European Journal of Medicinal Chemistry 95 (2015) 240–248

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of heterocycle-modified betulinic acid derivatives as antitumor agents

Hai-Wei Cui [a, 1], Yuan He [b, 1], Jinhua Wang [b], Wei Gao [a], Ting Liu [c], Min Qin [b], Xue Wang [b], Cheng Gao [a], Yan Wang [a], Ming-Yao Liu [b], Zhengfang Yi [b, *], Wen-Wei Qiu [a, *]

[a] Department of Chemistry, School of Chemistry and Molecular Engineering, East China Normal University, 500 Dongchuan Road, 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] Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, North Zhongshan Road, Shanghai 200062, China

ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form
11 March 2015
Accepted 20 March 2015
Available online 21 March 2015

Keywords:
Triterpenoids
Betulinic acid
Antitumor
Apoptosis
Migration

ABSTRACT

A series of novel heterocycle-modified betulinic acid (BA) derivatives were synthesized and investigated for their activity against the growth of eight non-drug resistant and one multidrug-resistant tumor cell line using a sulforhodamine B (SRB) assay. The most active compound **17** showed an average IC_{50} 1.19 μM, which was about 20 times more potent than the lead compound BA. It is amazing that for most synthetic saturated N-heterocycle derivatives, MCF-7/ADR was the most sensitive tumor cells, especially **17** showed the most potent antitumor activity (IC_{50} = 0.33 μM) on this multidrug-resistant tumor cell line, that was 117 times more potent than BA. Most of the tested compounds displayed less toxic on human fibroblasts (HAF) in comparison with the tumor cell lines. The cytometry and transwell migration assays were used to test the ability of **17** to induce apoptosis and inhibit metastasis on tumor cell lines respectively.

1. Introduction

Betulinic acid (BA, Scheme 1) is a type of pentacyclic triterpene acid that can be found in several species of plants, principally the white birch [1]. It has a variety of biological activities, such as anti-HIV [2], anti-inflammatory, antimalarial, antimicrobial, and especially antitumor activity [3–5]. BA was initially known for its highly selective antitumor activity against the human melanoma cells [6]. Subsequent studies revealed that this natural product had a broad inhibitory effects in various cancerous tumors, including neuroblastoma, medulloblastoma, Ewing's sarcoma [7], leukemia [8], brain-tumors [9], gliomas [10], colon carcinoma [11], lung, breast, prostate and cervical cancers [12]. The inhibitory mechanisms of BA in various cancerous cells remains to be unraveled detailedly, which have been reported to involve the inhibition of nuclear factor kappa B (NF-κB) [13], topoisomerases I and II [14], and cholesterol acyltransferases (ACAT-1 and ACAT-2) [15], activation of caspases and DNA fragmentation [16], and induction of apoptosis in a CD-95 and p53 independent manners [17,18].

