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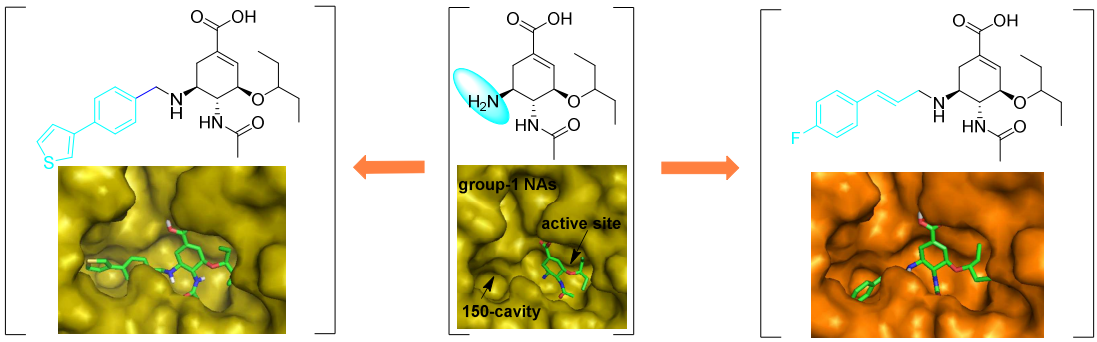

9a

IC_{50} (nM): 118.17 (N1, H5N1), 78.06 (N8, H5N8), 1442.6 (N2, H5N2), 34543.33 (N6, H5N6)

oseltamivir carboxylate

IC_{50} (nM): 20.61 (N1, H5N1), 8.81 (N8, H5N8), 16.22 (N2, H5N2), 14.42 (N6, H5N6)

11e

IC_{50} (nM): 33.26 (N1, H5N1), 33.02 (N8, 5N8), 16.81 (N2, H5N2), 45.46 (N6, H5N6)

Discovery of Novel "Dual-Site" Binding Oseltamivir Derivatives as Potent Influenza Virus Neuraminidase Inhibitors

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Abstract:

From our research group, it was noticed that oseltamivir derivatives targeting 150-cavity of neuraminidase enzyme (NA) could significantly increase antiviral activity. Thus, we further enriched the C5-NH_2 position of oseltamivir structure to obtain more potent oseltamivir derivatives. In this article a series of oseltamivir derivatives were synthesized by modifying C5-NH_2 position of oseltamivir. All the

compounds were evaluated for *in vitro* antiviral activity against H5N1 and H5N8. Encouragingly, compounds **9a** and **11e** were exhibited prominent activity, which is similar to oseltamivir carboxylate (**OSC**) and in NAs inhibitory assay, **11e** showed remarkable potency against N1 (H5N1), N2 (H5N2), N6 (H5N6) and N8 (H5N8). In addition, **11e** demonstrated low cytotoxicity and no obvious toxicity at the dose of 1500 mg/kg in mice. Molecular docking studies of **9a** and **11e** provided a plausible rationale for the high potency against group-1 NAs. This work provided new insights to design further neuraminidase inhibitors, which can help to investigate new potent inhibitors for group-1 and group-2 shortly.

Keywords: Influenza virus, Neuraminidase inhibitors, Oseltamivir derivatives, 150-cavity, Active site.

1. Introduction

Influenza is an acute respiratory infectious disease that can be life-threatening. According to the specific antigens of matrix protein (M1) and nuclear protein (NP), the influenza virus can be divided into four types: A, B, C, and D [1]. Among them, influenza A virus has the strongest and most frequent variability and the cause of influenza epidemic almost every year. Accordingly, influenza A virus is considered to be the main pathogens involved in influenza outbreaks. [2, 3]. During the outbreak of the influenza virus H1N1 in 2009, about 300,000 people died around the world which caused great social panic [4]. Globally, from 2013 to 2017, a total of 1161 people were infected with H7N9, 433 deaths and a mortality rate of 37.3% [5]. Each round of flu outbreaks will be unexpected, causing great social and economic loss. According to the World Health Organization (WHO) statistical data, there are about 290,000-650,000 death cases every year caused by influenza [2].

The influenza virus is a negative-sense, single-stranded RNA virus with a

lipid-containing envelope [6]. The viral membrane contains two important surface glycoproteins: neuraminidase (NA) and hemagglutinin (HA) [7]. They are prone to antigen transformation and cause mutation. NA contains 11 subtypes (N1-N11), HA contains 18 subtypes (H1-H18), and the influenza A viruses found so far are different combinations of HA and NA [8, 9, 10]. NA plays a crucial role in the whole replication cycle of the virus, which has been an attractive therapeutic target in the field of anti-influenza drugs [11]. At present, four kinds of neuraminidase inhibitors have been approved for clinical treatment, including Oseltamivir (Tamiflu) [12], Zanamivir (Relenza) [13], Peramivir (Rapivab) [14] and Laninamivir octanoate (Inavir) [15] (**Figure 1**). However, oseltamivir is only administered orally, also it is the earliest clinical first-line drug to treat influenza (approved in 1999). In recent years, the emergence of drug-resistant viral strains has restricted its clinical use, specially N1-H274Y mutant [16, 17, 18]. In addition, the emergence of other NA inhibitors resistant strains has also been reported. Therefore, there is an urgent need to develop a new generation of NA inhibitors to solve these issues.

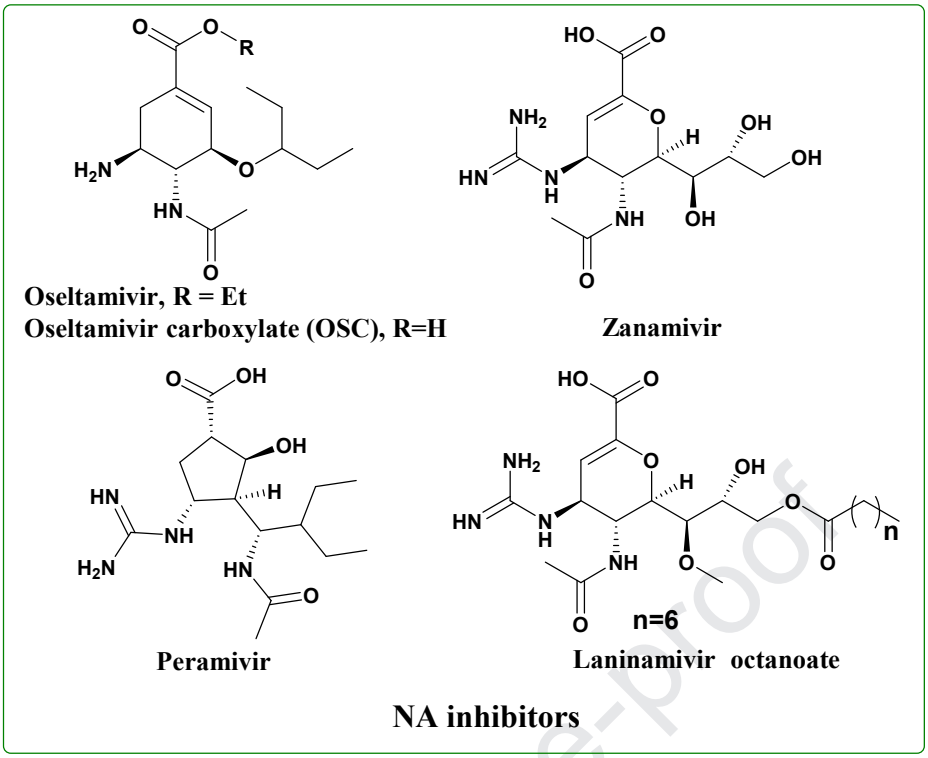


Figure 1. Structures of four neuraminidase inhibitors used in clinical for the treatment of flu.

The N1-N9 subtypes of influenza A virus can be divided into two groups: group-1 NAs, which include N1, N4, N5, and N8 subtypes, and group-2 which include N2, N3, N6, N7 and N9 subtypes [19]. X-ray crystallographic studies of NAs showed that the 150-loop of group-1 NAs is usually present in an open conformation while the 150-loop of group-2 NAs is present in a closed conformation [19]. Due to the unusual structure of these two groups of NAs 150-loop, a new cavity (150-cavity) with a volume of 10 Å × 5 Å × 5 Å is formed near the active site of group-1 NAs. But this cavity does not exist in group-2 NAs (**Figure 2**). In N2, for example, there is a key salt bridge between conservative residues Asp147 and His150, which is thought to be able to close 150-loop and control the formation of 150-cavity [20, 21]. Crystal structure studies indicate that the 150-loop of 09N1 is closed, and it does not have an open 150-cavity [22]. This study shows that the 150-loop of 09N1 is different from group-1 NAs but similar to group-2 NAs [20, 23, 24]. Inspired by this discovery, our group previously synthesized several compounds

such as **JMC01** and **JMC02** [11] (**Figure 3**) by replacing the oseltamivir C-5 amino group to target 150-cavity. From the results, it was found that, compared to oseltamivir, the activities of the compounds against N1 (H5N1) were significantly increased by nearly ten folds [11].

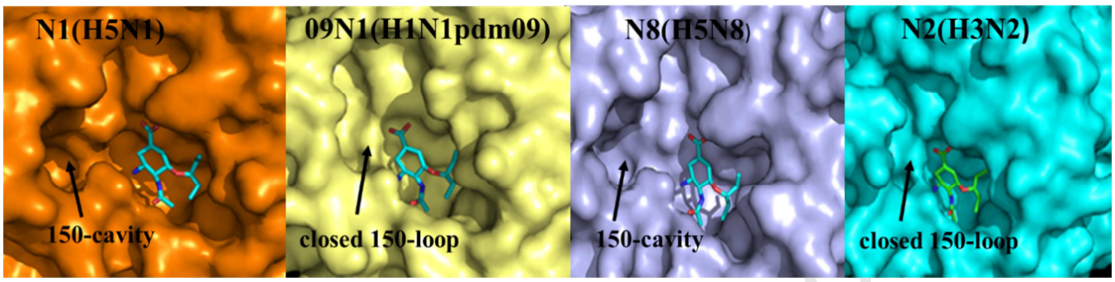


Figure 2. The crystal structures of representative group-1 NAs N1 (H5N1, PDB code: 2HU0), N8 (H5N8, PDB code: 2HT7), 09N1 (H1N1pdm09, PDB code: 3TI6), and group-2 NAs, N2 (H3N2, PDB code: 4GZP) bound with **OSC**. In surface representation, it can be seen that N1 and N8 have an open 150-cavity, but 09N1 and N2 have a closed 150-loop.

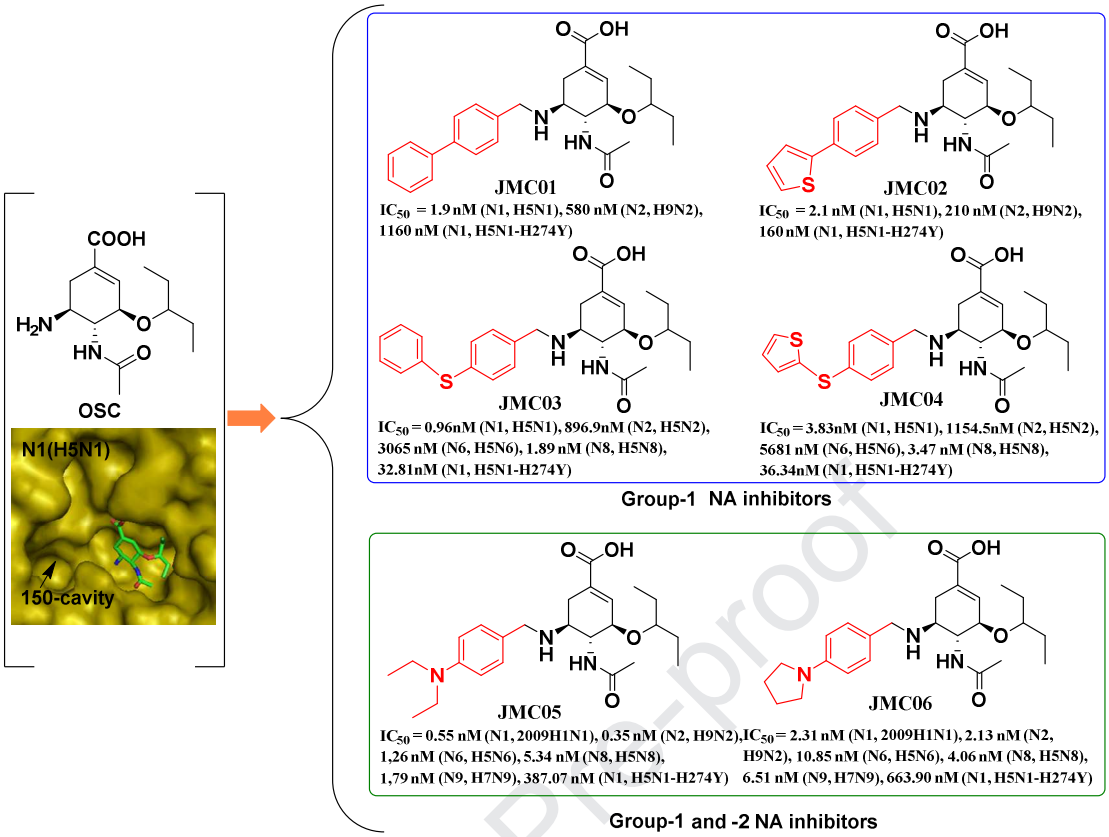


Figure 3. Previously designed (in our lab) oseltamivir derivatives as group-1 selective NA inhibitors (**JMC01-JMC04**) [11, 25] and group-1 and -2 NAs inhibitors (**JMC05** and **JMC06**) [26].

