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A novel influenza virus neuraminidase inhibitor AV5027

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ABSTRACT

A medium-sized focused library of novel Oseltamivir structural analogues with promising antiviral activity was successfully synthesized using a combinatorial approach. The synthesized compounds were then thoroughly evaluated in neuraminidase- and cell-based assays. As a result, (3*R*,4*R*,5*S*)-4-(2,2-difluoroacetylamino)-5-amino-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid (AV5027) was identified as novel Hit-compound with picomolar potency. QSAR analysis was carried out based on the obtained biological data. Computational modeling was performed using a 3D-molecular docking approach and classical regression analysis. The developed integral model demonstrated a sufficient prediction accuracy and tolerance to evaluate compounds based on their potential activity against neuraminidase (NA) at least within the scaffold. Several compounds from the series can be reasonably regarded as promising anti-influenza drug-candidates.

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1. Introduction

Influenza is one of the most abundant acute respiratory diseases affecting people worldwide of all age groups and social backgrounds. Frequent seasonal epidemics lead to increased morbidity and, in more severe cases, mortality on a global scale. Annually, up to 10% among the U.S. population is affected by symptomatic influenza infection. More than 220 K persons are hospitalized and 24 K deaths due to influenza-associated illness are reported (Hayden, 2002; Monto, 2008). The highest hospitalization rate is observed of aged population, children and young persons, about one per 1 K or higher in infants, persons age 65 (approx. 20% of deaths) and older as well as persons with chronic medical conditions (Griffin, 2013). In addition to available and well-distributed anti-influenza vaccines small-molecule compounds are currently described as promising therapeutics targeted against both viral types (A and B). Influenza virus H1N1 was comprehensively described in many papers from different viewpoints (for review see: Du et al., 2010a,b; Li et al., 2011; Wang et al., 2009a,b). During the last decade, various attempts have been made to develop effective NA inhibitors with a low level of resistance (Wang et al., 2009b; Du et al., 2007; Gong et al., 2009; Wang et al., 2007, 2010; Wei et al., 2006). Oseltamivir phosphate (OsP, Fig. 1) (Kim et al., 1997), also known as Tamiflu, is one of the most effective oral neuraminidase inhibitors with a prominent antiviral activity. OsP is the pro-drug of Oseltamivir carboxylate (OsC). With respect to the route of administration, cost of production and structure optimization, the development of novel small-molecule compounds targeted against influenza is reasonably attractive. Following this concept, we have synthesized a combinatorial library of novel OsC analogues with the general structures **1a–r** (Fig. 1) (Ivachtchenko, 2012, 2013).

2. Materials and methods

2.1. Cells and viruses

Madin-Darby Canine Kidney (MDCK) cells were grown in minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 5 mM L-glutamine, 25 mM HEPES, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, and 100 μg/ml kanamycin sulfate, in a humidified atmosphere of 5% CO_2. The influenza virus strains A/CA/07/09 (H1N1) pdm09 and A/Duck/MN/1525/81 (H5N1) were obtained from the WHO and Utah State University (Logan, Utah, USA). Purified N1 crystals from A/Pr/8/34 (H1N1), B/Taiwan/2/62 (H1N1), A/CA/07/09 (H1N1) viruses were obtained from American Type Culture Collection (Manassas, Virginia, USA),

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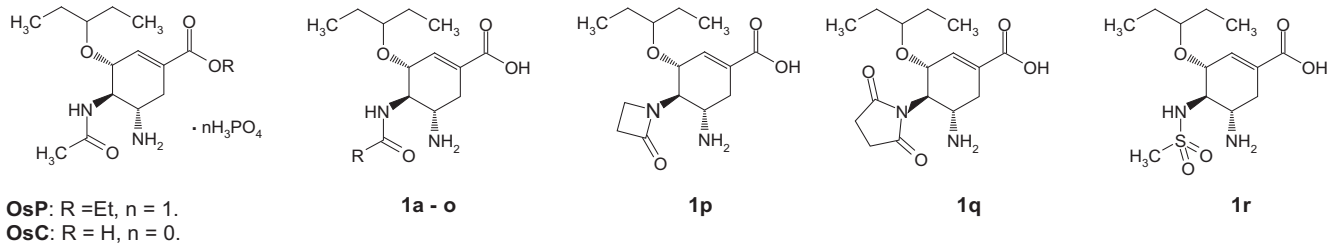


Fig. 1. OsP, OsC and compounds under investigation in this work (**1a**–**r**). **1**: R = H (**a**), CH_2=CH (**b**), CH≡C (**c**), *c*-H_7C_3 (**d**), FH_2C (**e**), F_2HC (**f**), F_3C (**g**), CF_3CH_2 (**h**), *n*- F_7C_3 (**i**), $CHF_2CF_2CF_2CF_2$ (**j**), *n*- F_9C_4 (**k**), OCH_2CH_3 (**l**), NH_2 (**m**), CH_2NH_2 (**n**), CH_2OH (**o**); R^1+R^2 = CH_2CH_2 (**p**), $CH_2CH_2C(O)$ (**q**).

and mouse-adopted A/Aichi/2/69 (H3N2) viruses were from collection of Ivanovsky Institute of Virology (Moscow, Russia).

2.2. Compounds and reagents

2.2.1. General and analytical chemistry

In all cases, the end of the reaction was determined by conversion of the substrate (LC–MS control). Evaporation of solvents from the resulting mixture and drying of the products were carried out at reduced pressure. Separation of reaction products was performed using a Shimadzu LC-8A HPLC system equipped with a Reprosil-pur C-18-AQ 10 μm 250 × 20 mm chromatographic column and Reprosil-Pur C-18-AQ 10 μm 50 × 20 mm precolumn, at a flow rate of 25 mL/min in a gradient mode with mobile phase MeCN + water + 0.05% CF3COOH.

