Steroids 125 (2017) 54–60

Contents lists available at ScienceDirect

Steroids

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

The synthesis and antitumor activity of lithocholic acid and its derivatives

Xiao-Long He[a], Yajing Xing[b], Xiang-Zhong Gu[c], Jie-Xin Xiao[a], Ying-Ying Wang[a], Zhengfang Yi[b], Wen-Wei Qiu[a,*]

[a] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Chemical Engineering, East China Normal University, Shanghai 200241, China
[b] Shanghai Key Laboratory of Regulatory Biology, Institute of Biomedical Sciences and School of Life Sciences, East China Normal University, Shanghai 200241, China
[c] Department of Research and Development, Jiangsu Jiaerke Pharmaceuticals Group Co Ltd., Zhenglu Town, Changzhou 213111, China

ARTICLE INFO

Keywords:
Lithocholic acid
Antitumor
Apoptosis
Migration
Breast cancer

ABSTRACT

In this paper, a new and concise synthetic route of lithocholic acid (LCA) using commercially available steroid source deoxycholic acid is reported. A series of amide derivatives of LCA were also synthesized and investigated for their activity against the growth of MCF-7 and MCF-7/ADR cells using the sulforhodamine B assay. For MCF-7, the most potent compound **20** showed a 20-fold higher antitumor activity than LCA. For MCF-7/ADR, the most potent compound **24** showed a 22-fold higher antitumor activity than LCA. The transwell migration assay of **20** was evaluated on MDA-MB-231 cells. The colony formation and apoptosis assays of **20** were performed on MCF-7 and MCF-7/ADR cell lines.

1. Introduction

Lithocholic acid (LCA) is a hydrophobic secondary bile acid, formed by bacterial 7-dehydroxylation of chenodeoxycholic acid and ursodeoxycholic acid [1,2]. Recently, LCA and its derivatives attracted the focus of research interests due to their variety of biological activities, such as α-2,3-sialyltransferase inhibition [3], protein tyrosine phosphatase 1B inhibition [4], vitamin D receptor modulation [5,6], TGR5 receptor activation [7], antibacterial and antifungal [8], anti-aging [9] and especially antitumor activity [10,11]. For instance, LCA not only has the ability to selectively kill the neuroblastoma cell lines while sparing normal neuronal cells [12], but also induces apoptosis selectively in androgen-dependent (LNCaP) and -independent (PC-3) prostate cancer cells [13]; LCA amphiphiles possesses potent anticancer activity against colon cancer cells [14]; amino acid conjugates of LCA can serve as antagonists of the EphA2 receptor as novel anti-angiogenetic agents [15].

Although, LCA and its derivatives play an increasingly important role in biological activity studies of drug discovery, the practical approach for the preparation of LCA is rarely reported. Willard et al. [16] provided LCA (50% overall yield) in 4 steps from methyl deoxycholate, and the key reactions are oxidation of the C12-hydroxy with CrO_3 and reduction of C12-semicarbazone with Na at 200 °C. Fujinori et al. [17] also used the methyl deoxycholate as the starting material for preparation of LCA (7.6% overall yield) in 4 steps, and the key step is producing the C11–C12 alkene by dehydration of the C12-hydroxy with $POCl_3$. Mckenzie et al. [18] reported the synthesis of LCA (23.5% overall yield) in 7 steps from commercially available deoxycholic acid, and the crucial steps are formation of C11–C12 alkene at 300 °C and hydrogenation of the double bond with PtO_2 and H_2. There are deficiencies in above three procedures, such as high temperature, non-environmental friendly or expensive reagents, and very low overall yields. Accordingly, exploitation of an economical and efficient synthetic route of LCA will promote the further development of LCA and its derivatives. Herein, we report an efficient and concise synthetic route of LCA (Scheme 1) and its derivatives (Scheme 2) using inexpensive reagents and commercially available steroid source deoxycholic acid. These synthetic compounds were evaluated for their in vitro activity against breast cancer cell lines (MCF-7 and MCF-7/ADR) using the sulforhodamine B (SRB) assay. The most potent derivative was screened to determine its inhibitory effects on tumor cell migration and colony formation ability, and its apoptotic behavior was also tested.

