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SYNTHESIS OF FLUORINATED HYDROXYCINNAMOYL DERIVATIVES OF ANTI-INFLUENZA DRUGS AND THEIR BIOLOGICAL ACTIVITY

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Abstract. The emergence of a novel pandemic influenza A (H1N1) strain in 2009 is an evident mark for the unremitting risk of respiratory viral diseases. Influenza outbreaks and development of desease and could be overcome by use of vaccines and antivirals. Currently, clinically applied influenza antivirals are limited to M2 ion channel blockers (amantadine and rimantadine) and neuraminidase inhibitors (oseltamivir and zanamivir). Development of resistant viral progeny though, is one of the major challenges to antiviral chemotherapy, in general. Resistant viral mutants are described to both classes of anti-influenza antivirals, as well. Therefore, the development of new antiviral agents for influenza treatment is a hot topic and a never ending task. Combining antiviral drugs with antioxidants in severe influenza-associated complications is of great therapeutic significance. Based on the prominent antioxidant activity of hydroxycinnamic acids, herein, we report the biological activity of newly synthesized amides obtained by coupling previously synthesized N-hydroxycinnamoyl amides of fluorinated amino acids and a fragment of oseltamivir.

Keywords: M2 ion channel blockers, neuraminidase inhibitors, N-hydroxycinnamoyl fluorinated amino acids amides

Introduction

Influenza, well-known as the flu, is an acute respiratory disease caused by the influenza viruses. Usually, influenza outbreaks in epidemic proportions or rarely in a pan-

demic form (Olsen et al., 2006). Despite of sporadic appearance, roughly three times per century, flu pandemic represents one of the most ravaging disasters (Stiver, 2004). This threat takes millions of human lives and has serious social and economic impacts worldwide (Stiver, 2004; Suzuki, 2005). Therefore, extensive efforts have been invested in attempting to control influenza infection.

Currently, two classes of anti-influenza drugs (Fig. 1.) are used for chemoprophylaxis and treatment of influenza virus infections: *M2 proton channel blockers* (amantadine (**1**) and rimantadine (**2**)) prevent virion uncoating; and the *neuraminidase inhibitors* - oseltamivir (**3**), zanamivir (**4**), peramivir (**5**) and laninamivir octanoate (**6**) inhibit viral neuraminidase (NA) that is responsible for virion release. In addition, another compound, i.e. the *nucleoside analogue* - ribavirin (**7**) is also capable to inhibit the replication of influenza viruses by inhibiting the virus-specific RNA polymerase, which is essential for viral replication (Beigel & Bray, 2008). Nowadays the clinical applications of aminoadamantanes have been limited in consequence of fast-spreading resistant viruses (Bright et al., 2005; Deyde et al., 2007; De Clercq, 2006). Moreover, M2 ion channel blockers inhibit the replication of influenza A virus only, and may also cause neurological side effects (Deogaonkar et al., 2011).

Being highly effective against both influenza A and B viruses, neuraminidase inhibitors have priority over aminoadamantanes, due to their less side effects and a better profile regarding drug resistance (Reece, 2007; Hurt et al., 2009). Today these antivirals are the best choice for treatment and prophylaxis of seasonal, avian and pandemic influenza (Nitsch-Osuch & Brydak, 2014).

On the other hand, it is known that oxidative stress gets involved in various human pathological conditions, such as cancer, rheumatoid arthritis, ischemic heart disease, ageing and diverse virus infectious diseases-AIDS, hepatitis, and influenza included.

In particular, during influenza viral infection, the depletion of endogenous antioxidant concentrations such vitamin E and glutathione have been observed (Hennet et al., 1992), whereas the levels of xanthine oxidase, generating superoxide radicals have been elevated (Oda et al., 1989).

Therefore, for reducing the oxidative stress during flu infection is necessary and antioxidant therapy is indicated. Many studies have shown that administration of antioxidants or combining them with anti-influenza drugs inhibits the proliferation of influenza virus (Oda et al., 1989; Mileva et al., 2000; Haidari et al., 2009; Uchide & Toyoda, 2011; Raju et al., 2000; Uchide & Toyoda, 2008a, b; Kumar et al., 2003; Christen et al., 1990; Hennet et al., 1992).

