European Journal of Medicinal Chemistry 123 (2016) 317–331

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Research paper

# Synthesis and cytotoxic activity of novel A-ring cleaved ursolic acid derivatives in human non-small cell lung cancer cells

Vanessa I.S. Mendes [a, b], Geoffrey A. Bartholomeusz [c], Mary Ayres [c], Varsha Gandhi [c, **, 1], Jorge A.R. Salvador [a, b, *, 1]

[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 Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

ARTICLE INFO

*Article history:*
Received 24 May 2016
Received in revised form
18 July 2016
Accepted 20 July 2016
Available online 22 July 2016

*Keywords:*
Triterpenoids
Ursolic acid
Lung cancer
Spheroid
Apoptosis
Autophagy

ABSTRACT

Ursolic acid (UA) is a pentacyclic triterpenoid with recognized anticancer properties. We prepared a series of new A-ring cleaved UA derivatives and evaluated their antiproliferative activity in non-small cell lung cancer (NSCLC) cell lines using 2D and 3D culture models. Compound **17**, bearing a cleaved A-ring with a secondary amide at $C_3$, was found to be the most active compound, with potency in 2D systems. Importantly, even in 3D systems, the effect was maintained albeit a slight increase in the $IC_{50}$. The molecular mechanism underlying the anticancer activity was further investigated. Compound **17** induced apoptosis via activation of caspase-8 and caspase-7 and via decrease of Bcl-2. Moreover, induction of autophagy was also detected with increased levels of Beclin-1 and LC3A/B-II and decreased levels of mTOR and p62. DNA synthetic capacity and cell cycle profiles were not affected by the drug, but total RNA synthesis was modestly but significantly decreased. Given its activity and mechanism of action, compound **17** might represent a potential candidate for further cancer research.

© 2016 Elsevier Masson SAS. All rights reserved.

## 1. Introduction

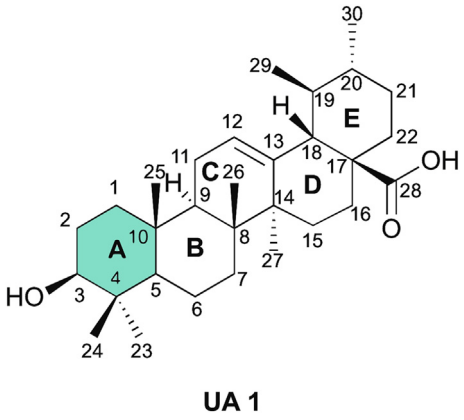
Lung cancer is a heterogeneous disease that is divided into two main types, non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), with different disease patterns and treatment strategies for each type. The most common type is NSCLC (85%), which includes such subclasses as adenocarcinoma, squamous cell carcinoma, and large cell carcinomas [1]. Despite advances in diagnostics and therapeutics, the outcome for patients with lung cancer remains poor [2,3]. According to GLOBOCAN 2012, the most commonly diagnosed cancer and the most common cause of death by cancer worldwide is lung cancer [4]. A substantial proportion of patients with lung cancer show advanced disease at the time of diagnosis, and 40% of patients with NSCLC have distant metastases [2,3].

Taking into account the complexity underlying lung cancer, it has been proposed the development of strategies that would target cancer as a complex disease, for example, the use of multifunctional drugs that would modulate the activity of different regulatory networks, affecting as many hallmarks of cancer as possible [5–7].