Based on our earlier discovery of high activity and high selectivity oseltamivir derivatives (**JMC01** and **JMC02**), the 150-cavity of NAs could be further explored to yield more potent and more anti-drug-resistant NA inhibitors. Therefore, structures of **JMC01** and **JMC02** were optimized by varying substituents on the amino group, and compounds **JMC03-JMC06** were obtained [25, 26]. **JMC03** and **JMC04** displayed antiviral activities similar to or better than **OSC** against H5N1, H5N2, H5N6, and H5N8. Both compounds were potent group-1 NAs-selective inhibitors which showed more potency than **OSC** against the N1, N8, and N1-H274Y mutant NAs [25]. Compounds **JMC05** and **JMC06** showed great activity against group-1 and group-2 NAs, especially for 09N1, N2, N6 and N9 subtypes. They also showed that the inhibitory activity of N1-H274Y and N2-E119V

variants increased by nearly 4 and 2.5 times, respectively compared to **OSC** [26].

In addition, the studies also showed that the amino acid residues of 150-loop and 150-cavity are quite flexible and have the possibility for further chemical modifications on the inhibitor structures [20, 24]. By analyzing the structures of the previously discovered active compounds, it was found that 5-amino substituents are benzyl derivatives with a single structural type (such as compounds **JMC01**, **JMC04**). Obviously, this cannot meet the needs of flexible 150-cavity, and the structure-activity relationship studies of substituted benzyl sites still needs to be further enriched. We expect to enhance the potency against both group -1 and -2 NAs and also improve the resistance for the mutant virus. Stimulated by the above analysis, we herein more diverse fragments of C5-NH_2 substituted oseltamivir derivatives were designed and synthesized

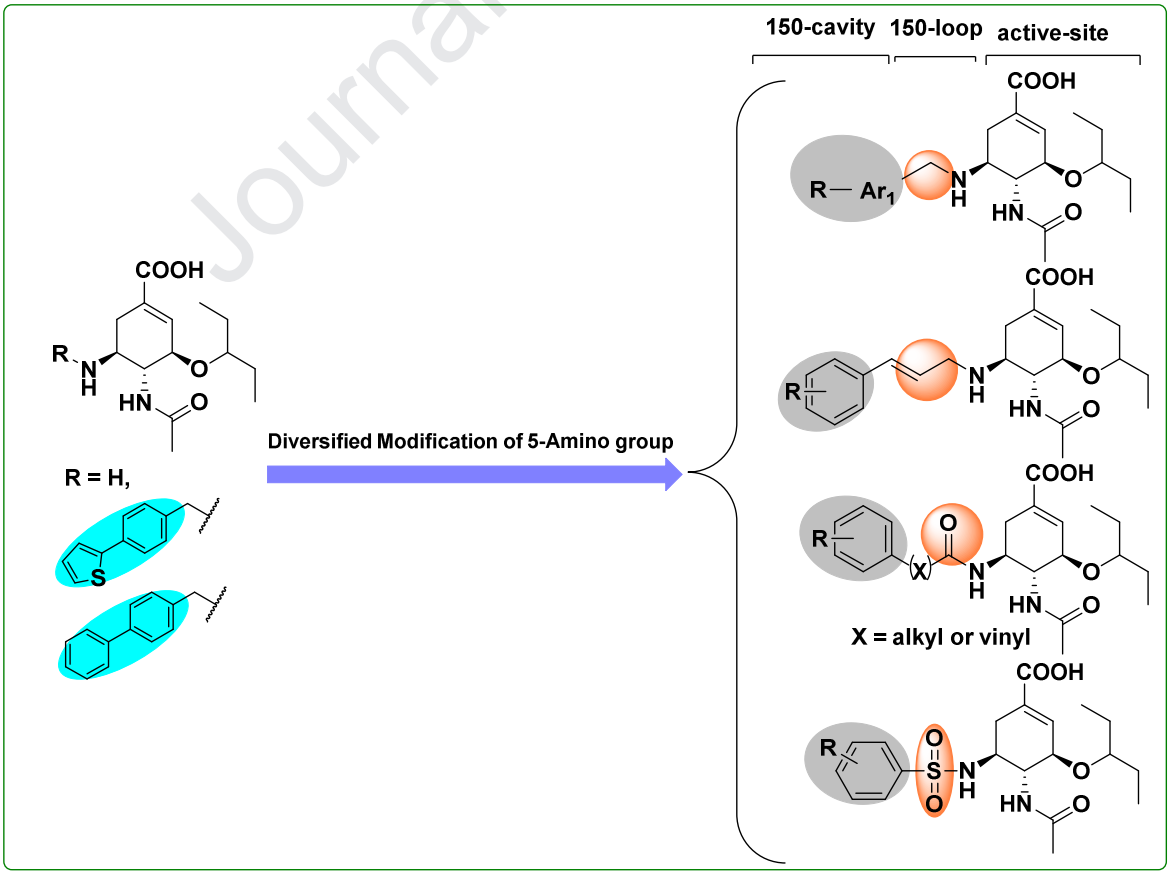


Figure 4. The newly designed compounds are targeting both the active site and 150-cavity. The benzene ring (silver) was replaced by substituted heterocycle and propenyl group,amido group and sulfonamide groups were introduced as linkers (pastel orange).

From the previous literature investigation, it is observed that the substitution of aromatic rings is beneficial to occupy the space of 150-cavity effectively [11, 25, 26]. Some aromatic rings have been proved to be well adapted to the 150-cavity, hydrophobic interaction, and van der Waals forces formed between aromatic rings and hydrophobic amino acid residues in 150-cavity [11, 25]. On the other hand, hydrogen-bond also formed between the amino group in the *N*,*N*-diethylamino fragment (**JMC05**) and either Gly147 (of N8, 67 of N9, 66 of 09N1), Thr 361 of N6 or Asp66 of N2 in the 150-cavity [26].

Therefore, we hypothesized that many multiple *N*-substituted groups of **OSC** may efficiently enter and enormously occupy the 150-cavity. Herein we report the design and synthesis of four series of novel oseltamivir derivatives: 1), we decided to incorporate some electron-withdrawing groups on the 5-amino substituted benzyl groups; 2) according to the bioisosterism principle, the benzene or thiophene ring (silver) in the lead compounds (**JMC01** and **JMC02**) were replaced by the substituted heterocycle; 3) the linker (pastel orange) between benzene ring substituent and oseltamivir nucleus was modified by introducing double bond, amide and sulfonamide group. Ultimately, a total of 38 compounds were designed and synthesized and their activity were evaluated (**Figure 4**).

2. Results and discussion

2.1. Chemistry

The synthetic routes for oseltamivir derivatives (**2a-2g, 4, 6, 9a-9h, 11a-11g, 13a-13b, 15a-15e, 17a-17g**) were conducted as illustrated in **Schemes 1-7**. All

derivatives were synthesized by well-established methods using commercially available oseltamivir phosphate as the primary starting material for all reactions. As shown in **Scheme 1**, oseltamivir phosphate was reacted with a series of different commercial aldehydes in the presence of $NaBH_3CN$ to obtain the key intermediates **1a-1g**. Subsequently, **1a-1g** were hydrolyzed in the presence of NaOH aqueous solution and acidified by HCl aqueous solution to afford the target compounds **2a-2g**.

Scheme 2 illustrates the synthesis of compounds **4** and **6**, oseltamivir phosphate was treated with less than 1eq and more than 2eq of commercially available thiophene-3-carboxaldehyde to obtain the intermediates **3** and **5** respectively by Borch Reductive Amination. The target compounds **4** and **6** were prepared by direct hydrolysis of the **3** and **5**.

As shown in **scheme 3**, aryl bromide or bromoaryl aldehyde was coupled with the corresponding formyl aryl boronic acid or aryl boronic acid to obtain the intermediates **7** *via* Suzuki reaction. Subsequently, target compounds **9a-9h** were prepared by using the same method as in **Scheme 1**.

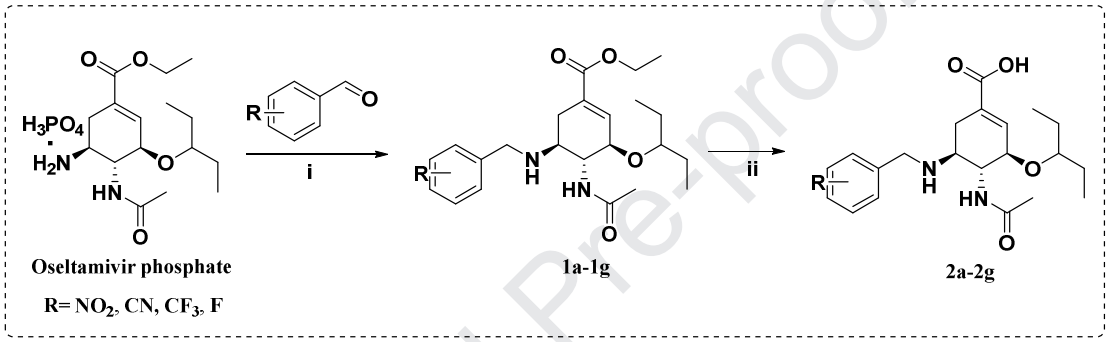
As outlined in **Scheme 4**, the intermediates **10a-10g** were prepared by Borch Reduction using different substituents of cinnamaldehyde reacted with oseltamivir phosphate in the presence of $NaBH_3CN$, then they were hydrolyzed to afford the target compounds **11a-11g**.

As shown in **scheme 5**, **12a** and **12b** were achieved by allowing condensation reaction between the [1, 1'-biphenyl]-4-carboxylic acid or benzoic acid and oseltamivir phosphate in the presence of DMAP in CH_2Cl_2 and Et_3N. And then they were hydrolyzed with 4 M NaOH aqueous solution and acidified with 2 M HCl aqueous solution to obtain the target compounds **13a** and **13b**.

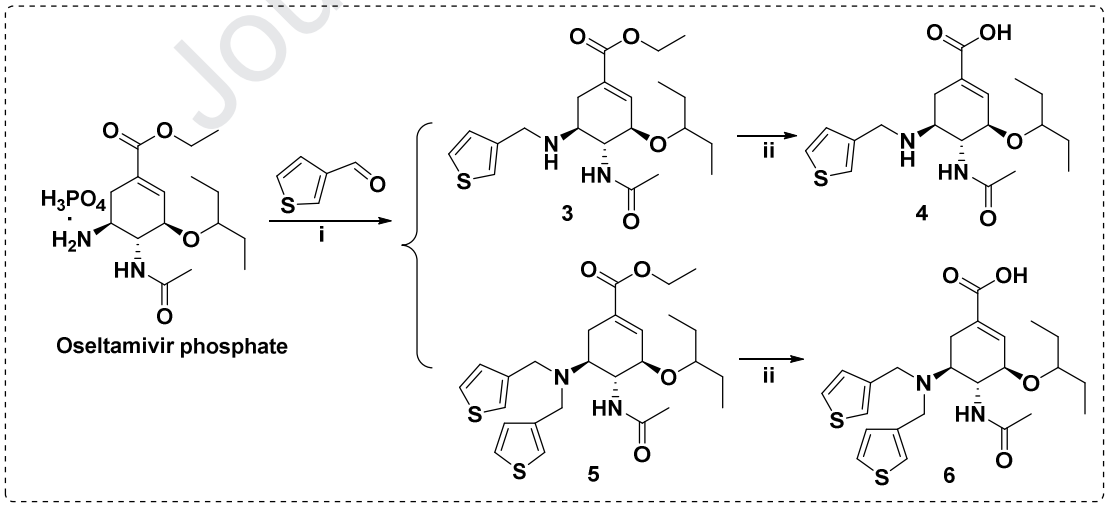
As shown in **Scheme 6**, the key intermediates **14a-14e** were obtained *via* the

same method as **scheme 5**. Subsequently, **14a-14e** were hydrolyzed by following the method as **scheme 1** to afford the target compounds **15a-15e**.