Hydroxycinnamic acids (e.g., ferulic, sinapic, caffeic) and their derivatives have been reported to possess a broad range of biological activities, including antioxidant proper-

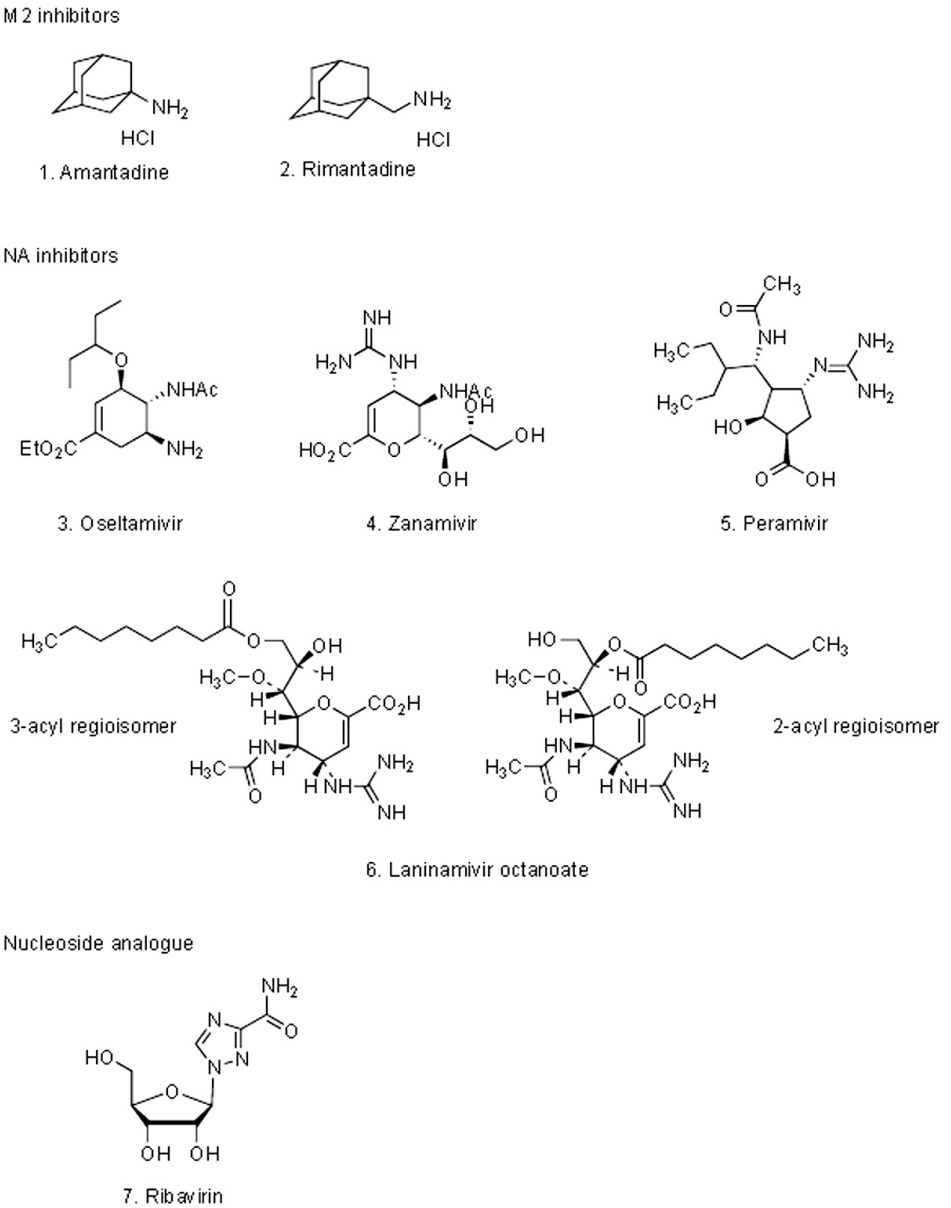


Fig 1. Anti-influenza drugs ((Uchide & Toyoda, 2011; Beigel & Bray, 2008)

ties (Razzaghi-Asl et al., 2013). Moreover, the introduction of cinnamoyl moiety has also been known to alter the potency, permeability, solubility or other parameters of a selected drug or pharmacophore. Consequently, this lead us to look for an anti-influenza activity of chemically combined antioxidant molecules - fluorinated hydroxycinnamic acid amides with anti-influenza drug-oseltamivir.

