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Research paper

Anti-herpetic and anti-dengue activity of abietane ferruginol analogues synthesized from (+)-dehydroabietylamine

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ABSTRACT

The abietane-type diterpenoid (+)-ferruginol (**1**), a bioactive compound isolated from several plants, has attracted much attention as consequence of its pharmacological properties, which includes antibacterial, antifungal, antimicrobial, cardioprotective, anti-oxidative, anti-plasmodial, leishmanicidal, anti-ulcerogenic, anti-inflammatory and antitumor actions. In this study, we report on the antiviral evaluation of ferruginol (**1**) and several analogues synthesized from commercial (+)-dehydroabietylamine. Thus, the activity against Human Herpesvirus type 1, Human Herpesvirus type 2 and Dengue Virus type 2, was studied. Two ferruginol analogues showed high antiviral selectivity index and reduced viral plaque-size in post-infection stages against both Herpes and Dengue viruses. A promising lead, compound **8**, was ten-fold more potent (EC_{50} = 1.4 μM) than the control ribavirin against Dengue Virus type 2. Our findings suggest that the 12-hydroxyabieta-8,11,13-triene skeleton, which is characteristic of the diterpenoid ferruginol (**1**), is an interesting molecular scaffold for development of novel antivirals. In addition, the cytotoxic and antifungal activities of the synthesized ferruginol analogues have also been investigated. ©20155 Elsevier Science. All rights reserved.

1. Introduction

Human Herpesvirus (HHV) types 1 and 2 are enveloped DNA viruses characterized for producing latent infection in sensory neurons and reactivation during periods of severe immunosuppression of the host. Many factors such as stress, fatigue, sexual relations with people who have active lesions, excess exposure to heat or cold, fever, laser treatments, local tissue trauma and nerve damage, can predispose to reactivation, especially in neonates, transplant and immunocompromised patients which tend to present aggressive and recurrent lesions [1]. Also, it has been reported that prolonged therapy with Acyclovir (ACV), and its analogues, has induced the emergence of drug-resistant virus strains. Human Herpesvirus predominantly develops resistance, almost 95%, as a result of mutations in the genes that encode the viral thymidine kinase (TK) [2].

However, mutations in the viral DNA polymerase can also cause this resistance. In immunocompromised patients, the presence of HHV species resistant to ACV, with a prevalence of 4–10%, has complicated their clinical management [3]. Moreover, Dengue virus (DENV) is an enveloped ssRNA virus. To date, there have been described four serotypes established in humans (DENV-1 to DENV-4) and recently, a fifth Dengue subtype that follows the sylvatic cycle has been identified [4]. Dengue is the most important mosquito-borne disease worldwide, since it is distributed over one hundred countries of the tropical belt and the tropics, both the Old and New World [5]. Disease manifestations cover a wide spectrum ranging from dengue with or without warning signs to severe dengue in which plasma leakage, haemorrhage and organ impairment can lead to death. In addition, unusual manifestations such as cardiomyopathy, liver failure and neurological disorders have been reported [6]. The major problem of this pathology is that there are no drugs approved for human treatment or vaccine available for prophylaxis. Also, antiviral drugs addressed to specific genes or proteins of the virus are not a good alternative due to the high

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mutation rate of RNA viruses, often because viral mutants are selected for drug resistance [7]. During the last years in Colombia, several reports had shown the high rates of evolution of DENV, possibly related with the new Asiatic-American genotypes found in Colombia and Brazil [8].

The aromatic abietane-type diterpenoids comprise a large number of natural metabolites of plant origin which possess a wide variety of biological effects [9]. These abietanes have been the target of several synthetic campaigns towards both the natural products and synthetic derivatives with interesting pharmacological properties [10]. Among these known bioactive compounds, (+)-ferruginol (**1**), some derivatives of (+)-dehydroabietic acid (**2**) and (+)-dehydroabietylamine (**3**) as well as (+)-jiadifenoic acid C (**4**) (Fig. 1) have shown promising results, including antiviral properties [10b,11].

The present study is a continuation of our research programs to discover bioactive diterpenoids [12]. It includes the synthesis of a focused library based on (+)-ferruginol (**1**) from commercially available (+)-dehydroabietylamine (**3**), where a series of phthalimides could have potential antiviral activity [13]. A fullerene diterpenoid hybrid was also envisaged as potential inhibitor [14]. Herein, we describe the antiviral activity of ferruginol (**1**) and some analogues (**5**–**14**) (Scheme 1) synthesized from dehydroabietylamine (**3**) against HHV-1 and HHV-2, and DENV-2. Our aim is to identify new antiviral drug candidates which act on cellular and/or molecular targets instead on viral proteins. In this way, the concerns about the viral mutants resistant to the treatments can be avoided. This approach has been recently called host-targeted antiviral in an evolutionary study of resistance of Dengue viruses under selective pressure of several antiviral agents [15].

