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**ORIGINAL ARTICLE**

# Design, synthesis, and biological activity of thiazole derivatives as novel influenza neuraminidase inhibitors

Yu Liu[1], Lei Zhang[1], Jianzhi Gong[1], Hao Fang[1], Ailin Liu[2], Guanhua Du[2], and Wenfang Xu[1]

*[1]Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong, China, and [2]Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, P.R. China*

**Abstract**

A series of novel influenza neuraminidase (NA) inhibitors based on thiazole core were synthesized and evaluated for their ability to inhibit NA of influenza A virus ($H_3N_2$). All compounds were synthesized in good yields starting from commercially available 2-amino-4-thiazole-acetic ester using a suitable synthetic strategy. These compounds showed moderate inhibitory activity against influenza A NA. The most potent compound of this series is compound **4d** ($IC_{50} = 3.43$ μM), which is about 20-fold less potent than oseltamivir, and could be used to design novel influenza NA inhibitors that exhibit increased activity based on thiazole ring.

**Keywords:** Neuraminidase inhibitor, thiazole, influenza



## Introduction

Despite advances in the understanding of molecular and cellular aspects of influenza, the disease remains the major cause of mortality and morbidity among patients with respiratory diseases.[1]

Influenza virus neuraminidase (NA, EC 3.2.1.18) is a major surface glycoprotein of the influenza A and B. Viral NA catalyses cleavage of the ketosidic linkage between terminal sialic acid (SA) and an adjacent sugar residue[2,3] via a oxocarbonium intermediate. Compounds that inhibit NA can protect the host from viral infection and retard its propagation and it is thought that rationally designed NA inhibitors are effective for the treatment of influenza. Also, because the structure of the active site of NA is highly conserved across all nine influenza A NA subtypes and influenza B subtypes, the NA inhibitors are well-tolerated against all influenza types.[4]

The NA inhibitors have currently emerged as promising therapeutics for the treatment of influenza.[5] Two such successful examples of structure-based drug design of NA inhibitors, Relenza (zanamivir, Glaxo Wellcome, Misslesex, UK/Biota, Notting Hill, Victoria, Australia) and Tamiflu (oseltamivir, Hoffman-La Roche, Basel, Switzerland/Gilead, Foster Ciy, CA), exhibit their antiviral effects through the inhibition of NA activities of the influenza A and B viruses and further underscored the importance of NA as a valid anti-influenza drug target.[6,7]

Oseltamivir is the predominant choice and is used worldwide for the treatment of influenza, but the generation and circulation of oseltamivir-resistant mutants of seasonal influenza, as well as H5N1 avian influenza, due to NA mutations of the viruses[8–13] after Tamiflu treatment of humans infected with viruses containing different NA subtypes, have become major concerns. Moreover, some attractive structural features of H5N1 avian influenza virus NA[14] have led us to search for new drug candidate and more effective antivirals.[15–17]

## Material and methods

### Design of novel NA inhibitors based on thiazole scaffold

Earlier crystallographic and ensuing SAR studies have revealed that the active site of NA can be divided into

*Address for Correspondence*: Wenfang Xu, Department of Medicinal Chemistry, School of Pharmacy, Shandong University, 44, West Culture Road, Ji'nan, 250012, P.R. China. E-mail: wfxu@yahoo.cn

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four major binding sites. Abu Hammad et al.[18] envisaged the binding compartment of NA as a shallow pouch comprised of four primary regions involved in binding with docked ligands (Figure 1). Wang et al.[19] have also derived an "airplane" model of the NA active site to summarize the basic structural requirements of a potent NA inhibitor. The first region (R1) is comprised of the positively charged guanidino groups of arginines 118, 292, and 371 and interacts with the carboxylate. Confronting R1 is a mainly hydrogen-bond-forming region (R2) consisting of the side chain of Arg 152 and the amidic carbonyl of Trp 178 and Asp 151. The negatively charged region R3 is comprised of Glu 119, Asp 151, and Glu 227, Glu 276, Glu 277, and Tyr 406 and interacts with the amino or guanidine. The hydrophobic region (R4) is situated adjacent to R2 and comprised of the side chains of Ala 246, Ile 222, Trp 178, and Arg 224 and binds to hydrophobic side chain. All binding regions were implicated in substrate recognition; however, Asp 151, Glu 277, and Tyr 406 are believed to play a critical role in the catalytic activity of NA.[20,21]

According to the studies on NA active site and SAR of published NA inhibitors, inhibition of the NA is mainly determined by the relative positions of the four substituents of the central ring.[22] For example, in oseltamivir, the four substituents are carboxyl ethyl ester, amino, acetamino, and alkyl.

In our previous study,[23,24] we have reported several kinds of novel NA inhibitors based on pyrrolidine and benzyl scaffold. In our recent screening, we found that the commercially available 2-amino-4-thiazole-acetic ester **1** exhibited substantial NA inhibition ($IC_{50}$ = 358 μM). Considering that there have not been thiazole derivatives reported as NA inhibitors, we chose compound **1** as the lead compound for further modification and optimization.

