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Research paper

Synthesis and anticancer activity of novel fluorinated asiatic acid derivatives

Bruno M.F. Gonçalves [a, b], Jorge A.R. Salvador [a, b, *], Silvia Marín [c, d], Marta Cascante [c, d, **]

[a] Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, 3000-548, Coimbra, Portugal
[b] Center for Neuroscience and Cell Biology, Coimbra, Portugal
[c] Department of Biochemistry and Molecular Biology, Faculty of Biology, Institute of Biomedicine of University of Barcelona, Diagonal 643, 08028 Barcelona, Spain
[d] Institute of Biomedicine of University of Barcelona (IBUB) and Associated Unit to CSIC, Barcelona, Spain

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ABSTRACT

A series of novel fluorinated Asiatic Acid (**AA**) derivatives were successfully synthesized, tested for their antiproliferative activity against HeLa and HT-29 cell lines, and their structure activity relationships were evaluated. The great majority of fluorinated derivatives showed stronger antiproliferative activity than **AA** in a concentration dependent manner. The most active compounds have a pentameric A-ring containing an α,β-unsaturated carbonyl group. The compounds with better cytotoxic activity were then evaluated against MCF-7, Jurkat, PC-3, A375, MIA PaCa-2 and BJ cell lines. Derivative **14** proved to be the most active compound among all tested derivatives and its mechanism of action was further investigated in HeLa cell line. The results showed that compound **14** induced cell cycle arrest in G0/G1 stage as a consequence of up-regulation of $p21^{cip1/waf1}$ and $p27^{kip1}$ and down-regulation of cyclin D_3 and Cyclin E. Furthermore, compound **14** was found to induce caspase driven-apoptosis with activation of caspases-8 and caspase-3 and the cleavage of PARP. The cleavage of Bid into t-Bid, the up-regulation of Bax and the down-regulation of Bcl-2 were also observed after treatment of HeLa cells with compound **14**. Taken together, these mechanistic studies revealed the involvement of extrinsic and intrinsic pathways in the apoptotic process induced by compound **14**. Importantly, the antiproliferative activity of this compound on the non-tumor BJ human fibroblast cell line is weaker than in the tested cancer cell lines. The enhanced potency (between 45 and 90-fold more active than **AA** in a panel of cancer cell lines) and selectivity of this new **AA** derivative warrant further preclinical evaluation.

1. Introduction

Natural products are an indispensable source of lead structures for development of new drugs [1–4]. Triterpenoids are one of the largest classes of natural products with over 20 000 known members, and they are synthesized in a wide spectrum of plants by cyclization of squalene [5]. Over the last decades, an unique range of pharmacological activities of pentacyclic triterpenoids have been reported, including their enormous chemopreventive and anti-neoplastic potential [5–9].

Asiatic acid (**AA**, 2α,3β,23-trihydroxyurs-12-ene-28-oic acid, Fig. 1) is a member of the ursane family, extracted mainly from the tropical medicinal plant *Centella Asiatica* [10]. This compound exhibits several pharmacological effects including hepatoprotective [11,12], neuroprotective and anti-alzheimer [13,14], antidiabetic, antihyperlipidemic [15,16], anti-inflammatory [17] and antioxidant [18]. Moreover, **AA** induces apoptotic cell death in several cancer cell lines [19–23], inhibits tumor cell proliferation [24], induces cell cycle arrest [25], inhibits TPA-induced tumor promotion in a rat model [26], increases sensitivity of colon cancer cells to treatment with camptothecin (CPT-11) [27], and exerts anti-angiogenic activity [28].

In recent years, some studies showed that chemical modification of **AA** can improve its anticancer activities. Jian-Fei et al.

* Corresponding author. Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, 3000-548, Coimbra, Portugal.
** Corresponding author. Department of Biochemistry and Molecular Biology, Faculty of Biology, Institute of Biomedicine of University of Barcelona, Diagonal 643, 08028 Barcelona, Spain.
E-mail addresses: salvador@ci.uc.pt (J.A.R. Salvador), martacascante@ub.edu (M. Cascante).

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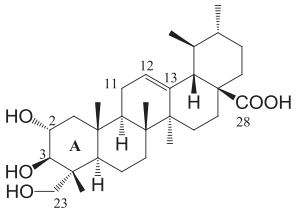

Fig. 1. Chemical structure of asiatic acid.

reported that the introduction of anilines formed amide bound in C-28 combined with a carbonyl moiety in C-11 significantly improved the anticancer activity [23]. Another study indicated that derivatives with substituted amide group at C-28 and acetylation of the C-2, C-3 and C-23 hydroxyl groups, have stronger cell growth inhibitory activity than **AA** against several cancer cell lines [29]. Furthermore, **AA** derivatives with a modified A-ring displayed good cytotoxicity against neoplasm P388D1 and melanoma Malme-3M cells [30]. However, the number of **AA** derivatives that have been synthesized and investigated with respect to their anticancer activity are still quite limited. Therefore there is a great interest in the synthesis and evaluation of new **AA** derivatives in order to develop new and more effective anticancer drugs.

Fluorine is a small and highly electronegative atom, and the incorporation of fluorine in organic molecules could improve the metabolic and chemical stability, increase lipid solubility and membrane permeability and enhance binding affinity of the drug to the molecular targets [31,32]. As a consequence of the desirable properties of organofluorine compounds, fluorine has become very important in the design and development of new drugs [32–35], which is reflected in the number of drugs on the market today that contain fluorine in its structure [36]. Moreover, our group recently reported the synthesis of a series of ursolic acid fluorolactone derivatives which demonstrated promising antiproliferative activities [37]. Taking into account these good results, in this paper we reported the synthesis of a series of fluorolactone and fluorolactam **AA** derivatives. All new derivatives were screened for their *in vitro* cytotoxic activity against cervical (HeLa) and colon (HT-29) cancer cell lines. The antiproliferative activities of the compounds with lower IC_{50} values were further screened against additional cancer cell lines (MCF-7, Jurkat, PC-3, MIA PaCa-2 and A375) and against non-tumoral human fibroblasts (BJ). Further studies were performed in order to investigate the potential mechanism of action of the most active compound **14** in HeLa cell.

