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Research paper

Design, synthesis, and biological evaluation of crenatoside analogues as novel influenza neuraminidase inhibitors

Bao-Long Chen [a], Ya-Jing Wang [b], Hong Guo [a], Guang-Yao Zeng [a, *]

[a] School of Pharmaceutical Sciences, Central South University, 172 Tongzipo Road, Yuelu District, Changsha 410013, PR China
[b] Hunan University of Chinese Medicine, PR China

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ABSTRACT

Natural products, especially derived from TCMH, have been found to exert antiviral effects against influenza virus. Crenatoside, a phenylethanoid glycoside from *Pogostemon cablin* Benth, which has been shown as a novel effective NA inhibitor previously, is considered as the leading compound for our further SARs studies. This work presented design, synthesis of novel crenatoside analogues from readily available D-Glucose and L-rhamnose in a convergent manner. Furthermore, their biological activities and SARs were also investigated. Especially, compound **2 h** showed impressive IC_{50} = 27.77 μg/mL against NAs, which is 3 folds more potent than the leading compound crenatoside (IC_{50} = 89.81 μg/mL). These results would promise their therapeutic potential for influenza disease.

1. Introduction

Influenza virus causes worldwide outbreaks in humans and animals every year with high morbidity and mortality. In the past century, severe influenza outbreaks took place, including the catastrophic H1N1 Spanish influenza in 1918 (caused more than 50 million deaths globally), the H2N2 Asian flu in 1957 (caused more than 1 million deaths globally), and the H3N2 Hong Kong flu in 1968 (caused ~0.5 million deaths globally) [1]. The battle between human and influenza has begun since ancient time, and will last for a long time.

The enveloped, negative-stranded influenza virus belongs to the family *Orthomyxoviridae*, which can be classified by the antigenic properties of glycopro-teins located at the surface of the virus, haemagglutinin (H) and neuraminidase (N). Sixteen subtypes have currently been defined with the haemagglutinin protein (H1–H16) and nine with the neuraminidase protein (N1–N9) accordingly [2]. Both haemagglutinin and neuraminidase are crucial for the replication and infectivity of influenza virus [3]. Many compounds against NP and HA targets were found [4,5]. For example, a new class of compounds featuring a camphor moiety has been discovered by Sokolova, which exhibits potent inhibitory activity against influenza A viruses [6]. Especially, neuraminidase (NA) cleaves the specific linkage of the sialic acid receptor, resulting in the release of the newly formed virions from the infected cells. Additionally, the neuraminidase may facilitate the early stage of influenza virus infection towards lung epithelial cells [7]. Hence, neuraminidase has been an attractive target for the development of novel anti-influenza drugs.

At present, the first-line drugs (oseltamivir and zanamivir) recommended for flu treatment are NA inhibitors. Both of them are sialic acid (Neu5Ac) analogues (Fig. 1). Though with tremendous success, the treatment doesn't seem to be optimistic due to the spontaneously arising and spreading of oseltamivir resistance among influenza virus [8]. Therefore, developing novel NA inhibitors to combat influenza virus is desirable.

Natural products, especially those derived from traditional Chinese medicine herbs (TCMH), are still the major source of innovative therapeutic agents for infectious diseases, cancer, lipid disorders and immunomodulation [9]. However, the structural complexity, small content and unfavourable pharmacokinetic properties of bioactive natural products limited their use in therapeutic field [10]. Thus it is desirable to initiate a SARs study to identify the drug candidate for further investigations.

Pogostemon cablin Benth, also known as “Guang-Huo-Xiang” in China, is an important TCMH that has been widely used for

* Corresponding author.
E-mail address: zenggy2013@csu.edu.cn (G.-Y. Zeng).

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treatment on common cold, nausea, diarrhoea, headaches and fever [11]. In previous study, we have identified crenatoside as a novel selective NA inhibitor from a collection of phenylethanoid glycosides isolated from *P. cablin* Benth. The study partially revealed a molecular basis for the anti-influenza activity of this TCMH. It also suggested that caffeoyl and 1, 4-dioxanyl moieties are critical for their inhibition and selectivity of NA from influenza A virus (H1N1) (Fig. 2). However, several defects of crenatoside, including its poor pharmacokinetic properties and small content in plants, limited its use as a drug candidate. In this paper, we presented the design, synthesis and biological evaluation of crenatoside analogues as novel NA inhibitors, which not only reveal the structure-activity relationships in crenatoside, but also provide drug candidate for further investigations.

2. Design of crenatoside analogues

Generally, the design of crenatoside analogues is based on the bioisosteric replacement and functionality "knock-out" strategy. In medicinal chemistry, bioisosteric replacement is a frequently used strategy in optimization to obtain the desired biological or physical properties of a compound. And functionality "knock out" is also a common protocol to clearly state the contributions of the structural motif to the biological activities. Specifically, we would like to replace the caffeoyl group with privileged drug scaffold and eliminate the rhamnose moiety to investigate the impact of these functional groups on the biological activities. Furthermore, MOE simulation showed that the bulky glucose 3′- rhamnose moiety impedes its binding with the NA (Fig. 3). Additionally, the substitution effect of C-3′ on the biological activity is still unexplored. Therefore, we would like to incorporate privileged drug scaffold, amino acid, onto the C-3′ position.

3. Chemistry

With considerations mentioned above, we initiated our synthesis of the crenatoside analogues. The compound **7** can be served as the versatile intermediate for the synthesis of crenatoside analogues. The retrosynthestic analysis of compound **7** is shown in Scheme 1. Compound **7** can be synthesized by intermediate **8** through C-3′ functionalization and reductive debenzylation. The intermediate **8** can be prepared by base-mediated intramolecular alkylation of compound **9**, which is routinely synthesized by glycosidation of compound **10** and phenylethanol. The activated precursor can be prepared by protection of C-5′, C6′ hydroxyl groups with benzyl acetide and activation of the anomeric carbon with phenyl sulfide of readily available D-glucose.

