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Original article

1,2,3-Triazole-containing derivatives of rupestonic acid: Click-chemical synthesis and antiviral activities against influenza viruses

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ABSTRACT

Two series of rupestonic acid derivatives, (1-substituted-1*H*-1,2,3-triazol-4-yl)methyl 2-((5*R*,8*S*,8a*S*)-3,8-dimethyl-2-oxo-1,2,4,5,6,7,8,8a-octahydroazulen-5-yl)acrylate and *N*-(1-substituted-1*H*-1,2,3-triazol-4-yl)methyl 2-((5*R*,8*S*,8a*S*)-3,8-dimethyl-2-oxo-1,2,4,5,6,7,8,8a-octahydroazulen-5-yl)acrylamide were easily and efficiently synthesized via click chemistry. These compounds were tested for their *in vitro* activities against various strains of influenza A virus (H1N1, oseltamivir resistant H1N1, H3N2) and influenza B virus. The results showed that nine compounds were active against the H1N1 strain of influenza A virus and among them the best one **14a**, was as active as the reference drugs, Oseltamivir and Ribavirin. Some of them were also active on the Oseltamivir resistant H1N1 strain. In regards to influenza B virus, twenty-one compounds over thirty were active and seven of them **7b**, **8b**, **9b**, **10a**, **11b**, **12b**, **13b** showed better activity than Ribavirin. The structure–activity relationship of these compounds is discussed on the basis of each type of the viruses studied. Furthermore, four best representative compounds **7b**, **10a**, **12b** and **14a** were evaluated in a plaque assay experiment using MDCK cells and RBV as control compound and the results showed that **7b**, **10a** and **12b** were better than RBV in inhibiting plaque formation, in good accordance with their anti-influenza B activities.

1. Introduction

Influenza is a serious public health problem that causes severe illnesses and deaths for higher risk populations [1]. It is an acute viral infection that spreads easily from person to person and anybody in any age group. An epidemic can take an economic toll through reduction of workforce productivity, and strain health services. Annual epidemics of influenza result in about 3–5 million cases of severe illness, and up to 500,000 deaths worldwide [2]. The influenza is caused by influenza viruses which can be classified into three types, A, B and C [3]. Type A influenza viruses are further divided into subtypes according to different combinations of virus surface proteins. Among many subtypes of influenza A viruses, currently H1N1 and H3N2 subtypes are circulating in human beings. The subtype H7N9 has been reported since a few weeks to have infected humans in China, but no evidence has been found for its capacity to be vehiculated from man to man. Influenza B viruses are normally found only in humans. Although influenza type B viruses can cause human epidemics, they have not caused pandemics. Type C influenza viruses are much less frequent than A and B [1].

To date, two types of small molecular drugs are available for the treatment of influenza. Adamantine (amantadine and remantadine) are effective against the type A viruses and the inhibitors of influenza neuraminidase, such as oseltamivir and zanamivir, are used for the treatment of both types A and B viruses [4]. However, the amantidine is strictly limited actually because of rapid emergence of the drug resistance and its CNS side effects [5]. Meanwhile, the oseltamivir resistant viruses have been reported as well [6]. Therefore, it is necessary to develop novel and effective drugs to overcome the limitations of the existing antiviral agents.

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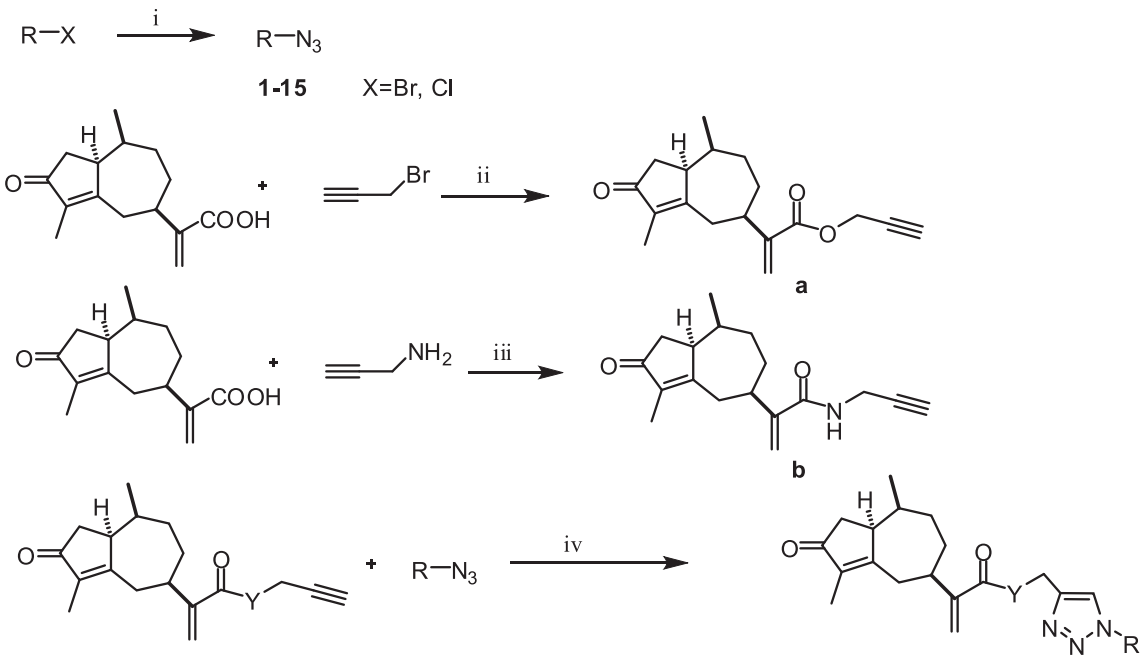

