RESEARCH ARTICLE

Conjugation to Polymeric Chains of Influenza Drugs Targeting M2 Ion Channels Partially Restores Inhibition of Drug-Resistant Mutants

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ABSTRACT: By attaching multiple copies of the influenza M2 ion channel inhibitors amantadine (**1**) and rimantadine (**2**) to polymeric chains, we endeavored to recover their potency in inhibiting drug-resistant influenza viruses. Depending on loading densities, as well as the nature of the drug, the polymer, and the spacer arm, polymer-conjugated drugs were up to 30-fold more potent inhibitors of drug-resistant strains than their monomeric parents. In particular, a 20% loading density and a short linker group on the negatively charged poly-L-glutamate resulted in one of the most potent inhibitors for **2**'s conjugates against drug-resistant influenza strains. Although full recovery of the inhibitory action against drug-resistant strains was not achieved, this study may be a step toward salvaging anti-influenza drugs that are no longer effective. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: antiinfectives; biodegradable polymers; conjugation; inhibition; drug resistance; polymeric drug carrier; polymeric drugs; polymers

INTRODUCTION

Influenza viruses commonly infect the respiratory tract in humans[1] and are a major cause of morbidity and mortality in the world.[2,3] Two of the four US Food and Drug Administration (FDA)-approved small-molecule anti-influenza drugs—the adamantane-class M2 ion channel inhibitors amantadine (**1**) and rimantadine (**2**) (Fig. 1)—are no longer recommended as therapeutics because nearly every circulating influenza A strain has evolved resistance to them.[2,4,5] These drugs block the M2 ion channels on the surface of the virus,[6–9] thereby preventing the flow of protons into the viral core (an essential step in the viral infection cycle).[2] Resistance to **1** and **2** is because of point mutations in the M2 ion channel protein, with the most common being the S31N in the interior of the channel.[2]

Because of the daunting challenges in discovering new anti-influenza drugs, it would be of great benefit to salvage older FDA-approved drugs that are impotent against newly emerged mutants. Previously, we have demonstrated that the attachment of multiple copies of the influenza neuraminidase inhibitor zanamivir to a flexible polymeric chain not only dramatically improves the potency against drug-sensitive strains, but also resurrects the inhibitory effect against zanamivir-resistant mutants.[10,11] This phenomenon appears to stem from two mechanisms. The first is multivalency, whereby several simultaneous interactions between polymer-attached zanamivir and its viral target result in a far greater avidity compared with the monomer's binding constant,[10,12,13] while also generating an increased drug concentration in the vicinity of the virus.[13] The second contributor to the improved potency is a novel mechanism of inhibition, blocking earlier stages of the viral cycle, which monomeric zanamivir lacks.[3]

Additional Supporting Information may be found in the online version of this article. Supporting Information

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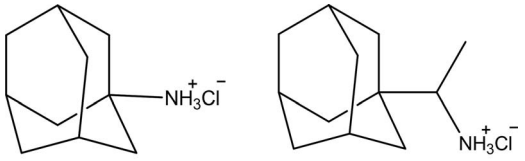

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Figure 1. Chemical structures of both FDA-approved adamantane-class M2 ion channel influenza A inhibitors: amantadine·HCl (**1**) and rimantadine·HCl (**2**).

Herein, we explore whether the approach of attaching multiple copies of influenza drugs to polymeric chains can boost the adamantane inhibitors' prowess against drug-resistant influenza mutants (as it did with zanamivir[10]).

MATERIALS AND METHODS

Materials

Amantadine·HCl (**1**; here and henceforth the bold number equally applies to a free base/acid and its salt), rimantadine·HCl (**2**), 3-amino-1-adamantanecarboxylic acid (**3**), 3-(1-aminoethyl) adamantan-1-ol·HCl (**7**), poly-L-glutamate Na salt (MW of 50–100 kDa), carboxymethylcellulose Na salt (CMC; MW of ~100 kDa), poly(acrylic acid) (MW of ~100 kDa), and all solvents and other reagents were purchased from Sigma–Aldrich Chemical Company (St. Louis, Missouri) and used without further purification unless otherwise specified. N-Hydroxysulfosuccinimide (sulfo-NHS) was obtained from Proteochem (Denver, Colorado). 5-azidopentanoic acid and 5-azidopentan-1-amine was obtained from Synthonix (Wake Forest, North Carolina), and 11-azido-3,6,9-trioxaundecanoic acid from TCI America (Portland, Oregon).