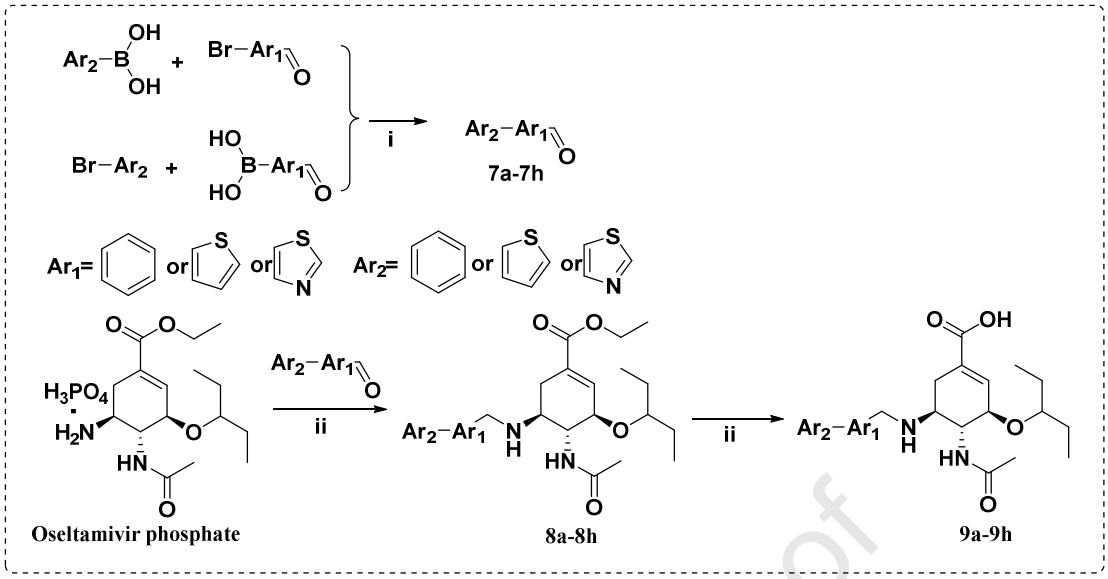
As outlined in **scheme 7**, the intermediates **16a-16g** were prepared by treating oseltamivir phosphate with a different substituted sulfonyl chloride. Further, **16a-16g** were hydrolyzed using the same method in **scheme 1** to afford the target compounds **17a-17g**.



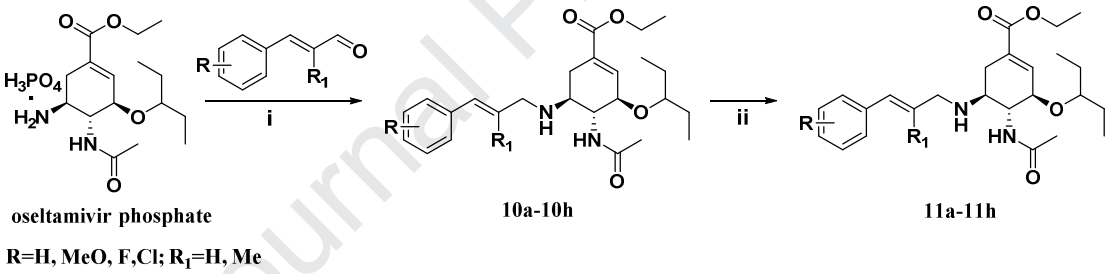
Scheme 1. Reagents and conditions: (i) $NaBH_3CN$, EtOH, MeOH, r.t.; (ii) NaOH, MeOH, H_2O, r.t, then HCl (2 mol/L).



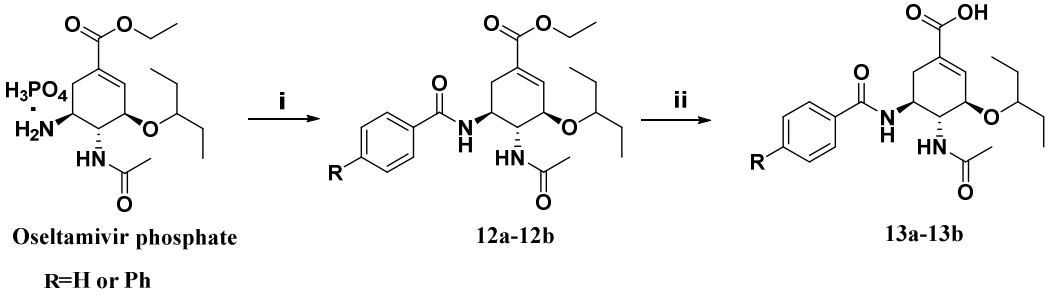
Scheme 2. Reagents and conditions: (i) $NaBH_3CN$, EtOH, MeOH, r.t.; (ii) NaOH, MeOH, H_2O, r.t., then HCl (2 mol/L).



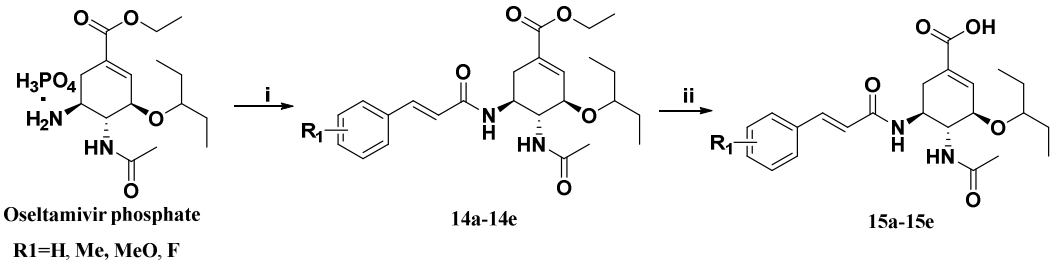
Scheme 3. Reagents and conditions: (i) DMSO, K_2CO_3, $Pd(PPh_3)_4$, N_2, 100^0C. (ii) $NaBH_3CN$, EtOH, MeOH, rt; (iii) NaOH, MeOH, H_2O, r.t., then HCl (2 mol/L).



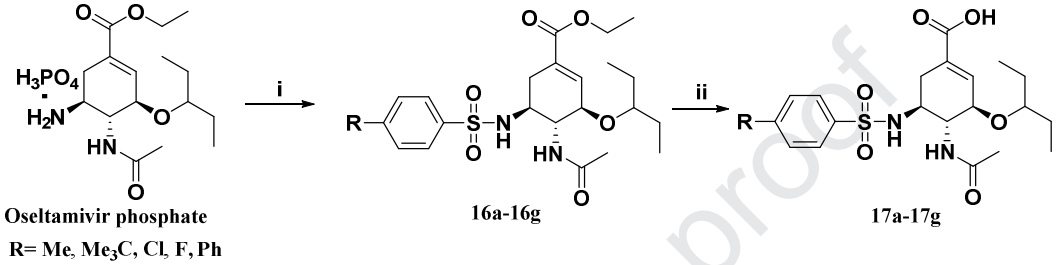
Scheme 4. Reagents and conditions: (i) $NaBH_3CN$, EtOH, MeOH, r.t.; (ii) NaOH, MeOH, H_2O, r.t., then HCl (2 mol/L).



Scheme 5. Reagents and conditions: (i) [1,1'-biphenyl]-4-carboxylic acid or benzoic acid, HBTU or TBTU, DMAP, Et_3N, CH_2Cl_2, rt; (ii) 4M NaOH, CH_3OH, rt, then HCl (2 mol/L).



Scheme 6. Reagents and conditions: (i) Substituted cinnamic acid, HBTU or TBTU, DMAP, Et_3N, CH_2Cl_2, rt; (ii) 4M NaOH, CH_3OH, rt, then HCl (2 mol/L).



Scheme 7. Reagents and conditions: (i) substituted benzenesulfonyl chloride, Et_3N, CH_2Cl_2, rt; (ii) 4M NaOH, CH_3OH, rt, then HCl (2 mol/L).

2.2. Biological Activity

2.2.1. Anti-Influenza Virus Activity in Cell Culture

In order to study the efficacy of newly synthesized compounds against influenza virus infection, we evaluated their inhibition abilities against avian influenza virus proliferation by cell-based assays (chicken embryo fibroblasts, CEFs), Using A/ goose/ Guangdong/ SH7/ 2013 (H5N1) and A/goose/ Jiangsu/ 1306/ 2014 (H5N8) represent influenza virus of group 1, A/chicken/Hebei/LZF/2014 (H5N2) and A/Duck/Guangdong/674/2014 (H5N6) represent influenza virus of group 2. **JMC02** oseltamivir carboxylic acid (**OSC**), Zanamivir (**ZAN**), and Ribavirin (**Rib**) were used as control drugs in parallel. The values of IC_{50} (effectiveness of inhibition of avian influenza virus proliferation) and CC_{50} (cytotoxicity) for the synthesized compounds were reported in **Table 1**. All compounds showed no significant cytotoxic activities at the highest test concentration (CC_{50} > 200 μM) and exhibited antiviral activity in CEFs. **OSC** and **ZAN** as neuraminidase inhibitors have high

potency to inhibit the proliferation of four influenza virus subtypes. Furthermore, they have different inhibitory abilities to N1 and N2 subtypes of influenza viruses, which is consistent with the literature reports [27]; **Rib** has moderate (H5N1, H5N6, H5N8) or poor (H5N2) potency against influenza virus proliferation. It is consistent with the results of our previous tests [25, 26], which proves that this method has good reliability.

We focused on the novel oseltamivir derivatives (**2a-2g**), where the thienyl group of the 4-benzyl moiety of **JMC02** was replaced with various electron-withdrawing groups containing nitro, cyano group and a fluorine atom. We also incorporated trifluoromethyl, nitro and fluorine atom at the *meta*-position of phenyl (**2a**, **2c**, **2f**) to explore the effect of withdrawing groups at different positions. The results showed that these oseltamivir derivatives were less potent than **OSC** and **ZAN**, especially **2a-2f** were almost lost the inhibition activity against H5N1 (IC_{50} > 10 μM). Further, when the electron-withdrawing group on the benzene ring was replaced by a bromine atom (**2g**), little improvement in antiviral activity was observed and it is still less than **OSC** and **ZAN**. Therefore, it concluded that electron-withdrawing groups would reduce the activity.

To explore whether the various substituents at C5-NH_2 position of oseltamivir will affect the activity, we introduced a thiophen-3-methyl (**4**) and two thiophen-3-methyl (**6**). The results exhibited that these two compounds have equal inhibition activities to H5N1, H5N2, and H5N6, and as for H5N8, the inhibition activity of compound **4** surpasses compound **6** more than ten times. However, these results were not prominent and still lower than **OSC** and **ZAN**.

Inspired by the structure of **JMC01-JMC04**, we designed and synthesized compounds **9a-9h**. The potency of **9a** against H5N1 and H5N6 is lower than that of **JMC02**, but encouragingly it displayed almost equal activity against H5N2 and H5N8. Also **9a** displayed a prominent selective inhibition to H5N1 and H5N8 compared to **OSC** and **ZAN**. It showed similar inhibition activity to **OSC** and **ZAN**

against H5N1 and H5N8 but it is less active against H5N2 and H5N6.

We further exchanged the positions of thiophene and benzene groups of compound **JMC02** to obtain **9b**, which slightly reduced the antiviral activity in contrast to **JMC02**. And we replaced phenyl of **JMC02** to thienyl to afford compound **9c**, which showed the activity against H5N1 and H5N6 was less than **JMC02**, especially to H5N1, it was observed nearly lacked inhibition activity. However, it exhibited similar potency against H5N2 and H5N8 as **JMC02**. The substituents at the C5-NH_2 position of **9d** and **9e** are 2-(*p*-tolyl)-thiazole and 2-methyl-5-phenylthiazole respectively, and **9d** and **9e** are a pair of isomers, both exhibited lower anti-viral activities than **OSC**, **ZAN** and **JMC02**.