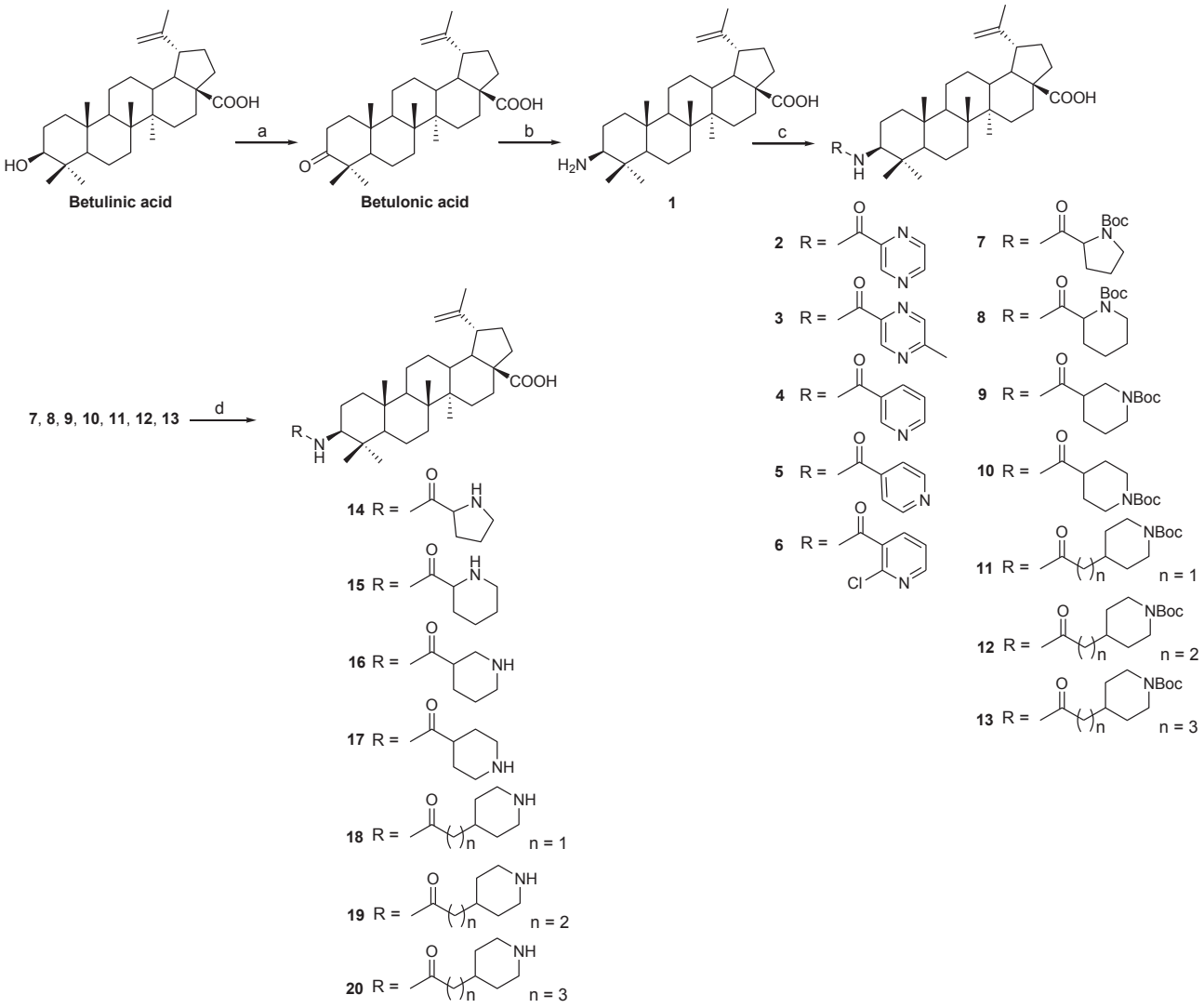
Previous studies suggested that introduction of the nitrogen-containing heterocyclic rings to the pentacyclic triterpenoids can significantly improve the biological activities [19–23]. Thus, in order to search for agents with high antitumor activity and selectivity, a series of BA derivatives were synthesized by introducing nitrogen-containing heterocycles at C-3 position with ester, and especially amide linkages (amides are more stable than esters in metabolism). Their inhibitory activities against the growth of eight non-drug resistant tumor cell lines and one multidrug-resistant breast cancer cell line MCF-7/ADR were evaluated using an SRB assay. BA and its derivatives were screened by flow cytometry to determine their apoptotic behaviors, and their inhibitory effects on tumor cell migration were also tested.

2. Chemistry

A series of heterocycle-modified BA derivatives with amide or ester linkage were synthesized according to the pathways

* Corresponding authors.
E-mail addresses: zfyi@bio.ecnu.edu.cn (Z. Yi), wwqiu@chem.ecnu.edu.cn (W.-W. Qiu).
[1] These authors contributed equally to this work.

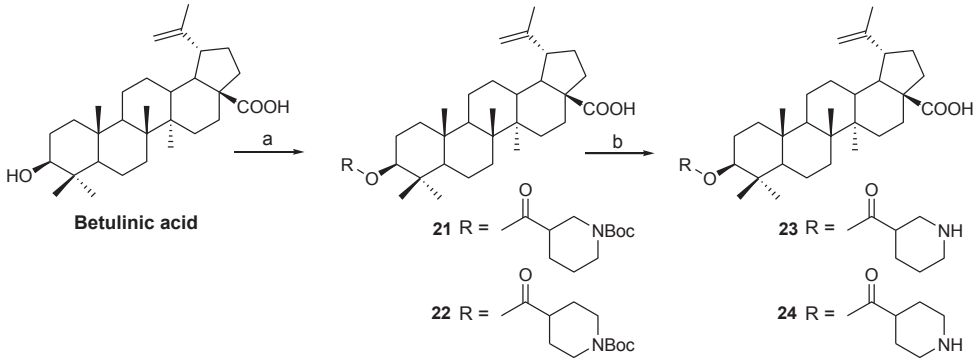
http://dx.doi.org/10.1016/j.ejmech.2015.03.048
0223-5234/



Scheme 1. Synthesis of betulinic acid derivatives **2**–**6** and **14**–**20**. Reagents and conditions: (a) IBX, DMSO, rt, 6 h, 90%; (b) $NaCNBH_3$, CH_3COONH_4, CH_3OH, rt, 12 h, 82%; (c) CDI, DMAP, CH_2Cl_2, rt, 6 h, 73–83% for **2**–**13**; (d) boron trifluoride etherate, Et_2O, rt, 10 min, 75–86% for **14**–**20**.

described in Schemes 1 and 2.

The heterocycle-modified BA derivatives with amide linkage were synthesized as shown in Scheme 1. Oxidation of BA with IBX gave betulonic acid. The important intermediate **1** was obtained by Borch reduction with sodium cyanoborohydride and ammonium acetate in methanol [24]. Unfortunately, the yields of preparing compounds **2**–**6** and intermediates **7**–**13** by condensation of **1** with heterocyclic carboxylic acids under various condensation



Scheme 2. Synthesis of betulinic acid derivatives **23** and **24**. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, rt, 4 h, 79% for **21** and 75% for **22**; (b) boron trifluoride etherate, Et_2O, rt, 10 min, 81% for **23** and 80% for **24**.