2. Results and discussion

2.1. Chemistry

AA has three hydroxyl groups at C-2, C-3 and C-23, an olefin group at C-12 and a carboxylic acid group at C-28. Structural modifications were carried out in these functional groups in order to obtain the semisynthetic derivatives and study the structure-activity relationships (SAR). The structures of all new synthesized derivatives were fully elucidated by infrared spectroscopy (IR), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. As shown in Scheme 1 the preparation of fluorinated **AA** derivatives started with the treatment of **AA** with selectfluor in a mixture of two inert solvents, nitromethane and dioxane, at 80 °C [37], to afford the 12β-fluorolactone derivative **1** in 61% yield. The presence of the 12β-fluorolactone moiety in derivatives (1–17), was confirmed by the observation, in ^{1}H NMR spectrum, of a double triplet or a double quartet at 4.85–5.00 ppm, with a coupling constant of around 45 Hz, typical for H-12 adjacent to β-fluorine. In addition, the characteristic ^{13}C NMR signal for C-12 was observed as a doublet ranging from 88.6 to 89.5 ppm, with a coupling constant of around 186 Hz. The ^{13}C signals for C-13 also appeared as a doublet ranging from 91.5 to 92.0 ppm with a coupling constant of around 14 Hz. These ^{1}H NMR and ^{13}C NMR signals are typical of the β-fluorine isomer [37].

Derivatives **2** and **3** were prepared in good yields by esterification of the three hydroxyl groups of compound **1** with acetic and butyric anhydrides respectively in tetrahydrofuran (THF) in the presence of 4-dimethylaminopyridine (DMAP) (Scheme 1).

Reaction of **1** with dry acetone and a catalytic amount of HCl in the presence of activated molecular sieves [38], afforded the acetonide derivative **4** (Scheme 1), which was oxidized with pyridinium dichromate (PDC) in dichloromethane to give the 2-oxo derivative **5** in 39% yield. The deprotection of acetonide **5** with HCl 1 M, in THF, followed by acetylation of C-3 and C-23 hydroxyl groups with acetic anhydride, gave the diacetylated compound **7** (Scheme 1).

We adapted a known procedure for the synthesis of compounds **8** and **9** [39]. Oxidation of compound **1** with $NaIO_4$ in methanol/water gave the lactol derivative **8**, in 59% yield, which opened in the presence of catalytic amounts of piperidine and acetic acid in benzene at reflux and underwent recyclization to give the α,β-unsaturated aldehyde derivative **9** in 62% yield (Scheme 2).

As depicted in Scheme 2 the ester derivatives **10**–**13** were obtained in moderate to good yields through reaction of compound **9** with acetic, butyric, benzoic and succinic anhydrides respectively. For the synthesis of cinnamic ester derivative **14**, compound **9** was treated with cinnamoyl chloride in dry benzene at 60 °C in the presence of DMAP affording the derivative **14** in 40% yield [40].

In previous studies, our group reported that introduction of imidazolyl and 2-methylimidazolyl moieties in pentacyclic triterpene structures could improve the *in vitro* anticancer activity of the parental compounds [37,41–45]. Hence we synthesized the imidazole carbamate derivative **15** and the 2-methylimidazole carbamate derivative **16** by reaction of compound **9** with 1,1′-carbonyldiimidzole (CDI) and 1,1′-carbonylbis-2'methlyimidazole (CBMI), respectively, in anhydrous THF at reflux temperature under inert atmosphere (N_2) (Scheme 2) [43,44]. The formation of carbamate derivative **15** was confirmed by three peaks in the ^{1}H NMR spectra at 8.11, 7.40 and 7.10 ppm that are characteristic for the three hydrogens of the imidazole ring [43]. In the case of 2-methylimidazole carbamate derivative **16**, only two proton peaks appeared in ^{1}H NMR at 7.29 and 6.87 ppm and an additional methyl peak was observed at 2.63 ppm [44].

Several studies have reported the pharmaceutical importance of the presence of a nitrile group in organic molecules [46,47]. Actually more than 30 nitrile containing drugs are used in clinic for diverse therapeutic indications, including treatment of cancer [46]. Thus, the α,β-unsaturated aldehyde **9** was treated with iodine in ammonia water at room temperature, affording the α,β-unsaturated nitrile derivative **17** (Scheme 2) in 57% yield [48]. The conversion of aldehyde into nitrile, in compound **17**, was confirmed by the specific IR absorption for the C≡N vibrations at 2210 cm^{-1}, in combination with the signal for the quaternary carbon attached to the nitrogen observed in the ^{13}C NMR spectrum at 117.2 ppm.

Acetylation of **AA** with acetic anhydride in THF and in the presence of DMAP yielded the triacetate derivative **18** quantitatively (Scheme 3). Treatment of **18** with oxalyl chloride in dichloromethane, afforded the corresponding 28-acyl chloride, which reacted without purification with ammonia, methylamine or amino acids methyl ester hydrochloride to give the corresponding amide derivatives **19** and **20**, and amino acid derivatives **21** and **22** [49,50]. Amides **19**–**22** reacted with selectfluor in a mixture of two inert solvents, nitromethane and dioxane, at 80 °C, to afford the