The terminal aromatic rings of **JMC01**, **JMC02** and **9a**, were re-positioned from the *para*-position to the *meta*-position of the benzene ring to obtain **9f**, **9g** and **9h** respectively. Among these compounds, **9f** displayed similar potency to **OSC** and **ZAN** for H5N1, but lower than that of **JMC02**. In contrast, **9f** showed weaker activity against H5N1, H5N2 and H5N6 strains compared to **OSC**, **ZAN** and **JMC02** while, **9g** and **9h** displayed similar potency to **OSC**, **ZAN** and **JMC02** against H5N8. Their activities against H5N1, H5N2 and H5N6 were slightly reduced compared to **OSC** and **ZAN**. The activity of **9h** was similar to **JMC02** for H5N2 and H5N6 inhibition, but slightly less for H5N1. The activity of compound **9g** against H5N1, H5N2 and H5N6 was slightly less compared to **JMC02**.

To enrich the structure-activity relationship studies, we synthesized phenylpropene derivatives to modify C5-NH_2 of **OSC** (compounds **11a-11g)**. However, **11b** and **11c**, **11e-11g** exhibited slightly reduced anti-H5N1 activity compared to **OSC** and **ZAN**. **11d** showed considerably decreased potency against H5N1compared to other compounds; **11a** displayed similar potency to **OSC** of H5N1.

Notably, from **11a-11g**, compound **11e** was the most potent compound against

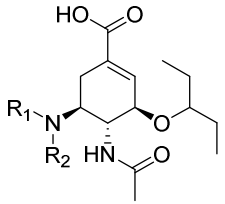
H5N2, H5N6 and H5N8 strains except **11b** (the IC_{50} of **11b was** 0.065μM against H5N2). Moreover, **11e** showed comparable activity to **JMC02** against H5N2 and H5N8 and displayed greater potency than **JMC02** against H5N6. **11b** showed similar potency to **JMC02** against H5N2, H5N6, and H5N8. Compounds **11a**, **11c**, **11d**, **11f** and **11g** were all less potent than **JMC02**, **OSC**, and **ZAN** for inhibition of H5N2, H5N6 and H5N8, displayed moderate ability to inhibit viral proliferation.

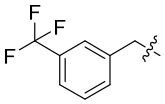
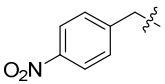
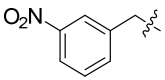
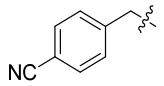
Further, we inserted an acyl or sulfonyl between the position of C5-NH_2 and aromatic group to obtain compounds **13a**, **13b**, **15a**-**15e**, and **17a**-**17g** respectively. Unfortunately, the anti-viral activities of these compounds were sharply reduced or lost.

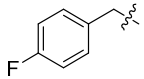
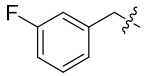
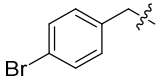
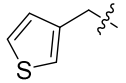
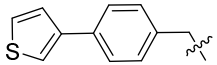
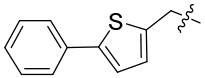
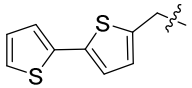
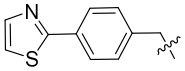
Overall, the arylmethyl and phenylpropenyl substituted derivatives at the C5-NH_2 position of **OSC** displayed greater activities towards H5N1, H5N2, H5N6, and H5N8 compared to amide and sulfonamide derivatives. Among arylmethyl substituted **OSC** derivatives, the stronger the electron-withdrawing ability of the substituent on the aryl group, the weaker the ability to inhibit virus proliferation. And, the anti-viral activities of **2a**-**2f** were less than **2g**. Biaryl group substituents consist of benzene and thiophene have similar ability to inhibit viral proliferation regardless of their relative positions. When the compounds were substituted by smaller groups of dithiophene or thiazole, the ability to inhibit H5N1 were significantly reduced.

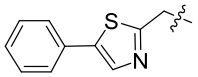
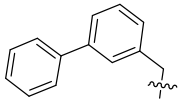
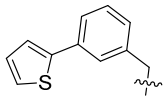
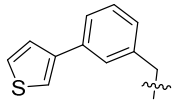
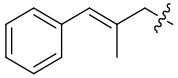
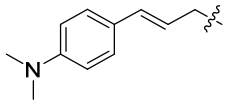
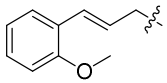
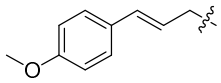
Among the phenylpropenyl substituted derivatives at the C5-NH_2 position of **OSC**, most compounds have moderate inhibitory ability against the four strains except individual compounds that have antiviral activity close to **OSC** and **ZAN** for some strains, such as **11a** to H5N1.

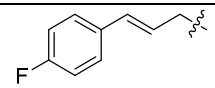
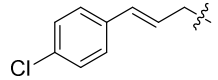
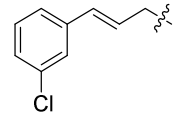
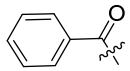
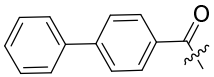
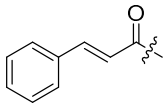
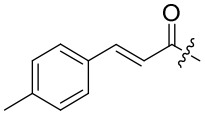
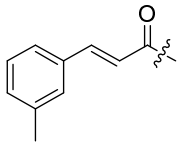
Table 1. Anti-Influenza Virus Activity and Cytotoxicity of Oseltamivir Derivatives in CEFs.

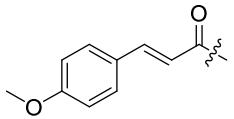
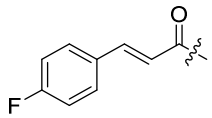
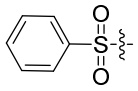
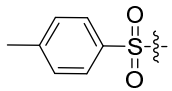
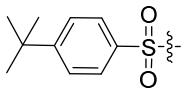
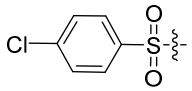
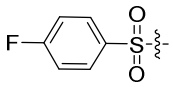


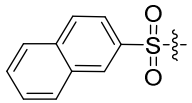
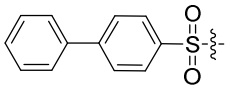
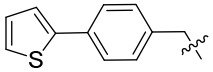
Compound	R_1	R_2	IC_{50}[a] values (μM) against influenza virus				CC_{50}[b]
			H5N1[c]	H5N2[d]	H5N6[e]	H5N8[f]	
			Group-1	Group-2	Group-2	Group-1	
2a		H	＞10	0.34±0.02	3.17±0.23	0.75±0.073	> 200[g]
2b		H	＞10	0.21±0.0045	2.16±0.025	0.42±0.022	>200
2c		H	＞10	0.24±0.063	2.16±0.35	0.70±0.19	>200
2d		H	＞10	0.38±0.089	＞10	0.68±0.21	>200

2e		H	＞10	0.11±0.087	4.57±0.13	0.52±0.094	>200
2f		H	＞10	0.14±0.012	3.87±0.34	0.49±0.091	>200
2g		H	2.53±0.099	0.11±0.000071	1.99±0.32	0.25±0.017	>200
4		H	＞10	0.13±0.029	3.58±0.069	0.081±0.0032	>200
6			＞10	0.15±0.011	3.03±0.24	0.88±0.27	>200
9a		H	**0.88±0.018**	**0.10±0.025**	**5.5±0.21**	**0.051±0.0019**	**>200**
9b		H	1.59±0.037	0.12±0.0084	3.02±0.32	0.098±0.013	>200
9c		H	＞10	0.083±0.0081	7.12±0.00	0.072±0.011	>200
9d		H	＞10	0.20±0.0051	3.23±0.27	0.059±0.018	>200

9e		H	＞10	0.41±0.043	4.29±0.11	0.49±0.045	>200
9f		H	0.84±0.094	0.23±0.0011	4.49±0.017	0.65±0.13	>200
9g		H	0.97±0.35	0.22±0.025	6.71±0.00	0.089±0.0038	>200
9h		H	1.25±0.23	0.070±0.0079	2.62±0.36	0.056±0.036	>200
11a		H	0.79±0.25	0.16±0.013	3.07±0.26	0.61±0.077	>200
11b		H	1.29±0.27	0.065±0.0014	2.78±0.0042	0.88±0.11	>200
11c		H	1.20±0.15	0.13±0.0012	3.07±0.26	0.089±0.012	>200
11d		H	7.56±0.48	0.21±0.029	4.07±0.057	0.64±0.15	>200

11e		H	**3.4±1.24**	**0.094±0.023**	**0.79±0.29**	**0.077±0.013**	**>200**
11f		H	0.96±0.011	0.24±0.0095	3.93±0.37	0.53±0.029	>200
11g		H	2.99±0.42	0.29±0.00	5.52±0.34	0.13±0.023	>200
13a		H	＞10	＞10	＞10	＞10	>200
13b		H	＞10	＞10	＞10	3.54±0.59	>200
15a		H	＞10	＞10	＞10	5.32±0.48	>200
15b		H	＞10	＞10	＞10	4.76±0.50	>200
15c		H	＞10	＞10	＞10	4.37±0.16	>200

15d		H	＞10	＞10	＞10	8.48±0.66	>200
15e		H	＞10	＞10	＞10	5.16±0.44	>200
17a		H	＞10	＞10	＞10	4.82±0.23	>200
17b		H	＞10	＞10	＞10	3.12±0.59	>200
17c		H	＞10	＞10	＞10	5.29±0.56	>200
17d		H	＞10	＞10	＞10	4.76±0.31	>200
17e		H	＞10	＞10	＞10	4.25±0.39	>200

17f		H	＞10	＞10	＞10	3.23±0.39	>200
17g		H	＞10	＞10	＞10	6.79±1.35	>200
JMC02			0.29±0.044	0.079±0.021	2.39±0.14	0.053±0.0069	>200
OSC	---		0.69±0.013	0.0042±0.000025	0.097±0.0034	0.037±0.0028	>200
ZAN	---		0.42±0.026	0.013±0.00078	0.097±0.0034	0.041±0.0017	>200
Rib	---		4.01±0.31	＞10	6.68±0.34	1.96±0.046	>200

[q] IC_{50}: Concentration of a compound required to achieve 50% inhibition of influenza virus proliferation in CEFs, presented as the mean ± standard deviation (SD).

[b] CC_{50}: Concentration required to reduce the viability of mock-infected cell cultures by 50%, as determined by the CCK-8 method.

[c] A/goose/Guangdong/SH7/2013. [d] A/Chicken/Hebei/LZF/2014. [e] A/duck/Guangdong/674/2014. [f] A/goose/Jiangsu/1306/2014.

[g] Highest tested concentration.

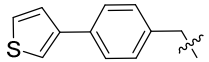
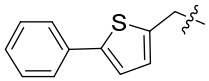
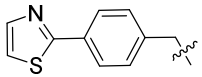
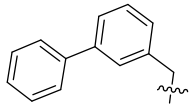
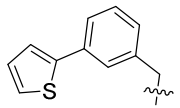
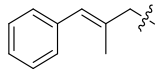
2.2.2. *In Vitro* Inhibitory Activities of Neuraminidases

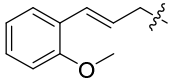
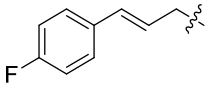
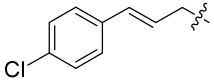
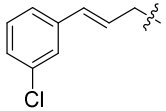
We selected 10 representative compounds for further enzyme assay against influenza neuraminidases N1 (H5N1), N2 (H5N2), N6 (H5N6), N8 (H5N8), and N1-H274Y to validate their binding target. **OSC** and **ZAN** were used as control drugs. The results were summarized in **Table 2**, and each data was measured after three parallel tests. From the test results, we found that **OSC** and **ZAN** displayed very high activity against the four wild-type NAs, and it was clear that **OSC** and **ZAN** did not show equal potency to NAs but have some selectivity. For example, **OSC** displayed less potency against N1 (H5N1) than **ZAN**, but showed more activity against N2 (H5N2), and **ZAN** exhibited high activity against N1-H274Y mutant but **OSC** did not, which was consistent with reported data [11, 28]. This reflected the reliability of the activity test method which we have established.

