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Caffeic acid derivatives: A new type of influenza neuraminidase inhibitors

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ABSTRACT

Recently, many natural products, especially some plant-derived polyphenols have been found to exert antiviral effects against influenza virus and show inhibitory activities on neuraminidases (NAs). In our research, we took caffeic acid which contained two phenolic hydroxyl groups as the basic fragment to build a small compound library with various structures. The enzyme inhibition result indicated that some compounds exhibited moderate activities against NA and compound **15d** was the best with IC_{50} = 7.2 μM and 8.5 μM against N2 and N1 NAs, respectively. The 3,4-dihydroxyphenyl group from caffeic acid was important for the activity according to the docking analysis. Besides, compound **15d** was found to be a non-competitive inhibitor with K_i = 11.5 ± 0.25 μM by the kinetic study and also presented anti-influenza virus activity in chicken embryo fibroblast cells. It seemed promising to discover more potent NA inhibitors from caffeic acid derivatives to cope with influenza virus.

Influenza virus, especially the highly pathogenic avian influenza A (H5N1) virus is a great threat to human. In recent years, the worldwide spread of H5N1 avian influenza virus in birds and the increasing cases of bird-to-human transmission get heightened concern. The viral surface protein, neuraminidase (NA) plays an important role in the life cycle of influenza virus and has been discovered as an important target for anti-influenza drugs design. Oseltamivir, one kind of NA inhibitors is currently the first line defense drug against influenza. However, more and more influenza virus strains are resistant to oseltamivir, such as the seasonal H1N1 viruses[1] and avian H5N1 strains.[2] In 2009, peramivir was urgently authorized in America to cope with the novel swine-origin H1N1 (A) influenza virus that was also resistant to oseltamivir. At present, this drug has been approved in many countries, but it is mainly administered intravenously, which is very inconvenient for patients.

According to recent studies, it seems hard to discover novel NA inhibitors which would be better than oseltamivir simply based on the NA active site. However, many natural products, especially some small plant-derived polyphenols have been found to exert antiviral effects against influenza virus and show inhibitory activities on NAs (Fig. 1).[3,4] Unlike the classical ones, the structures of these compounds are various and interestingly, most of them act as noncompetitive NA inhibitors, indicating that they likely inhibit the NA activity by binding on some other sites of the enzyme, rather than the catalytic center.

Caffeic acid, which is abundant in nature has a variety of potential pharmacological effects in vitro and in vivo, such as anti-inflammatory, anti-cancer and antiviral activities.[5] Some natural products containing the fragment of caffeic acid, like chlorogenic acid and its analogue also show inhibitory activities against influenza NAs.[6] A simple compound, caffeic acid phenethyl ester (CAPE, Fig. 2) is found to have anti-mitogenic, anti-carcinogenic, anti-inflammatory and especially anti-influenza virus properties in vitro.[7] In 2012, Liu et al. reported some novel dual-targeted bifunctional zanamivir anti-influenza drugs formed by conjugation of zanamivir with caffeic acid for simultaneous inhibition of influenza virus NA and suppression of pro-inflammatory cytokine. These conjugates provided remarkable protection of cells and mice against influenza infections.[8]

Till now, except some natural products, there are no synthetic compounds designed to target influenza NA based on caffeic acid. Therefore, in our study, we took caffeic acid as the basic fragment to design and synthesize a small compound library of caffeic acid derivatives. Through determining the inhibitory effects on the N1 and N2 NAs of influenza virus, we discovered a new kind of NA inhibitors with better activities than the natural products.

Compounds were synthesized using methods presented in Schemes 1–3. Scheme 1 presented the synthesis of compounds **3a**–**3f**. In order to increase the yields of target compounds, caffeic acid was first acetylated to give (*E*)-3-(3,4-diacetoxyphenyl)acrylic acid (compound **1**) that had a relatively better solubility in

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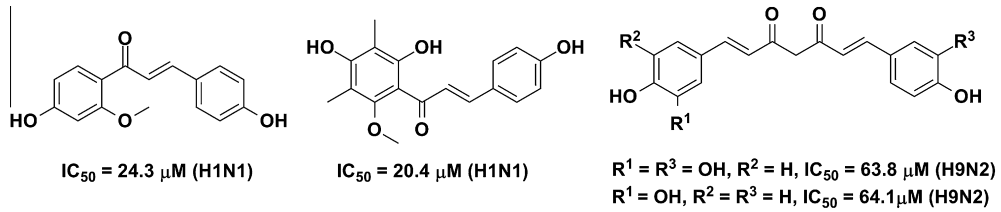


Figure 1. Plant-derived polyphenols with inhibitory activities against influenza NAs.