With the retrosynthetic analysis in mind, we then started the synthetic endeavours. Starting from commercially available D-glucose, we obtained compound **12** by fully acetylation of hydroxyl group with Ac_2O in the presence of sodium acetate in good yield. Sequential BF3·Et2O catalysed theo glycosylation, and Zemplen deacetylation, led to the glucosinolate **13**, of which the C-4′, C6′ hydroxyl groups were selectively protected with O-benzylidene group [12] and C2′, C3′ hydroxyl groups were protected as diacetate

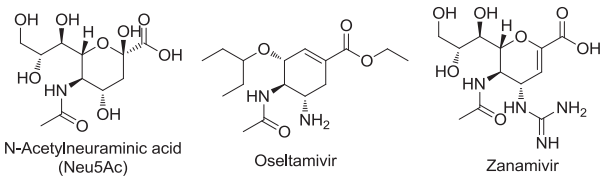


Fig. 1. The structure of Neu5Ac, oseltamivir and zanamivir.

to reach intermediate **15**. Subsequently, we focused on the challenging glycosidation. After multiple attempts, we found that C-2′ acetylate promoted stereoselective glycosidation of compound **15** with phenylethanol **16** under AgOTf/NIS/-40 °C conditions. This gave our target compound **17** as diastereoisomers in moderate yield [13]. In this protocol, phenylethanol **16** was prepared by reaction of styrene oxide with hydrobromide as racemic isomers, which would help our investigation of C-7 configuration on the biological activities. Finally, deacetylation with NaOMe followed by intramolecular O-alkylation in the presence of NaH yields compound **7** as diastereoisomers (Scheme 2).

We then performed synthesis of analogues. On one hand, debenzylidenation of compound **7** in the presence of 80%HOAc afforded isomers **1a** and **1b**, which was then separated by HPLC. The stereochemistry of **1a** and **1b** was elucidated by the difference of the coupling constant for H-7/H-8 (J_7-8β = 3.6 Hz for α configuration) [14]. The stereochemistry of C-7 of analogues is also assigned in the same way. On the other hand, L-rhamnose conjugated analogues **1c** and **1d** were synthesized in a convergent manner. Compound **20** was prepared through a three-step protocol from L-rhamnose according to the routine procedure. Subsequent glycosylation of compound **20** with the compound **7** in the presence of TMSOTf at −30 °C gave compound **21** in good yield [15], which was then debenzylidenated and deacetylated to provide isomers **1c** and **1d** [16] (Scheme 3).

As mentioned above, a series of C-3′ substituted analogues were also synthesized. Specifically, we would like to attach cinnamoyl group and amino acid at C-3′ position. Compound **7** was esterified with different cinnamic acid derivatives, followed by debenzylidenation and deacetylation to provide compounds **2a**–**2 h** in moderate yield [17]. Similarly, compounds **3a**–**3d** synthesized by esterification and simultaneous removal of benzylidenation and Boc groups using TFA/CH_2Cl_2 (Scheme 4).

4. Experimental

4.1. General remarks

^1H and ^{13}C NMR spectra were recorded on a Brucker AV 400 spectrometer or 500 using tetramethylsilane (TMS) as an internal standard; J-values are in Hz. Mass spectra were recorded by ESI-HRMS analysis, which was provided by Agilent 6520 Q-TOF LC/MS spectrometer (Agilent, Germany). Solvent for anhydrous reaction should be processed before use. Commercially obtained reagents were used without further purification. All reactions were monitored by thin-layer chromatography (TLC) with Huang hai GF_{254} silica gel coated plates.

4.2. Procedure for the preparation of the unknown compounds

4.2.1. 2-β-bromo-Benzeneethanol, 4, 6-O-Benzylidene-2, 3-O-acetyl-β-D- glucopyranose (17)

A suspension of compound **15** (88.8 mg, 0.2 mmol) and compound **16** (60 mg, 0.3 mmol) in dry CH_2Cl_2 (5 mL) containing activated 4 Å molecular sieves (80 mg) was stirred under an atmosphere of argon at RT for 30 min. After cooling to −40 °C, NIS (54 mg, 0.24 mmol) and a solution of AgOTf in toluene (18 mg, 2 ml) was added and the resulting mixture was stirred from −40 °C to RT for 2.5 h. The reaction was quenched with 0.3 ml Et_3N and diluted with CH_2Cl_2 (10 mL). The solution was washed with brine and the organic layer was dried over Na_2SO_4 and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash silica gel column chromatography to give compound **17** (58 mg, 55.0%) as a white powder.

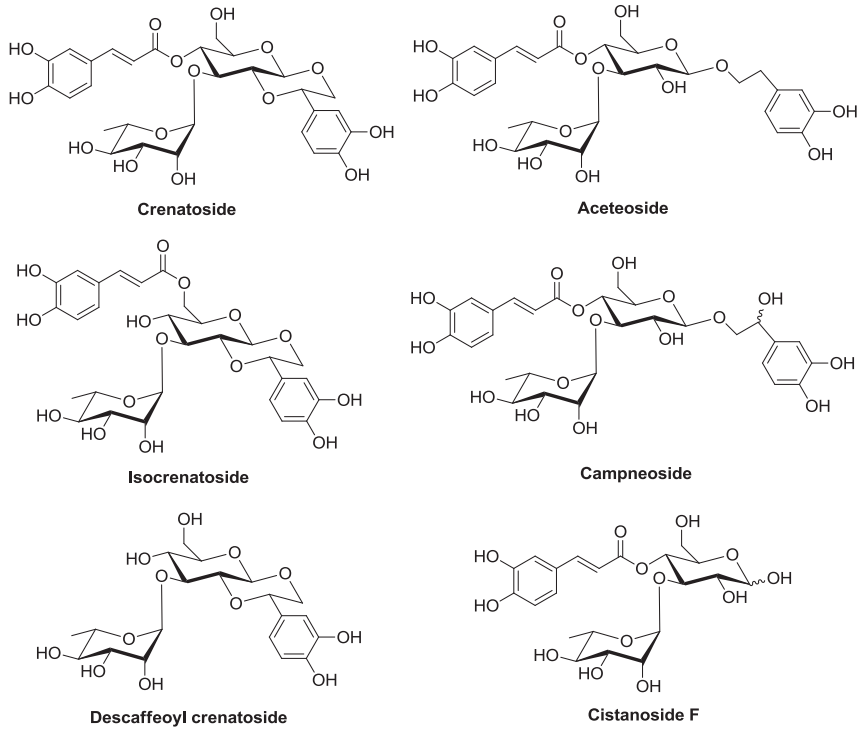


Fig. 2. Phenylethanoid glycosides isolated from *P. cablin* Benth.