Natural products represent an interesting platform for such strategy, because multitargeted effects have been reported and they have been evolutionarily select to bind to biological macromolecules [8]. However, natural products did not undergo evolutionary selection to serve as human therapeutics and thus need to be optimized to have the desired potency, selectivity, and pharmacokinetic properties to become clinically useful drugs. Optimization of the basic scaffold to improve these properties can be accomplished by a semisynthetic approach [8]. Ursolic acid (**UA, 1**) (Fig. 1), a pentacyclic triterpenoid present in a large variety of medicinal herbs and other plants [9], could be used as one such scaffold. **UA (1)** antitumor activity has been demonstrated in several cancer cell lines, namely breast, lung, pancreatic, and prostate cancers [10,11]. The antitumor activity of **UA (1)** has been attribute to induction of apoptosis [12–14] and differentiation [15,16]; inhibition of invasion [17], progression [18], and angiogenesis [19,20]; promotion of chemosensitization [21]; and

* Corresponding author. Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, 3000-548, Coimbra, Portugal.
** Corresponding author.
*E-mail addresses:* vgandhi@mdanderson.org (V. Gandhi), salvador@ci.uc.pt (J.A.R. Salvador).
[1] Equal contributions.

http://dx.doi.org/10.1016/j.ejmech.2016.07.045
0223-5234/© 2016 Elsevier Masson SAS. All rights reserved.




UA 1

**Fig. 1.** Chemical structure of ursolic acid 1.

induction of cell cycle arrest [22,23]. Given their promising anticancer activity, low toxicity, and commercial availability, several structural modifications of the **UA (1)** backbone have been explored. The chemical modifications performed so far have focused mostly on the alcohol group at position $C_3$, on the unsaturation on C-ring at position $C_{12(13)}$, and on the carboxylic acid at position $C_{28}$. Most semisynthetic derivatives obtained have shown improved cytotoxic activity in several cancer cell lines compared with **UA (1)** [10,24]. Some **UA (1)** derivatives have been tested in the A549 lung cancer cell line, e.g., introduction of a piperazine and thiourea moiety at $C_{28}$ and benzylidine derivatives at $C_2$, among other modifications [25–30]. However, further investigation is needed to develop and synthesize new **UA (1)** derivatives that could act as agents against lung cancer.

In this study, we prepared a series of derivatives with a modified A-ring, using **UA (1)** as the starting material. The *in vitro* antitumor activities were tested against NSCLC cell lines, in monolayer and spheroid culture models. The most active compound, compound **17**, was selected for further experiments and to determine its mechanism of action in the H460 cell line.

## 2. Results and discussion

### 2.1. Chemistry

A series of **UA (1)** derivatives with a modified A-ring were synthetized as outlined in Schemes 1 and 2, and their structures were fully elucidated. The introduction of nitrogen-containing groups was explored, namely the formation of a lactam, several amides, and a nitrile group. Nitrogen-containing groups offer versatile properties that could improve the biological and pharmacokinetic profile of compounds. Amide bonds, for example, play a major role in the elaboration and composition of biological systems, representing the main chemical bonds that link amino acid building blocks together to form proteins [31–33]. According to one survey, amides are present in 25% of known pharmaceutical [34].

The synthetic route began with the formation of 12β-fluoro-13,28β-lactone (**2**) via reaction of **UA (1)** with Selectfluor® fluorinating reagent in a mixture of nitromethane and dioxane at 80 °C, with yields above 60% [35]. The fluorination reaction was selected as the first step of the synthetic scheme, because some functional groups synthesized can also be fluorinated, resulting in a mixture of fluorinated compounds. The presence of fluorine, a small and highly electronegative atom, in key positions of a biologically active molecule has been shown to improve the metabolic and chemical stability, membrane permeability, and protein-binding affinity of the agent [36,37]. The introduction of the 12β-fluorolactone was confirmed by the presence in the $^1$H NMR spectra of a double triplet or double quartet at 4.55–5.00 ppm, with a coupling constant of around 45 Hz, characteristic of the geminal proton for the β-fluorine. In the $^{13}$C NMR spectra, we observed a doublet for the signal of $C_{12}$ (88.50–89.50 ppm) and of $C_{13}$ (91.50–92.00), with coupling constants of 186 and 14 Hz, respectively. This profile of $^1$H and $^{13}$C NMR spectra is characteristic of the β-isomer [35].

