European Journal of Medicinal Chemistry 134 (2017) 133–139

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Inhibition of influenza virus infection by multivalent pentacyclic triterpene-functionalized per-*O*-methylated cyclodextrin conjugates

Zhenyu Tian [a, 1], Longlong Si [a, 1], Kun Meng [a], Xiaoshu Zhou [a], Yongmin Zhang [b], Demin Zhou [a], Sulong Xiao [a, *]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China
[b] Sorbonne Universités, UPMC Univ Paris 06, Institut Parisien de Chimie Moléculaire, CNRS UMR 8232, 4 place Jussieu, 75005 Paris, France

ARTICLE INFO

Article history:
Received 17 January 2017
Received in revised form
22 February 2017
Accepted 31 March 2017
Available online 2 April 2017

Dedicated to Professor Lihe Zhang on the Occasion of His 80th Birthday.

Keywords:
Per-*O*-methylated cyclodextrin
Pentacyclic triterpene
Influenza virus
Hemagglutinin
Multivalent effect

ABSTRACT

Multivalent ligands that exhibit high binding affinity to influenza hemagglutinin (HA) trimer can block the interaction of HA with its sialic acid receptor. In this study, a series of multivalent pentacyclic triterpene-functionalized per-*O*-methylated cyclodextrin (CD) derivatives were designed and synthesized using 1, 3-dipolar cycloaddition click reaction. A cell-based assay showed that three compounds (**25**, **28** and **31**) exhibited strong inhibitory activity against influenza A/WSN/33 (H1N1) virus. Compound **28** showed the most potent anti-influenza activity with IC_{50} of 4.7 μM. The time-of-addition assay indicated that compound **28** inhibited the entry of influenza virus into host cell. Further hemagglutination inhibition (HI) and surface plasmon resonance (SPR) assays indicated that compound **28** tightly bound to influenza HA protein with a dissociation constant (K_D) of 4.0 μM. Our results demonstrated a strategy of using per-*O*-methylated β-CD as a scaffold for designing multivalent compounds to disrupt influenza HA protein-host receptor protein interaction and thus block influenza virus entry into host cells.

1. Introduction

Influenza A viruses are enveloped viruses belonging to the family *Orthomyxoviridae*. They can cause both annual epidemics and occasional pandemics and remain an enormous clinical and public health challenge worldwide [1]. Based on the antigenic major surface glycoproteins hemagglutinin (HA; 18 subtypes) and neuraminidase (NA; 11 subtypes), they are divided into different subtypes [2]. Currently available options to fight against the respiratory system attack by influenza A viruses include both vaccines and antiviral agents. However, the three types of available vaccines, inactivated influenza vaccine, live attenuated influenza vaccine, and recombinant influenza vaccine, have moderate efficacy that varies seasonally due to constant viral evolution [3]. The biggest challenge in the development of anti-influenza therapeutics is the rapid emergence of drug-resistant strains due to the high mutation rate [4–7]. Influenza A virus infection begins with the binding of HA protein to its cellular receptor, sialic acid (SA)-linked glycoproteins, followed by fusion of the viral and endosomal membranes. Consequently, effective blocking of the interaction between HA and SA could lead to suppression of viral infection [8,9].

Structurally, HA is composed of a head domain (HA1), which contains the receptor-binding site, and a stem domain (HA2/HA1) [10,11]. It is synthesized as a single polypeptide precursor protein (HA0). Three copies of HA assemble into a noncovalent trimer which mediates influenza A virus infection by attaching to SA receptors on the cell surface to facilitate virion internalization [10,12]. Once inside the acidic endosome, a conformational change in the trimeric structure occurs, exposing a fusion peptide and allowing endosomal escape of the viral RNA. Low-angle neuron scattering studies have shown that the surface glycoproteins, predominantly HA trimers, constitute 40–60% of the virus mass [13,14]. The high density of HA on the surface of influenza virions results in specific high-avidity binding of the virus to the mucosal membranes [15]. This process provides important clues for the design of efficient multivalent ligands to block the viral protein-host receptor interactions [16].

Cyclodextrins (CDs) are cyclic oligosaccharides, containing a relatively hydrophobic central cavity and hydrophilic outer surface

* Corresponding author.
E-mail address: slxiao@bjmu.edu.cn (S. Xiao).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ejmech.2017.03.087
0223-5234/

[17]. They possess unique ability to act as molecular containers by entrapping guest molecules in their internal cavity [18,19]. The unique steric accessibility and acidity of the three types of hydroxyls in CDs have been taken into account to conceive efficient position-selective and face-selective chemical functionalization methodologies [20]. Over the past decades, a variety of multivalent conjugates based on CD scaffold involving heptylmannoside-functionalized glycoconjugate [21], mannose-functionalized amphiphilic β-CDs [22], and other biorecognizable sugar ligand-functionalized CDs [23], have been prepared and shown to be effective modulators of carbohydrate-protein interactions. In our previously study, a series of pentacyclic triterpenes were grafted onto the primary face of CD to explore the interactions between multiple pentacyclic triterpenes and HA protein; a dramatic multivalent effect for their anti-influenza virus entry activity has been found [24].

In spite of the progress, the design of multivalent ligands with optimized biological properties still remains a challenge considering the complexity of the multivalent ligand-receptor interactions [25]. Recently, per-*O*-methylated CDs and their derivatives have attracted considerable attention due to their improved solubility in water and organic solvents [26,27]. In addition, they show strong binding ability to anionic tetraarylporphyrin [28] and can be used to functionalize the bovine serum albumin (BSA) surface for inducing self-assembly [29]. However, to our knowledge, no multivalent compound has been designed based on per-*O*-methylated CD. As a natural step in our efforts towards the development of novel antiviral inhibitors [24,30], we prepared multivalent pentacyclic triterpene-functionalized per-(2,3-di-*O*-methyl)-α-, β- or γ-CD conjugates and evaluated their *in vitro* anti-influenza virus activities.