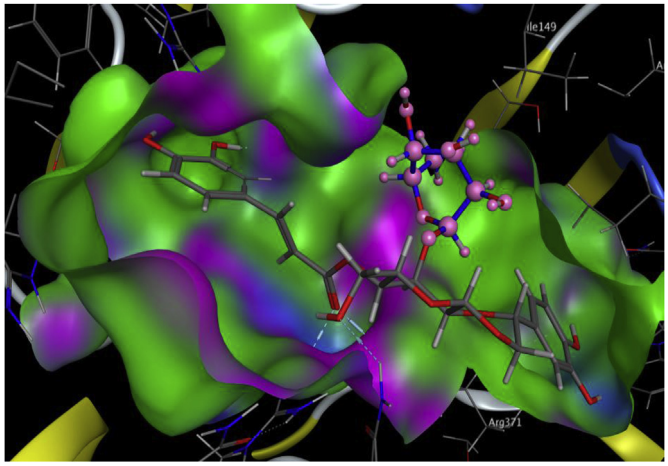


Fig. 3. Docking result:crenatoside in the active site of NA(PDB ID:3TI6). The red part is rhamnose molecules. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.2.2. β-D-Glucopyranose 4, 6-O-Benzylidene-1, 2-O-[(2α/β)-2-benzene-1, 2-ethanediyl] (7)

A solution of compound **17** (267 mg, 0.5 mmol) in MeOH containing NaOMe (1.0 mmol) was stirred at RT for 2 h. Then the mixture was neutralized by Amberlite IR-120 (H^+ form), filtered, and the filtrate was concentrated. The residue was dissolved in dry THF (15 mL) and NaH (84 mg, 7 mmol). The mixture was stirred under an atmosphere of argon at RT for 5 h, and then refluxed overnight. The solution was neutralized by 1 M/L HCl, and then extracted with dichloromethane and the organic layer was dried over Na_2S0_4 and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash silica gel column chromatography to give compound **7** (83.6 mg, 45.2%) as a white powder.

Compound 7 (7α): m.p. 185–187 °C; **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.35–7.62 (m, 10H), 5.49 (s, 1H), 4.89–4.90 (d, J = 3.6 Hz, 1H), 4.67–4.68 (m, 2H), 4.36–4.40 (dd, J = 4.8, 10.8 Hz), 4.27–4.31 (dd, J = 3.6, 12.4 Hz, 1H), 3.76–3.96 (m, 2H, 8-H), 3.61–3.67 (m, 1H), 3.37–3.54 (m, 2H). **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 137.32, 136.82, 129.35, 128.74, 128.38, 128.12, 127.99, 126.28, 102.18, 99.66, 81.34, 73.15, 72.75, 70.86, 68.41, 68.19, 67.09.

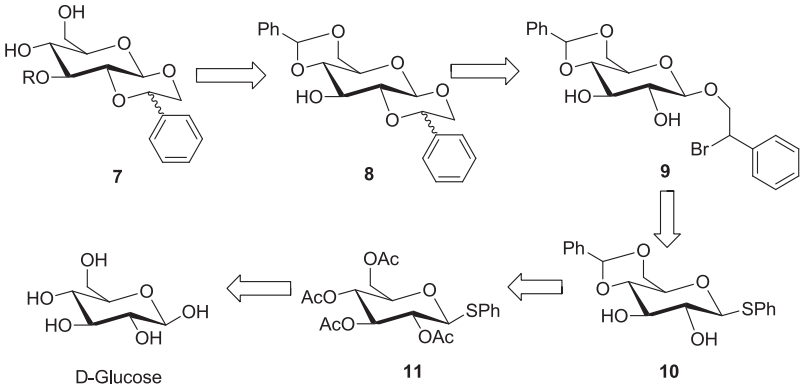
Compound 7 (7β): m.p. 182–184 °C; **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.35–7.55 (m, 10H), 5.59 (s, 1H), 4.76–4.80 (dd, J = 3.2, 10.8 Hz, 1H), 4.57–4.59 (d, J = 7.6 Hz, 1H), 4.42–4.46 (dd, J = 4.4, 10.4 Hz, 1H), 4.09–4.13 (dd, J = 3.2, 12.4 Hz, 1H), 3.86–4.06 (m, 2H), 3.48–3.53, 3.73–3.79 (m, 2H), 3.66–3.76 (m, 2H). **^{13}C-NMR** (125 MHz, $CDCl_3$): δ 136.83, 136.24, 129.36, 128.65, 128.61, 128.38, 126.60, 126.32, 102.21, 98.67, 81.30, 80.22, 78.03, 72.09, 70.82, 68.50, 68.28.