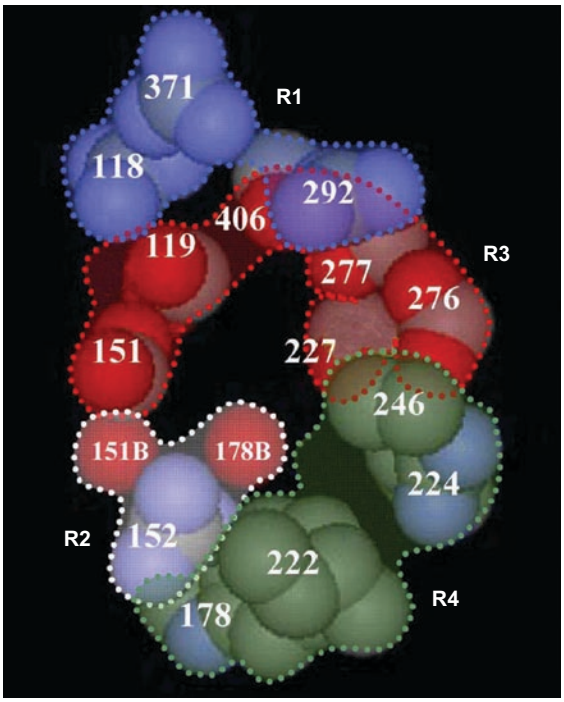



Figure 1. A presentation of binding pocket of neuraminidase (NA) showing the different binding regions. The crater of the binding pocket is the middle gap.[17]

To contain the different substituents to interact with the four binding pockets of the NA active sites, we used the following chemical modifications: (1) compound **1** were coupled with a few kinds of amino acids with different hydrophobic side chains to introduce the amide and alkyl, aiming to form hydrogen bond with R2 and interact with the hydrophobic region R4, respectively; (2) carboxyl ethyl ester was kept or converted to carboxylic group, aiming to interact with R1; (3) BOC-protected amino group was kept or be converted to free amino group, aiming to interact with R3.

Then we will describe the synthesis, biological activity, and the docking study of the thiazole derivates as NA inhibitors.

## Chemistry

All reactions were carried out by standard techniques for the exclusion of moisture. Solvents were distilled prior to use and flash chromatography was performed using silica gel (60 Å, 200 ± 300 mesh). All reactions were monitored by thin-layer chromatography on 0.25-mm silica gel plates (60GF-254) and visualized with UV light, or iodine vapour. Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Proton nuclear magnetic resonance ($^1$H NMR) spectra were determined on a Brucker Avace 600 spectrometer using trimethylsilane (TMS) as an internal standard. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from TMS. High-resolution mass spectral (HRMS) data are reported as *m*/*z* (relative intensity).

### 2-(tert-Butoxycarbonylamino)acetic acid (*N*-BOC-L-glycine) (2)

The title compound was prepared as described by Nielsen et al.[25]

### General procedure for the synthesis of 3a–9a

Compound **2** (10 mmol) was dissolved in anhydrous $CH_2Cl_2$ and 1 equiv of $Et_3N$ was added to the solution. Then TBTU (11 mmol) was added slowly to the above solution under ice-bath. After stir for 10 min at 0°C, compound **1** (12 mmol) was added to the mixture and adjust to pH 7 with $Et_3N$. After about 4 h stirring at room temperature, the mixture was filtrated and washed with 10% citric acid, brine, and saturated $NaHCO_3$ solution, and then was dried with $Na_2SO_4$. The solvent was evaporated to give a residue that was chromatographed on a silica column to give the title compound **3a** (yield: 85%) as a white solid, m.p. 154–156°C. HRMS: calcd for $C_{14}H_{21}N_3O_5S$ $[M+H]^+$ 344.1235. Found: 344.1286. $^1$H NMR (DMSO-$d^6$): δ 1.18 (t, 3H, *J* = 7.2 Hz), 1.39 (s, 9H), 3.68 (s, 2H), 3.8 (d, 2H, *J* = 6.0 Hz), 4.08 (q, 2H, *J* = 7.2 Hz), 6.97 (s, 1H), 7.14 (t, 1H, *J* = 6.0 Hz), 12.2 (s, 1H).

Compounds **4a**–**7a** were prepared following the general procedure as described above.

***(S)-Ethyl 2-(2-(2-(tert-butoxycarbonylamino)propanamido) thiazol-4-yl)acetate (4a)***

A white solid, yield 81%, m.p. 140–142°C. HRMS: calcd for $C_{15}H_{23}N_3O_5S$ [M+H]$^+$ 358.1392. Found: 358.1489. $^1$H NMR (DMSO-$d^6$): δ 1.18 (t, 3H, $J$=7.2 Hz), 1.24 (d, 3H, $J$=7.2 Hz), 1.39 (s, 9H), 3.682 (s, 2H), 4.08 (q, 2H, $J$=7.2 Hz), 4.20 (m, 1H), 6.98 (s, 1H), 7.22 (d, 1H, $J$=6.6 Hz), 12.18 (s, 1H).

***Ethyl 2-(2-((2S,3R)-2-(tert-butoxycarbonylamino)-3-methylpentanamido)thiazol-4-yl)acetate (5a)***

A white solid, yield 87%, m.p. 64–66°C. HRMS: calcd for $C_{18}H_{29}N_3O_5S$ [M+H]$^+$ 400.1861. Found: 400.2092. $^1$H NMR (DMSO-$d^6$): δ 0.83 (m, 6H), 1.16 (t, 3H), 1.24 (m, 1H), 1.37 (t, 9H), 1.45(m, 1H), 1.78 (m, 1H), 3.69 (s, 2H), 4.05(q, 1H), 4.07 (q, 2H), 6.99 (s, 1H), 7.10 (d, 1H), 12.20 (s, 1H).