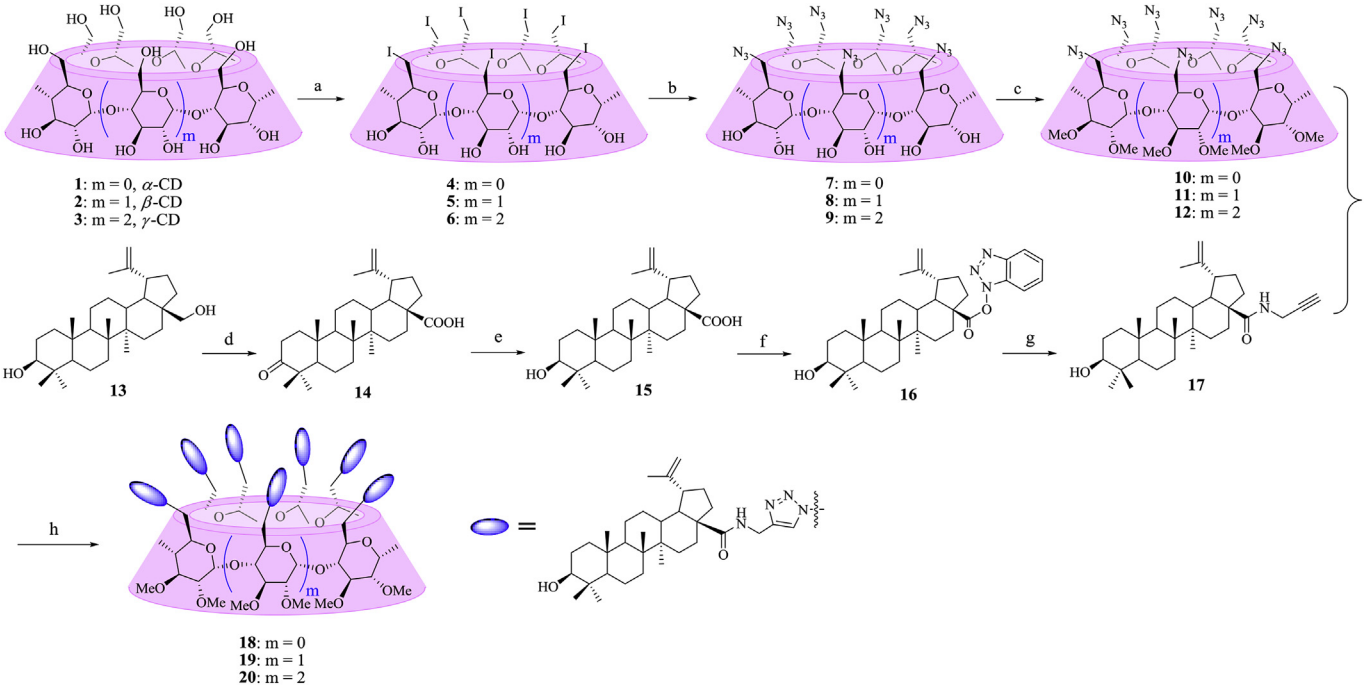
2. Results and discussion

2.1. Chemistry

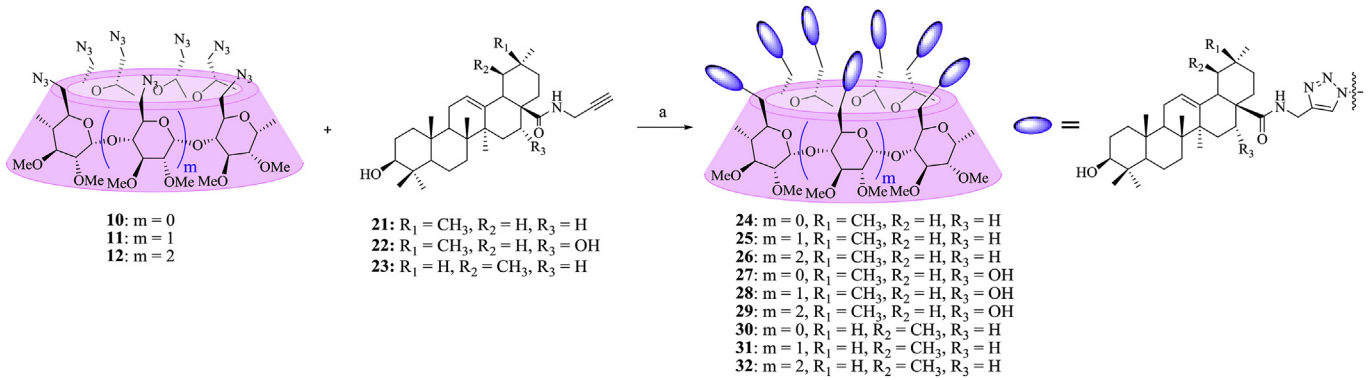
Synthesis of the multivalent betulinic acid (BA)-CD conjugates has been performed as shown in Scheme 1. We selected α-, β- and γ-CD as the central building blocks to describe the structurally diverse collection of multivalent conjugates. At first, compounds **4**–**6** were synthesized from the commercially available α-, β- and γ-CD by primary face-selective functionalization according to the procedure described by Ashton et al. [31] This was followed by nucleophilic substitution with sodium azide in *N*,*N*-dimethylformamide to provide the intermediate per-(6-azide-6-deoxy)-CDs (**7**–**9**) in quantitative yield, which was used without further purification in the next step. Methylation of crudes **7**–**9** with CH_3I in the presence of NaH afforded the per-(6-azide-6-deoxy-2,3-di-*O*-methyl)-CDs (**10**–**12**) in 65–77% yield. The synthesis of alkynyl-functionalized BA derivative **17** was performed from betulin **13** with 49% yields in four steps according to the reported method [32]. In the last step, coupling of **17** with azide functionalized compounds **10**–**12** via click reaction in THF/H_2O (1:1) in the presence of a catalytic amount of copper sulfate and *L*-sodium ascorbate yielded the multivalent BA-functionalized per-*O*-methylated CD conjugates **18**–**20**, respectively.