It was found that the biarylmethyl-substituted derivatives at the position of 5-amino group showed significant inhibition for group-1 NAs (H5N1 and H5N8). The order of their potency is **9f** > **9g** > **9a** >**9b** >**9d**, which is consistent with the sequence of anti-H5N1 strain activity at their cellular level. But these five compounds showed poor activities to inhibit group-2 NAs (H5N2 and H5N6), only at the micromolar level, this is because N2 and N6 do not have the opened 150-loop. Among the phenylpropenyl substituted derivatives at the 5-amino group, **11a**, **11f** and **11g** showed significant selective inhibition of group-1 NAs relative to **OSC** and **ZAN**. Compounds **11a** (H5N1, IC_{50} = 57.66 nM), **11f** (H5N1, IC_{50} = 22.39 nM), and **OSC** (H5N1, IC_{50} = 20.61 nM), which have comparable activities, cannot form good interactions to N2 and N6 due to the closed conformation of 150-loop. Overall, the inhibitory effects of these three compounds for the four wild-type NAs (H5N1, H5N2, H5N6 and H5N8) were weaker than the **OSC**. These results were consistent with their trend in antiviral activity in cells. **11c** and **11e** showed strong potency to all four wild-type NAs, and their inhibitory activity against H5N2 was even comparable to **OSC** (**11c**, IC_{50} = 21.37 nM; **11e**, IC_{50} = 16.81 nM; **OSC**, IC_{50} = 16.22 nM). They did not show significant selective inhibition, which were also consistent with their cellular activities.From the results, it was concluded that the inhibitory activity of the above 10 representative compounds was weaker than **OSC** against N1-H274Y [A/Anhui/1/2005 (H5N1-H274Y)].

In conclusion, the test results of the representative compounds indicated that most of them have obvious selective inhibition of group-1 NAs, and the selectivity of biarylmethyl group at C5-NH_2 position derivatives are particularly noticeable. The phenylpropenyl group substituted derivatives, such as **11a**, **11f**, and **11g**, also have significant group-1 NAs selective inhibition. Moreover, **11a** and **11f** inhibitory activities against N1 have achieved a similar level as **OSC**. **11c** and **11e** have strong inhibitory effects against four wild-type NAs, especially for N2 which have similar activity to **OSC**. Unfortunately, the inhibition of the selected representative compounds against N1-H274Y was significantly reduced. This could be due to that the substitution group at the position of C5-NH_2 of **OSC** affects the binding mode of the compounds with the mutant enzyme.

Table 2. Neuraminidase (NA) Inhibition of Oseltamivir Derivatives in Chemiluminescence-Based Assay

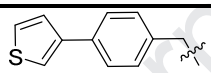
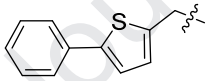
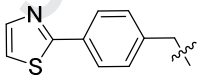
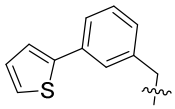
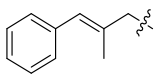
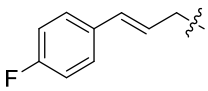
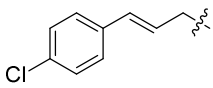
Compound	R_1	R_2	NA-Inhibitory Activity, IC_{50} (nM)[a]				
			H5N1[b]	**H5N2**[c]	**H5N6**[d]	**H5N8**[e]	**H5N1-H274Y**[f]
			Group-1	Group-2	Group-2	Group-1	Group-1
9a		H	**118.17±9.56**	**1442.6±121.99**	**34543.33±2849.81**	**78.06±10.64**	**10260±493.25**
9b		H	264.03±5.35	1100.66±103.24	1743.67±350.44	90.06±28.19	31213.33±2826.42
9d		H	391.5±37.68	7881.67±1061.17	9595±784.88	108.57±9074	＞100000
9f		H	53.99±5.73	13700±854.34	39073.33±3780.86	83.13±10.08	2922.67±333.41
9g		H	74.82±3.45	11996.67±1803.62	16110±339.41	55.25±4.76	7384.33±1007.34
11a		H	57.66±2.38	952.45±89.87	5622.33±466.57	92.61±1.76	5994±278.60

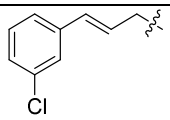
11c		H	254.2±11.79	21.37±0.14	155.43±20.25	62.09±8.22	10431±670.39
11e		H	**33.26±2.16**	**16.81±0.73**	**45.46±9.17**	**33.02±8.78**	**5270.33±210.02**
11f		H	22.39±1.28	255.33±43.24	809.05±140.36	174.4±18.98	8336.5±848.09
11g		H	129.28±9.98	869.03±159.98	10674±1592.40	309±44.97	28340±1936.28
OSC	H	H	20.61±0.73	16.22±0.91	14.42±0.25	8.81±0.10	1826.5±234.05
ZAN	----	----	1.66±0.44	33.66±2.99	24.23±1.59	9.99±0.46	12.02±0.29

[a] Concentration required to reduce NA activity to 50% of the control NA activity (IC_{50}). Values are the mean of three experiments, presented as the mean ± standard deviation (SD). [b] A/goose/Guangdong/SH7/2013. [c] A/Chicken/Hebei/LZF/2014. [d] A/duck/Guangdong/674/2014. [e] A/goose/Jiangsu/1306/2014. [f] A/Anhui/1/2005.

We also tested compounds 9a, 9b, 9d, 9g, 11a, 11e, 11f, and 11g inhibit N1 (H1N1pdm09) and N2 (H3N2) to validate their binding target by chemiluminescence-based assay. OSC was used as positive control for inhibition. The values of IC_{50} of the selected oseltamivir derivatives and the positive control drugs are summarized in **Table 3**. But all the tested compounds exhibited weaker potency against N1 (0.087 μM-1.67 μM) and N2 (0.24 μM-17.69 μM) compared to OSC (N1: 0.041 μM, N2: 0.016 μM), which can be easily explained by the structural features of N1 (H1N1pdm09) and N2 (H3N2). The closed 150-loop and the lack of 150-cavity in N1 (H1N1pdm09) and N2 (H3N2) (Figure 2) indicate that the cavity would not well accommodate the selected oseltamivir derivatives bearing large groups at C-5-NH_2 position.

Table 3. N1 (H1N1pdm09) and N2 (H3N2) Inhibition of Oseltamivir Derivatives in Chemiluminescence-Based Assay

Compound	R_1	R_2	NA-Inhibitory Activity, IC_{50} (μM)	
			N1 (H1N1pdm09)[a]	N2 (H3N2)[b]
			Group-1	Group-2
9a		H	0.48±0.029	12.22±0.66
9b		H	0.31±0.12	0.91±0.06
9d		H	1.67±0.48	10.21±0.11
9g		H	0.60±0.066	7.64±1.49
11a		H	0.43±0.16	4.64±0.09
11e		H	0.43±0.15	3.47±0.39
11f		H	0.84±0.0039	1.56±0.16

11g		H	0.087±0.0056	17.69±1.51
OSC	H	H	0.041±0.0082	0.016±0.0004

[a] A/California/04/2009. [b] A/Babol/36/2005.

2.3. Molecular Docking

To further study the binding modes of the best active compounds in the 150-cavity of Group-1 NAs, we selected **9a** and **11e** for molecular docking with N1 (PDB code: 2HU0) and N8 (PDB code: 2HT7), respectively. The Surflex-Dock module of SYBYL-X 1.3, PyMOL and Ligplot softwares were used for docking and graphic display respectively.

As shown in **Figure 5**, it can be observed from A, C, E, and G that the oseltamivir carboxylic acid part and the position of C-5-NH_2 substituent of **9a** and **11e** occupy the active sites and 150-cavities of N1 and N8, respectively, and the oseltamivir carboxylic acid moieties of **9a** and **11e** were highly coincident with the **OSC**. The above two points are the basis of the effective inhibition of N1 and N8 by **9a** and **11e**, and also consistent with the idea of two-site binding design. Because the N1 and N8 structures are highly similar, the binding modes and interactions between **9a** and **11e** are also very similar.

The interactions between the part of C5-NH_2 substituents of **9a** and **11e** (thiophenylbiphenyl and p-fluorophenylpropenyl) and the 150-cavities of N1 and N8 are shown in figure **B**, **D**, **F**, **H**: The hydrophobic amino acids or amino acid residues with hydrophobic side chains (Arg118, Gly147, Val149, Asp151 Arg156 and Thr439 for N1，Glu119, Gly147, Asp151 Arg156 and Thr439 for N8) in N1 and N8 150-cavities interact with the thiophene biphenyl to form hydrophobic and van der Waals interactions. The guanidine group in Arg181 and the benzene group in thiophene biphenyl are parallel to each other, forming cation-π interactions. These interactions closely bind thiophene biphenyl to 150-cavity. However, from figures **B** and **F**, it was found that the hydrogen bonds formed by the C-5 amino group of **9a** with Glu119 and Asp151 disappeared, possibly because the presence of

thiophenebiphenyl changes the conformation of oseltamivir carboxylic acid ring in NAs, and weakened the binding force of **9a** to the active site to some extent. In addition, the whole conformation changes occurred when the molecule **9a** combined with N1-H274Y, which reduced the inhibitory activity to N1-H274Y. Similarly, the hydrophobic amino acids in 150-cavity of N1 and N8 or the amino acid residues containing hydrophobic side chains (Arg118,Gln136 Gly147, Val149, Asp151 and Arg156 for N1 , Asp151 Arg156 and Thr439 for N8) interacted with *p*-fluorophenylene propylene groups in **11e** to form hydrophobicity and van der Waals interactions. Although the propene group was beneficial to the cross-linking of *p*-fluorophenylpropene group with 150-cavity, the interaction between propene group and 150-loop was weak. In summary, the ability of **11e** to inhibit N1 and N8 is similar to that of OSC. Because of the slender *p*-fluorophenylpropenyl group, **11e** may have little effect on the change of the conformation of oseltamivir carboxylic acid in N8, so the hydrogen bond between C5-NH_2 and Glu119 of N8 exists, but the hydrogen bond in N1 disappears. In addition, the interaction between *p*-fluorophenylpropenyl and 150-cavity is weak, so the inhibitory effect of **11e** on N1-H274Y is not enhanced.

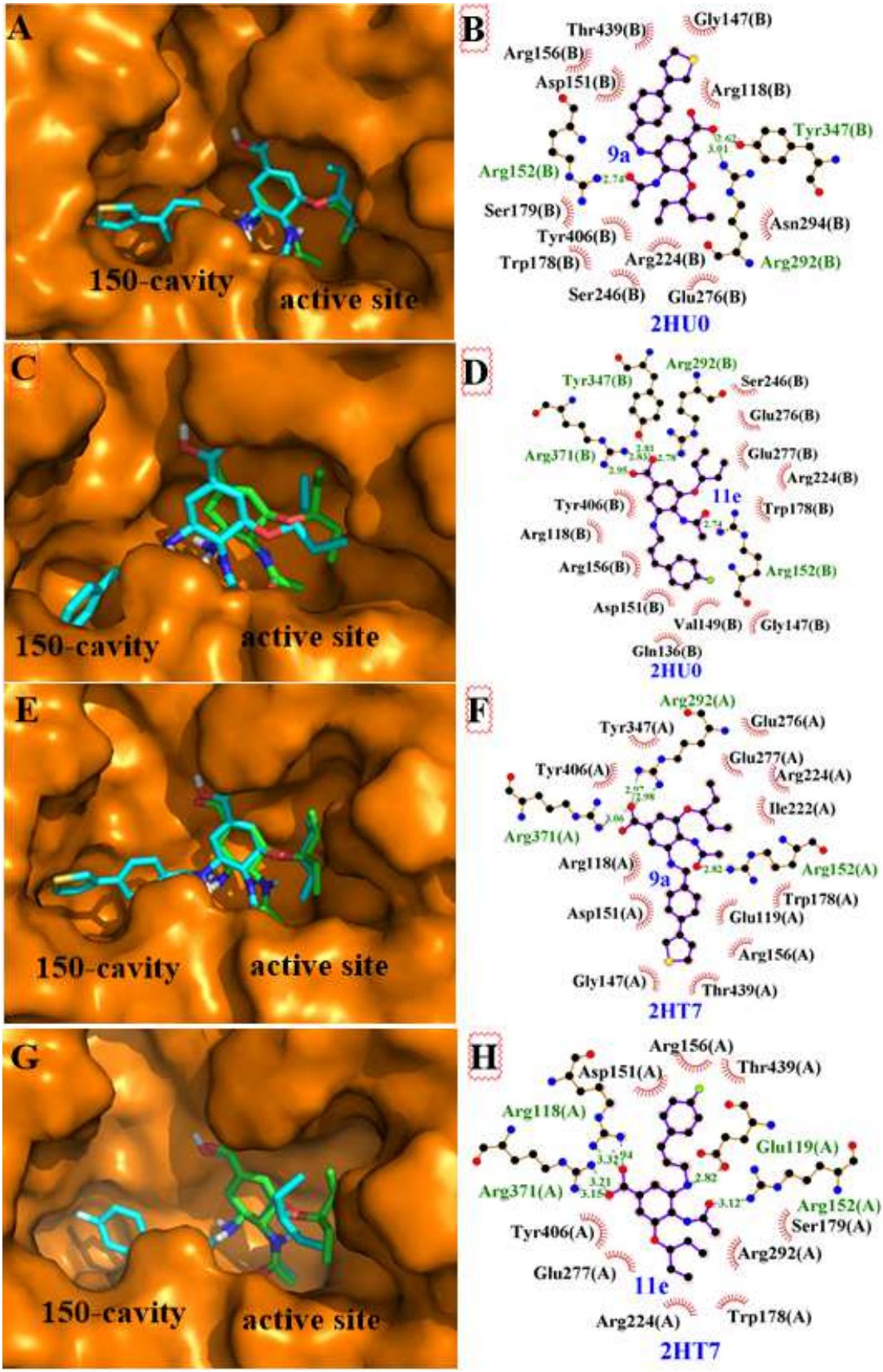


Figure 5. Docking results of compounds **9a** (blue, **A**) and **11e** (blue, **C**) with N1 (PDB code: 2HU0), compounds **9a** (blue, **E**) and **11e** (blue, **G**) with N8 (PDB code: 2HT7) compared with the

binding mode of **OSC** (yellow) and the hydrophobic amino acids in 150-cavity of N1 and N8 or the amino acid residues containing hydrophobic side chains (Arg118, Gly147, Asp151 Arg156 and Thr439 for N1; Glu119, Gly147, Asp151 Arg156 and Thr439 for N8), interacted with *p*-fluorophenylene propylene groups in **9a** (**B**, **F**), and (Arg118,Gln136 Gly147, Val149, Asp151 and Arg156 for N1， Asp151 Arg156 and Thr439 for N8), interacted with *p*-fluorophenylene propylene groups in **11e** (**D**, **H**) to form hydrophobicity and van der Waals interactions.