*4.2.3. β-D-Glucopyranose 1, 2-O-[(2α/β)-2-benzene-1, 2-ethanediyl] (**1a** and **1b**)*

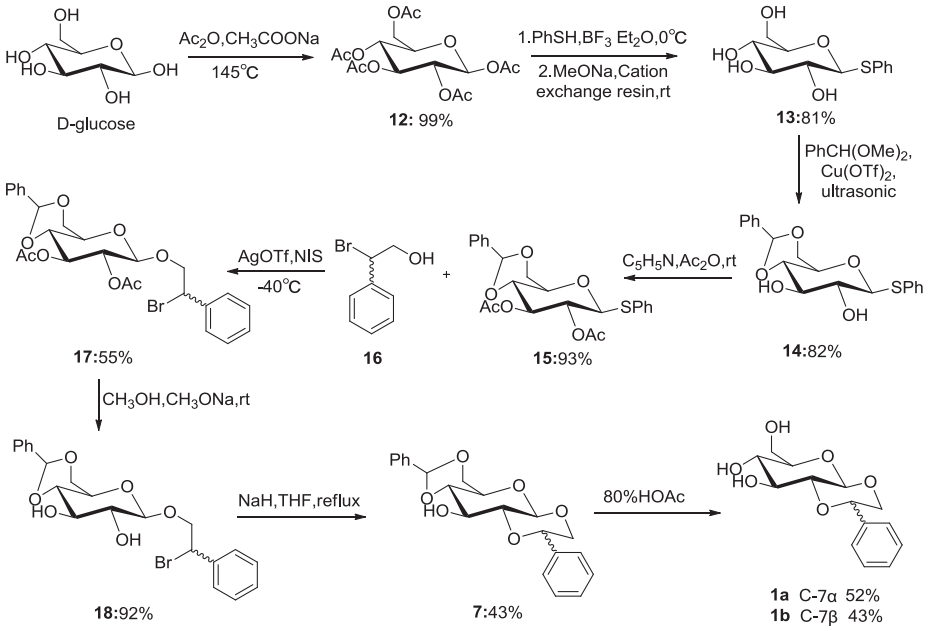
A suspension of **7** (370 mg, 0.1 mmol) in 80% HOAc (20 mL) was heated to 90 °C with stirring till all starting material was consumed. The solvent was removed under reduced pressure, the residue was purified by flash column chromatography to give 220 mg of **1a and 1b**, 78% yield. Then HPLC is used to separate, afforded the **1a** and **1b** respectively.

Compound 1a (7α): m.p. 217–219 °C; **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 7.54–7.29 (m, 5H), 5.22–5.23 (s, 1H), 5.14–5.15 (s, 1H), 4.82–4.83 (d, J = 3.5 Hz, 1H), 4.61–4.63 (s, 2H), 4.52–4.55 (d, J = 12.0 Hz, 1H), 4.39–4.40 (d, J = 7.5 Hz, 1H), 4.07–4.10 (dd, J = 4.0, 13.0 Hz, 1H), 3.41–3.67 (m, 2H), 3.33–3.37 (m, 1H), 3.04–3.09 (m, 1H), 3.24–3.28 (m, 1H), 2.92–2.96 (m, 1H). **^{13}C-NMR** (125 MHz, DMSO-d_6): δ 139.21, 128.77, 127.90, 127.71, 99.11, 79.07, 73.77, 73.38, 71.66, 70.74, 66.68, 61.11; **ESI-HRMS**: $C_{14}H_{18}O_6Na$ for +, calculated 305.1001, found 305.0972.

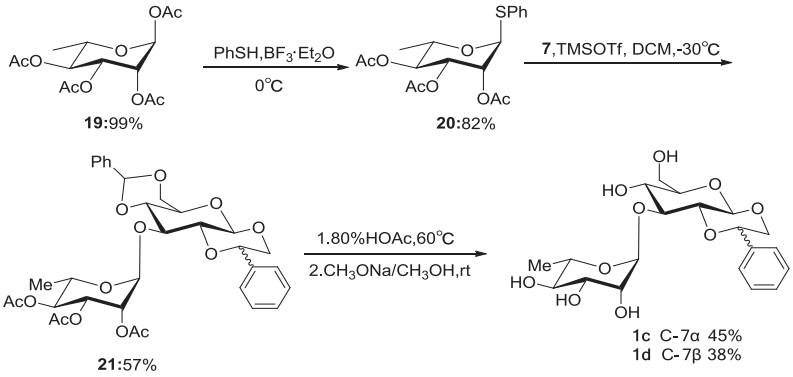
Compound 1b (7β): m.p. 269–271 °C; **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 7.31–7.43 (m, 5H) 5.19–5.22 (m, 2H), 4.69–4.71 (m,