Similarly, the synthesis of alkynyl-functionalized pentacyclic triterpenes derivatives **21**–**23** were performed from commercially available oleanolic acid (OA), echinocystic acid (EA) and ursolic acid (UA) with high yields in two steps. Subsequent conjugation with **10**–**12** in a similar manner as described in Scheme 1 afforded conjugates **24**–**32** in yields ranging from 43 to 65% (Scheme 2).

The structures of pentacyclic triterpene-functionalized per-*O*-methylated CD conjugates **18**–**20** and **24**–**32** were characterized by



Scheme 1. *Reagents and conditions*: (a) Ph_3P, I_2, DMF, 80 °C, overnight; (b) NaN_3, H_2O, 60 °C, 12 h; (c) CH_3I, NaH, DMF; (d) H_2CrO_4, acetone, 0 °C to rt, 18 h; (e) $NaBH_4$, THF, rt, 2.5 h; (f) TBTU, DIPEA, THF; (g) 2-propargylamine, K_2CO_3, DMF, 1 h; (h) $CuSO_4$, Na-*L*-ascorbate, THF-H_2O (1:1, V/V), 100 °C, microwave.



Scheme 2. *Reagents and conditions*: (a) $CuSO_4$, Na-*L*-ascorbate, THF-H_2O (1:1, V/V), 100 °C, microwave.

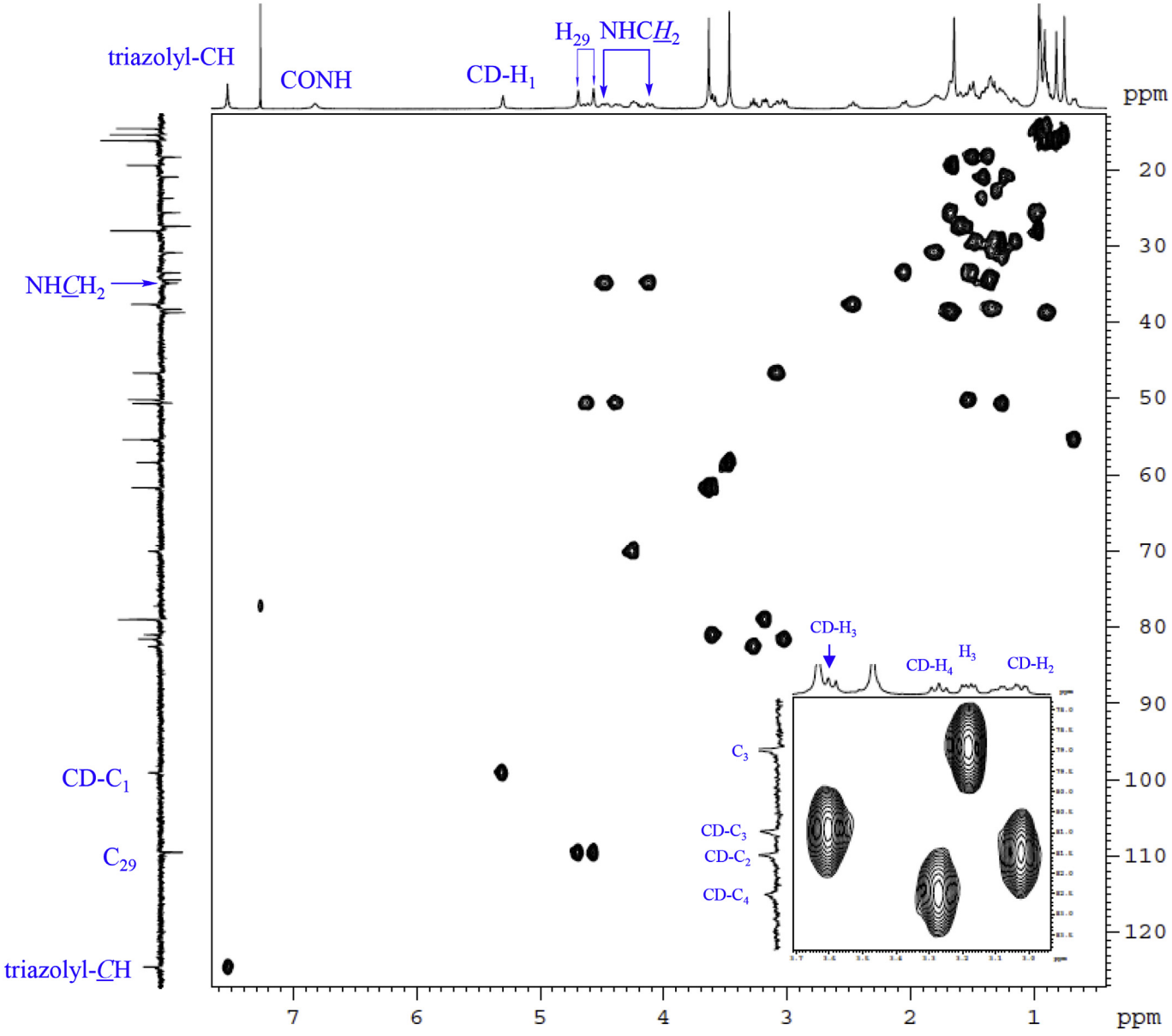


Fig. 1. A portion of the 400 MHz HSQC ($CDCl_3$, 25 °C) spectrum of **18**, with the 1D ^1H and ^{13}C NMR spectra along the top and the side, respectively.