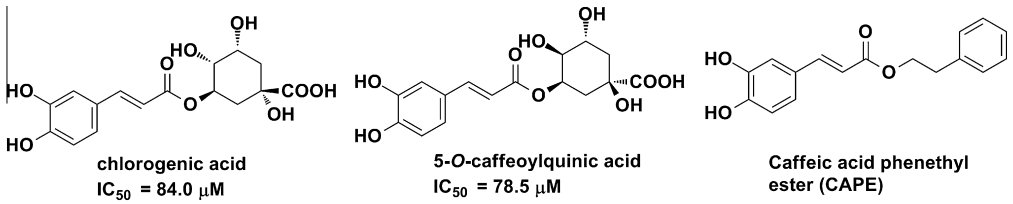
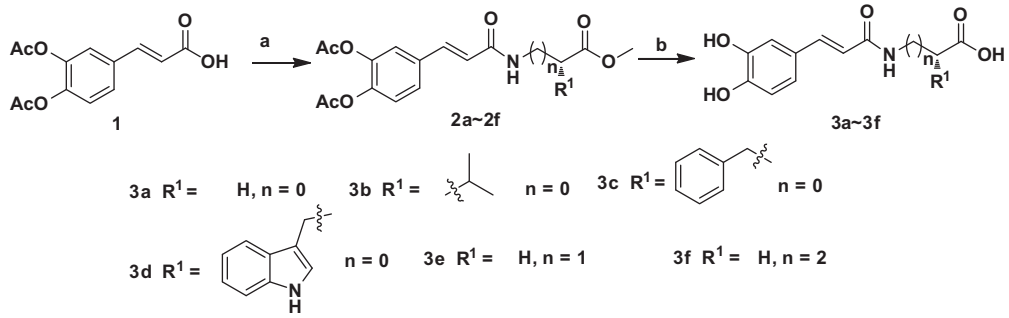


Figure 2. Caffeic acid derivatives with activities against influenza NA or influenza virus.

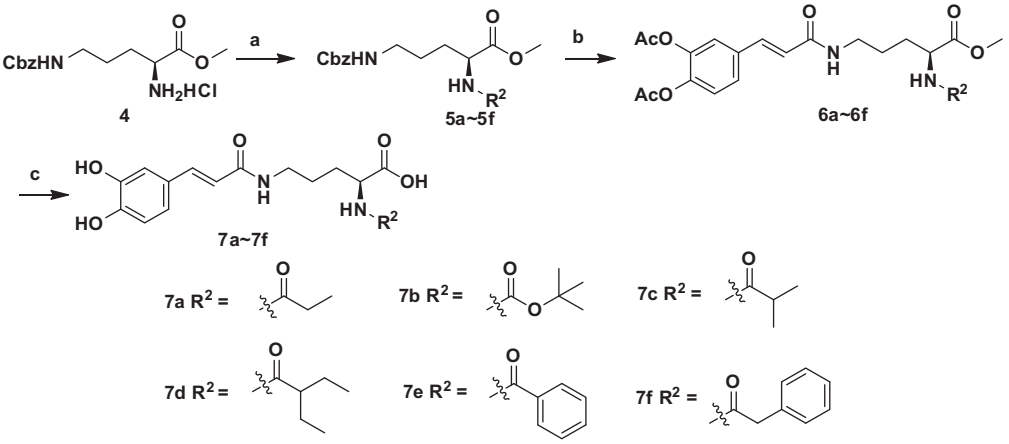
common organic solvents. This compound was then converted to acyl chloride and reacted with different amino acid methyl esters in THF. After hydrolysis with NaOH, compounds **3a**–**3f** were obtained with acceptable yields.

L-Ornithine that contained two amino groups, a carboxyl group and a relative long carbon chain can be modified with caffeic acid and some other structures. In Scheme 2, the α-NH_2 of N′-Cbz-L-ornithine methyl ester hydrochloride (**4**) was first acylated to introduce different fragments on the skeleton of L-ornithine. Under hydrogen atmosphere, the Cbz group was removed with Pd/C, therefore exposing the other NH_2 that was acylated with compound **1**. After hydrolysis with NaOH, compounds **7a**–**7f** were obtained.

In Scheme 3, compounds **11a**–**11e** bearing cinnamic acid group were prepared for making a comparison with compounds **12a**–**12e** on their NA inhibitory activities. The starting material, 2-amino-4-nitrophenol (compound **8**) was acylated with different acyl chloride followed by reduction with Pd/C in the presence of H_2.



Scheme 1. Reagents and conditions: (a) (1) $(COCl)_2$, DMF, anhydrous THF, rt; (2) amino acid methyl esters, $NaHCO_3$, THF, rt; and (b) NaOH, CH_3OH, H_2O, rt.



Scheme 2. Reagents and conditions: (a) RCOCl, $NaHCO_3$, THF, H_2O, rt; (b) (1) H_2, Pd/C, CH_3COOH, CH_3OH, rt; (2) compound **1**, $(COCl)_2$, DMF, anhydrous THF; (3) $NaHCO_3$, THF, H_2O, rt; and (c) NaOH, CH_3OH, H_2O, rt.