Scheme 1. Retrosynthetic analysis of compound **7.**

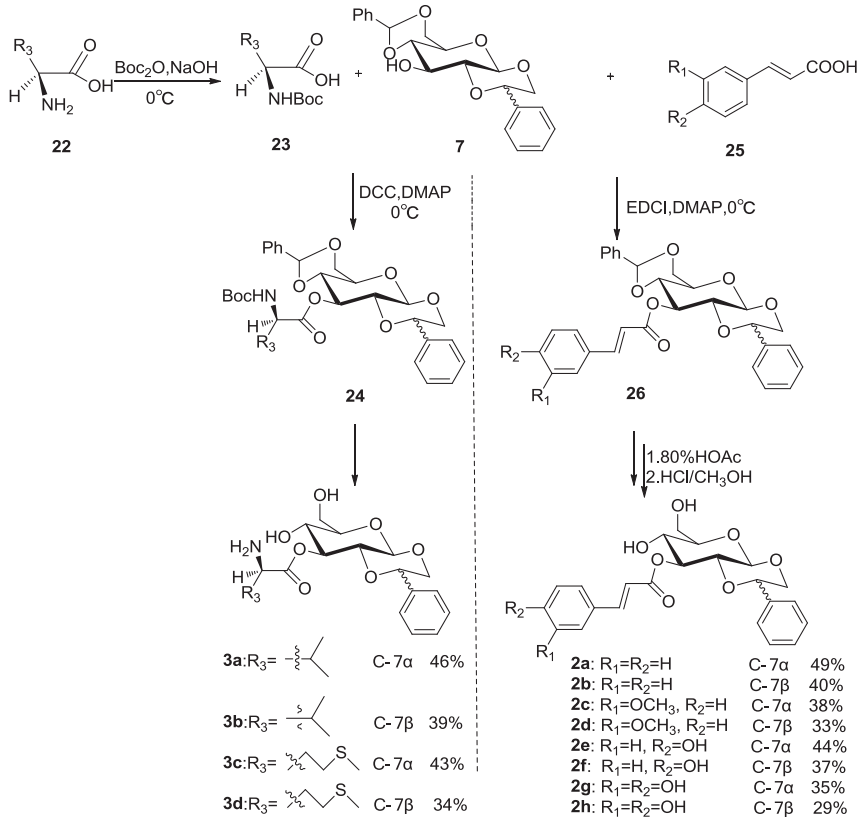


Scheme 2. Synthesis of key intermediate **7** and **1a, 1b.**



Scheme 3. Synthesis of target molecules **1c**–**1d.**

2H), 4.63–4.66 (dd, J = 2.5, 10.5 Hz, 1H), 4.37–4.38 (d, J = 7.5 Hz, 1H), 3.98–4.01 (dd, J = 4.0, 12.0 Hz, 1H), 3.71–3.74 (m, 1H), 3.48–3.55 (m, 1H), 3.55–3.59 (m, 1H), 3.40–3.44 (m, 1H), 3.18–3.22 (m, 1H), 3.30–3.34 (m, 1H), 3.04–3.08 (m, 1H, 4′-H). **^{13}C-NMR** (125 MHz, DMSO-d_6): δ 137.85, 128.70, 128.53, 127.12, 98.27, 79.97, 79.16, 76.87, 73.75, 71.02, 70.87, 61.23; **ESI-HRMS**: $C_{14}H_{18}O_6Na$ for +, calculated 305.1001, found 305.0977.



Scheme 4. Synthesis of target molecules **2a**–**h**,**3a**–**d.**

4.2.4. 1,2-O-[(2α/β)-2-benzene-1,2-ethanediyl] 4,6-0-benzylidene-3-O-(2,3,4- tri-O-acetyl-α-L-rhamno-pyranosyl)-β-D-glucopyranose (21)

A suspension of compound **7** (111 mg, 0.3 mmol) and compound **20** (119.25 mg, 0.36 mmol) in dry CH_2Cl_2 (5 mL) containing activated 4 Å molecular sieves (80 mg) was stirred under an atmosphere of argon at RT for 30 min. After cooling to −30 °C, NIS (90 mg, 0.4 mmol) and TMSOTf (88.8 mg, 0.4 mmol) was added and the resulting mixture was stirred from −30 °C to RT for 4 h. The reaction was quenched with 0.3 ml Et3N and diluted with CH_2Cl_2 (10 mL). The solution was washed with brine and the organic layer was dried over Na_2S0_4 and filtered. The filtrate was concentrated in vacuo and the residue was purified by flash silica gel column chromatography to give compound **21** (109 mg, 56.7%) as a pale yellow oily matter.

Compound 21 (7α) ^{1}H-NMR (500 MHz, $CDCl_3$**):** δ 7.32–7.57 (m, 10H), 5.51 (s, 1H), 5.31–5.32 (dd, *J* = 2.0, 4.0 Hz, 1H), 5.25–5.27 (m, 2H), 5.01–5.05 (m, 1H), 4.86 (d, *J* = 3.0 Hz, 1H), 4.68–4.70 (d, *J* = 13.0 Hz, 1H), 4.61–4.63 (d, *J* = 8.0 Hz, 1H), 4.37–4.40, 4.25–4.29 (dd, *J* = 4.0, 10.5 Hz), 4.31–4.34 (m, 1H), 4.15–4.21 (m, 1H), 3.77–4.01 (m, 2H), 3.58–3.65 (m, 2H), 3.37–3.41 (m, 1H), 2.21 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 0.94–0.95 (d, *J* = 6.0 Hz, 3H).

Compound 21 (7β) ^{1}H-NMR (500 MHz, $CDCl_3$**):** δ 7.30–7.50 (m, 10H), 5.63 (s, 1H), 5.35–5.36 (dd, *J* = 1.5, 3.0 Hz), 5.26–5.29 (dd, *J* = 3.5, 10.0 Hz, 1H), 5.01–5.05 (d, *J* = 1.5 Hz, 1H), 4.96–5.01 (m, 1H), 4.78–4.8 (dd, *J* = 2.5, 10.5 Hz, 1H), 4.55–4.57 (d, *J* = 8.0 Hz, 1H), 4.44–4.47, 4.26–4.29 (m, 2H), 4.31–4.34 (m, 1H), 4.13–4.16 (m, 1H), 3.77–3.93 (m, 2H), 3.67–3.73 (m, 2H), 3.60–3.63 (m, 1H), 2.06 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 0.96–0.97 (d, *J* = 6.0 Hz, 3H).

4.2.5. 1, 2-O-[(2α/β)-2-benzene-1, 2-ethanediyl] 3-O-(2, 3, 4-tri-O-acetyl-α-L- rhamno - pyranosyl)-β-D-glucopyranose (1c and 1d)

A suspension of **21** (624 mg, 1.0 mmol) in 80% HOAc (20 mL) was heated to 90 °C with stirring till all starting material was consumed. The solvent was removed under reduced pressure. A solution of the residue in MeOH containing NaOMe (2.0 mmol) was stirred at RT for 2 h. Then the mixture was neutralized by Amberlite IR-120 (H^+ form), filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography to give 302 mg of **1c and 1d**, 71% yield. Then HPLC is used to separate, afforded the **1c and 1d** respectively.

Compound 1c (7α): m.p. 173–175 °C; **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 7.31–7.49 (m, 5H), 5.12 (s, 1H), 4.81 (s, 1H), 4.63 (s, 2H), 4.53 (s, 1H), 5.08 (s, 1H), 4.85 (d, *J* = 2.5 Hz, 1H), 4.56–4.59 (d, *J* = 12.5 Hz, 1H), 4.45–4.56 (d, *J* = 8.0 Hz, 1H, 1′-H), 4.09–4.12 (dd, *J* = 3.5, 12.5 Hz, 1H), 3.86–3.91 (m, 1H), 3.70 (s, 1H), 3.46–3.64 (m, 4H), 3.31–3.33 (m, 1H), 3.09–3.22 (m, 2H), 2.95–2.99 (m, 1H), 1.10–1.11 (d, *J* = 6.5 Hz, 3H). **^{13}C-NMR** (125 MHz, DMSO-d_6): δ 138.86, 128.85, 127.86, 127.78, 100.76, 98.33, 78.86, 77.28, 74.63, 72.50, 71.60, 71.17, 70.91, 68.86, 68.54, 66.13, 60.96, 18.32; **ESI-HRMS**: $C_{20}H_{28}O_{10}Na$ for +, calculated 451.1580, found 451.1550.