one and two dimensional NMR and MALDI-TOF spectroscopy. Fig. 1 represents the HSQC spectrum of compound **18** as an example. In the aromatic region, the signal at 7.53 ppm was assigned to triazole-CH according to the ^{1}H-^{1}H and ^{1}H-^{13}C correlation spectra. The assignment of NH proton at 6.82 ppm was confirmed as there is no correlation for NH by HSQC spectral analysis. With a C_6-symmetry in the molecule, compound **18** showed only one set of CD-H_1 signal appearing at 5.31 ppm and one CD-C_1 signal appearing at 99.09 ppm. Similar observation was also made for other proton and carbon signals due to the C_6-symmetry. The MALDI-TOF MS of compound **18** showed a sodium adduct ion at *m/z* 4273.9, which further confirmed it was a hexavalent conjugate.

Fig. 2 presents the characteristic portions (80–180 ppm) of the ^{13}C NMR spectra (in $CDCl_3$) of conjugates **18**, **27**, and **30**. The signals have been fully assigned with the aid of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC spectra. To our surprise, the other hexavalent conjugate **24** showed significantly differences in NMR spectra from its analogs (**18**, **27** and **30**). The 1D NOE and 2D-ROESY spectra of **24** clearly indicated that there was conformational exchange in solution (SI Figs. 1 and 2).

2.2. Biological assays

2.2.1. Anti-influenza virus activity assay

Antiviral activity of the newly synthesized multivalent per-(2,3-di-*O*-methyl)-α-, β- or γ-CD-based pentacyclic triterpene conjugates (**18**–**20** and **24**–**32**) was tested against influenza A/WSN/33 virus that was propagated in MDCK cells by the CellTiter-Glo assay and the cytopathic effect (CPE) reduction assay [33]. As shown in Table 1, no significant cytotoxicity was observed at the concentration of 100 μM for all the conjugates. Except compound **29**, the relationship between anti-influenza A/WSN/33 virus activity and CD scaffold was similar to our previously results [24], *i.e.*, β-CD is the best candidate for the construction of the multivalent conjugates and the smaller or larger CDs would decrease the anti-influenza virus activity. Two γ-CD derivatives (**26** and **32**) showed

Table 1
Inhibitory activities of multivalent conjugates **18**–**20** and **24**–**32** against infection of MDCK cells by influenza A/WSN/33 virus.

Compound	CC_{50} (μM)[a]	IC_{50} (μM)[b]	Compound	CC_{50} (μM)[a]	IC_{50} (μM)[b]
Oseltamivir[c]	>200	16.5 [24]			
18	>100	14.5 ± 1.74	**27**	>100	52.4 ± 7.86
19	>100	22.8 ± 1.48	**28**	>100	4.7 ± 0.52
20	>100	23.0 ± 1.59	**29**	>100	30.9 ± 4.33
24	>100	48.0 ± 5.04	**30**	>100	40.3 ± 3.63
25	>100	9.9 ± 0.79	**31**	>100	6.5 ± 1.24
26	>100	>100	**32**	>100	>100

[a] 50% cytotoxicity concentration.
[b] Concentration inhibiting viral replication by 50%. The values are means of at least three independent determinations; the corresponding standard deviations are noted.
[c] Positive control.

the weakest activity with IC_{50} over 100 μM. In the case of β-CD derivatives (**19**, **25**, **28** and **31**), the effect of the four pentacyclic triterpenes on the anti-influenza A/WSN/33 virus activity was in the order: EA > UA > OA > BA. But, interestingly, the relationship was reversed for α-CD and γ-CD derivatives. Compound **28** exhibited the most potent anti-influenza A/WSN/33 virus activity with an IC_{50} of 4.7 ± 0.52 μM, a CC_{50} > 100 μM and a selectivity index (SI) of >21.3 (Table 1 and Fig. 3). Another compound **31** also showed strong anti-influenza virus activity with an IC_{50} of 6.5 ± 1.24 μM and a selectivity index (SI) of >15.4.

Compared with our previous work on pentacyclic triterpene-CD conjugates [24], we can see clearly that the methylation or acetylation of C-2 and C-3 hydroxys of CD has significant effect on their anti-influenza virus activity. The acetylation decreased the antiviral activity by ~1.4–4.2 fold, while the methylation decreased the potent by ~1.7–8.9 fold, even with two γ-CD conjugates diminished activity (IC_{50} > 100 μM), indicating that the alkyl of the hydroxyls of CD conjugates will decrease their antiviral activity.

