RESEARCH ARTICLE

Conjugating Drug Candidates to Polymeric Chains Does Not Necessarily Enhance Anti-Influenza Activity

ALYSSA M. LARSON,[1] HONGMEI WANG,[2] YANG CAO,[3] TAIJIAO JIANG,[4] JIANZHU CHEN,[5] ALEXANDER M. KLIBANOV[1,6]

[1]Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

[2]Department of Applied Chemistry, China Agricultural University, Beijing 100193, People's Republic of China

[3]College of Life Science, Sichuan University, Chengdu 610064, People's Republic of China

[4]Institute of Biophysics, National Laboratory of Biomacromolecules, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

[5]Department of Biology and David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

[6]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 2 April 2012; revised 8 June 2012; accepted 13 June 2012

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23253

ABSTRACT: Using the plaque reduction assay, relatively simple bicyclic quinone molecules, as well as multiple copies thereof covalently attached to a long polyglutamate-based polymeric chain, were examined as new inhibitors of various naturally occurring strains of influenza A virus. The polymer-conjugated inhibitors were found to have a far greater potency (for some as high as two orders of magnitude when a long spacer arm was employed) than their corresponding parent molecules against the human Wuhan influenza strain. However, such polymeric inhibitors failed to exhibit higher potency compared with their small molecule predecessors against the human Puerto Rico and avian turkey influenza strains. These observations, further explored by means of molecular modeling, reveal the previously unrecognized unpredictability of the benefits of multivalency, possibly because of poor accessibility of the viral targets to polymeric agents. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: polymeric drugs; structure–activity relationship; drug design; conjugation; anti-infectives; polymeric drug carrier; influenza; inhibitor

INTRODUCTION

Influenza A virus is highly transmissible and kills over 250,000 people worldwide each year. In the United States alone, some 20% of the population contracts the virus annually, leading to countless missed days of work and school and tens of billions of dollars in associated costs.[1,2] The two US Food and Drug Administration-approved drugs for the treatment of influenza infections, Oseltamivir (Tamiflu™) and Zanamivir (Relenza™), have fallen short of expectations due to their mediocre activity in reducing the symptoms and duration of the infection, as well as emerging resistance in clinical isolates.[3–5] Thus, new, more effective anti-influenza therapeutic agents are greatly needed.

One proposed strategy for generating more potent inhibitors of influenza is to utilize the benefits of multivalency.[2,4,6–11] Conjugating multiple copies of influenza inhibitors to a flexible polymeric chain has been shown to result in multivalent interactions between the polymer-attached inhibitors and the viral surface receptor proteins.[2,4,6–11] These enhanced interactions, in turn, lead to a much stronger binding compared with that of the small molecule parents stemming from favorable entropic factors; in addition, H_2O-swollen polymeric chains may sterically hinder physical contacts between the virus and the target cell.[6]

The foregoing benefits of multivalency for binding to influenza virus have been demonstrated for viral

Correspondence to: Alexander M. Klibanov (Telephone: +617-253-3556; Fax: +617-252-1609; E-mail: klibanov@mit.edu)

surface proteins with the natural ligand of hemagglutinin, *N*-acetylneuraminic (sialic) acid, and with the neuraminidase inhibitor Zanamivir.[2,4,6–11] However, both of these compounds are structurally complex, requiring many-step syntheses to become amenable to attachment to polymeric chains in order to investigate the effect of multivalency.[2,4] They are also difficult to modify selectively and thus not optimal for structure–activity relationship (SAR) studies. In the present work, we instead have employed simple organic molecules[12] with anti-influenza properties to investigate the SAR of multivalency. In particular, we have assessed whether the aforementioned potential benefits of multivalency invariably translate into greater anti-influenza activity.

MATERIALS AND METHODS

Materials

All small molecule inhibitors except for **13** (Figure 1), poly-L-glutamate Na salt (50–100 kDa), solvents, and reagents were purchased from Sigma–Aldrich Chemical Company (St. Louis, Missouri) and used without further purification. Dialysis membranes [3500 kDa molecular weight (MW) cutoff] were from Spectrum Laboratories (Rancho Dominguez, California) and PD-10 desalting columns were from GE Healthcare (Buckinghamshire, United Kingdom).