Compound 1d (7β): m.p. 217–219 °C; **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 7.31–7.38 (m, 5H), 5.23 (s, 1H), 4.74 (s, 1H), 4.63 (s, 2H), 4.48 (s, 1H), 5.02 (s, 1H), 4.70–4.72 (dd, *J* = 2.5, 10.5 Hz, 1H), 4.42–4.43 (d, *J* = 8.0 Hz, 1H), 4.03–4.06 (dd, *J* = 2.5, 12.0 Hz, 1H), 3.89–3.95 (m, 1H), 3.16–3.75 (m, 9H), 1.11–1.12 (d, *J* = 6.0 Hz, 3H). **^{13}C-NMR** (125 MHz, DMSO-d_6) δ 137.78, 128.81, 128.37, 126.39, 100.31, 97.64, 80.92, 79.09, 76.70, 76.34, 72.45, 71.12, 71.03, 70.85, 68.69, 68.58, 61.12, 18.35; **ESI-HRMS**: $C_{20}H_{28}O_{10}Na$ for +, calculated 451.1580, found 451.1549.

4.2.6. The general procedure of synthesis of 2a–2h

To a soln of different cinnamic acid analogues (0.5 mmol) and compound **7** (0.4 mmol) were added EDCI (0.5 mmol) and a catalytic amount of DMAP at 0 °C. The soln was stirred for 24 h. The solution was washed with brine and the organic layer was dried

over Na_2S0_4 and filtered. The organic layer was concentrated and applied to a column of silica gel to give **26**.

A suspension of **26** (0.5 mmol) in 80% HOAc (20 mL) was heated to 55 °C with stirring till all starting material was consumed. The solvent was removed under reduced pressure, the residue was purified by flash column chromatography. Then a solution of the product in MeOH containing HCl (1.0 mmol) was stirred at 0 °C till all starting material was consumed. The solvent was removed under reduced pressure, then use of silica gel or Lipophilic Sephadex LH-20 to Separation of this epimers.

Compound 2a (7α): m.p. 145–147 °C; **^{1}H-NMR (**400 MHz, CD_3OD): δ 7.72–7.76 (d, *J* = 16.0 Hz, 1H), 7.29–7.65 (m, 10H), 6.58–6.62 (d, *J* = 16.0 Hz, 1H), 5.21–5.25 (m, 1H), 4.79–4.80 (d, *J* = 3.2 Hz, 1H), 4.62–4.66 (m, 2H), 4.23–4.27 (dd, *J* = 3.6, 12.8 Hz), 3.70–3.90 (m, 2H), 3.25–3.60 (m, 3H). **^{13}C-NMR (**100 MHz, CD_3OD): δ 166.76, 145.27, 137.98, 134.34, 130.26, 128.72, 128.14, 127.94, 127.46, 117.43, 98.91, 78.24, 74.74, 72.14, 71.20, 68.30, 66.17, 60.68; **ESI-HRMS**: $C_{23}H_{25}O_7$ for +, calculated 413.4404, found 413.4693.

Compound 2b (7β): m.p. 113–115 °C; **^{1}H-NMR (**500 MHz, CD_3OD): δ 7.71–7.74 (d, *J* = 16.0 Hz, 1H), 7.26–7.58 (m, 10H), 6.55–6.58 (d, *J* = 16.0 Hz, 1H), 5.33–5.37 (m, 1H), 4.70–4.73 (dd, *J* = 3.0, 10.5 Hz, 1H), 4.61–4.63 (d, *J* = 8.0 Hz, 1H), 3.94–4.06 (dd, *J* = 3.0, 12.0 Hz, 2H), 3.79–3.82 (dd, *J* = 5.0, 12.0 Hz, 1H), 3.74–3.78 (m, 1H), 3.47–3.69 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 166.81, 145.13, 136.87, 134.38, 130.07, 128.58, 128.01, 127.85, 127.78, 125.91, 117.49, 98.10, 78.44, 77.64, 77.03, 74.66, 71.43, 68.42, 60.79; **ESI-HRMS**: $C_{23}H_{25}O_7$ for +, calculated 413.4404, found 413.4567.

Compound 2c(7α): m.p. 116–118 °C; **^{1}H-NMR (**400 MHz, CD_3OD): δ 7.65–7.69 (d, *J* = 15.6 Hz, 1H), 7.30–7.53 (m, 9H), 6.39–6.43 (d, *J* = 15.6 Hz, 1H), 5.15–5.23 (m, 1H), 4.80–4.81 (d, *J* = 3.6 Hz, 1H), 4.63–4.67 (m, 2H), 4.24–4.28 (dd, *J* = 4.0, 12.8 Hz, 1H), 3.69–3.89 (m, 2H), 3.22–3.58 (m, 3H). **^{13}C-NMR (**100 MHz, CD_3OD): δ 167.39, 159.99, 145.52, 137.98, 129.85, 128.11, 127.41, 125.77, 115.48, 113.469, 98.93, 78.26, 74.49, 72.13, 71.25, 68.32, 66.14, 60.67; **ESI-HRMS**: $C_{23}H_{25}O_8$ for +, calculated 429.1549, found 429.1530.