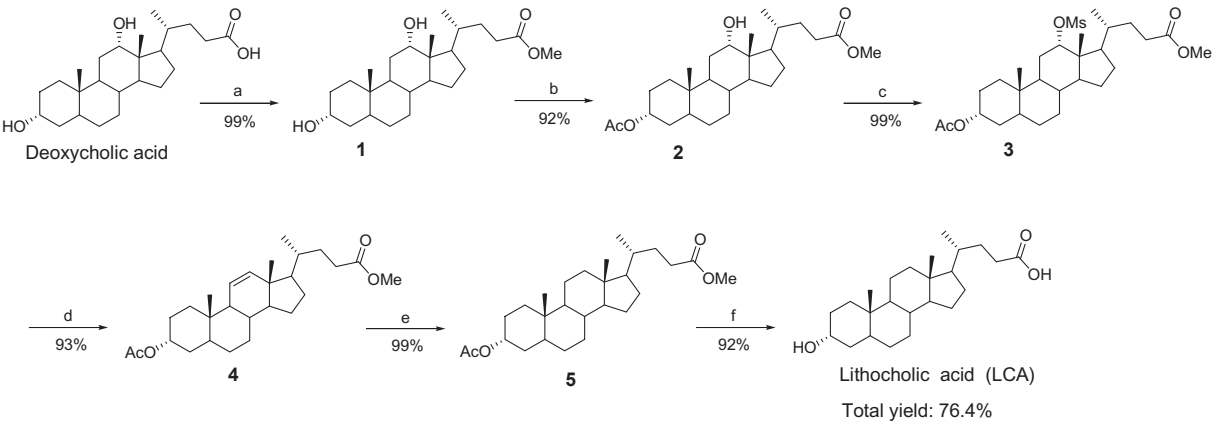
2. Experimental

2.1. General procedures

Starting steroid, deoxycholic acid was purchased from Aladdin Company, China. All the other reagents and chemicals were purchased from commercial suppliers and used without further purification unless otherwise stated. When needed, the reactions were carried out in oven-dried glassware under a positive pressure of dry N_2. Column

* Corresponding author.
E-mail address: wwqiu@chem.ecnu.edu.cn (W.-W. Qiu).

http://dx.doi.org/10.1016/j.steroids.2017.06.009
Received 13 April 2017; Received in revised form 14 May 2017; Accepted 19 June 2017
Available online 23 June 2017
0039-128X/



Scheme 1. Reagents and conditions: (a) CH_3OH, H_2SO_4, reflux, 99%; (b) Ac_2O, Py, DMAP, CH_2Cl_2, rt, 92%; (c) MsCl, Py, CH_2Cl_2, rt, 99%; (d) CH_3COOK, DMPU, 130 °C, 93%; (e) H_2 (5 MPa), Pd, THF, CH_3OH, 70 °C, 99%; (f) K_2CO_3, THF, CH_3OH, 70 °C, 92%.

chromatography was performed on silica gel (QinDao, 200–300 mesh) using the indicated eluents. Thin-layer chromatography was carried out on silica gel plates (QinDao) with a layer thickness of 0.25 mm. Melting points were determined using the MEL-TEMP 3.0 apparatus and uncorrected. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker AM-400 spectrometer with $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard. All chemical shift values were reported in units of δ (ppm). The following abbreviations were used to indicate the peak multiplicity: *s* = singlet; *d* = doublet; *t* = triplet; *m* = multiplet; *br* = broad. High-resolution mass data were obtained on a Bruker microOTOF-Q II spectrometer.