2.4. Safety Assessment

A single dose toxicity test of compound **11e** was carried out in Kunming mice. After intragastric administration **11e** at a dose of 1.5 g/kg. No obvious differences were found in the weight gain between the Vehicle control and experiment groups (**Figure 6**). None of the male or female mice receiving 1.5 g/kg of **11e** developed any symptoms, such as death, lethargy, clonic convulsion, hunched posture, piloerection. And they lived for the one study week prior to being sacrificed (**Table 4**).

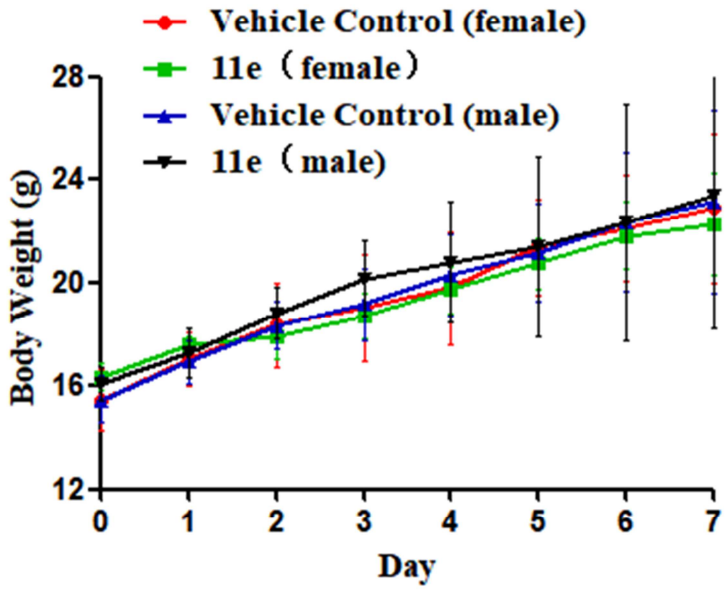


Figure 6. Bodyweight-time profiles in Kunming mice following intragastric administration **11e** (1.5 g/kg, femal, (green); male, (black)) and Vehicle control (femal, (red); male, (blue)).

Table 4. After Administration of 11e the Clinical Behaviors in Mice

Dose of **11e** (g/kg)	Clinical behaviors	Day, number of mice										
		1					2	3	4	5	6	7
		10min	30min	1h	3h	6h						
Female mice												
Vehicle control	No abnormality	5	5	5	5	5	5	5	5	5	5	5
	Death	0	0	0	0	0	0	0	0	0	0	0
	Lethargy	0	0	0	0	0	0	0	0	0	0	0
	Clonic convulsion	0	0	0	0	0	0	0	0	0	0	0
	Hunched posture	0	0	0	0	0	0	0	0	0	0	0
	Piloerection	0	0	0	0	0	0	0	0	0	0	0
1.5	No abnormality	5	5	5	5	5	5	5	5	5	5	5
	Death	0	0	0	0	0	0	0	0	0	0	0
	Lethargy	0	0	0	0	0	0	0	0	0	0	0
	Clonic convulsion	0	0	0	0	0	0	0	0	0	0	0
	Hunched posture	0	0	0	0	0	0	0	0	0	0	0
	Piloerection	0	0	0	0	0	0	0	0	0	0	0
Male mice												
Vehicle control	No abnormality	5	5	5	5	5	5	5	5	5	5	5

	Death	0	0	0	0	0	0	0	0	0	0	0
	Lethargy	0	0	0	0	0	0	0	0	0	0	0
	Clonic convulsion	0	0	0	0	0	0	0	0	0	0	0
	Hunched posture	0	0	0	0	0	0	0	0	0	0	0
	Piloerection	0	0	0	0	0	0	0	0	0	0	0
1.5	No abnormality	5	5	5	5	5	5	5	5	5	5	5
	Death	0	0	0	0	0	0	0	0	0	0	0
	Lethargy	0	0	0	0	0	0	0	0	0	0	0
	Clonic Convulsion	0	0	0	0	0	0	0	0	0	0	0
	Hunched posture	0	0	0	0	0	0	0	0	0	0	0
	Piloerection	0	0	0	0	0	0	0	0	0	0	0

3. Conclusion

Based on the previous studies, a series of novel oseltamivir derivatives were designed, synthesized, and evaluated by modifying the C5-NH_2 position of **OSC**. The results of activity showed that the amide and sulfonamides derivatives obtained by modifying C5-NH_2 group were lost the ability to inhibit the proliferation of avian influenza virus. There are two main reasons for this: 1) After the C5-NH_2 was converted into amide or sulfonamide groups, it lost alkalinity and cannot form hydrogen bond with amino acids near 150-loop (such as Glu119 and Asp151 in N1); 2) The rigidity of amide or sulfonamide leads to the change of the whole conformation of the molecule when it binds to NAs, and the compound cannot bind effectively with NAs, thus losing its inhibitory activity on NAs and avian influenza virus.

Strong electron-withdrawing groups may affect the binding of compounds to 150-cavity, such as **2a**-**2f**, which have weak inhibitory effects on the four subtypes of avian influenza virus, especially for H5N1. Most of the derivatives modified at C5-NH_2 position of **OSC** by biaromatic groups have selective inhibitory effects on avian influenza virus and NAs, however introducing polar atoms at any aromatic ring, especially near the C5-NH_2 group (**9e**), would reduce their inhibitory activity. The elongated and conjugated phenylpropenyl group is favorable for binding to 150-cavity, some of the derivatives modified by phenylpropenyl have a strong inhibitory effect on group-1 avian influenza virus and NAs, comparable to **OSC**. Generally, the inhibitory activity of the selected compounds on NAs at the enzyme level was consistent with that on the avian influenza virus at the cellular level. The docking results of **9a** and **11e** with N1 and N8 molecules showed that thiophenyl and *p*-fluorophenylpropenyl could effectively bind to 150-cavity, increasing part of the hydrophobic interactions. The existence of the two substituents changed the conformation of the molecule binding to NAs and weakened the hydrogen bond between C5-NH_2 group and Glu119 or Asp151. Therefore the inhibitory effects of these compounds on H5N1 and H5N8 or N1, N8 and N1-H274Y mutant were similar to **OSC** or slightly weakened.

4. Experimental Section

4.1. Chemistry

The main reagent Oseltamivir phosphate was provided by Shandong Qidu Pharmaceutical Co., Ltd. The other reagents and chemicals were bought from commercial suppliers such as Aladdin, TCI and Sino Pharm Chemical Reagent Co., Ltd. With purities at least 97%. The solvents were obtained from commercial suppliers. HRMS analysis was performed using an Agilent 6520 Q-TOF LC/MS spectrometer (Agilent, Germany). ^{1}H-NMR and ^{13}C-NMR were determined by Bruker AV-400 NMR in solvent DMSO-d_6 and CD_3OD, TMS was used as internal standard, chemical displacement was expressed by δ, coupling constants (J) were expressed in hertz (Hz). The melting points of all compounds were measured by the micro melting point instrument (RY-1G, Tianjin TianGuang Optical Instrument). The reaction process was monitored by thin layer chromatography (TLC) with silica gel GF 254 for TLC (Merck), and the product spots were observed under ultraviolet light (254 nm). The products were separated by Flash column chromatography in silica gel (200-300

mesh), purchased from Qingdao Haiyang Chemical Company. When necessary, solvents were purified and dried in standard methods.

4.1.1 General procedure for the synthesis of compounds 1a-1g.

The solution of oseltamivir phosphate (0.82 g, 2.0 mmol) and a kind of aldehyde (2.4 mmol, 1.2 equiv) in 30 mL methanol and ethanol (V : V = 2 : 1) was stirred at room temperature for half an hour. And then, $NaBH_3CN$ (0.31 g, 5.0 mmol, 2.5 equiv) was added slowly to the solution. After that, the mixture was stirred for 6 hours at room temperature. The solvent was removed under reduced pressure, water (30 mL) was added to the residue and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with saturated sodium chloride (2 × 30mL) and dried with anhydrous $MgSO_4$and concentrated under reduced pressure. The concentrated crude was purified by flash column chromatography to obtain the corresponding pure intermediates **1a-1g**.

Ethyl(3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(trifluoromethyl)benzyl)amino)cyclohex-1-ene-1-carboxylate (1a). 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, J = 8.9 Hz, 1H), 7.69 (s, 1H), 7.64 – 7.49 (m, 3H), 6.64 (s, 1H), 4.13 (q, J = 6.6 Hz, 2H), 4.02 (d, J = 6.4 Hz, 1H), 3.86 (d, J = 14.0 Hz, 1H), 3.74 (dd, J = 20.3, 11.4 Hz, 2H), 2.80 – 2.69 (m, 1H), 2.64 (d, J = 17.5 Hz, 1H), 2.11 (s, 2H), 1.85 (s, 3H), 1.50 – 1.34 (m, 4H), 1.21 (t, J = 6.9 Hz, 3H), 0.82 (dt, J = 14.0, 7.1 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.99, 166.28, 143.53, 138.42, 132.32, 129.47, 129.36 (q, $^2J_{CF}$ = 33.0), 129.17, 124.87 (q, $^1J_{CF}$ = 270.4), 124.67 (q, $^3J_{CF}$ = 3.5), 123.63 (q, $^3J_{CF}$ = 3.5), 81.34, 75.72, 60.79, 55.21, 54.51, 49.44, 30.92, 26.09, 25.66, 23.39, 14.52, 9.89, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-nitrobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (1b). 77% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (d, J = 8.5 Hz, 2H), 7.80 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 8.4 Hz, 2H), 6.63 (s, 1H), 4.13 (q, J = 7.0 Hz, 2H), 4.01 (d, J = 7.3 Hz, 1H), 3.87 (q, J = 15.0 Hz, 2H), 3.72 (q, J = 9.1 Hz, 1H), 2.72 (dt, J = 14.8, 7.5 Hz, 1H), 2.64 (dd, J = 17.6, 4.1 Hz, 1H), 2.22 (s, 1H), 2.08 (dd, J = 17.2, 9.3 Hz, 1H), 1.86 (s, 3H), 1.42 (qt, J = 14.0, 7.0 Hz, 4H), 1.21 (t, J = 7.0 Hz, 3H), 0.82 (dt, J = 14.3, 7.3 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.05, 166.26, 150.45, 146.69, 138.45, 129.25, 129.09, 123.68, 81.34, 75.72, 60.80,