The inhibitory activity of compound **28** against influenza A/WSN/33 virus was confirmed by direct observation under a

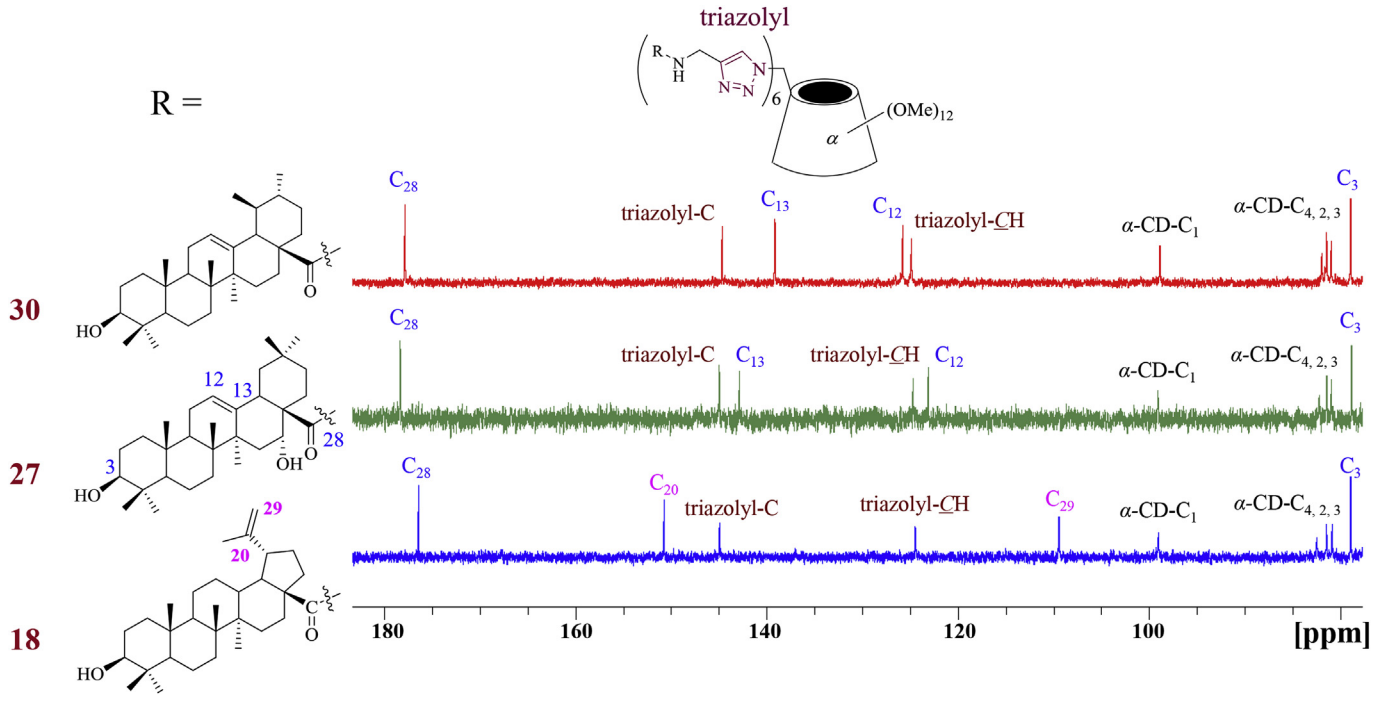


Fig. 2. Characteristic portions of the 400-MHz ^{13}C NMR spectra of **18**, **27** and **30** recorded in $CDCl_3$ at 298K.

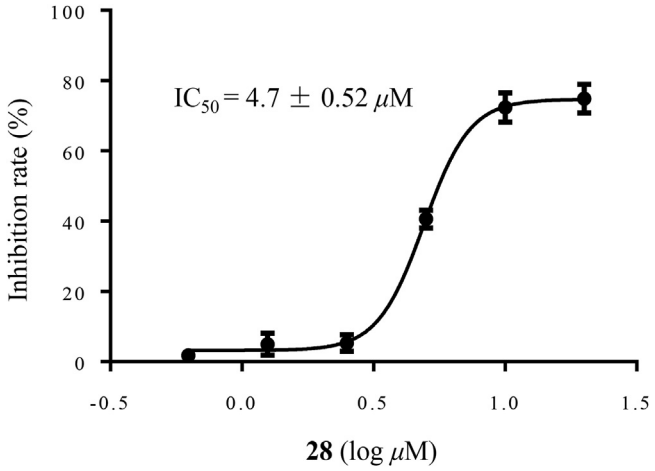


Fig. 3. Inhibition curve of compound **28**. Concentrations of **28** were 0.625 μM, 1.25 μM, 2.5 μM, 5 μM, 10 μM, and 20 μM. Each concentration was tested in triplicate, and the results are expressed as the mean and standard deviation.

microscope, which showed that **28** significantly reduced the CPE induced by influenza virus infection compared with the DMSO control (SI Fig. 3).

2.2.2. Mechanistic exploration

To explore the mechanism of the antiviral effect of compound **28**, we firstly used the time-of-addition assay to estimate which stage(s) of the influenza virus life cycle was inhibited by compound **28**. The results showed that the production of progeny virus was markedly decreased when compound **28** was present during the period from 0 to 2 h (entry stage) or from 0 to 10 h (the whole virus lifecycle), as compared with DMSO control, indicating that compound **28** exhibited the inhibitory effect at the early stage of virus infection (0–2 h) (SI Fig. 4). On the contrary, compound **28** had no inhibitory effects on viral production when added 2 h post-infection or later. Based on these data, we concluded that compound **28** was only effective at viral entry step [24].

