European Journal of Medicinal Chemistry 85 (2014) 95–106

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Synthesis and biological evaluation of andrographolide analogues as anti-cancer agents☆

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ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form
23 July 2014
Accepted 24 July 2014
Available online 24 July 2014

Keywords:
Andrographolide
C14 ester analogues
Epoxy diastereomers
Anti-cancer
HEK-293
MCF-7
Normal cells
Apoptosis

ABSTRACT

A new family of andrographolide analogues were synthesized and screened *in vitro* against kidney (HEK-293) and breast (MCF-7) cancer cells. The anti-cancer effects of the active analogues (**2b**, **2c** and **4c**) were determined by multiple cell based assays such as MTT, immunostaining, FACS, western blotting and transcriptional inhibition of NF-κB activity. Importantly, these compounds were found to possess higher anti-cancer potency than andrographolide and low toxicity to normal (VERO and MCF-10A) cells. Increased level of Bax/Bcl-xL ratio, caspase 3, and sub G1 population, higher expression level of tumor suppressor protein p53 and lower expression level of NF-κB suggested potent apoptotic property of the active analogues. Data revealed that the andrographolide derivative-mediated cell death in cancer cells was p53 dependent.

1. Introduction

Andrographis paniculata Nees (Acanthaceae) is considered as one of the most important medicinal plants in India, China and other Asian countries due to its popular use in traditional systems of medicines [1]. Andrographolide **1** (Fig. 1), a major phytoconstituent of the plant, has been recognized as an important pharmacophore because of its key role as inducer of apoptosis against different types of cancers [2] in addition to other pharmacological effects [3] (e.g., anti-viral [3a], anti-inflammatory [3b], antimalarial [3c], anti-hyperglycemic [3d], immunostimulatory [3e] etc.). However, despite its impressive biological activities, the major drawback of andrographolide is poor oral bioavailability [4] making it difficult to prepare formulations for clinical use. Thus, only well-designed derivatives of andrographolide might have the potential to be developed as anti-cancer chemotherapeutic agents. Indeed, a growing interest has been observed in recent times for designing, synthesizing and subsequently screening different analogues of andrographolide in order to discover lead(s) having better pharmacological profile than the parent compound. Towards this endeavor, few promising compounds having ester functionality at C14 of **1** have recently been identified by Stanslas [5a] (14-acetylandrographolide), Nanduri [5b] (14-cinnamoyl-8,17-epoxy-andrographolide), Rajagopal [5c] (DRF 3188), and us [5d] (14-succinylandrographolide). In our previous report [5d], we also established the important role of the α-alkylidene-γ-butyrolactone moiety of andrographolide to account for its cytotoxicity in human leukemic cell lines (U937, K562 and THP1). In continuation of our work [5d,6] in lead identification from bioactive natural products, we therefore became interested to check the anti-cancer potential of the ester analogs of **2** and their epoxy derivatives **3**–**4** as well

Abbreviations: AG, andrographolide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DAPI, 4,6-diamidino-2-phenylindole; PARP, poly(-ADP-ribose)polymerase.

☆ This paper is dedicated to Dr. Pradeep Kumar Dutta, a former scientist and head of Chemistry Division, Indian Institute of Chemical Biology, Kolkata 700032, India.

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http://dx.doi.org/10.1016/j.ejmech.2014.07.088
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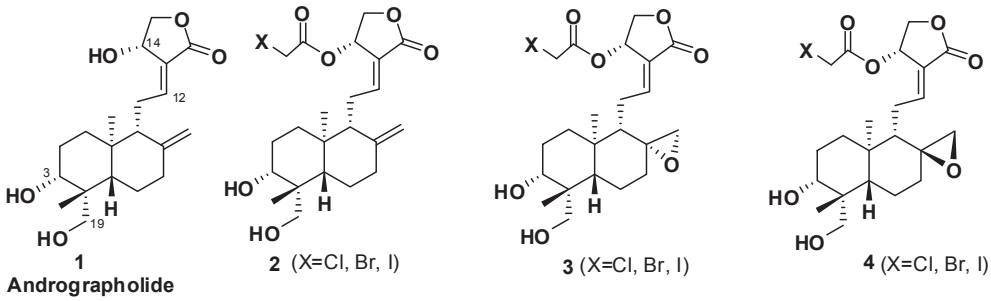

Fig. 1. Andrographolide and its designed analogues.

(Fig. 1). The rationale for choosing the aforesaid compounds was primarily the following: (a) an ester moiety at C14 of andrographolide has been shown to be important for cytotoxicity [5]; (b) the ester moiety (or the halogen in the side chain of the ester moiety) of compounds (**2**–**4**) may serve as good leaving group to facilitate the binding with intracellular glutathione (GSH) [7], thereby triggering suicide of the cell leading to apoptosis; (c) esters might act like prodrugs, being hydrolyzed by esterases or under physiological pH to release andrographolide **1** (or its epoxy derivatives) *in vivo* [8]. Besides, compounds **3**–**4** differing in their epoxide configurations could be important for structure–activity relationship (SAR) studies [9].