***(S)-Ethyl 2-(2-(2-(tert-butoxycarbonylamino)-3-methylbutanamido)thiazol-4-yl) acetate (6a)***

A yellow liquid, yield 79%. HRMS: calcd for $C_{22}H_{38}N_4O_7S$ [M+H]$^+$ 386.1705. Found: 386.1777. $^1$H NMR (DMSO-$d^6$): δ 0.85–0.91 (m, 6H, $J$=7.2 Hz),1.17–1.38 (s, 9H), 1.96 (m, 1H), 3.62 (s, 2H), 4.01 (m, 1H), 4.08 (q, 2H), 6.99 (s, 1H), 7.07 (d, 1H, $J$=7.2 Hz), 12.21 (s, 1H).

***(S)-Ethyl 2-(2-(2-(tert-butoxycarbonylamino)-4-methylpentanamido)thiazol-4-yl) acetate (7a)***

A white solid, yield 83%, m.p. 68–70°C. HRMS: calcd for $C_{18}H_{29}N_3O_5S$ [M+H]$^+$ 400.1861. Found: 400.1967. $^1$H NMR (DMSO-$d^6$): δ 1.18 (t, 3H), 1.38 (s, 9H), 1.82–1.91 (m, 2H), 2.046 (m, 3H), 2.41–2.54 (m, 2H), 3.69 (s, 2H), 4.08 (q, 2H), 4.23 1(q, 1H), 6.99 (s, 1H), 7.27 (s, 1H),12.27 (s, 1H).

***(S)-Ethyl 2-(2-(2-(tert-butoxycarbonylamino)-4-(methylthio) butanamido)thiazol-4-yl) acetate (8a)***

A yellow liquid, yield 85%. HRMS: calcd for $C_{17}H_{27}N_3O_5S_2$ [M+H]$^+$ 418.1426. Found: 418.1491. $^1$H NMR (DMSO-$d^6$): δ 1.17 (t, 3H), 1.19 (s, 9H), 1.93 (m, 3H), 2.14 (m, 2H), 2.56 (m, 2H), 3.72 (s, 2H), 4.08 (q, 2H), 4.23 (q, 1H), 6.99 (s, 1H), 7.27 (d, 1H), 12.27 (s, 1H).

***(S)-Ethyl 2-(2-(2-(tert-butoxycarbonylamino)-3-phenylpropanamido)thiazol-4-yl) acetate (9a)***

A white solid, yield 78%, m.p. 116–118°C; HRMS: calcd for $C_{21}H_{27}N_3O_5S$ [M+H]$^+$ 434.1705. Found: 434.1857. $^1$H NMR (DMSO-$d^6$): δ 1.18 (t, 3H), 1.31 (s, 9H), 2.80 (dd, 1H), 2.99 (dd, 1H), 3.69 (s, 2H), 4.09 (q, 2H), 4.40 (br, 1H), 7.00 (s, 1H), 7.20 (t, 1H), 7.28 (t, 2H),7.35 (d, 2H), 12.41 (s, 1H).

## General procedure for the synthesis of 3b–9b

To a solution of compound **3a** (10 mmol) in dry EtOAc at 0°C was added a solution of EtOAc (10 mL) saturated with dry HCl gas. The reaction solution was stirred at 0°C for 2 h, and then the temperature is raised to room temperature and the reaction proceeds for 5 h before being concentrated *in vacuo*. The product **3b** was recrystallized with MeOH and EtOAc from the residue as a white solid. Yield 90%, m.p. 97–99°C. HRMS: calcd for $C_9H_{13}N_3O_3S$ (M+H): 244.0711. found: 244.0753; $^1$H NMR (DMSO-$d^6$): δ 1.18 (t, 3H, $J$=7.2 Hz), 3.68 (s, 2H), 3.80 (d, 2H, $J$=6.0 Hz), 4.08 (q, 2H, $J$=7.2 Hz), 6.97 (s, 1H), 7.14 (t, 1H, $J$=6.0 Hz), 12.20 (s, 1H).

Compounds **4b**–**9b** were prepared following the general procedure as described above.

***(S)-Ethyl 2-(2-(2-aminopropanamido)thiazol-4-yl)acetate (4b)***

A white solid, yield 93%, m.p. 145–147°C. HRMS: calcd for $C_{10}H_{15}N_3O_3S$ [M+H]$^+$ 258.0868. Found: 257.0817. $^1$H NMR (DMSO-$d^6$): δ 12.18 (s, 1H), 8.52 (s, 3H), 7.10 (d, 1H), 4.20 (m, 1H), 4.08 (q, 2H), 3.68 (s, 2H), 1.24 (d, 3H), 1.18 (t, 3H).

***Ethyl 2-(2-((2S,3R)-2-amino-3-methylpentanamido)thiazol-4-yl)acetate (5b)***

A white solid, yield 89%, m.p. 178–180°C. HRMS: calcd for $C_{13}H_{21}N_3O_3S$ [M+H]$^+$ 300.1337. Found: 300.1439. $^1$H NMR (DMSO-$d^6$): δ 0.85–0.93 (m, 6H), 1.13 (m, 1H), 1.19 (t, 3H), 1.57 (m, 1H), 1.98 (m, 1H), 3.73 (s, 2H), 3.94 (q, 1H), 4.09 (q, 2H), 7.09 (s, 1H), 8.67 (d, 3H), 12.20 (s, 1H).