After oxidation by Jones reagent, the treatment of 3-oxo-derivative **3** with $NaN_3$ in glacial acetic acid and sulfuric acid afforded lactam **4**, while the treatment of compound **3** with *m*-chloroperbenzoic acid (*m*-CPBA) in $CHCl_3$ afforded lactone **5**, with yields around 60% (Scheme 1). For the formation of N-derivatives, lactone **5** was cleaved using *p*-toluenesulfonic acid monohydrate in $CH_2Cl_2$ to give **6** (Scheme 2). The cleavage of A-ring with formation of a carboxylic acid and unsaturation at $C_{4(23)}$ position was confirmed by the presence in the $^1$H NMR spectrum of two singlets for the protons at position 23 at 4.88 and 4.67 ppm. In the $^{13}$C NMR spectrum, the carbon of the carboxylic acid was observed at 179.71 ppm, close to the carbonyl carbon signal of the fluorolactone (179.02 ppm). Unsaturation at $C_{4(23)}$ was observed at 146.85 ppm and 114.13 ppm, which was determined as a quaternary carbon ($C_4$) and as a secondary carbon ($C_{23}$) by $^{13}$C and DEPT-135 NMR spectra.

Compound **6** was first treated with oxalyl chloride and then reacted with cold 25% ammonium aqueous solution to generate the primary amide **7**. The introduction of the amide function in compound **7** was confirmed in the $^1$H NMR spectrum by a broad singlet at 5.80 ppm for the proton of the amide function. The carbonyl carbon of the amide group was observed in the $^{13}$C NMR spectrum at 176.14 ppm.

The reagent T3P® (propylphosphonic anhydride solution, 50 wt % in THF) was used to prepare several amide derivatives (**9**–**17**) and the nitrile derivative (**8**) (Scheme 2). T3P is an efficient coupling reagent that has been employed for the conversion of carboxylic acids to aldehydes and amides, amides to nitriles, and formamides to isonitriles, as well as for the synthesis of heterocycles, Weinreb amides, β-lactams, hydroxamic acids, acyl azides, esters, imidazopyridines, and dihydropyrimidinones [38–40]. The free carboxylic acid group of compound **6,** in the presence of T3P (50 wt% in THF) and $Et_3N$ (triethylamine) in THF in an ice bath, reacted with several amines to yield the respective amide derivatives (**9**–**17**). Compound **8** was obtained by reducing the amide **7** with T3P (50 wt% in THF) in a mixture of THF/EtOAc and $Et_3N$ at 77 °C for 5 h. In the $^1$H NMR spectra, we observed a broad singlet or triplet for the proton of the several amides around the chemical shifts of 5.7 and 5.3 ppm. The carbonyl carbon of the amide was observed in the region of 173 ppm in the $^{13}$C NMR spectra. The nitrile derivative **8** was confirmed in the $^1$H NMR spectrum by the disappearance of the proton signal of the NH group and in the $^{13}$C NMR spectrum by the disappearance of the carbonyl carbon signal at 173 ppm and appearance of the carbon signal of the nitrile group at 120.39 ppm.

### 2.2. Biological studies

#### 2.2.1. Evaluation of in vitro antitumor activity

The anti-tumor activities of **UA (1)** and all the newly synthesized derivatives against NSCLC cells (H460, H322, H460 LKB1$^{+/+}$) were evaluated using the CellTiter-Blue® assay. A culture medium containing 0.15% dimethyl sulfoxide (DMSO) served as negative control. The cell lines were treated with increasing concentrations of each compound, and the $IC_{50}$ values (half-maximal inhibitory concentration) were determined after 72 h of incubation. The values for $IC_{50}$ are summarized in Table 1.

As shown in Table 1, the introduction of the fluorolactone moiety into the **UA (1)** backbone, compound **2**, was not critical for

Download English Version:

https://daneshyari.com/en/article/7797833

Download Persian Version:

https://daneshyari.com/article/7797833

Daneshyari.com