Results

Chemistry

The global effort at finding potent anti-influenza drugs is of great concern for combating influenza virus infections.

Since it is well known that influenza induces oxidative stress, an increasing number of publications suggest that antioxidant therapy could be used as a potent approach to severe influenza-associated complications, especially.

In this regard, antioxidant molecules of hydroxycinnamoyl 6-fluorotryptophan amides, were chemically modified with an oseltamivir moiety.

Oseltamivir-based analogues have been synthesized via the route outlined in Scheme 1.

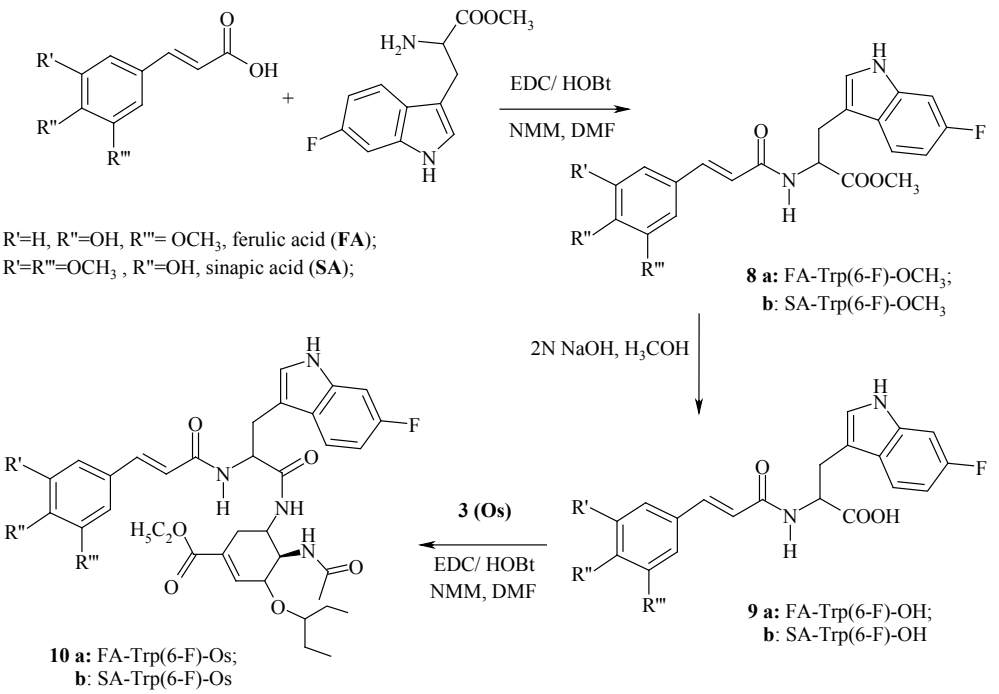
Following previously reported procedures (Stoykova et al., 2013), fluorinated hydroxycinnamoyl amides (feruloyl- and sinapoyl 6-fluoro-tryptophan amides (**8a,b**)) were synthesized through solution-phase EDC/HOBt coupling (Scheme 1) and *E*-π diastereoisomers were consistent with literature NMR values of *J* constant (Stoykova et al., 2013).

Subsequent deprotection of the ester group of compounds (8a,b) by saponification with 2N NaOH in MeOH gave 9a,b in yields greater than 80% (Scheme 1). The target oseltamivir analogues (**10a**,b) were obtained by condensation using EDC/HOBt method in yields of 45% (10b) and 73% (10a). The structure of amides (**10a**,b) were confirmed by their melting points, IR and MS with electrospray ionization (ESI pos. and neg.). As shown in Fig. 2 the positive-ion ESI-MS spectrum of feruloyl compound (10a) (Mw=692.8) clearly reveals that the base peak at m/z 715.3 corresponds to $[M+Na]^+$ and the negative-ion ESI-MS spectrum gives a deprotonated molecular ion peak at $[M–H]^-$ m/z 691.2.

Comparing with 10a, the difference in the spectrum of sinapoyl amide (10b, Fig.3.) (Mw=722.8) is due to an additional methoxyl group. Under positive-ion conditions, an intense $[M+Na]^+$ occurs at m/z 745.3, while the deprotonated molecular ion peak $[M–H]^-$ is observed at m/z 721.2 in negative-ion spectrum.

