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Research paper

Discovery and evolution of aloperine derivatives as novel anti-filovirus agents through targeting entry stage

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ABSTRACT

Preventing filoviruses in the entry stage is an attractive antiviral strategy. Taking aloperine, a Chinese natural herb with an endocyclic skeleton, as the lead, 23 new aloperine derivatives were synthesized and evaluated for their anti-filovirus activities including ebola virus (EBOV) and marburg virus (MARV) using pseudotyped virus model. Structure-activity relationship (SAR) analysis indicated that the introduction of a 12*N*-dichlorobenzyl group was beneficial for the potency. Compound **2e** exhibited the most potent anti-EBOV and anti-MARV effects both *in vitro* and *in vivo*. It also displayed a good pharmacokinetic and safety profile *in vivo*, indicating an ideal druglike feature. The primary mechanism study showed that **2e** could block a late stage of viral entry, mainly through inhibiting cysteine cathepsin B activity of host components. We consider compound **2e** to be a promising broad-spectrum anti-filovirus agent with the advantages of a unique chemical scaffold and a specific biological mechanism.

1. Introduction

An extremely contagious pathogen ebola virus (EBOV), along with the marburg virus (MARV), constitutes the filovirus family [1], and both of them can cause a lethal hemorrhagic fever disease in human beings and animals [2]. For the EBOV alone, the most recent EBOV outbreak in West African caused more than 28000 infectious patients and 11300 death tolls [3]. Worse still, new flare-ups have occurred several times after the EBOV breakout was over [4]. As for MARV, since its first identification in 1968, it has triggered two large outbreaks, with high lethalities of 83% in the Democratic Republic of the Congo and 90% in Angola respectively [5]. Although outbreaks have predominantly occurred in central Africa to date, the potential for imported cases or bioterrorism in non-African countries cannot be ignored [6]. Both EBOV and MARV infections have become a tremendous threat to public health. However, although a lot of efforts have been made to battle diseases infected by EBOV and MARV, there are no clinically approved drugs available to treat the contagious patients up to now [7]. Therefore, small molecule drug candidates to effectively cure the diseases caused by EBOV or MARV are urgently needed.

Viral entry is an essential process in the viral life cycle and has become an attractive target for discovering and searching new antiviral candidates, with an advantage of reducing the chances to drug-resistance [8]. EBOV infection is mediated by its 676-residue envelope glycoprotein (EBOV-GP) that transfers the viral to enter into host cells, thereby providing a potential target for the screening of small molecules against EBOV [9].

In the past few years, our group has been dedicating to the search of novel antiviral candidates from Tradition Chinese Medicine such as *Sophora alopecuroides*, and a library of quinolizidine alkaloids was constructed in our laboratory [10–18]. In order to find the lead compounds against EBOV, this library was then screened with a pseudotyped EBOV virus model (namely pHIV-EBOVGP-Fluc) [19], taking sertraline (**1**, Fig. 1) as the positive control [20]. Luckily, aloperine (Fig. 1), a Chinese natural product with a unique endocyclic skeleton, demonstrated a good anti-EBOV activity with a half maximal effective concentration (EC_{50}) value of 12.4 μM and a

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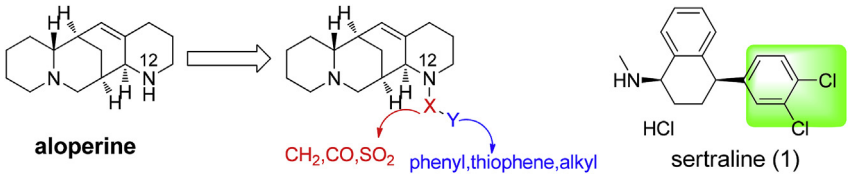


Fig. 1. Chemical structures of aloperine, **1** and the modification sites in aloperine.

selective index (SI) value of over 16.1. Furthermore, it also showed a potential activity against pseudotyped MARV virus (namely pHIV-MARVGP-Fluc) [21], with an EC_{50} value of 10.2 μM and a SI value of over 19.6. Its unique chemical scaffold and biological activity against filoviruses spurred us to further explore the structure-activity relationship (SAR) of its kind so as to develop a novel family of anti-filovirus candidates with a specific mechanism of action.

Therefore, as illustrated in Fig. 1, taking aloperine as the lead, SAR study was mainly focused on the variations of the 12-side chain, by which several series of target compounds including 12*N*-benzyl, 12*N*-benzoyl and 12*N*-sulfonyl aloperine derivatives were designed, synthesized and evaluated for their *in vitro* anti-EBOV and anti-MARV activities respectively. Furthermore, *in vivo* anti-EBOV and anti-MARV effects, pharmacokinetics (PK) and safety profiles as well as primary mechanism of the representative compound were carried out in the present study.