Despite several reports on the structural modifications of andrographolide [10], chemo-selective functionalizations at C14 hydroxyl are limited in number [5,11]. In this report, we describe the synthesis of novel C14-ester analogues **2**–**4** and their cytotoxic effects (*in vitro*) on kidney (HEK-293) and breast (MCF-7) cancer cells. We have also demonstrated the apoptotic properties of the most active analogues and compared their cytotoxicity with that in normal cells.

2. Results and discussion

2.1. Chemistry

Andrographolide (~100 g) was isolated from the leaves of *A. paniculata* and used as starting material for derivatization. The synthetic pathways used in the present work are outlined in Scheme 1. We chose to carry out chemoselective esterification of andrographolide at C14 hydroxy, which is allylic in nature. Towards this objective, the other hydroxyls were converted (Scheme 1) into 3,19-isopropylidene derivative **5** by treatment with 2,2-dimethoxypropane and *p*-toluenesulfonic acid (cat.). Compound **5** was initially reacted with chloroacetyl chloride in dry THF in the presence of pyridine (1.5 eqv.) and 4-dimethylaminopyridine (cat.) to afford the intermediate ester **6a** with 66% yield. The corresponding bromo-ester **6b** was obtained (63% yield) by reacting bromoacetyl bromide with **5**, while the iodo derivative **6c** was prepared (65% yield) by treating **6a** with sodium iodide in acetone at rt for 3 h. The isopropylidene moiety of products **6a**–**c** was then removed by exposing the products to aqueous acetic acid (3:7), affording the targeted compounds **2a**–**c** which were then purified (>95%) by HPLC separations. Thereafter, we directed our efforts for chemoselective epoxidation of the exocyclic double bond ($\Delta^{8(17)}$) of intermediates **6a**–**c**. Towards this end, compound **6a** was treated with *m*-chloroperbenzoic acid (*m*-CPBA) in dry dichloromethane to furnish a diastereomeric mixture of epoxides **7a** and **8a** with 51% yield. Pleasingly, this mixture was separated through silica gel (100–200 mesh) column chromatography (**7a**:**8a** = 2:3). This reaction protocol was subsequently applied on compounds **6b** and **6c**, which afforded the corresponding diastereomeric epoxides **7b**/**8b** (54%, **7b**:**8b** = 1:2) and **7c**/**8c** (52%, **7c**:**8c** = 2:5), respectively (Scheme 1). Thereafter, the isopropylidene moiety of the epoxides (**7a**–**c** and **8a**–**c**) was removed using aqueous acetic acid (3:7) leading to the formation of the targeted products **3a**–**c** and **4a**–**c** as shown in Scheme 1. These compounds were finally purified (>95%) using HPLC separations.

As epoxidation of intermediate **6** resulted in a diastereomeric mixture, we tried to make this reaction stereoselective by changing the strategy. Pleasingly, replacing intermediate **6** with **5** for epoxidation using *m*-CPBA ensured a totally stereoselective formation of epoxide **9** with 76% yield (Scheme 2). The absolute stereochemistry at C8 of product **9** was found to be *S* by single crystal X-ray analysis (see Fig. S1 of the Supplementary material). Notably, a previous study [12] on this reaction failed to get the expected epoxide **9**; instead, a deprotected product was isolated. The facial selectivity operating in β-epoxidation of compound **5** is possibly influenced by the hydroxyl group which takes up β-orientation by rotation around the C_{11}–C_{12} single bond (for energy minimized conformations see Fig. S2 of the Supplementary Material) to become proximate to the reactive double bond and directs (through hydrogen bonding [13]) the approach of *m*-chloroperbenzoic acid towards it. However, attempted esterification at C14 hydroxy of **9** employing chloroacetyl chloride, pyridine and DMAP (cat.), as shown in Scheme 2, did not furnish ester **8a**; instead, an intractable solid resulted. After having this disappointing result, we performed this reaction using bromoacetyl bromide; this resulted in the formation of the expected product **8b**, but with a discouraging yield of 35% only (Scheme 2). Due to the poor yield and lack of the consistency of the reaction for esterification of epoxide **9**, the former strategy (Scheme 1) seemed to be a better option for the generation of such analogues. However, the structure determination of epoxide **8b** from the latter study (Scheme 2) helped us to identify the stereochemical outcomes of the conversion of intermediate **6** described in Scheme 1 unambiguously. In ^{1}H NMR, the olefinic proton (C12–H) signal appeared as triplet (t) at around δ 6.99 ppm for diastereomers **7**, but δ 7.15 ppm in other diastereomers **8**. The stereochemical assignments were further supported by NOESY analysis.

2.2. Biology

2.2.1. Anticancer properties of the synthesized compounds

To check the anticancer effects of the synthesized compounds, we initially performed an MTT assay in human embryonic kidney cancer cells (HEK-293) and compared its effect with the normal monkey kidney cells (VERO). VERO cells are non cancerous in nature and are derived from the normal kidney epithelial cells. Cells