***(S)-Ethyl 2-(2-(2-amino-3-methylbutanamido)thiazol-4-yl) acetate (6b)***

A white solid, yield 91%, m.p. 138–140°C. HRMS: calcd for $C_{12}H_{19}N_3O_3S$ [M+H]$^+$ 286.1181. Found: 286.1233. $^1$H NMR (DMSO-$d^6$): δ 0.97 (m, 6H, $J$=7.2 Hz), 1.19 (m, 3H), 2.11 (m, 1H), 3.62 (s, 2H), 3.87 (m, 1H), 4.08 (q, 2H), 4.37 (br, 1H), 6.99 (s, 1H), 8.56 (d, 3H, $J$=4.2 Hz), 12.21 (s, 1H).

***Ethyl 2-(2-((2S,3R)-2-amino-3-methylpentanamido)thiazol-4-yl)acetate (7b)***

A white solid, yield 88%, m.p. 151–153°C. HRMS: calcd for $C_{13}H_{21}N_3O_3S$ [M+H]$^+$ 300.1337. Found: 300.1453. $^1$H NMR (DMSO-$d^6$): δ 0.91 (t, 6H, $J$=6.0 Hz), 1.66–1.68 (m, 3H), 3.72 (s, 2H), 3.89 (br, 1H), 4.03 (m, 1H), 4.09 (m, 2H), 7.10 (s, 1H), 8.59 (s, 3H), 12.70 (s, 1H).

***(S)-Ethyl 2-(2-(2-amino-4-(methylthio)butanamido)thiazol-4-yl)acetate (8b)***

A white solid, yield 85%, mp = 162–164°C. HRMS: calcd for $C_{12}H_{19}N_3O_3S_2$ (M+H): 318.0901. Found: 318.0952. $^1$H NMR (DMSO-$d^6$): δ 2.06 (m, 3H), 2.14 (m, 2H), 2.56 (m, 2H), 3.72 (s, 2H), 3.83 (br, 1H), 4.09 (q, 2H), 4.13 (q, 1H), 7.10 (s, 1H), 8.64 (s, 3H), 12.80 (s, 1H).

***(S)-Ethyl 2-(2-(2-amino-3-phenylpropanamido)thiazol-4-yl) acetate (9b)***

A white solid, yield 92%, m.p. = 238–239°C. HRMS: calcd for $C_{16}H_{19}N_3O_3S$ [M+H]$^+$ 334.1181. Found: 334.1252. $^1$H NMR (DMSO-$d^6$): δ 1.19 (t, 3H), 3.11 (dd, 1H), 3.20 (t, 1H), 3.61 (q, 2H), 3.72 (s, 2H), 4.26 (m, 1H), 7.08(s, 1H), 7.25 (s, 1H), 7.27 (t, 1H), 7.33 (t, 2H), 7.55 (d, 2H ), 12.79 (s, 1H).

## General procedure for the synthesis of 3c–9c

LiOH hydrate (12 mmol) was added in portions to a solution of **3b** (10 mmol) in dioxane/$H_2O$ (3:2) (20 mL). The

reaction mixture was stirred at room temperature until all the starting material had been consumed. The dioxane was evaporated and water was added. The aqueous layer was washed twice with $CH_2Cl_2$, neutralized with $KHSO_4$ (12 mmol), then extracted with $CH_2Cl_2$, washed once with brine, and was dried with $Na_2SO_4$. The solvent was evaporated to give a residue that was chromatographed on a silica column to give the title compound **3c** (yield: 72%) as a white solid. Yield 82%, m.p. 161–163°C. HRMS: calcd for $C_{12}H_{17}N_3O_5S$ $[M+H]^+$ 316.0966. Found: 316.0964. $^1H$ NMR (DMSO-$d^6$): δ 1.39 (s, 9H), 3.59 (s, 2H), 3.80 (d, 2H, $J$ = 6.0 Hz), 7.14 (t, 1H, $J$ = 6.0 Hz), 6.95 (s, 1H), 12.15 (s, 1H), 12.15 (s, 1H).

Compounds **4c**–**9c** were prepared following the general procedure as described above.

***(S)-2-(2-(2-(tert-Butoxycarbonylamino)propanamido)thiazol-4-yl)acetic acid (4c)***

A white solid, yield 78%, m.p. 159–160°C. HRMS: calcd for $C_{13}H_{19}N_3O_5S$ $[M+H]^+$ 330.1179. Found: 330.1124. $^1H$ NMR (DMSO-$d^6$): δ 1.24 (d, 3H), 1.39 (s, 9H), 3.59 (s, 2H), 4.19 (d, 2H), 6.95 (s, 1H), 7.21 (t, 1H, $J$ = 6.0 Hz), 12.15 (s, 1H), 12.16 (s, 1H).

***2-(2-((2S,3R)-2-(tert-Butoxycarbonylamino)-3-methylpentanamido)thiazol-4-yl) acetic acid (5c)***

A white solid, yield 82%, m.p. 122–124°C. HRMS: calcd for $C_{16}H_{25}N_3O_5S$ $[M+H]^+$ 372.1548. Found: 372.1594. $^1H$ NMR (DMSO-$d^6$): δ 0.83 (m, 6H), 1.27 (m, 1H), 1.37 (t, 9H), 1.46 (m, 1H), 1.75 (m, 1H), 3.60 (s, 2H), 4.07 (q, 1H),M 6.95 (s, 1H), 7.09 (d, 1H), 12.18 (s, 1H), 12.38 (s, 1H).