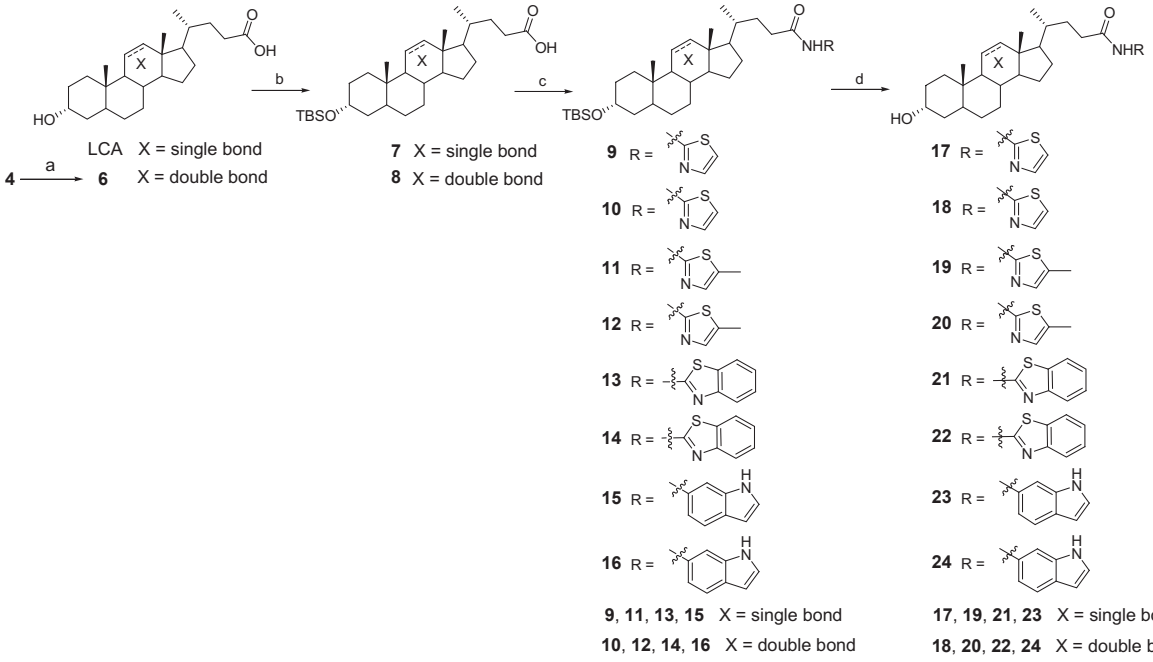
2.2. Chemical synthesis

2.2.1. Methyl 3α,12α-dihydroxy-5β-cholan-24-oate (1)

To a solution of deoxycholic acid (20 g, 50.94 mmol) in CH_3OH (300 mL) was added H_2SO_4 (5 mL). The reaction mixture was refluxed for 2 h and then concentrated. The residue was dissolved in DCM (300 mL). Organic layer was washed with 5% aqueous NaOH and brine, dried over anhydrous Na_2SO_4 and concentrated to give compound **1** (20.6 g, 99%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.97 (s, 1H), 3.66 (s, 3H), 3.64–3.57 (m, 1H), 0.97 (d, J = 6.0 Hz, 3H), 0.91 (s, 3H), 0.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.8, 73.1, 71.7, 51.5, 48.2, 47.2, 46.5, 42.1, 36.4, 36.0, 35.3, 35.2, 34.1, 33.6, 31.1, 30.9, 30.3, 28.6, 27.5, 27.1, 26.1, 23.7, 23.1, 17.2, 12.7.

2.2.2. Methyl 3α-acetoxy-12α-hydroxy-5β-cholan-24-oate (2)

To a solution of **1** (20.6 g, 50.66 mmol) in DCM (300 mL) was added acetic anhydride (6 mL, 63.33 mmol), pyridine (8.2 mL, 101.32 mmol) and DMAP (309 mg, 2.53 mmol). The reaction mixture was stirred for 2 h at room temperature and then poured into 2M HCl (150 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (petroleum ether/AcOEt, 5/1 v/v) to give **2** (20.9 g, 92%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.72–4.64 (m, 1H), 3.97 (s, 1H), 3.64 (s, 3H), 2.00 (s, 3H), 0.95 (d, J = 6.4 Hz, 3H), 0.90 (s, 3H), 0.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.8, 170.8, 74.4, 73.2, 51.6, 48.4, 47.5, 46.6, 42.0, 36.1, 35.2, 35.0, 34.2, 33.7, 32.3, 31.2, 31.0, 28.8, 27.5, 27.1, 26.6, 26.1, 23.7, 23.2, 21.6, 17.4, 12.8.



Scheme 2. Reagents and conditions: (a) K_2CO_3, THF, CH_3OH, 70 °C, 87%. (b) TBSCl, imidazole, DMF, rt, 95%; (c) amines, EDCI, HOBt, DMAP, CH_2Cl_2, rt, 61–83%; (d) TBAF, THF, 60 °C, 88–93%.