2. Results and discussion

2.1. Chemistry

The synthesis of (+)-ferruginol (**1**) and analogues **5**–**14** from commercial (+)-dehydroabietylamine (**3**) was performed as outlined in the Scheme 1. Compounds **1** and **5**–**9** were synthesized as reported in the literature using a Friedel–Crafts acylation and a Baeyer–Villiger oxidation as key steps [16]. Then, compound **6** was used as starting material for the preparation of compounds **10** and **11**. Later, compound **11** was converted into compounds **12**–**14** as described recently in the literature [17]. Thus, the synthesis starts with the introduction of the phthalimide group on (+)-dehydroabietylamine (**3**), followed by Friedel–Crafts acylation and oxidation under Baeyer-Villiger conditions to afford acetate **5** in 72% overall yield. Hydrolysis of the acetate group in **5** gave phenol **8** in high yield, while overall deprotection of **5** afforded the aminophenol **6** in 75% yield. Compound **6** was the intermediate of three separate approaches. Firstly, tosylation under standard conditions gave compound **7** in 90% yield. Secondly, oxidative deamination of

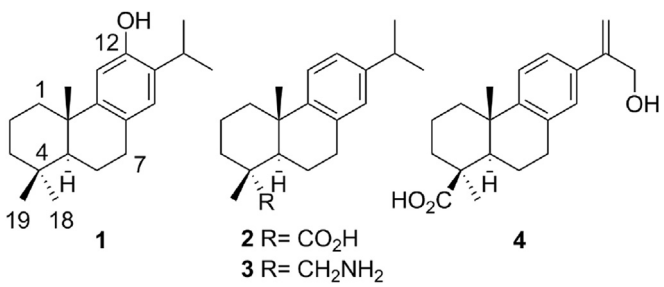

Fig. 1. Examples of bioactive aromatic abietane diterpenoids.

6 gave 18-oxoferruginol (**9**) in moderate yield (50%), which was converted into ferruginol (**1**) by Wolff-Kishner reduction (90% yield). And finally, the treatment of **6** with tetrachlorophthalic anhydride (TCPA) afforded phenol **10** in 75% yield, which was acetylated with acetic anhydride in pyridine to give acetate **11** in quantitative yield. Acetate **11** was oxidized at C-7 with excess of *t*-BuOOH as oxidant and CrO_3/pyridine mixture as a catalyst in DCM, the yield of ketone **12** was 66%. Subsequently, the reaction of **12** with *p*-tosylhydrazide yielded the corresponding *p*-tosylhydrazone **13** (77% yield). The fullerene-terpenoid hybrid **14** was obtained by the treatment with NaOMe of **13** in anhydrous pyridine for 20 min at room temperature followed by addition of a solution of C_{60} in chlorobenzene and heating at 70 °C for 24 h. This gave compound **14** in ca. 30% yield. All the compounds showed spectroscopic data in agreement with the assigned structures and purity >95%.

2.2. Biological evaluation

The synthesized compounds **1** and **5**–**14** (Scheme 1) were evaluated for antiviral activity against HHV-1, HHV-2 and DENV-2 (see Table 1). Two ferruginol analogues, compounds **8** and **9** (18-oxoferruginol), showed relevant activity.

In particular, the ferruginol analogue **8** showed moderate activity against HHV-1 ($Rf = 1 \times 10^2$) at a concentration of 14.5 μM, high activity against HHV-2 ($Rf = 1 \times 10^3$) and reduction of cytopathic effect during DENV-2 infection, comparable to control of untreated cells, at concentration of 29.0 μM. Compound **9** (18-oxoferruginol) also showed high activity against HHV-2 ($Rf = 1 \times 10^3$) at a concentration of 41.7 μM, however, its ability to exert reduction of cytopathic effect in the presence of DENV-2 infection was lower than that of compound **8**. Moreover, compound **9** did not show activity against HHV-1.

In 2007, Wen and co-workers reported the antiviral potential of certain diterpenoid derivatives, including the abietane-type diterpenoid ferruginol (**1**), during *in vitro* coronavirus infection [18]. However, at present, the antiviral activity of the abietane class of compounds has not been much investigated, especially the study of derivatives and analogues; therefore, the activity against HHV-1, HHV-2 and DENV-2 reported in this study is to our knowledge, the first report of antiviral activity of ferruginol analogues. Also, as the compounds **8** and **9** are active against two different viruses, such as herpesvirus and dengue virus, our findings suggest that these molecules probably have a broad-spectrum of antiviral activity against enveloped viruses with DNA and RNA genome.

Later on, to check the specific antiviral activity of these compounds, we carried out the evaluation of other biological activities such as cytotoxic and antifungal, because is well known that ferruginol (**1**) has promising bioactivities, such as antifungal, antibacterial, miticidal antiplasmodial, antileishmanial, nematicidal, and antitumor, among others [9]. In Table 2, it is shown the cytotoxic activity on Vero cells as well as on the tumor cells HeLa, Jurkat and U937 of ferruginol **1** and analogues **5**–**14**.

Compounds **1**, **6**, **7**, **9**, **10** and **12** produced a dose-dependent inhibition on the growth of the three tumor cell lines: Jurkat, U937 and HeLa, as well as the Vero cell line, with R^2 (coefficient of linear regression) > 0.8. Most of these compounds showed cytotoxic activity against at least one tumor cell line at concentrations below 30 μM. However, only compound **10** against HeLa and Jurkat cell lines, and compound **12** against the three tumor cell lines, showed a great SI (SI ≥ 5), being compound **12** the most selective agent against tumor cell lines respect the non-tumor cell line (SI > 43.8, >26.0 and > 38.5, for HeLa, Jurkat and U937, respectively). The compounds **5**, **8**, **11**, **13** and **14** were not active against the tumor cell lines at the tested concentrations and hence they were not evaluated on the Vero cell line.