 eq), and stirring continued at room temperature for 2 h. After completion (by TLC), DCM (15 mL) and H_2O (15 mL) were added. The organic phase was separated, washed with aq. Na_2CO_3 and brine, dried over Na_2SO_4, and concentrated in vacuo to give yellow residue, and the residue was subjected to chromatographic separation on silica gel (30 g) with petroleum ether/acetone/triethylamine (100:30:1 v/v/v).

2.1.1. Compound *3a*

3a (77 mg, 55%) mp 199–201 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.83 (dd, 1H, J = 9.6, 2.4), 7.22 (s, 1H), 6.25 (d, 1H, J = 9.6), 4.98 (brs, 1H), 3.27 (q, 2H, J = 6.0, 5.4), 2.75 (t, 2H, J = 6.0), 2.65 (q, 2H, J = 6.9), 1.10 (t, 3H, J = 7.2), 0.93 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.6, 156.7, 148.7, 147.0, 122.9, 115.5, 85.5, 70.8, 51.5,49.1, 48.6, 44.0, 42.5, 41.1, 40.8, 37.0, 36.0, 35.3, 33.0, 30.9, 29.9, 28.9, 26.6, 25.5, 21.6, 23.9, 21.5, 16.7, 15.4; MS (ESI) m/z: 501.4 $[M+H]^+$; HRMS (ESI): calcd for $C_{29}H_{45}N_2O_5$ $[M+H]^+$ 501.3323, found 501.3310.

2.1.2. Compound *3b*

3b (89 mg, 60%) mp 198–200 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.84 (dd, 1H, J = 9.9, 2.4), 7.22 (d, 1H, J = 2.4), 6.25 (d, 1H, J = 9.6), 4.98 (br s, 1H), 3.23 (br d, 2H, J = 5.4), 2.55 (m, 6H), 1.02 (t, 6H,

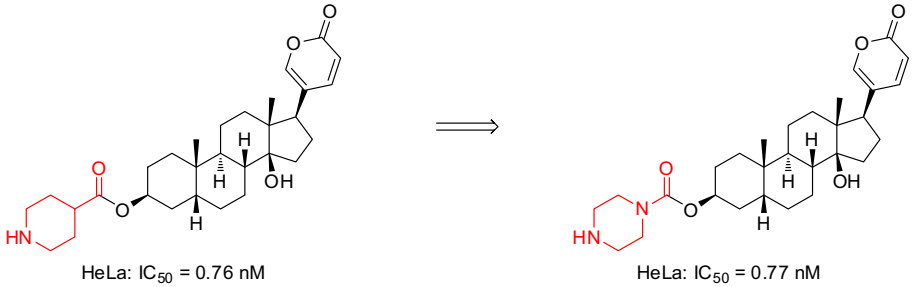


Fig. 2. The design of bufalin-3-yl carbamate derivatives.