***(S)-2-(2-(2-(tert-Butoxycarbonylamino)-3-methylbutanamido) thiazol-4-yl)acetic acid (6c)***

A white solid, yield 85%, m.p. 173–175°C. HRMS: calcd for $C_{20}H_{34}N_4O_7S$ $[M+H]^+$ 358.1392. Found: 358.1453. $^1H$ NMR (DMSO-$d^6$): δ 1.25 (m, 6H, $J$ = 7.2 Hz), 1.38 (s, 9H), 2.98 (m, 1H), 3.60 (s, 2H), 4.01 (m, 1H), 6.95 (s, 1H), 7.05 (d, 1H, $J$ = 7.2 Hz),12.17 (s, 1H), 12.38 (s, 1H).

***(S)-2-(2-(2-(2-(tert-Butoxycarbonylamino)-4-methylpentanamido)thiazol-4-yl)acetic acid (7c)***

A white solid, yield 78%, m.p. 138–140°C. HRMS: calcd for $C_{16}H_{25}N_3O_5S$ $[M+H]^+$ 372.1548. Found: 372.1595. $^1H$ NMR (DMSO-$d^6$): δ 0.88 (t, 6H, $J$ = 7.2 Hz), 1.27 (m, 2H), 1.37 (s, 9H), 1.52 (m, 1H), 1.62 (t, 1H, $J$ = 6.6 Hz), 3.33 (s, 2H), 4.21 (m, 1H), 6.95 (s, 1H), 7.15 (d, 1H, $J$ = 7.2 Hz), 12.23 (s, 1H), 12.34 (s, 1H).

***(S)-2-(2-(2-(2-(tert-Butoxycarbonylamino)-4-(methylthio) butanamido)thiazol-4-yl)acetic acid (8c)***

A white solid, yield 86%, m.p. 153–155°C. HRMS: calcd for $C_{15}H_{23}N_3O_5S_2$ $[M+H]^+$ 390.1113. Found: 390.1161. $^1H$ NMR (DMSO-$d^6$): δ 1.26 (t, 3H), 1.83 (s, 9H), 1.90 (m, 2H), 2.03 (m, 3H), 3.59 (m, 2H), 3.69 (s, 2H), 4.08 (q, 2H), 4.23 (q, 1H), 6.95 (s, 1H), 7.25 (d, 1H), 12.24 (s, 1H), 12.57 (s, 1H).

***(S)-2-(2-(2-(tert-Butoxycarbonylamino)-3-phenylpropanamido)thiazol-4-yl)acetic acid (9c)***

A white solid, yield 83%, m.p. = 180–182°C. HRMS: calcd for $C_{19}H_{23}N_3O_5S$ $[M+H]^+$ 406.1392. Found: 406.1448. $^1H$ NMR (DMSO-$d^6$): δ 1.19 (t, 3H), 1.30 (s, 9H), 2.51 (t, 1H), 2.51 (dd, 1H), 3.60 (s, 2H), 4.38 - 4.42 (m, 1H), 6.96 (s, 1H),7.20 (s, 1H), 7.25 (t, 1H), 7.28 (t, 2H), 7.34 (d, 2H), 12.39 (s, 2H).

## General procedure for the synthesis of 3d–9d

To a solution of compound **3c** (10 mmol) in dry EtOAc at 0°C was added a solution of EtOAc (10 mL) saturated with dry HCl gas. The reaction solution was stirred at 0°C for 2 h, and then the temperature is raised to room temperature and the reaction proceeds for 5 h before being concentrated *in vacuo*. The product **3d** was recrystallized with MeOH and EtOAc from the residue as a white solid. Yield 88%, m.p. 185–187°C. HRMS: calcd for $C_7H_9N_3O_3S$ $[M+H]^+$ 216.0398. Found: 216.0344. $^1H$ NMR (DMSO-$d^6$): δ 3.62 (s, 2H), 3.86 (q, 2H), 7.04 (s, 1H), 8.53 (t, 3H).

Compounds **4d**–**9d** were prepared following the general procedure as described above.

***(S)-2-(2-(2-Aminopropanamido)thiazol-4-yl)acetic acid (4d)***

A white solid, yield 90%, m.p. 101–103°C. HRMS: calcd for $C_8H_{11}N_3O_3S$ $[M+H]^+$ 230.0655. Found: 230.0607. $^1H$ NMR (DMSO-$d^6$): δ 1.47 (d, 3H, $J$ = 7.2 Hz), 4.08 (m, 1H), 3.63 (s, 2H), 7.06 (s, 1H), 8.51 (d, 3H, $J$ = 4.2 Hz), 12.70 (s, 1H).

***2-(2-((2S,3R)-2-Amino-3-methylpentanamido)thiazol-4-yl) acetic acid (5d)***

A white solid, yield 88%, m.p. 172–174°C. HRMS: calcd for $C_{11}H_{17}N_3O_3S$ $[M+H]^+$ 272.1024. Found: 272.1080. $^1H$ NMR (DMSO-$d^6$): δ 0.83 (m, 6H), 1.27 (m, 1H), 1.46 (m, 1H), 1.75 (m, 1H), 3.60 (s, 2H), 4.07 (q, 1H), 6.95 (s, 1H), 7.09 (d, 1H), 8.59 (s, 3H), 12.38 (s, 1H).