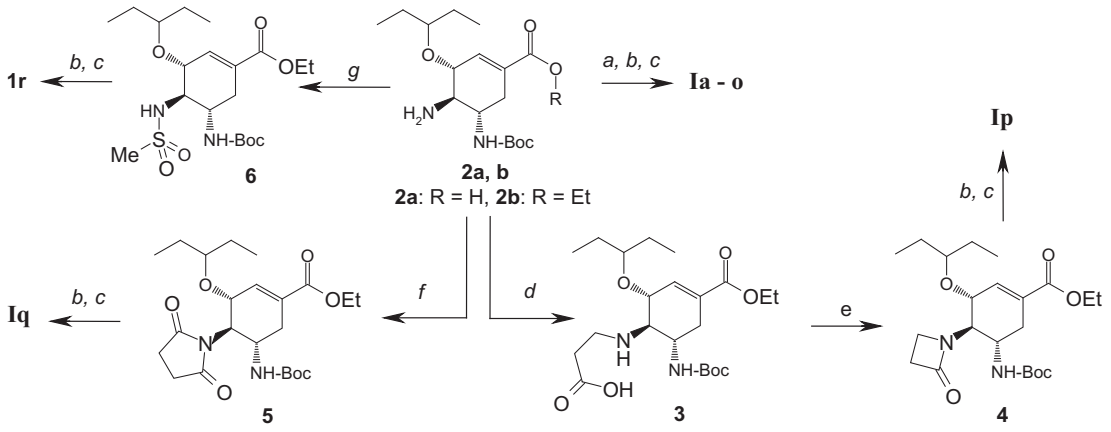
^{1}H NMR spectra of the investigated compounds were recorded in solutions of DMSO-d_6 or CDCl3, respectively, using a Bruker DPX-400 spectrometer (400 MHz, 27 °C). LC–MS spectra were obtained using a Shimadzu LC-8A HPLC system equipped with a Waters XBridge C18 3.5 mm column (4.6 × 150 mm), PE SCIEX API 150 EX mass detection and Shimadzu spectrophotometric detector (λmax 220 and 254 nM. Purity of the synthesized compounds was determined using the LC–MS method with UV detector at the absorption wavelength of 254 nM. Purity of the compounds was more than 95%.

2.2.2. General synthetic procedure

Trifloroacetate (TFA) of OsC was prepared by basic hydrolisis of OsP, obtained from Airsea Pharmaceutical Ltd., and TFA of (3*R*,4*R*,5*S*)-4,5-diamino-3-(1-ethylpropoxy)cyclohex-1-enecarboxylic acid (**1a**) – by acidic hydrolisis of OsP. Lanamivir (LA) was obtained from AK Scientific Inc. TFAs of N(4)-substituted (3*R*,4*R*,5*S*)-4,5-diamino-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acids (TFA of **1a**–**r**) were synthesized starting from (3*R*,4*R*,5*S*)-4-amino-5-(Boc-amino)-3-(1-ethyl-propoxy)-cyclohex-1-enecarboxylic acid (**2a**) and its ethyl ester **2b** (Konno et al., 2008; Morita et al., 2008) according to Scheme 1.

2.2.2.1. TFA of (3R,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)cyclohex-1-enecarboxylic acid (TFA of OsP). OsP (500 mg) was dissolved in 5 mL 5% lithium hydroxide solution in 1:1 dioxane–water. The resulting mixture was stirred for 1 h at room temperature. After the reaction was completed, the solvent was evaporated using a rotavapor, the residue was then treated with dioxane, filtered and rotavaped again. The resulting dry product was subjected to preparative HPLC to give TFA of OC. LC-MS (ESI) $[M+H]^+$ 285. ^{1}H NMR (DMSO-d_6) δ 12.79 (br, 1H), 8.06 (d, J = 9.6 Hz, 1H), 8.02 (br, 2H), 6.64 (s, 1H), 4.15 (m, 1H), 3.79 (q, J = 11.2 Hz, 1H), 3.39 (m, 1H), 3.28 (m, 1H), 2.74 (m, 1H), 2.31 (m, 1H), 1.88 (s, 3H), 1.42 (m, 4H), 0.83 (t, J = 7.6 Hz, 3H), 0.79 (t, J = 7.6 Hz, 3H).

2.2.2.2. TFA of (3R,4R,5S)-5-amino-3-(1-ethylpropoxy)-4-formamidocyclohex-1-enecarboxylic acid (TFA of 1a). A mixture of acid **2a** (250 mg, 0.73 mmol), formic acid (0.5 mL) and molecular sieves 3 Å (1 g) in 15 mL of toluene was refluxed for 12 h. The mixture was filtered and precipitated, then washed with ethanol and the combined filtrate was rotavaped. The resulting residue was dissolved in ethyl acetate, dried over anhydrous magnesium sulfate, filtered and rotavaped. The residue was dissolved in 3 M HCl in dioxane (3 mL) and stirred at room temperature. The reaction was monitored by TLC and LC–MS. After the reaction was completed, the mixture was rotavaped and analyzed using HPLC to give TFA of **1b** with 75% yield. LC-MS (ESI) $[M+H]^+$ 271. ^{1}H NMR (DMSO-d_6) δ 8.26 (d, J = 9.2 Hz, 1H), 8.18 (s, 1H,) 8.06 (s, 1H), 6.65 (s, 1H), 4.18 (q, J = 8 Hz, 1H), 3.86 (q, J = 10 Hz, 1H), 3.56 (s, 1H), 2.77 (m, 1H), 2.30 (m, 1H), 1.43 (m, 4H), 0.82 (m, 6H).



Scheme 1. The common synthetic strategy.