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Synthesis, anti-inflammatory activity and modeling studies of cycloartane-type terpenes derivatives isolated from *Parthenium argentatum*

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ABSTRACT

The 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema model in mice determined the anti-inflammatory activities in vivo of argentatins A, B and D, the main cycloartenol-type triterpenes present in *Parthenium argentatum*. Our results showed that argentatin B ($ED_{50} = 1.5 \times 10^{-4}$ mmol/ear) and argentatin A ($ED_{50} = 2.8 \times 10^{-4}$ mmol/ear) were more potent anti-inflammatory agents than indomethacin ($ED_{50} = 4.5 \times 10^{-4}$ mmol/ear), the reference drug. Based on these findings, we decided to evaluate 13 derivatives of argentatins A and B. All the derivatives showed anti-inflammatory activity in the TPA-induced edema model in mice. The most active compound was 25-nor-cycloart-3, 16-dione-17-en-24-oic acid, obtained from argentatin A ($ED_{50} = 1.4 \times 10^{-4}$ mmol/ear). Argentatin B was assayed as inhibitor of COX-2 activity one of the key enzymes involved in the TPA assay. The results showed that argentatin B at 15 μM doses inhibited 77% COX-2 activity. Docking studies suggest that argentatin B interacts with Arg 120, a key residue for COX-2 activity.

1. Introduction

During the last two decades, a wealth of information has pointed to the deregulated inflammatory response as the cause of most chronic diseases, including different types of cancer.[1,2] The identification of transcription factors such as NF-κB, AP-1 and STAT3 and their gene products such as tumor necrosis factor, interleukins-1, -6, chemokines, cyclooxygenase-2, 5-lipooxygenase, matrix metalloproteases, and vascular endothelial growth factor, adhesion molecules and others have provided the molecular basis for the role of inflammation in cancer.[1] These pathways have been implicated in transformation, survival, proliferation, invasion, angiogenesis, metastasis, chemo-resistance, and radio-resistance of cancer, so much so that survival and proliferation of most types of cancer stem cells themselves appear to be dependent on the activation of these inflammatory pathways.[3,4]

Natural products have been investigated as anti-inflammatory agents, for example several cycloartane glycosides, isolated from *Astragalus membranaceus* have recently explored for anti-inflammatory properties showing significant inhibition of NO production.[5] Other example are terpenoids isolated from *Krameria pauciflora*, these compounds unselectively inhibited cyclooxygenases 1 and 2.[6]

In order to achieve greater therapeutic relevance it seems valuable that an anticancer compound could also have anti-inflammatory activity.[1,7] Taking into consideration this idea and based on our previous reports on the anti-proliferative effect against several human cancer cell lines of the argentatins A (**1**), B (**2**) and D (**3**) (Fig. 1) and some of their derivatives,[8,9] in this work we tested the anti-inflammatory activities of **1**–**3** as well as 13 derivatives (Table 1 and Scheme 1) in the TPA-induced edema in mice model. Docking studies for argentatin B is presented along with COX-2 inhibitory activity for selected compounds in this work.

2. Results and discussion

2.1. Chemistry

Argentatin A (**1**), B (**2**) and D (**3**) were obtained from the resin of *Parthenium argentatum* (Gray) as previously reported and

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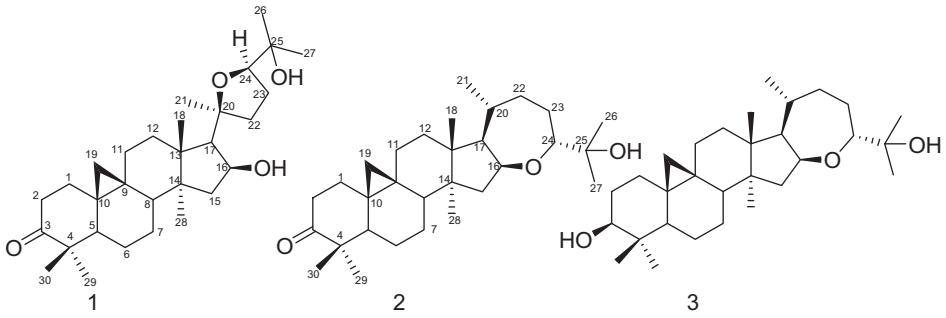

Figure 1. Structures of argentatins A (**1**), B (**2**) and D (**3**).