***(S)-2-(2-(2-Amino-3-methylbutanamido)thiazol-4-yl)acetic acid (6d)***

A white solid, yield 92%, m.p. 194–196°C. HRMS: calcd for $C_{10}H_{15}N_3O_3S$ $[M+H]^+$ 258.0868. Found: 258.0909. $^1H$ NMR (DMSO-$d^6$): δ 1.25 (m, 6H, $J$ = 7.2 Hz), 2.98 (m, 1H), 3.60 (s, 2H), 4.01 (m, 1H), 6.95 (s, 1H), 7.05 (d, 1H), 8.55 (s, 3H), 12.38 (s, 1H).

***((S)-2-(2-(2-Amino-4-methylpentanamido)thiazol-4-yl)acetic acid (7d)***

A white solid, yield 88%, m.p. 205–206°C. HRMS: calcd for $C_{11}H_{17}N_3O_3S$ $[M+H]^+$ 272.1024. Found: 272.1070. $^1H$ NMR (DMSO-$d^6$): δ 0.91 (t, 6H), 1.66–1.68 (m, 3H), 3.63 (s, 2H), 3.74 (s, 1H), 4.09 (m, 1H), 7.10 (s, 1H), 8.59 (s, 3H), 12.70 (s, 1H).

***(S)-2-(2-(2-Amino-4-(methylthio)butanamido)thiazol-4-yl) acetic acid (8d)***

A white solid, yield 90%, m.p. 190–192°C. HRMS: calcd for $C_{10}H_{15}N_3O_3S_2$ $[M+H]^+$ 290.0588. Found: 290.0624. $^1H$ NMR (DMSO-$d^6$): δ 2.06 (m, 3H), 2.15 (m, 2H), 2.56 (m, 2H),

3.72 (s, 2H), 4.09 (q, 1H), 4.15 (m, 1H), 7.10 (d, 1H), 8.74 (s, 3H), 12.34 (s, 1H).

#### *(S)-2-(2-(2-Amino-3-phenylpropanamido)thiazol-4-yl)acetic acid (9d)*

A white solid, yield 88%, m.p. 225–227°C. HRMS: calcd for $C_{14}H_{15}N_3O_3S$ $[M+H]^+$ 306.0868. Found: 306.0914. $^1H$ NMR (DMSO-$d^6$): δ 3.11–3.20 (m, 2H), 3.61 (dd, 2H), 3.72 (s, 1H), 4.26 (t, 1H), 4.26 (t, 1H), 7.08 (s, 1H), 7.25 (s, 1H), 7.27 (t, 2H), 7.33 (d, 2H), 8.55 (s, 3H), 12.79 (s, 1H).

### NA inhibition assay

NA inhibitory activity was determined by the commercial NA inhibitory screening kit (Beyotime Institute of Biotechnology, Jiangsu, China). Although the NA for enzyme assay is not originated from avian influenza A/H5N1 strain, their sequences are highly conserved. Therefore, this kit is suitable for the high-throughput screening of NA inhibitors *in vitro*.

The compound 2′-(4-methylumbelliferyl)-*a*-D-acetyl neuraminic acid (MUNANA) is the substrate of NA. And cleavage of this substrate by NA produces a fluorescent product, which can emit an emission wavelength of 460 nm with an excitation wavelength of 355 nm. The intensity of fluorescence can reflect the activity of NA sensitively.

The reaction mixture containing the buffer, NA enzyme, test compounds, or oseltamivir carboxylate (which was prepared according to literature method[26]) and the substrate were incubated at 37°C. Terminate the reaction by adding 150 μL stop solution to all wells including the blank row. Read the plate within 20 min of adding stop solution detecting fluorescence using an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The $IC_{50}$ was calculated by plotting percent inhibition versus the inhibitor concentration and determination of each point was performed in duplicate. The data are expressed as the mean of three independent experiments.[27]