Scheme 1. Synthesis of 1,2,3-Triazole-containing derivatives of rupestonic acid. Reagents and conditions: i) NaN_3, DMSO; ii) K_2CO_3, DMF; iii) EDCI, HOBt, DCM; iv) $CuSO_4$/Na-Ascorbate, DCM/H_2O.

Natural products represented and will represent an important source of lead compounds for drug discovery [7]. *Artemisia rupestris* L. is a well-known medicinal plant in Xinjiang, China and has been traditionally used for detoxification, anti-hypersusceptibility and protecting liver, also as antitumor, antibacterial and antiviral agents [8]. It is the main ingredient in Compound Yizhihao Granule (Fufang Yizhihao Keli, No. Z20026711), which is prescribed to treat colds in China since more than 10 years. 2-((5*R*,8*S*,8a*S*)-3,8-dimethyl-2-oxo-1,2,4,5,6,7,8,8a-octahydroazulen-5-yl)acrylic acid (rupestonic acid, R.A.), isolated from *A. rupestris* L. [9], is a guaiane type sesquiterpene with multifunctional groups. It shows anti-influenza virus activities and low toxicity (IC_{50} = 25.8 μM and TC_{50} = 4653 μM against influenza H3N2 virus) [10]. In order to improve its efficacy, our research group has long been involved in synthesizing and testing rupestonic acid derivatives against influenza A and B viruses. The rupestonic acid derivatives, bearing a 1,3,4-triazoles [10] or isoxazole moiety [11] we developed recently, exhibit a good *in vitro* inhibitory activity against influenza A and B viruses. Unfortunately, the high toxicity associated has limited their further development. Hence, it remains a challenge for us to design and synthesize new derivatives with higher efficiency and less toxicity against influenza virus.

1,2,3-Triazole has been frequently used in drugs with a large spectrum of therapeutic properties, such as anti-allergic, antifungal, antibacterial and antiviral (against HIV or influenza virus) activities [12–17]. Recent innovations in click chemistry have made 1,4-disubstituted-1,2,3-triazoles easily accessible from alkynes and azides, through Huisgen 1,3-dipolar cycloaddition [18]. This copper (I) catalyzed reaction is mild and very efficient, requiring no protecting groups and no purification in many cases with the reaction rate accelerated up to 10^7 times. This has put it in a class of its own and enabled many novel applications [19], in particular in the synthesis of drug-like molecules to accelerate drug discovery process [20].

Starting from rupestonic acid, we report in this work the synthesis of its 1,2,3-triazole-containing derivatives and their *in vitro* biological activities against influenza viruses. The structure–activity relationship of these compounds is discussed on the basis of each type of the viruses studied. Furthermore, in a plaque assay experiment three representative compounds were shown to be better inhibitors than RBV, in good accordance with their anti-influenza B activities.

2. Results and discussion

2.1. Chemistry

The synthetic route for the title compounds is shown in Scheme 1. Rupestonic acid was isolated from raw roots of A. *rupestris* L. in a global yield of about 0.1%. It reacted with 3-bromoprop-1-yne or prop-2-yn-1-amine to afford the compounds **a** or **b** containing a terminal alkyne function. In parallel, substituted benzyl and alkyl azides (**1**–**15**) were prepared according to the experimental procedures described in the literature [21] and characterized by the absorption at 2100 cm^{-1} in the IR spectra, corresponding to the azide function. They were used without further purification. The compounds **a** and **b** were then submitted to the copper-catalyzed [3 + 2] cycloaddition with the azides (**1**–**15**) in the presence of $CuSO_4$ and sodium ascorbate in a non-homogeneous mixture of solvents (CH_2Cl_2/H_2O, 1:1, v/v) to give the rupestonic acid derivatives **1-15a** and **1-15b** in moderate to good yields (35%–74%).