55.01, 54.60, 49.41, 30.99, 26.08, 25.64, 23.51, 14.53, 9.90, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((3-nitrobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (1c). 75% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.22 (s, 1H), 8.08 (d, J = 9.6 Hz, 1H), 7.78 (dd, J = 15.3, 8.4 Hz, 2H), 7.60 (t, J = 7.9 Hz, 1H), 6.63 (s, 1H), 4.13 (q, J = 7.0 Hz, 2H), 4.01 (d, J = 8.2 Hz, 1H), 3.93 – 3.78 (m, 2H), 3.72 (q, J = 9.1 Hz, 1H), 3.45-3.3(m, 2H), 2.75 – 2.61 (m, 2H), 2.09 (dd, J = 16.9, 8.9 Hz, 1H), 1.87 (s, 3H), 1.42 (tp, J = 15.0, 7.3 Hz, 4H), 1.21 (t, J = 7.1 Hz, 3H), 0.81 (dt, J = 14.4, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.07, 166.27, 148.26, 144.61, 138.46, 135.03, 129.93, 129.13, 122.77, 121.92, 81.37, 75.76, 60.81, 55.02, 54.56, 49.02, 30.99, 26.09, 25.65, 23.45, 14.54, 9.90, 9.42.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-cyanobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (1d). 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (t, J = 8.8 Hz, 3H), 7.52 (d, J = 8.2 Hz, 2H), 6.63 (s, 1H), 4.13 (q, J = 7.0 Hz, 2H), 4.00 (d, J = 8.2 Hz, 1H), 3.91 – 3.66 (m, 3H), 3.42 – 3.35 (m, 1H), 2.70 (q, J = 9.5 Hz, 1H), 2.62 (dd, J = 17.4, 4.9 Hz, 1H), 2.15 (s, 1H), 2.11 – 1.99 (m, 1H), 1.85 (s, 3H), 1.51 – 1.33 (m, 4H), 1.21 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 14.7, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.03, 166.26, 148.06, 138.45, 132.48, 129.08, 119.50, 109.64, 81.33, 75.69, 60.80, 54.94, 54.60, 49.65, 30.96, 26.08, 25.64, 23.51, 14.54, 9.91, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-fluorobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (1e). 68% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.80 (d, J = 9.1 Hz, 1H), 7.33 (dd, J = 8.6, 5.8 Hz, 2H), 7.17 – 7.06 (m, 2H), 6.63 (s, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.00 (d, J = 8.2 Hz, 1H), 3.79 – 3.60 (m, 3H), 3.39 – 3.35 (m, 1H), 2.71 (td, J = 9.7, 5.5 Hz, 1H), 2.63 (dd, J = 17.4, 4.9 Hz, 1H), 2.11 – 2.01 (m, 1H), 1.94 (s, 1H), 1.85 (s, 3H), 1.51 – 1.33 (m, 4H), 1.22 (t, J = 7.1 Hz, 3H), 0.81 (dt, J = 14.9, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.04, 166.29, 161.48 (d, $^{1}J_{CF}$ = 240.1), 138.42, 137.77 (d, ^{4}J = 2.9 Hz), 130.08 (2C, d, $^{3}J_{CF}$ = 7.9 HZ), 129.13, 115.22 (2C, d, $^{2}J_{CF}$ = 20.9 HZ), 81.32, 75.70, 60.80, 54.79, 54.54, 49.36, 30.93, 26.07, 25.63, 23.48, 14.54, 9.91, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((3-fluorobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (1f). 66% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ

7.81 (d, *J* = 9.1 Hz, 1H), 7.39 – 7.29 (m, 1H), 7.14 (t, *J* = 7.8 Hz, 2H), 7.03 (td, *J* = 8.7, 2.2 Hz, 1H), 6.63 (s, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 4.01 (d, *J* = 8.0 Hz, 1H), 3.84 – 3.65 (m, 3H), 3.43 – 3.36 (m, 1H), 2.76 – 2.58 (m, 2H), 2.16 – 1.98 (m, 2H), 1.86 (s, 3H), 1.52 – 1.32 (m, 4H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.82 (dt, *J* = 14.6, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.03, 166.29, 162.72 (d, $^{1}J_{CF}$ = 240.4), 144.99 (d, $^{4}J_{CF}$ = 6.8 HZ), 138.44, 130.35 (d, $^{3}J_{CF}$ = 8.2 HZ), 129.10, 124.15, 114.76 (d, $^{2}J_{CF}$ =20.8 HZ), 113.63 (d, $^{2}J_{CF}$ = 20.8 HZ), 81.33, 75.68, 60.80, 54.82, 54.61, 49.48, 30.92, 26.08, 25.64, 23.48, 14.54, 9.91, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-bromobenzyl)amino)-3-(pentan-3-yloxy) cyclohex-1-ene-1-carboxylate (1g). 62% yield. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.49 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.3 Hz, 2H), 6.78 (s, 1H), 4.21 (q, *J* = 7.1 Hz, 2H), 4.07 (d, *J* = 7.9 Hz, 1H), 4.00 – 3.88 (m, 2H), 3.77 (d, *J* = 13.3 Hz, 1H), 3.39 (p, *J* = 5.5 Hz, 1H), 2.96 (td, *J* = 10.0, 5.4 Hz, 1H), 2.83 (dd, *J* = 17.6, 5.2 Hz, 1H), 2.27 (ddt, *J* = 15.2, 9.6, 2.6 Hz, 1H), 2.00 (s, 3H), 1.59 – 1.43 (m, 4H), 1.29 (t, *J* = 7.1 Hz, 3H), 0.94 – 0.84 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.68, 166.26, 137.70, 137.37, 131.30, 130.16, 128.60, 120.87, 82.07, 75.43, 60.70, 54.24, 54.12, 48.57, 29.19, 25.76, 25.30, 21.77, 13.11, 8.50, 8.18.

4.1.2. General procedure for the synthesis of compounds 2a-2g.

At room temperature, the intermediates **1a-1g** (0.8 mmol) were stirred respectively in a mixed solution of methanol (30 mL) and 16% sodium hydroxide solution (10 mL) for 6 h. Then the mixture was concentrated to remove methanol. The residue was dissolved in water (30 mL), and then acidified with dilute hydrochloric acid (2 mol/L) to adjust pH to 4-5. The solvent was extracted with ethyl acetate and tetrahydrofuran (V : V = 2 : 1, 4 × 30 mL), combined the organic layer, washed with saturated sodium chloride solution (2 × 30 mL), dried with anhydrous $MgSO_4$, filtered and removed the organic solvent under reduced pressure. The target compounds **2a-2g** were obtained by crystallization of the residue in isopropyl ether.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(trifluoromethyl)benzyl)a mino)cyclohex-1-ene-1-carboxylic acid (2a). White powder, 63% yield, mp: 135.8–136.8°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.71 (s, 1H), 9.90 (s, 1H), 9.53 (s, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 7.99 (s, 1H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.8 Hz,

1H), 7.66 (t, *J* = 7.7 Hz, 1H), 6.66 (s, 1H), 4.30 (s, 3H), 4.05 (q, *J* = 8.9 Hz, 1H), 3.53 – 3.46 (m, 1H), 2.96 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.77 – 2.65 (m, 1H), 1.91 (s, 3H), 1.42 (qq, *J* = 14.1, 8.8, 7.9 Hz, 4H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.33, 167.24, 138.05, 137.98, 135.00, 133.86, 130.05, 129.62 (q, $^{2}J_{CF}$ = 31.7), 128.11,127.58 (q, $^{3}J_{CF}$ = 3.6)，125.97 (q, $^{3}J_{CF}$ = 3.5)，124.52 (q, $^{1}J_{CF}$ = 270.6), 81.60, 74.88, 54.79, 50.93, 45.23, 26.06, 25.87, 25.50, 23.89, 9.83, 9.31. HRMS calcd for $C_{22}H_{29}F_3N_2O_4$ $[M + H]^+$: 443.2152. Found: m/z 443.2150.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-nitrobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2b). White powder, 62% yield, mp: 210.4–220.9°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (d, *J* = 8.7 Hz, 2H), 8.02 (d, *J* = 9.1 Hz, 1H), 7.62 (d, *J* = 8.7 Hz, 2H), 6.56 (s, 1H), 4.03 (d, *J* = 8.1 Hz, 1H), 3.89 (q, *J* = 15.1 Hz, 3H), 3.72 (q, *J* = 9.1 Hz, 2H), 2.73 (td, *J* = 10.1, 5.4 Hz, 1H), 2.64 (dd, *J* = 17.5, 4.1 Hz, 1H), 2.04 (dd, *J* = 17.4, 9.6 Hz, 1H), 1.87 (s, 3H), 1.42 (ddp, *J* = 26.8, 13.3, 6.7, 6.3 Hz, 4H), 0.82 (dt, *J* = 15.1, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.18, 169.35, 150.10, 146.70, 136.89, 136.83, 130.80, 129.35, 123.70, 81.26, 76.05, 55.14, 54.78, 49.22, 31.23, 26.13, 25.63, 23.54, 9.94, 9.41. HRMS calcd for $C_{21}H_{29}N_3O_6$ $[M + H]^+$: 420.2129. Found: m/z 420.2131.

(3*R*,4*R*,5*S*)-4-acetamido-5-((3-nitrobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2c). White powder, 60% yield, mp: 111.3–113.0°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.74 (s, 1H), 8.44 (s, 1H), 8.22 (d, *J* = 7.7 Hz, 1H), 8.17 – 8.02 (m, 1H), 7.96 (d, *J* = 6.6 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 6.65 (s, 1H), 6.54 (s, 0.5H), 4.24 (d, *J* = 12.3 Hz, 3H), 4.01 – 3.88 (m, 1H), 3.47 – 3.35 (m, 2H), 2.88 (d, *J* = 14.2 Hz, 1H), 2.57 (s, 0.5H), 1.91 (s, 3H), 1.43 (tt, *J* = 12.6, 6.8 Hz, 4H), 0.85 (t, *J* = 7.2 Hz, 3H), 0.80 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.14, 167.42, 148.16, 137.95, 136.97, 130.40, 128.48, 125.03, 123.64, 81.54, 75.08, 54.82, 51.87, 49.05, 45.88, 26.07, 25.54, 23.82, 9.85, 9.35. HRMS calcd for $C_{21}H_{29}N_3O_6$ $[M + H]^+$: 420.2129. Found: m/z 420.2128.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-cyanobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2d). White powder, 64% yield, mp: 110.1–112.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.70 (s, 1H), 8.17 (d, *J* = 9.0 Hz, 1H), 7.91 (d, *J* = 8.2

Hz, 2H), 7.77 (d, *J* = 8.2 Hz, 2H), 6.65 (s, 1H), 4.35 – 4.22 (m, 3H), 3.99 (q, *J* = 8.9 Hz, 1H), 3.45– 3.35（m，1H） 2.91 (dd, *J* = 17.1, 4.7 Hz, 1H), 2.72 – 2.59 (m, 1H), 1.91 (s, 3H), 1.42 (tq, *J* = 14.3, 7.0 Hz, 4H), 1.04 (d, *J* = 6.1 Hz, 1H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.31, 167.25, 138.00, 132.86, 131.46, 128.08, 119.01, 111.89, 81.57, 74.89, 67.77, 54.64, 51.24, 45.61, 26.05, 25.50, 23.94, 23.27, 9.85, 9.32. HRMS calcd for $C_{22}H_{29}N_3O_4$ [M + H]$^+$: 400.2231. Found: m/z 400.2233.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-fluorobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2e). White powder, 59% yield, mp: 170.2–172.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.17 (d, *J* = 9.1 Hz, 1H), 7.61 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.27 (t, *J* = 8.9 Hz, 2H), 6.65 (s, 1H), 4.30 – 4.13 (m, 3H), 4.06 – 3.95 (m, 1H), 3.42 – 3.37 (m, 3H), 2.92 (dd, *J* = 17.2, 4.9 Hz, 1H), 2.75 – 2.59 (m, 1H), 1.92 (s, 3H), 1.52 – 1.32 (m, 4H), 0.82 (dt, *J* = 19.2, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.26, 167.25, 162.78 (d, $^1J_{CF}$ = 243.8 HZ) 138.03, 133.01 (2C, d, $^3J_{CF}$ = 8.5 HZ), 128.71, 128.05, 115.90 (2C, d, $^2J_{CF}$ = 21.3 HZ), 81.56, 74.92, 54.32, 51.05, 45.19, 26.03, 25.98, 25.47, 23.96, 9.86, 9.30. HRMS calcd for $C_{21}H_{29}FN_2O_4$ [M + H]$^+$: 393.2184. Found: m/z 393.2185.