The transmembrane viral envelope protein HA plays a crucial role at the early stage of influenza virus infection. Therefore, we investigated whether compound **28** targeted HA protein using the hemagglutination inhibition (HI) assay. In this assay, three-fold serial dilutions of compound **28** were made from 0.4 to 10 μM. In the RBC-only control, the RBCs did not agglutinate (Fig. 4A, lane 1, lower row). When RBCs were mixed with influenza virus, hemagglutination was detected (Fig. 4A, lane 1, upper row). Inhibition of hemagglutination was observed for compound **28** at concentrations of 0.4–10 μM in a dose-dependent manner (Fig. 4A, lane 2–5, upper row). In parallel, inhibition of hemagglutination was also observed for anti-HA antibody at concentrations of 0.1–2.7 μg/mL (Fig. 4B). The HI assays implied that compound **28** and anti-HA antibody have the same target, HA. Surface plasmon resonance (SPR) experiments were subsequently performed with a Biacore 200 optical biosensor to determine the binding affinity of compound **28** with HA. As shown by SPR responses in Fig. 4C, compound **28** showed specific binding to HA protein immobilized on the CM5 chip and exhibited dose-dependent responses. The calculated equilibrium dissociation constant (K_D) values for

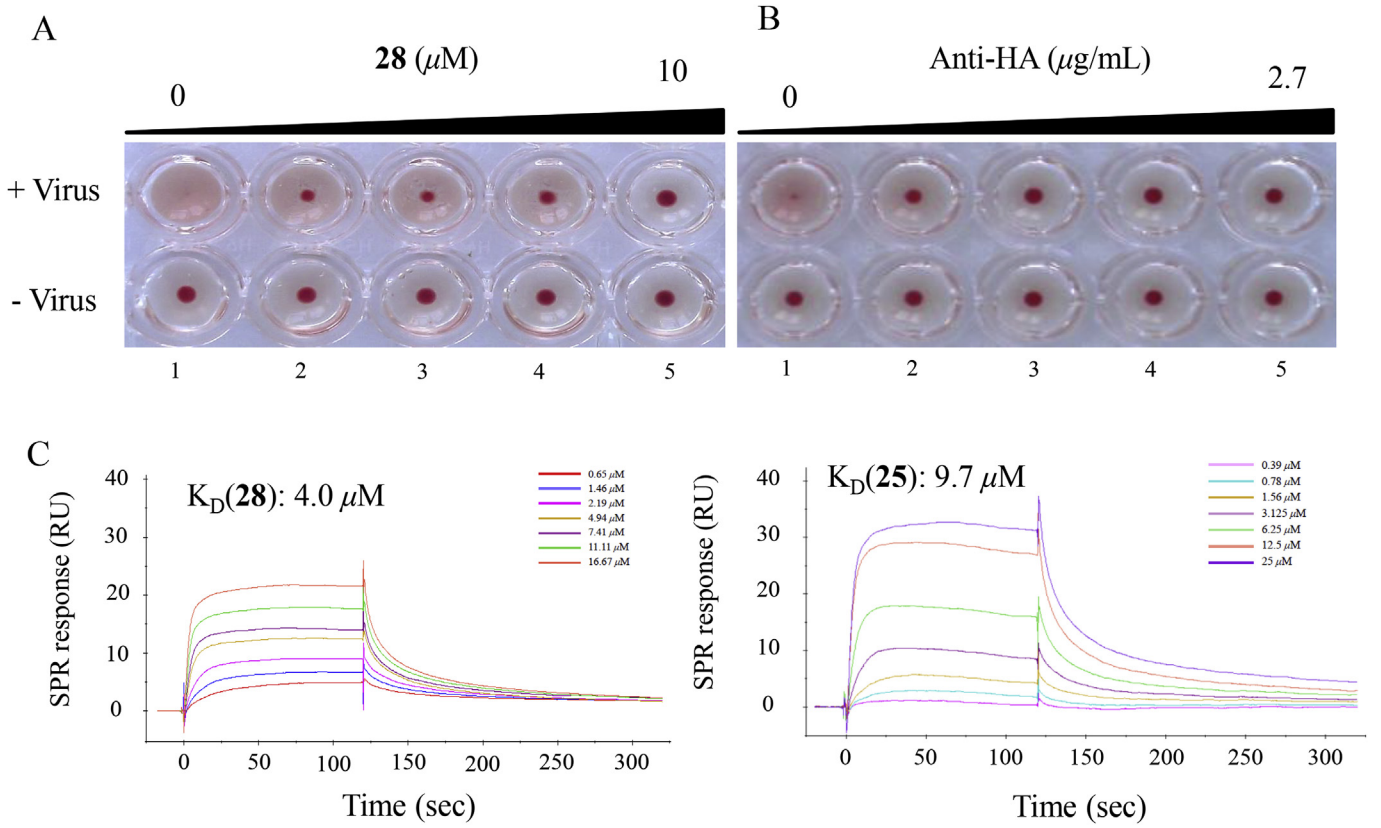


Fig. 4. Interactions of HA with compound **28**. (A–B). HI assay was performed to compare compound **28** *vs*. anti-HA antibody in inhibiting influenza virus-induced aggregation of chicken erythrocytes. (C). SPR assay to determine the affinity of compounds **25** and **28** to HA protein immobilized on a CM5 sensor chip. The K_D values of compounds **25** and **28** are 9.7 and 4.0 μM, respectively.