Evaluation of anti-influenza activity in vitro

Preliminary antiviral activities of the synthesized compounds (10a,b) against influenza A (H3N2) were evaluated in vitro through their ability to prevent cytopathic effects



Scheme 1. Synthesis

(CPE) in influenza A virus (H3N2) infected Madin-Darby canine kidney (MDCK) cells. Briefly, monolayer MDCK cells were inoculated with 0.1 mL virus suspension containing 100 $CCID_{50}$. After 1 h at 37^0C for virus adsorption the innoculum was washed out and replaced by 0.1 mL of noncytotoxic dilutions of the newly synthesized compounds. Cells that were not inoculated with virus were left for cell controls (with only maintenance medium) and toxicity controls (with respective dilution of the compound in the maintenance medium). Cells inoculated with virus but not treated with compound were left for virus controls. Following 48 h incubation (when virus specific cytopathic effect destroyed 100% of the cells in the virus control wells), the neutral red staining procedure was applied and the percentage of CPE inhibition, if present, was calculated using the following formula:

$$\% \text{ CPE} = (OD_{\text{test sample}} - OD_{\text{virus control}})/(OD_{\text{toxicity control}} - OD_{\text{virus control}}) - 100.$$

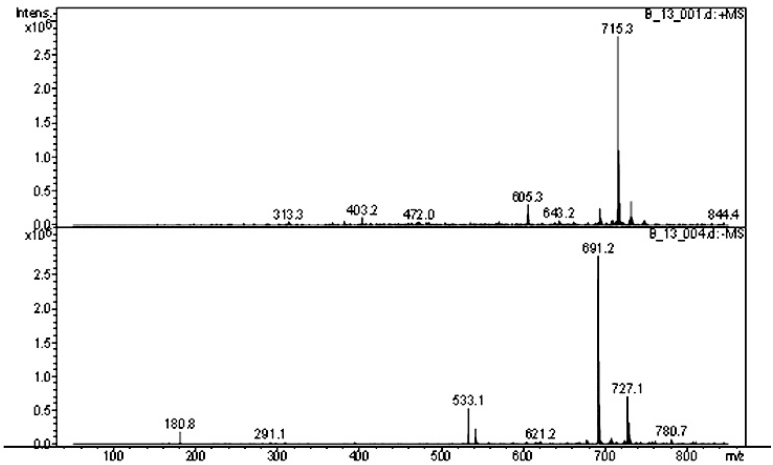


Fig. 2.

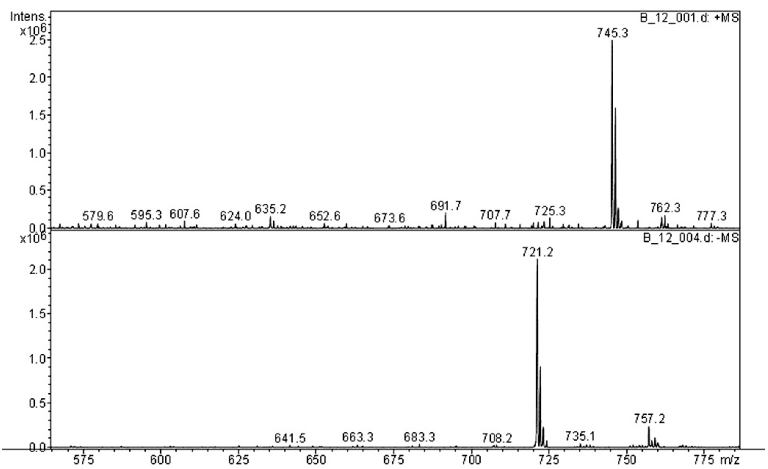


Fig. 3.

Despite expectations, the newly synthesized compounds did not provoke enhanced antiviral activity against the replication of influenza virus A (H3N2).

Conclusions

In conclusion, two novel oseltamivir-based derivatives (**10a,b**) were obtained, comprising of an oseltamivir skeleton linked to hydroxycinnamoyl fluorinated amides. The

antiviral effect of the covalently bonded hybride structures against influenza *A* (*H3N2*) were evaluated *in vitro.* It has been shown that the examined compounds did not provoke synergistic anti-influenza effects.

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