Syntheses

Synthesis of 1-Linker-Azide (6)

Linker addition to **3** was carried out as described by Wanka et al.[14] Briefly, 300 mg (1.5 mmol) of **3** and 715 mg (6.7 mmol) of Na_2CO_3 were suspended in a mixture of 10 mL of H_2O and 5 mL of acetone, followed by stirring and placing in an ice bath. Next, Fmoc-Cl (426 mg, 1.6 mmol) in 5 mL of acetone was added over 30 min with an addition funnel. The reaction mixture was incubated at room temperature (RT) overnight and then heated to 50°C for 2 h to evaporate acetone. To purify the product, the reaction was poured over ice (35 g) and extracted thrice with diethyl ether. The aqueous layer was then acidified to pH 5 and extracted thrice with ethyl acetate. The ethyl acetate portions were combined, washed with H_2O, and dried over Na_2SO_4 to afford an off-white powder of Fmoc-3-amino-1-adamantanecarboxylic acid (**4**) (~40% yield). R_f on TLC (thin layer chromatography) silica plate of 0.47 in 10:1 (v/v) CH_2Cl_2:MeOH. ^{1}H NMR **4** ([D_8] tetrahydrofuran (THF)) δ (400 MHz): 1.65 (2H, d, C*H*$_2$-**1**), 1.72 (2H, s, C*H*$_2$-**1**), 1.83 (4H, s, C*H*$_2$-**1**), 1.95 (4H, s, C*H*$_2$-**1**), 2.07 (H, s, C*H*-**1**), 2.14 (H, s, C*H*-**1**), 4.18 (1H, t, C*H*-Fmoc), 4.27 (2H, d, C*H*$_2$-Fmoc), 7.25 (2H, t, C*H*-aromatic-Fmoc), 7.3 (2H, t, C*H*-aromatic-Fmoc), 7.6 (2H, d, C*H*-aromatic-Fmoc), 7.8 (2H, d, C*H*-aromatic-Fmoc).

To synthesize **5**, **4** (220 mg, 0.53 mmol) was dissolved in 5 mL of dry THF. To that, O-benzotriazole-*N,N,N′,N′*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) (200 mg, 0.53 mmol) was added, followed by 65 μL (0.53 mmol) of 5-azidopentan-1-amine and 68 μL (0.5 mmol) of Hünig's base. The reaction mixture was stirred at RT overnight and then heated to 60°C for 1 h. After cooling, 3 mL of brine was added, and the mixture was extracted with $CHCl_3$ thrice. The organic phases were combined, washed with 1 M HCl, 5% $NaHCO_3$, H_2O, and brine, and then further purified on a silica gel column with 10:1 (v/v) CH_2Cl_2:methanol mobile phase to afford **5**[14] (~55% yield). R_f on TLC silica plate of 0.82 in 10:1 (v/v) CH_2Cl_2:MeOH. ^{1}H NMR **5** ($CDCl_3$) δ (400 MHz): 1.35 (2H, m, C*H*$_2$-linker), 1.47(2H, m, C*H*$_2$-linker), 1.6 (4H, m, C*H*$_2$-**1**, C*H*$_2$-linker), 1.78 (4H, s, C*H*$_2$-**1**), 1.85 (2H, d, C*H*$_2$-**1**), 1.95 (2H, d, C*H*$_2$-**1**), 2.05 (2H, s, C*H*$_2$-**1**), 2.18 (2H, s, C*H*-**1**), 3.2 (2H, dd, C*H*$_2$-linker), 3.24 (2H, t, C*H*$_2$-linker), 4.18 (1H, t, C*H*-Fmoc), 4.3 (2H, d, C*H*$_2$-Fmoc), 7.25 (2H, t, C*H*-aromatic-Fmoc), 7.3 (2H, t, C*H*-aromatic-Fmoc), 7.6 (2H, d, C*H*-aromatic-Fmoc), 7.7 (2H, d, C*H*-aromatic-Fmoc).

To generate the deprotected final **1**-linker-azide (**6**) for attachment to poly-L-glutamate, **5** (75 mg, 0.14 mmol) was dissolved in 1.2 mL of dry acetonitrile and cooled to 0°C. Diethylamine (1.2 mL) was added, and the reaction mixture was stirred for 1 h at 0°C and RT for 24 h. The reaction mixture was then extracted with H_2O at pH 3, and the product (**6**) was recovered from the aqueous phase[14] (~20% yield). R_f on TLC silica plate of 0.12 in 10:1 (v/v) CH_2Cl_2:MeOH. ^{1}H NMR **6** ($CDCl_3$) δ (400 MHz): 1.27 (2H, m, C*H*$_2$-linker), 1.45(2H, m, C*H*$_2$-linker), 1.52 (2H, m, C*H*$_2$-linker), 1.61 (2H, s, C*H*$_2$-**1**), 1.7 (2H, d, C*H*$_2$-**1**), 1.78 (4H, d, C*H*$_2$-**1**), 1.83 (2H, d, C*H*$_2$-**1**), 1.88 (2H, s, C*H*$_2$-**1**), 3.1 (2H, t, C*H*$_2$-linker), 2.25 (2H, s, C*H*-**1**), 3.25 (2H, t, C*H*$_2$-linker).

Synthesis of 2-Linker-Azides (11 and 12)

To obtain an organic solvent soluble free base, 3-(1-aminoethyl)adamantan-1-ol·HCl was suspended in CH_2Cl_2 and washed with 1 M NaOH. The resultant organic layer was rotary evaporated, and the isolated white powder of 3-(1-aminoethyl)adamantan-1-ol (**7**) was Boc protected for subsequent chemical modification. To this end, a solution of di-*tert*-butyl

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