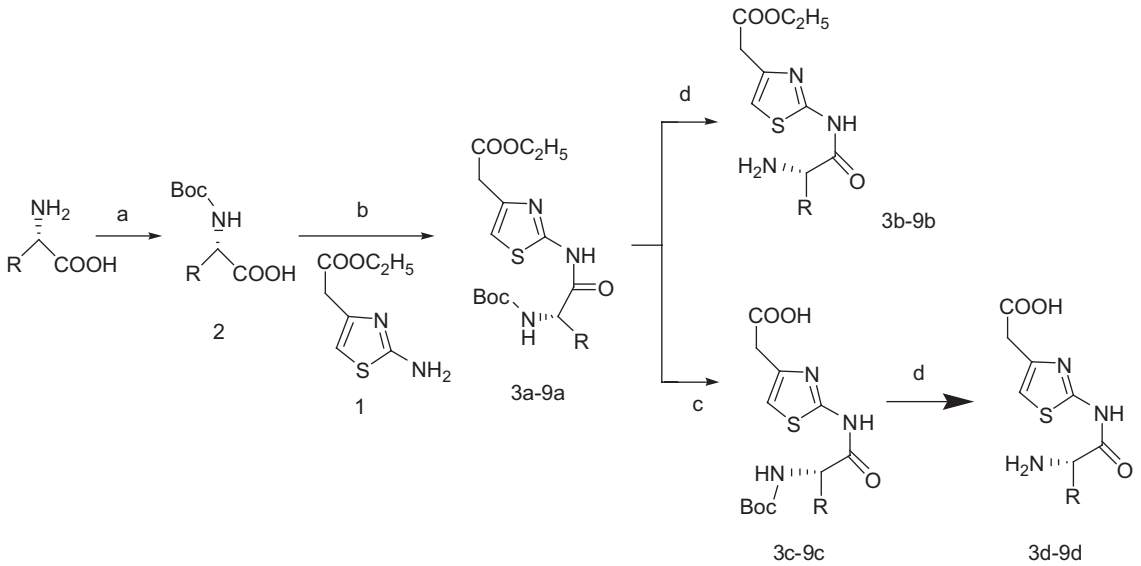
## Result and discussion

### Chemistry

The synthetic route of target compounds was shown in Scheme 1. Different amino acids with hydrophobic side chains are first protected by $BOC_2O$, and then the BOC-protected amino acids **2** were coupled with the lead compound **1** using TBTU as the coupling agent. Compounds **3a**–**9a** were obtained and the BOC group was easily cleaved by using 6 N HCl in EtOAc to provide compounds **3b**–**9b**. The esters of **3a**–**9a** were hydrolyzed to carboxylic acid by $LiOH/H_2O$ in dioxane to obtain compounds **3c**–**9c**. Then through deprotecting the BOC– group using 6 N HCl in EtOAc, compounds **3d**–**9d** were obtained.

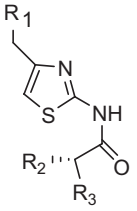
### NA inhibition assay

All the target compounds (28 compounds) were tested for their ability to inhibit NA. Preliminary results showed that most of the compounds (25 compounds) displayed enhanced inhibitory activities compared with the lead compound (Table 1). It seems that the compounds with proper hydrophobic side chain show better inhibitory activity as three such compounds (**4d**, **5d**, and **8d**) exhibit good activity (3.43, 4.27, and 7.78 μM, respectively) and the methyl group is the most suitable. As expected, compounds with –COOH group showed better activities than those with –COOEt group. Compounds with $–NH_2$ group showed increased activities than those with BOC–NH– group. On the other hand, the incorporation of natural amino acids would




Scheme 1. Reagents and conditions: a. $(BOC)_2O$/THF; b. TBTU, $Et_3N$/anhydrous $CH_2Cl_2$, 0°C; c. 1 M LiOH, dioxane/$H_2O$; and d. HCl/anhydrous EtOAc.

Table 1. The structure and *in vitro* inhibitory activities of target compounds against neuraminidase (NA).



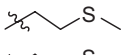
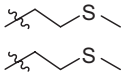
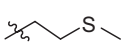
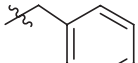
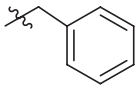
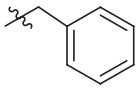
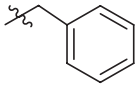

| Compound | R1 | R2 | R3 | IC50 (μM) |
|---|---|---|---|---|
| **1** | | | | 358 |
| **3a** | –COOEt | –BOC–NH– | H | 411 |
| **3b** | –COOEt | $-NH_2-$ | H | 313 |
| **3c** | –COOH | –BOC–NH– | H | 201 |
| **3d** | –COOH | $-NH_2-$ | H | 128 |
| **4a** | –COOEt | –BOC–NH– | $-CH_3$ | 68.4 |
| **4b** | –COOEt | $-NH_2-$ | $-CH_3$ | 22.8 |
| **4c** | –COOH | –BOC–NH– | $-CH_3$ | 19.5 |
| **4d** | –COOH | $-NH_2-$ | $-CH_3$ | 3.43 |
| **5a** | –COOEt | –BOC–NH– |  | 40.3 |
| **5b** | –COOEt | $-NH_2-$ |  | 36.6 |
| **5c** | –COOH | –BOC–NH– |  | 16.3 |
| **5d** | –COOH | $-NH_2-$ |  | 4.27 |
| **6a** | –COOEt | –BOC–NH– |  | 147 |
| **6b** | –COOEt | $-NH_2-$ |  | 40.2 |
| **6c** | –COOH | –BOC–NH– |  | 38.3 |
| **6d** | –COOH | $-NH_2-$ |  | 10.4 |
| **7a** | –COOEt | –BOC–NH– |  | 135 |
| **7b** | –COOEt | $-NH_2-$ |  | 141 |
| **7c** | –COOH | –BOC–NH– |  | 127 |
| **7d** | –COOH | $-NH_2-$ |  | 19.1 |
| **8a** | –COOEt | –BOC–NH– |  | 81.1 |
| **8b** | –COOEt | $-NH_2-$ |  | 45.2 |
| **8c** | –COOH | –BOC–NH– |  | 42.6 |
| **8d** | –COOH | $-NH_2-$ |  | 7.78 |
| **9a** | –COOEt | –BOC–NH– |  | 95.9 |
| **9b** | –COOEt | $-NH_2-$ |  | 221 |

Table 1. continued on next page

Table 1. Continued.

| Compound | R1 | R2 | R3 | IC50 (μM) |
|---|---|---|---|---|
| **9c** | –COOH | –BOC–NH– |  | 34.2 |
| **9d** | –COOH | $–NH_2–$ |  | 21.4 |
| Oseltamivir carboxylate | | | | 0.17 |

generate compounds with similar or better inhibitory activities compared with the parent compound.