Compound 2d (7β): m.p. 98–100 °C; **^{1}H-NMR(**500 MHz, CD_3OD): δ 7.62–7.65 (d, *J* = 16.0 Hz, 1H), 6.77–7.41 (m, 9H), 6.33–6.37 (d, *J* = 16.0 Hz, 1H), 5.32–5.36 (m, 1H), 4.67–4.70 (dd, *J* = 3.0, 10.5 Hz, 1H), 4.60–4.61 (d, *J* = 7.5 Hz, 1H), 3.94–4.06 (m, 2H), 3.80–3.83 (dd, *J* = 5.0, 12.0 Hz, 1H), 3.74–3.78 (m, 1H), 3.47–3.69 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 167.50, 159.79, 145.46, 136.85, 129.86, 128.03, 127.79, 125.94, 125.81, 115.40, 113.81, 98.11, 78.43, 77.66, 76.98, 74.43, 71.42, 68.49, 60.85; **ESI-HRMS**: $C_{23}H_{25}O_8$ for +, calculated 429.1549, found 429.1521.

Compound 2e (7α): m.p. 135–137 °C; **^{1}H-NMR (**500 MHz, CD_3OD): δ 7.64–7.68 (d, *J* = 15.5 Hz, 1H), 6.84–7.51 (m, 8H), 6.42–6.45 (d, *J* = 15.5 Hz, 1H), 5.18–5.22 (m, 1H), 4.79–4.80 (d, *J* = 3.5 Hz, 1H), 4.63–4.65 (m, 2H), 4.23–4.27 (dd, *J* = 3.5, 12.5 Hz, 1H), 3.92 (s, 1H), 3.70–3.88 (m, 2H), 3.23–3.56 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 167.38, 149.32, 148.02, 145.80, 137.99, 128.11, 127.44, 127.38, 126.33, 122.82, 115.12, 114.01, 110.34, 98.94, 78.27, 74.25, 72.14, 71.25, 68.35, 66.17, 60.69, 55.08; **ESI-HRMS**: $C_{24}H_{27}O_9$ for +, calculated 459.1655, found 459.1624.

Compound 2f (7β): m.p. 139–141 °C; **^{1}H-NMR (**500 MHz, CD_3OD): δ 7.61–7.64 (d, *J* = 16.0 Hz, 1H), 6.77–7.35 (m, 8H), 6.37–6.40 (d, *J* = 16.0 Hz, 1H), 5.32–5.36 (m, 1H), 4.68–4.71 (dd, *J* = 2.5, 10.0 Hz, 1H), 4.60–4.62 (d, *J* = 7.5 Hz, 1H), 3.94–4.07 (dd, *J* = 3.0, 12.0 Hz, 2H), 3.84 (s, 3H), 3.79–3.83 (dd, *J* = 5.0, 12.0 Hz, 1H), 3.74–3.78 (m, 1H), 3.46–3.67 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 167.43, 149.12, 147.86, 145.71, 136.85, 128.01, 127.78, 126.33, 125.94, 122.73, 115.00, 114.09, 110.29, 98.11, 78.45, 77.69, 77.02, 74.44, 71.41, 68.51, 60.83, 55.00; **ESI-HRMS**: $C_{24}H_{27}O_9$ for +, calculated 459.1655, found 459.1616.

Compound 2 g (7α): m.p. 81–83 °C; **^{1}H-NMR (**500 MHz, CD_3OD): δ 7.59–7.62 (d, *J* = 16.0 Hz, 1H), 6.98–7.51 (m, 8H), 6.32–6.35 (d, *J* = 16.0 Hz, 1H), 5.17–5.21 (m, 1H), 4.80 (d, *J* = 3.5 Hz, 1H), 4.63–4.66 (m, 2H), 4.23–4.27 (dd, *J* = 3.5, 12.5 Hz, 1H), 3.70–3.88 (m, 2H), 3.22–3.55 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 167.41, 148.29, 145.90, 145.47, 137.97, 128.12, 127.40, 126.35, 121.63, 115.17, 113.81, 113.65, 98.91, 78.24, 74.50, 72.13, 71.27, 68.34, 66.12, 60.70; **ESI-HRMS**: $C_{23}H_{25}O_9$ for +, calculated 459.1499, found 445.1469.

Compound 2 h (7β): m.p. 80–82 °C; **^{1}H-NMR (**500 MHz, CD_3OD): δ 7.57–7.60 (d, *J* = 16.0 Hz, 1H), 6.76–7.37 (m, 8H), 6.30–6.33 (d, *J* = 16.0 Hz, 1H), 5.30–5.34 (m, 1H), 4.69–4.72 (dd, *J* = 2.5, 10.5 Hz, 1H), 4.60–4.61 (d, *J* = 7.5 Hz, 1H), 3.93–4.09 (dd, *J* = 3.0, 12.0 Hz, 2H), 3.78–3.81 (dd, *J* = 5.5, 12.0 Hz, 1H), 3.72–3.76 (m, 1H), 3.44–3.68 (m, 3H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 167.48, 148.16, 145.7 9, 145.37, 136.88, 128.00, 127.76, 126.38, 125.90, 121.59, 115.07, 113.77, 113.73, 98.11, 78.45, 77.67, 76.98, 74.42, 71.41, 68.46, 60.81; **ESI-HRMS**: $C_{23}H_{25}O_9$ for +, calculated 459.1499, found 445.1464.

4.2.7. The general procedure of synthesis of 3a–3d

To a soln of different Boc protected amino acids (0.5 mmol) and compound **7** (0.4 mmol) were added DCC (1.5 mmol) and a catalytic amount of DMAP at 0 °C. The soln was stirred for 36 h. The solution was washed with brine and the organic layer was dried over Na_2S0_4 and filtered. The organic layer was concentrated and applied to a column of silica gel to give **24**.

Compound **24** (0.5 mmol) was treated with TFA/CH_2Cl_2 (15 mL) for 1.5 h at room temperature. The mixture was concentrated in vacuo, and the residue was separate by Lipophilic Sephadex LH-20, provided single configuration of **3a**–**3d**.