Table 1
The anti-inflammatory activities of triterpenes **1**–**16**

Compound	ED_{50} (CI) (mg/ear)	ED_{50} (10^{-4} mmol/ear)
1	0.13 (0.1, 0.17) $r = 0.971$	2.8
2	0.07(0.06, 0.08) $r = 0.963$	1.5
3	0.35(0.30, 0.42) $r = 0.982$	7.6
4	0.36 (0.26, 0.49) $r = 0.985$	6.5
5	0.17 (0.15, 0.21) $r = 0.999$	3.1
6	0.26 (0.18, 0.38) $r = 0.995$	5.3
7	ND	ND
8	0.57 (0.42,0.82) $r = 0.997$	12.1
9	0.28 (0.16,0.41) $r = 0.937$	6.5
10	0.11 (0.10,0.13) $r = 0.978$	2.3
11	0.06 (0.05,0.08) $r = 0.990$	1.4
12	ND	ND
13	0.08 (0.06,0.11) $r = 0.942$	1.5
14	0.45 (0.31,0.65) $r = 0.999$	9.6
15	0.12 (0.10,0.15) $r = 0.949$	2.5
16	0.22 (0.17,0.28) $r = 0.979$	4.7
Indomethacin	0.16 (0.15, 0.18) $r = 0.951$	4.5

Dose causing 50% of anti-inflammatory effect (ED_{50}) was calculated from a regression equation (r) using significant data ($P < 0.05$) after a dose–response curve ($n = 4$–8). CI = confidence interval. ND = it was not determined.

identified by comparison of its physical and spectroscopic constants (mp, ^{1}H and ^{13}C NMR) with those reported in literature.[8–10]

The **4**–**6**, **8**–**10** and **12**–**15** derivatives were synthesized as previously reported.[8–10] The lactam **7** was synthesized from the oxime **6** by a Beckman rearrangement. The derivative **11** was obtained from **9** by reflux of KOH in ethanol and the lactone **16** was synthesized from **2** by a Baeyer–Villiger rearrangement. All derivatives were purified by conventional procedures and characterized by spectroscopic and analytical methods (see Section 4).

2.2. Biological activity

2.2.1. Anti-inflammatory and COX-2 inhibition assays

The 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced edema model was used to evaluate the anti-inflammatory activity of **1**–**3**.[11–15] Our results showed that these triterpenes presented a dose-related anti-inflammatory activity. Furthermore, **2** was approximately two-fold more potent than indomethacin (Table 1). Based on these encouraging results, we synthesized the derivatives **4**–**11** from argentatin A (**1**) as well as the derivatives **12**–**16** from argentatin B (**2**) (Scheme 1).

The anti-inflammatory activity of each derivative was evaluated in vivo using the same experimental conditions employed for the evaluation of **1**–**3**. According to our results, all synthesized derivatives showed anti-inflammatory activity. The doses of all compounds are in $\times 10^{-4}$ mmol/ear. The derivatives **4**–**9** were less potent as anti-inflammatory agents than the parent compound, argentatin A (**1**), IC_{50} value for **7** was not determined (Table 1). However, the derivative **5** was more potent than indomethacin, the reference drug. It is interesting to note that by changing a hydroxyl group as in **1** by a ketone group at C-16 as in **8,** the activity of the later decreased more than four times compared to the activity of **1**. On the other hand, the activity of **10** compared with that of **1** is about the same. Thus, the presence of the β-hydroxyl group at C-3 as in **10** is not relevant for the anti-inflammatory activity. The opening of the five member ring of **1** produce the carboxylic acid **11**, which is the most active compound of the series, indicating the possibility that open chain derivatives could be more active (Table 1).

With respect to argentatin B (**2**) derivatives (Scheme 1), none of the structural modifications rendered more active compounds, remaining the parent compound **2** the most active of this series, (Tables 1). IC_{50} value for compound **12** was not determined.

It is well known that COX-2 is one of the principal enzymes involved in the TPA-induced edema.[16] In order to gain knowledge about the mechanism of action of the compounds reported in this work, we evaluated the inhibitory profile of compounds **2**, **11** and **13** on COX-2 activity. Notably, our results showed that at 15 μM compound **2** inhibited 78.77% of the enzyme's activity. Surprisingly, compound **11**, the most active in the TPA in vivo assay, did not show inhibition of COX-2. Similarly, compound **13** is among the most active compounds in the TPA assay but inhibits only on 12% at 100 μM. These results support the known complexity of inflammatory processes, where modulation of COX-2 activity plays a key role but other biological mechanisms cannot be ruled out.[17] Previous studies indicated that the derivative **13** showed a good anti-proliferative activity against some human cancer lines,[9] thus **13** has remarkable anti-inflammatory and anti-proliferative activities.

2.2.2. NO production in peritoneal macrophages

When stimulated in vitro by lipopolysaccharide (LPS), macrophages produce a number of cytokines and other inflammatory mediators, including TNFα and nitric oxide (NO), both of which contribute directly to the ability of macrophages to kill invading bacteria and tumor cells and to the pathogenesis of septic shock.

Thus, we evaluated the effects of **1** and **2** on the production of NO from mouse peritoneal macrophages. Macrophages (Mϕ) showed an enhanced nitrite release when they were stimulated with LPS (Table 2). Our results showed that **1**, at 31 and 100 μM significantly reduce the nitrite release by LPS-treated macrophages. However **2** was active only at 100 μM. A similar behavior was observed when **1** and **2** were evaluated on the production of NO from resting macrophages.

However, the results of macrophage viability determined in the MTT assay showed that both **1** and **2** decreased viability on 63% and 53%, respectively at 50 μM doses. These results suggest that the decrease on NO production shown by compounds **1** and **2** are due to the influence on cell viability induced by these triterpenes.