In order to determine the interaction between thiazole derivatives and the NA active site, compound **4d** was docked into the active sites of NA (PDB entry: 2HU4) using SYBYL 7.0 and optimized using Powell's method with the Tripos force field with convergence criterion set at 0.05 kcal/(Å·mol), and assigned with Gasteiger-Hückel method. The docking study performed using Sybyl/FlexX module, the residues in a radius of 7.0 Å around oseltamivir in the co-crystal structure (PDB ID: 2HU4) were selected as the active site. The binding of compound **4d** in the active site of NA is shown in Figure 2, and we found that the –COOH group of the target compounds interacts with the positively charged region R1 of NA active site by making charge–charge interactions with Arg 292 and Arg 371 of this subsite. This correlates with the fact that compounds with –COOH group showed better activities than those with –COOEt group. The reason for which the –COOH group is important for development of novel inhibitors is that it is well-established that two or three Arg residues in the immediate vicinity of the carboxylic group of NA inhibitors play a key role in orienting and stabilizing various inhibitors.[28,29]

The $–NH_2$ group binds to the negatively charged region R3 by interaction with Asp 151 and Glu 119. The –CO–NH– group forms hydrogen bond with Asp 151 of the region R2. In addition, the –COOH group of compound **4d** could also interact with Tyr 347 and Tyr 406 residues, the $–NH_2$ group could interact with Arg 156 residue by hydrogen bond for stabilizing the interaction. Also, as shown in Figure 3, the methyl group may accommodate a small hydrophobic pocket next to region R3 instead of the major hydrophobic region R4. And considering the actual activities, we can conclude that the methyl group is just the most suitable for this small hydrophobic pocket. Although R2 and R4 could not be occupied well, the result still indicated that compound **4d** could be a lead compound to develop new thiazole-based derivatives as novel NA inhibitors.

## Conclusion

A series of novel influenza NA inhibitors based on core were synthesized and evaluated for their ability to inhibit NA of influenza A virus.

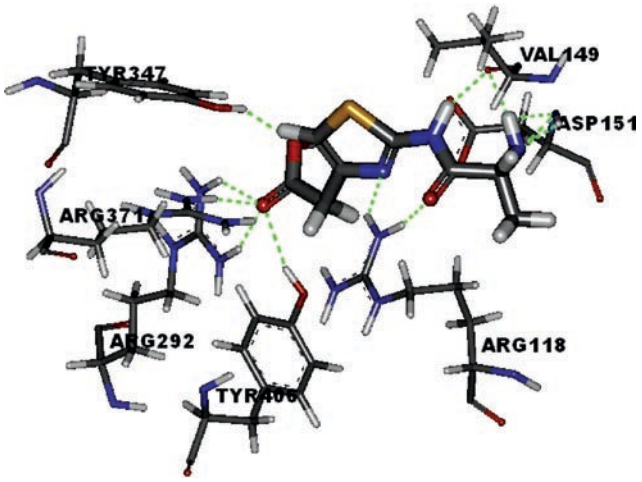



Figure 2. Flex X-docked result of compound **4d** in the active site of neuraminidase (PDB ID: 2HU4). The yellow lines and numbers show the potential hydrogen bonds and bond length.

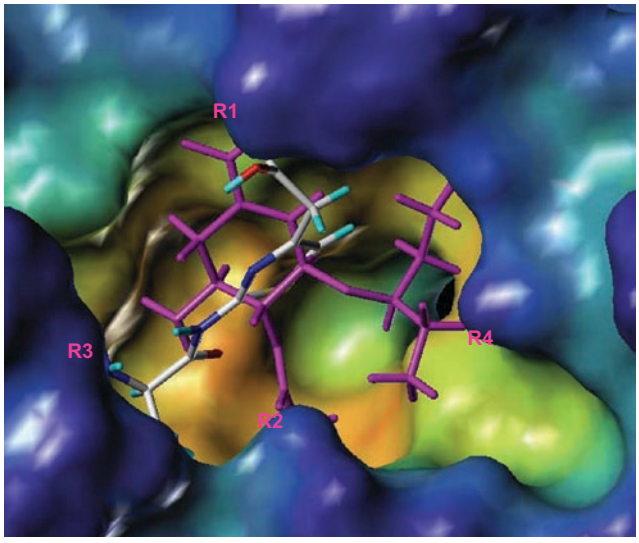



Figure 3. Docking result of compound **4d** shown by SYBYL 7.0 (zanamivir shown in magenta line).

In conclusion, we have described the synthesis and properties of a series of thiazole derivatives as influenza NA inhibitors. Several compounds were shown to possess moderate influenza NA inhibitory activity, although in all cases, measured activity was lower than that of oseltamivir. The most potent compound of the

series is compound **4d** ($IC_{50}$ = 3.43 μM), about 20-fold less potent than oseltamivir.

The binding of compound **4d** in the active site of NA is shown in Figure 3. Although the four regions of the active site of NA were not occupied as well as oseltamivir to establish a consistent binding orientation and the inhibitory activity is not so potent, our study still indicated that thiazole derivatives can show potent NA inhibitory activity and this finding could be used to design novel influenza NA inhibitors that exhibit increased activity based on thiazole ring. Both NA enzyme inhibition and X-ray crystallography data suggest that the strategy of designing an inhibitor of NA that binds to the highly conserved active site of the NA achieves the desired goal of activity against all influenza NA subtypes, N1–N9, and influenza B viruses.[30,31]

## Declaration of interest

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