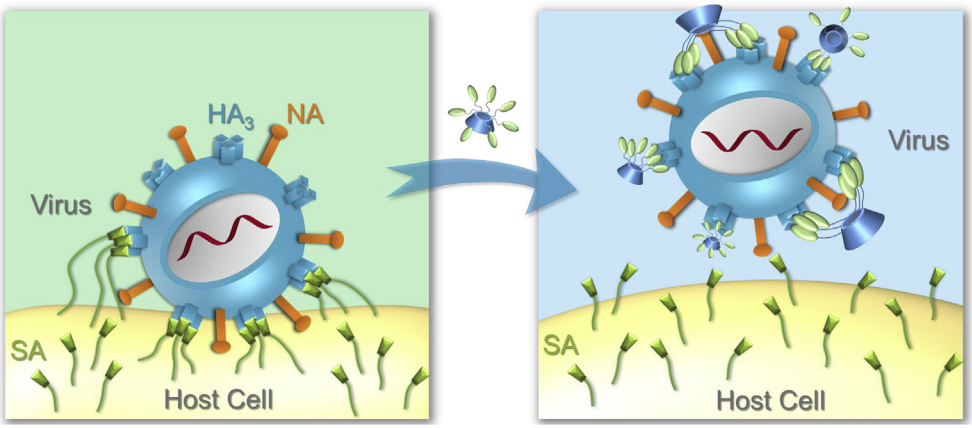


Fig. 5. A proposed mechanism for multivalent pentacyclic triterpene-functionalized per-*O*-methylated CD conjugates mediated blocking of influenza virus entry, wherein the multivalent pentacyclic-triterpenes tightly bind to viral homotrimeric HA protein, thus disrupting the attachment of viruses to host cells.

compounds **28** and **25** were determined to be 4.0 and 9.7 μM, respectively, based on the 1:1 Langmuir binding model. Therefore, compound **28** showed higher affinity to HA protein than its analog **25**.

3. Conclusion

In the present study, we designed and synthesized a series of pentacyclic triterpene-functionalized per-(2,3-di-*O*-methylated) α-, β- and γ-CD conjugates via click chemistry and tested their anti-influenza A/WSN/33 (H1N1) virus activity in MDCK cells. No significant cytotoxicity was observed at the concentration of 100 μM for all the conjugates in MDCK cells. Two conjugates **28** and **31** exhibited the highest anti-influenza virus activity with IC_{50} at micromole level and SI > 15. Mechanism investigation indicated that they showed high affinity to influenza HA protein with K_D at μM level, thus disrupting the interaction of influenza HA with the SA-linked glycoprotein receptor (Fig. 5). This work, as well as our previous study on multivalent pentacyclic triterpene-CD conjugates [24], provides the basis for the design of multivalent compounds based on CD scaffold as anti-influenza virus entry inhibitors and might pave the way for a variety of practical applications for other virus.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grants Nos. 81573269, 81373271, 21572015 and 81361168002). We thank Dr. Zhong at the Centre of Medical and Health Analysis, Peking University for MALDI-TOF experiments.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2017.03.087.

References

[1] P.R.S. Lagace-Wiens, E. Rubinstein, A. Gumel, Influenza epidemiology-past, present, and future, Crit. Care Med. 38 (2010) E1–E9.
[2] M.L. Shaw, P. Palese, Orthomyxoviridae, in: D.M. Knipe, P.M. Howley (Eds.), Fields Virology, sixth ed., ippincott Williams & Wilkins, Philadelphia, 2013.
[3] F. Carrat, A. Flahault, Influenza vaccine: the challenge of antigenic drift, Vaccine 25 (2007) 6852–6862.
[4] A.C. Hurt, H.T. Ho, I. Barr, Resistance to anti-influenza drugs: adamantanes and neuraminidase inhibitors, Expert Rev. Anti-Infect. Ther. 4 (2006) 795–805.
[5] R.A. Bright, M.J. Medina, X.Y. Xu, G. Perez-Oronoz, T.R. Wallis, X.H.M. Davis, L. Povinelli, N.J. Cox, A.I. Klimov, Incidence of adamantane resistance among influenza a (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern, Lancet 366 (2005) 1175–1181.
[6] E. van der Vries, F.F. Stelma, C.A.B. Boucher, Emergence of a multidrug-resistant pandemic influenza a (H1N1) virus, N. Engl. J. Med. 363 (2010) 1381–1382.
[7] A.C. Hurt, The epidemiology and spread of drug resistant human influenza viruses, Curr. Opin. Virol. 8 (2014) 22–29.
[8] A. Basu, A. Antanasijevic, M.X. Wang, B. Li, D.M. Mills, J.A. Ames, P.J. Nash, J.D. Williams, N.P. Peet, D.T. Moir, M.N. Prichard, K.A. Keith, D.L. Barnard, M. Caffrey, L.J. Rong, T.L. Bowlin, New small molecule entry inhibitors targeting hemagglutinin-mediated influenza a virus fusion, J. Virol. 88 (2014) 1447–1460.
[9] K. Das, J.M. Aramini, L.C. Ma, R.M. Krug, E. Arnold, Structures of influenza a proteins and insights into antiviral drug targets, Nat. Struct. Mol. Biol. 17 (2010) 530–538.
[10] J.J. Skehel, D.C. Wiley, Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin, Annu. Rev. Biochem. 69 (2000) 531–569.
[11] A. Impagliazzo, F. Milder, H. Kuipers, M.V. Wagner, X.Y. Zhu, R.M.B. Hoffman, R. van Meersbergen, J. Huizingh, P. Wanningen, J. Verspuij, M. de Man, Z.Q. Ding, A. Apetri, B. Kukrer, E. Sneekes-Vriese, D. Tomkiewicz, N.S. Laursen, P.S. Lee, A. Zakrzewska, L. Dekking, J. Tolboom, L. Tettero, S. van Meerten, W.L. Yu, W. Koudstaal, J. Goudsmit, A.B. Ward, W. Meijberg, I.A. Wilson, K. Radosevic, A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen, Science 349 (2015) 1301–1306.
[12] M. Waldmann, R. Jirmann, K. Hoelscher, M. Wienke, F.C. Niemeyer, D. Rehders, B. Meyer, A nanomolar multivalent ligand as entry inhibitor of the hemagglutinin of avian influenza, J. Am. Chem. Soc. 136 (2014) 783–788.
[13] W. Weis, J.H. Brown, S. Cusack, J.C. Paulson, J.J. Skehel, D.C. Wiley, Structure of the influenza-virus hemagglutinin complexed with its receptor, sialic-acid, Nature 333 (1988) 426–431.
[14] M.L. Huang, M. Cohen, C.J. Fisher, R.T. Schooley, P. Gagneux, K. Godula, Determination of receptor specificities for whole influenza viruses using multivalent glycan arrays, Chem. Commun. 51 (2015) 5326–5329.
[15] S. Cusack, R.W.H. Ruigrok, P.C.J. Krygsman, J.E. Mellema, Structure and composition of influenza-virus - a small-angle neutron-scattering study, J. Mol. Biol. 186 (1985) 565–582.
[16] S.K. Choi, Synthetic multivalent molecules: concepts and biomedical applications, Wiley-Interscience, New York, 2004.
[17] G. Crini, Review: a history of cyclodextrins, Chem. Rev. 114 (2014) 10940–10975.
[18] G. Becket, L.J. Schep, M.Y. Tan, Improvement of the in vitro dissolution of praziquantel by complexation with alpha-, beta- and gamma-cyclodextrins, Int. J. Pharm. 179 (1999) 65–71.
[19] S.V. Kurkov, T. Loftsson, Cyclodextrins, Int. J. Pharm. 453 (2013) 167–180.
[20] A.R. Khan, P. Forgo, K.J. Stine, V.T. D'Souza, Methods for selective modifications of cyclodextrins, Chem. Rev. 98 (1998) 1977–1996.
[21] A. Sivignon, X.B. Yan, D.A. Dorta, R. Bonnet, J. Bouckaert, E. Fleury, J. Bernard, S.G. Gouin, A. Darfeuille-Michaud, N. Barnich, Development of heptylmannoside-based glycoconjugate antiadhesive compounds against adherent-invasive escherichia coli bacteria associated with crohn's disease, Mbio 6 (2015) e01298-15.
[22] Z. Ye, Q. Zhang, S.T. Wang, P. Bharate, S. Varela-Aramburu, M.J. Lu, P.H. Seeberger, J. Yin, Tumour-targeted drug delivery with mannose-functionalized nanoparticles self-assembled from amphiphilic beta-cyclodextrins, Chem.-Eur. J. 22 (2016) 15216–15221.
[23] A. Martinez, C.O. Mellet, J.M.G. Fernandez, Cyclodextrin-based multivalent glycodisplays: covalent and supramolecular conjugates to assess carbohydrate-protein interactions, Chem. Soc. Rev. 42 (2013) 4746–4773.