2. Chemistry

As described in Schemes 1 and 2, all the newly synthesized compounds were prepared with commercially available aloperine as the starting material with purity over 95%, which was purchased from the Yanchi Dushun Biological and Chemical Co. Ltd. (Shanxi, China). As depicted in Scheme 1, the 12*N*-benzyl aloperine derivatives (**2a**–**i**) were gained through *N*-alkylation by an equimolar amount of benzyl halide in CH_3CN using K_2CO_3 as the base in yields of 70–87% [15]. Similarly, the 12*N*-benzoyl aloperine derivatives (**3a**–**c**) were obtained by *N*-acylation in CH_2Cl_2 with 80–85% yields. The synthesis of 12*N*-sulfonyl aloperine derivatives (**4a**–**e**) was similar to that of **3** series, in which different sulfonyl chlorides were applied instead in yields of 80–87%.

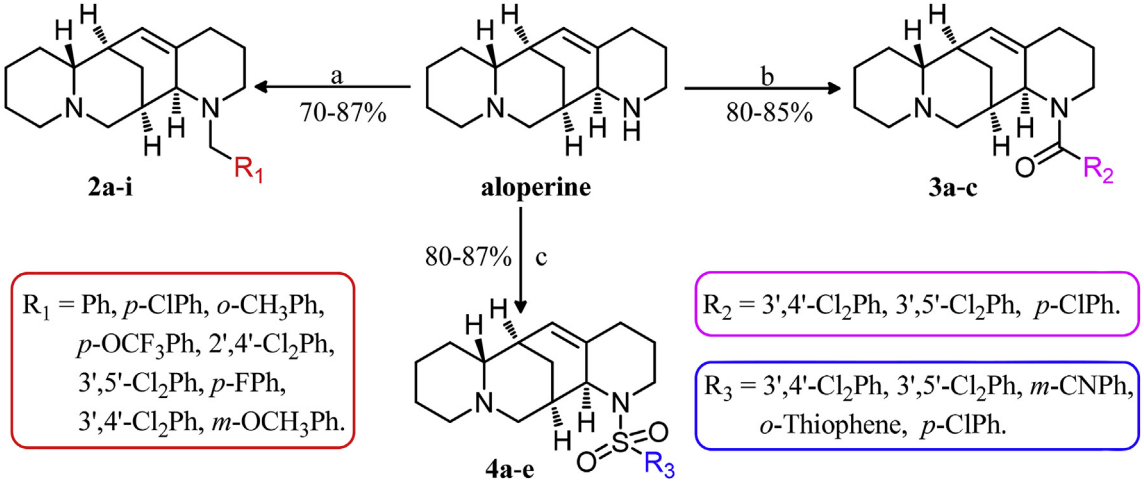
The synthetic route of 12*N*-alkyl aloperine derivatives (**8a**–**f**) was displayed in Scheme 2. Treatment of different substituted phenylacetic acids or phenylpropionic acids **5a**–**f** with $SOCl_2$ gave the intermediates **6a**–**f** as substituted phenylacetyl chlorides or phenylpropionyl chlorides in good yields. The key intermediates **7a**–**f** were acquired via the *N*-acylation of aloperine with **6a**–**f** in moderate yields. The desired products **8a**–**f** were obtained by reduction with $LiAlH_4$ in yields of 65–75% [18]. All the final products were purified by flash column chromatography on silica gel with CH_2Cl_2/CH_3OH as the gradient eluent.

3. Results and discussion

3.1. SAR analysis for anti-EBOV activity

As reported earlier [19], the constructed pHIV-EBOVGP-Fluc was composed with EBOV-GP and envelope-defective strain of HIV-1 containing firefly luciferase reporter gene (pSG3.cmv.Fluc). Similarly, a pHIV-MARVGP-Fluc model [21] was also applied to test the anti-MARV activities. The *in vitro* antiviral evaluations were performed in embryonic kidney (HEK) 293T cells, taking **1** as the positive reference [20]. The potency against pHIV-EBOVGP-Fluc or pMARV-EBOVGP-Fluc of each tested compound was evaluated by the combination of its EC_{50} and SI value that was calculated as a ratio of half maximal cytotoxic concentration (CC_{50}) to EC_{50}. The structures, anti-EBOV and anti-MARV activities as well as cytotoxicities of all the newly synthesized aloperine derivatives were displayed in Table 1.

The SAR analysis for anti-EBOV activity was first focused on the influence of different types of substituents at the 12-position, by which 12*N*-benzyl (**2a**–**i**), 12*N*-benzoyl (**3a**–**c**) and 12*N*-sulfonyl (**4a**–**e**) aloperine derivatives were generated and evaluated. As shown in Table 1, generally speaking, compounds **2a**–**i** showed apparently higher anti-EBOV activities than the other two series



Scheme 1. Synthetic Route of Target Compounds **2a**–**i**, **3a**–**c** and **4a**–**e**. Reagents and conditions for the chemical synthesis: (a) R_1X, MeCN, K_2CO_3, r.t., 2 h; (b) R_2COCl, CH_2Cl_2, K_2CO_3, r.t., 0 °C, 2 h; (c) R_3SO_2Cl, CH_2Cl_2, K_2CO_3, .r.t., 0 °C, 2 h.