Syntheses

Synthesis of 6-(Hydroxymethyl)Naphthalene-1,4-Dione (13)

It was carried out as described by Antonini et al.[13]

Synthesis of 2a, 2b, 5a, 5b, 8, 11, and 14

Conjugation was carried out via a Steglich esterification with minor deviations from a reported procedure.[14] Specifically, poly-L-glutamate Na salt was converted to poly(L-glutamic acid) by dissolution in double-distilled (dd) H_2O, lowering the pH to 1, and washing with 0.10 M HCl to remove free salts before an overnight lyophilization. Lyophilized poly(L-glutamic acid) (20 mg, 0.16 mmol) was dissolved in 0.80 mL of dry dimethylformamide (DMF), followed by the addition of *N,N′*-dicyclohexylcarbodiimide (DCC) [4.3 mg (0.021 mmol) in 0.30 mL of DMF for a ~10% derivatization], an anti-influenza agent (0.050 mmol in 0.20 mL of DMF), pyridine (10 μL, 0.12 mmol), and a catalytic quantity of 4-dimethylaminopyridine (DMAP) in 0.40 mL of DMF with vigorous stirring. The solution was stirred overnight at room temperature. The polymer was then isolated by precipitation in chloroform, washed with fresh chloroform to remove the unreacted anti-influenza agent, converted to the Na salt, and dialyzed against ddH_2O in a 3500-Da MW cutoff dialysis membrane for 24 h to remove free salts and

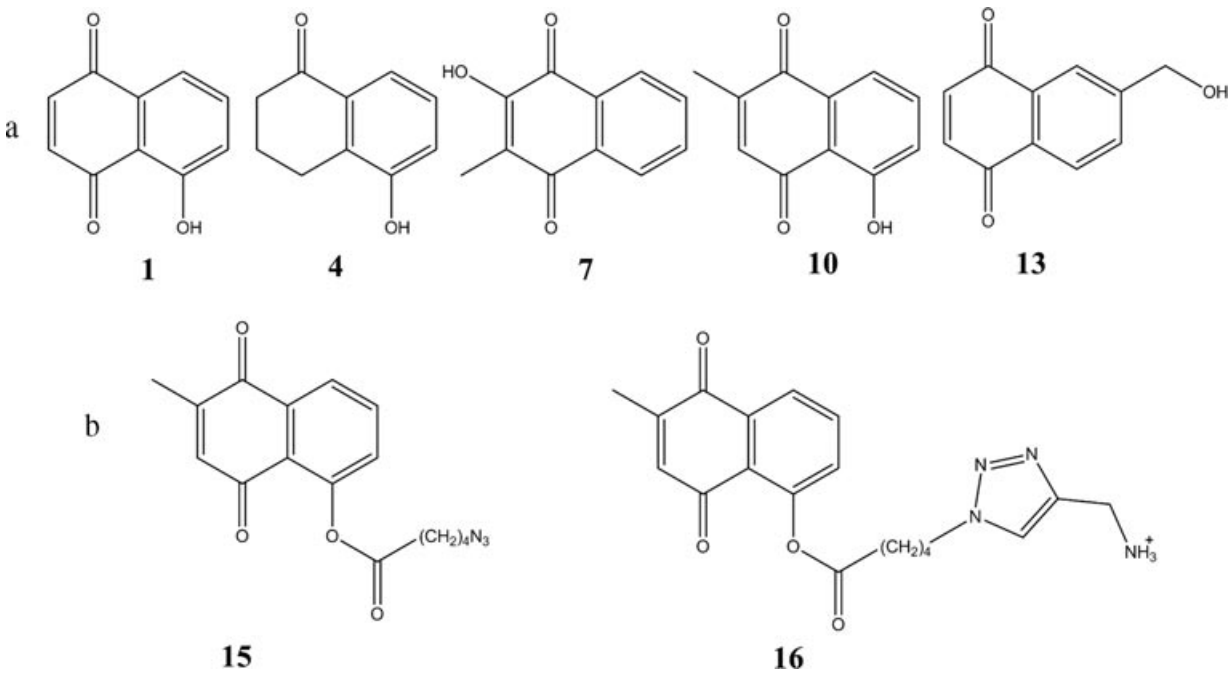

Figure 1. Chemical structures of (a) 5-hydroxynaphthalene-1,4-dione (**1**) and its analogs **4**, **7**, **10**, and **13** used in this study; (b) modified **10** with an azide-terminated spacer arm (**15**) for use in conjugating to propargylamine-derivatized poly-L-glutamate and **10** derivatized with a spacer arm (**16**) for investigation of the dependence of IC_{50} on the presence of the spacer arm by itself with no polymer.

 DOI 10.1002/jps