Compound 3a (7α): m.p. 113–115 °C; **^{1}H-NMR (**400 MHz, CD_3OD): δ 7.34–7.57(m, 5H), 5.19–5.24 (m, 1H), 4.79–4.80 (d, *J* = 3.6 Hz, 1H), 4.63–4.65 (d, *J* = 7.6 Hz, 1H), 4.56–4.59 (d, *J* = 12.8 Hz, 1H), 3.28–4.28 (m, 7H), 2.25–2.33 (m, 1H), 1.03–1.12 (d, *J* = 6.8 Hz, 6H). **^{13}C-NMR (**100 MHz, CD_3OD): δ 168.67, 137.72, 128.40, 128.02, 98.86, 78.05, 76.56, 72.55, 70.21, 68.21, 66.67, 60.53, 58.05, 29.57, 17.07, 16.91; **ESI-HRMS**: $C_{19}H_{28}NO_7$ for +, calculated 382.1866, found 382.1839.

Compound 3b (7β): m.p. 85–87 °C; **^{1}H-NMR (**400 MHz, CD_3OD): δ 7.31–7.37 (m, 5H), 5.34–5.38 (m, 1H), 4.69–4.73 (dd, *J* = 2.4, 10.4 Hz, 1H), 4.61–4.63 (m, 1H), 3.44–4.09 (m, 8H), 2.21–2.28 (m, 1H), 0.92–0.95(m, 6H). **^{13}C-NMR (**400 MHz, CD_3OD): δ 168.59, 136.52, 128.06, 126.07, 97.74, 78.23, 77.30, 77.17, 76.39, 71.31, 67.85, 60.58, 58.00, 29.70, 16.79, 16.37; **ESI-HRMS**: $C_{19}H_{28}NO_7$ for +, calculated 382.1866, found 382.1839.

Compound 3c (7α) ^{1}H-NMR (500 MHz, CD_3OD): δ 7.30–7.54 (m, 5H), 5.36–5.39 (m, 1H), 4.71–4.74 (d, *J* = 3.2 Hz, 1H), 3.45–4.63 (m, 9H), 1.82–2.90 (m, 7H). **^{13}C-NMR (**125 MHz, CD_3OD): δ 168.91, 136.54, 128.45, 128.19, 128.07, 126.28, 126.04, 97.73, 78.21, 77.22, 77.22, 76.62, 71.36, 67.88, 60.56, 51.75, 29.80, 27.94, 13.43; **ESI-HRMS**: $C_{19}H_{28}NO_7S$ for +, calculated 414.1586, found 414.1592.

Compound 3d (7β) ^{1}H-NMR (400 MHz, CD_3OD): δ 7.32–7.57 (m, 5H), 5.18–5.23 (m, 1H), 4.79–4.82 (dd, *J* = 2.4, 10.4 Hz, 1H), 3.23–4.70 (m, 9H), 2.04–3.07 (m, 7H). **^{13}C-NMR (**400 MHz, CD_3OD): δ 169.05, 137.74, 128.45, 128.29, 128.08, 127.90, 127.81, 98.75, 78.04, 76.77, 72.42, 70.40, 68.12, 66.44, 60.53, 51.49, 29.79, 28.80, 13.71; **ESI-HRMS**: $C_{19}H_{28}NO_7S$ for +, calculated 414.1586, found 414.1592.

5. Results and discussions

With crenatoside analogues in hand, we then determined their inhibitory activities by subjecting them onto Neuraminidase

Table 1
In vitro inhibitory effects of compounds against influenza A NAs.

Sample	1000ug/mLInhibitory rateμg/ml	IC_{50}μg/ml
1a	3.9	NT
1b	16.82	NT
1c	8.28	NT
1d	11.72	NT
2a	17.55	NT
2b	29.82	NT
2c	91.24	42.91
2d	95.89	29.64
2e	64.98	97.95
2f	66.69	78.03
2g	94.08	29.91
2h	97.42	27.77
3a	16.58	NT
3b	25.32	NT
3c	88.76	89.43
3d	90.33	78.12
Crenatoside	89.24	89.81
Isocrenatoside	86.33	115.84

All compounds were tested in DMSO wells per dilution. NT: not tested.

Inhibitors Screen Kit, with crenatoside and is crenatoside as references. Based on the biological results (Table 1), the structure-activity relationships (SARs) of the leading compound, crenatoside, were discussed.

As summarized in Table 1, there are some interesting SARs. It is interesting that the analogues **1a**–**1d,** with the core structure of crenatoside, do not show the activity of neuraminidase inhibitors. It's suggesting that the core structure of crenatoside does not guarantee the inhibitory activity of neuraminidase. This may be explained by the spatial effect of the molecule.

It is found that compounds **2a**–**2 h** are generally more potent than the leading compound crenatoside. Among them, compound **2 h** was the most potent one, with $IC_{50} = 27.77$ μg/ml against NAs. Carefully inspection into the structure of compounds **2a**–**2 h** revealed that their activity increases along with the number of phenolic hydroxyl groups on the benzene ring. When substituted with methoxy group on the C-3′ phenyl moiety, the activity decreased significantly. It is therefore proposed that the hydrogen donor substitution at the C-3′ phenyl moiety is favourable for the activity.

Generally, the NA inhibitory activities of compounds **3a**–**3b** were better than those of compounds **3c**–**3d**. It seemed that compounds with methionine groups were superior to those compounds containing valine groups. The docking analysis performed by MOE simulation indicated that the amino group of **3c**–**3d** showed hydrogen bonding interactions with residues Arg371 and Arg118. To some extent, this can explain why compounds **3c**–**3d** were potent than compounds **3a**–**3b**.

The effect of C-7 configuration on the activity was also examined. We found that C-7α analogues are less potent than C-7β ones, which indicated that the C-7 configuration exerts a significant effect on the inhibitory activity of neuraminidase.

In sum, we presented design, synthesis and SARs studies of crenatoside as novel NA inhibitors. This work disclosed some interesting SARs of phenylethanoid glycosides derivatives, which not only enrich the NA inhibitor reservoir, but also facilitate further development and promise their therapeutic potential for the rising challenge of influenza diseases.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.12.031.

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