[24] S. Xiao, L. Si, Z. Tian, P. Jiao, Z. Fan, K. Meng, X. Zhou, H. Wang, R. Xu, X. Han, G. Fu, Y. Zhang, L. Zhang, D. Zhou, Pentacyclic triterpenes grafted on cd cores to interfere with influenza virus entry: a dramatic multivalent effect, Biomaterials 78 (2016) 74–85.
[25] M.L. Lepage, J.P. Schneider, A. Bodlenner, P. Compain, Toward a molecular lego approach for the diversity-oriented synthesis of cyclodextrin analogues designed as scaffolds for multivalent systems, J. Org. Chem. 80 (2015) 10719–10733.
[26] B. Casu, M. Reggiani, G.R. Sanderson, Methylated cycloamyloses (cyclodextrins) and their inclusion properties, Carbohydr. Res. 76 (1979) 59–66.
[27] J. Szejtli, Cyclodextrin and Their Inculsion Complex, Akadémiai Kiado, Budapest, 1982.
[28] K. Kano, R. Nishiyabu, R. Doi, Novel behavior of *O*-methylated beta-cyclodextrins in inclusion of meso-tetraarylporphyrins, J. Org. Chem. 70 (2005) 3667–3673.
[29] H. Kitagishi, K. Kashiwa, K. Kano, Functionalization of a protein surface with per-*O*-methylated beta-cyclodextrin, Biopolymers 97 (2012) 11–20.
[30] W. Xiong, J. Zhao, H. Yu, X. Li, S. Sun, Y. Li, Q. Xia, C. Zhang, Q. He, X. Gao, L. Zhang, D. Zhou, Elevated expression of AKR1C3 increases resistance of cancer cells to ionizing radiation via modulation of oxidative stress, PLoS One 9 (2014) e111911.
[31] P.R. Ashton, R. Koniger, J.F. Stoddart, D. Alker, V.D. Harding, Amino acid derivatives of beta-cyclodextrin, J. Org. Chem. 61 (1996) 903–908.
[32] S. Xiao, Q. Wang, L. Si, Y. Shi, H. Wang, F. Yu, Y. Zhang, Y. Li, Y. Zheng, C. Zhang, C. Wang, L. Zhang, D. Zhou, Synthesis and anti-HCV entry activity studies of beta-cyclodextrin-pentacyclic triterpene conjugates, ChemMedChem 9 (2014) 1060–1070.
[33] J.W. Noah, W. Severson, D.L. Noah, L. Rasmussen, E.L. White, C.B. Jonsson, A cell-based luminescence assay is effective for high-throughput screening of potential influenza antivirals, Antivir. Res. 73 (2007) 50–59.