(3*R*,4*R*,5*S*)-4-acetamido-5-((3-fluorobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2f).White powder, 56% yield, mp: 157.1–158.7°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.16 (d, *J* = 9.0 Hz, 1H), 7.52 – 7.42 (m, 2H), 7.38 (d, *J* = 7.7 Hz, 1H), 7.24 (td, *J* = 8.5, 2.2 Hz, 1H), 6.65 (s, 1H), 4.30 – 4.15 (m, 3H), 4.00 (q, *J* = 8.9 Hz, 1H), 3.44 – 3.35 (m, 3H), 2.91 (dd, *J* = 17.1, 4.8 Hz, 1H), 2.73 – 2.58 (m, 1H), 1.92 (s, 3H), 1.42 (th, *J* = 14.0, 7.1 Hz, 4H), 0.84 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.25, 167.26, 162.38 (d, $^1J_{CF}$ = 242.1), 138.02, 135.36, 131.06 (d, $^3J_{CF}$ = 8.1 HZ), 128.09, 126.69, 117.32 (d, $^2J_{CF}$ = 22.0 HZ), 116.06 (d, $^2J_{CF}$ = 21.1 HZ), 81.56, 74.90, 68.73, 54.46, 51.23, 45.54, 27.88, 26.04, 25.49, 23.94, 22.21, 9.85, 9.31. HRMS calcd for $C_{21}H_{29}FN_2O_4$ [M + H]$^+$: 393.2184. Found: m/z 393.2185.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-bromobenzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (2g). White powder, 59% yield, mp: 105.7–108.5°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.62 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H),

6.84 (s, 1H), 4.35 (d, *J* = 13.2 Hz, 1H), 4.28 – 4.12 (m, 3H), 3.64 – 3.52 (m, 1H), 3.45 (p, *J* = 5.6 Hz, 1H), 3.01 (dd, *J* = 17.3, 5.2 Hz, 1H), 2.63 (dd, *J* = 17.1, 10.0 Hz, 1H), 2.05 (s, 3H), 1.61 – 1.47 (m, *J* = 6.8 Hz, 4H), 0.91 (q, *J* = 7.5 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): *δ* 173.47, 136.67, 132.03, 131.43, 130.67, 127.96, 124.72, 123.25, 82.27, 74.62, 55.05, 51.76, 46.90, 26.20, 25.74, 25.22, 21.98, 8.42, 8.17. HRMS calcd for $C_{21}H_{29}BrN_2O_4$ $[M + H]^+$: 453.1383. Found: m/z 453.1381.

4.1.3. General procedure for the synthesis of compound 3

Thiophene-3-formaldehyde (1.6 mmol, 0.8 equiv.) was added to the solution of 30 mL methanol and ethanol (V : V = 2 : 1) of oseltamivir phosphate (0.82 g, 2.0 mmol, 0.8 equiv.). The reaction solution was stirred for 0.5 h at room temperature, and then $NaBH_3CN$ (0.31 g, 5.0 mmol, 2.5 equiv.) was added and tirred for 6 hours at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with saturated sodium chloride (2 × 30mL) and dried with anhydrous $MgSO_4$. The $MgSO_4$ was removed by filtration and concentrated under reduced pressure. The crude product was purified by flash column chromatography to obtain the corresponding intermediate **3**.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((thiophen-3-ylmethyl)amino)cyclohex-1-ene-1-carboxylate (3). 77% yield, ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 7.80 (d, *J* = 9.1 Hz, 1H), 7.46 (dd, *J* = 4.8, 3.0 Hz, 1H), 7.28 – 7.20 (m, 1H), 7.04 (d, *J* = 4.8 Hz, 1H), 6.64 (s, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 4.00 (d, *J* = 7.8 Hz, 1H), 3.81 – 3.61 (m, 3H), 3.40 – 3.35 (m, 1H), 2.73 (td, *J* = 9.7, 5.3 Hz, 1H), 2.65 (dd, *J* = 17.6, 4.6 Hz, 1H), 2.14 – 1.98 (m, 1H), 1.97 – 1.73 (m, 4H), 1.41 (th, *J* = 14.2, 7.0 Hz, 4H), 1.22 (t, *J* = 7.1 Hz, 3H), 0.81 (dt, *J* = 15.2, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): *δ* 170.04, 166.30, 142.65, 138.44, 129.13, 128.23, 126.30, 121.62, 81.32, 75.69, 60.80, 54.69, 54.56, 45.49, 30.87, 26.07, 25.63, 23.48, 14.56, 9.92, 9.41.

4.1.4. General procedure for the synthesis of compound 4

The compound **4** was synthesized with the same procedure reported above as 4.1.2 and the raw material of the reaction was compound 3.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((thiophen-3-ylmethyl)amino)c

yclohex-1-ene-1-carboxylic acid (4). White powder, 61% yield, mp: 171.2–176.0°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.14 (s, 1H), 7.66 (s, 1H), 7.58 (s, 1H), 7.34 – 7.21 (m, 1H), 6.63 (s, 1H), 4.31 – 4.06 (m, 3H), 3.94 (q, J = 9.2 Hz, 1H), 3.39 (p, J = 5.3 Hz, 1H), 3.27 (s, 1H), 2.86 (dd, J = 17.1, 4.4 Hz, 1H), 2.63 – 2.52 (m, 1H), 1.91 (s, 3H), 1.54 – 1.29 (m, 4H), 0.81 (dt, J = 18.7, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.11, 167.37, 137.98, 133.88, 129.22, 128.26, 127.23, 126.80, 81.51, 74.92, 54.08, 51.65, 41.55, 26.61, 26.03, 25.48, 23.90, 9.87, 9.32. HRMS calcd for $C_{19}H_{28}N_2O_4S$ $[M + H]^+$: 381.1843. Found: m/z 381.1842.

4.1.5. General procedure for the synthesis of compound 5

To a solution of oseltamivir phosphate (0.82 g, 2.0 mmol) in 30 mL of methanol and ethanol (V : V = 2 : 1), thiophene-3-carbaldehyde (4.4 mmol, 2.2 equiv.) was added at room temperature. The reaction solution was stirred for 0.5 h at this temperature, and then $NaBH_3CN$ (0.62 g, 10 mmol, 5 equiv.) was added stirred for 8 hours at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with saturated sodium chloride (2 × 30 mL), dried with anhydrous $MgSO_4$, filtered and concentrated after removing $MgSO_4$. The concentrated product was purified by flash column chromatography, and the corresponding intermediate **5** was obtained.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(bis(thiophen-3-ylmethyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (5). 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (d, J = 8.8 Hz, 1H), 7.43 (dd, J = 4.8, 3.0 Hz, 2H), 7.31 – 7.25 (m, 2H), 7.01 (d, J = 4.7 Hz, 2H), 6.58 (s, 1H), 4.14 (q, J = 7.1 Hz, 2H), 3.98 (t, J = 7.7 Hz, 2H), 3.69 (d, J = 14.1 Hz, 2H), 3.53 (d, J = 14.1 Hz, 2H), 2.86 (td, J = 10.5, 4.6 Hz, 1H), 2.50 (s, 2H), 2.31 – 2.17 (m, 1H), 1.91 (s, 3H), 1.44 (dq, J = 18.9, 6.4 Hz, 4H), 1.22 (t, J = 7.1 Hz, 3H), 0.81 (dt, J = 14.3, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.18, 166.25, 141.81, 138.77, 129.54, 128.53, 126.11, 122.37, 81.51, 77.04, 60.82, 56.72, 52.53, 48.63, 26.03, 25.57, 24.45, 23.64, 14.58, 9.92, 9.39.

4.1.6. General procedure for the synthesis of compound 6

The synthetic method was similar to that of compound **2**, and the raw material is compound **5**.

(3*R*,4*R*,5*S*)-4-acetamido-5-(bis(thiophen-3-ylmethyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (6). White powder, 53% yield mp: 110.0–111.7°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.28 (s, 1H), 7.78 (d, J = 8.6 Hz, 1H), 7.43 (dd, J = 4.8, 3.0 Hz, 2H), 7.33 – 7.24 (m, 2H), 7.01 (d, J = 4.7 Hz, 2H), 6.55 (s, 1H), 4.05 – 3.87 (m, 2H), 3.69 (d, J = 14.1 Hz, 2H), 3.53 (d, J = 14.1 Hz, 2H), 2.85 (td, J = 10.7, 4.8 Hz, 1H), 2.51 (s, 2H), 2.27 – 2.12 (m, 1H), 1.91 (s, 3H), 1.42 (ddq, J = 18.9, 13.6, 6.9 Hz, 4H), 0.83 (t, J = 6.8 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.18, 167.97, 141.88, 138.27, 130.15, 128.52, 126.10, 122.32, 81.47, 77.17, 56.66, 52.57, 48.67, 26.04, 25.54, 24.55, 23.65, 9.94, 9.39. HRMS calcd for $C_{24}H_{32}N_2O_4S_2$ $[M + H]^+$: 477.1876. Found: m/z 477.1877.

4.1.7. General procedure for the synthesis of compounds 7a-7h

To a solution of bromoarylaldehyde or aryl bromide (1 equiv.) in dimethyl sulfoxide, the corresponding aryl boronic acid or formyl aryl boronic acid (1.1 equiv.), tetrakis(triphenylphosphine)palladium (0.05 equiv.) and potassium carbonate (1 equiv.) were added. Replace the air in the mixture system with nitrogen for 10 minutes. After heating at 120°C for 12 hours, the reaction mixture was cooled to room temperature and then poured into cold water (120 mL). Ethyl acetate (3 × 40 mL) was used for extraction. The organic phase was combined, then washed with saturated sodium chloride (50 mL) and water (50 mL), and the organic phase was dried with anhydrous $MgSO_4$. After filtering and removing $MgSO_4$, the solvent was removed under reduced pressure and the crude product was obtained. The corresponding product **7a-7h** were obtained from the crude product purified by flash chromatography.

4-(thiophen-3-yl)benzaldehyde (7a). 53% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 1H), 8.14 (dd, J = 2.8, 1.5 Hz, 1H), 8.00 – 7.94 (m, 4H), 7.71 (qd, J = 5.1, 2.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 192.94, 141.08, 140.60, 135.15, 130.71, 128.15, 127.00, 126.72, 124.13.

5-phenylthiophene-2-carbaldehyde (7b). 62% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.93 (s, 1H), 8.05 (d, J = 4.0 Hz, 1H), 7.84 – 7.79 (m, 2H), 7.76 (d, J = 4.0 Hz, 1H), 7.53 – 7.42 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.52, 153.08, 142.47, 139.67, 132.90, 130.04, 129.84, 126.68, 126.36, 125.76.

[2,2'-bithiophene]-5-carbaldehyde (7c). 67% yield, ^{1}H NMR (400 MHz,

Methanol-d_4): δ 9.81 (s, 1H), 7.81 (d, J = 4.0 Hz, 1H), 7.50 (dd, J = 5.1, 1.1 Hz, 1H), 7.44 (dd, J = 3.7, 1.1 Hz, 1H), 7.36 (d, J = 4.0 Hz, 1H), 7.10 (dd, J = 5.1, 3.7 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 183.36, 147.00, 141.58, 138.28, 135.69, 128.15, 127.11, 126.06, 124.26.

4-(thiazol-2-yl)benzaldehyde (7d). 56% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 8.19 (d, J = 8.2 Hz, 2H), 8.03 (d, J = 8.1 Hz, 3H), 8.01 – 7.92 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.05, 166.12, 144.98, 138.34, 137.31, 130.88, 127.21, 122.71, 40.19.

5-phenylthiazole-2-carbaldehyde (7e).

Phenylboric acid (2.42 g, 20 mmol), 5-bromothiophene-2-formaldehyde (3.84 g, 20 mmol), potassium carbonate (4.1 g, 30 mmol), tetra-triphenylphosphine palladium (0.08 g), DMSO (30 ml) were added to the flask, protected by nitrogen, and heated to 120°C. Stir for 12 hours. The reaction mixture was added to 150 ml water, extracted with ethyl acetate (3 ×50 ml), dried with anhydrous $MgSO_4$, filtered, evaporated under reduced pressure and separated by flash column chromatography to obtain the target compounds.

[1,1'-biphenyl]-3-carbaldehyde (7f). 55% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.11 (s, 1H), 8.21 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.91 (d, J = 7.6 Hz, 1H), 7.76 (d, J = 8.4 Hz, 2H), 7.70 (t, J = 7.7 Hz, 1H), 7.52 (t, J = 7.5 Hz, 2H), 7.43 (t, J = 7.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.70, 139.34, 137.31, 133.11, 130.32, 129.58, 128.55, 128.43, 127.29.

3-(thiophen-2-yl)benzaldehyde (7g). 63% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 8.16 (s, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.6 Hz, 1H), 7.69 – 7.62 (m, 3H), 7.20 (dd, J = 5.0, 3.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.51, 137.37, 135.08, 131.52, 130.51, 129.20, 128.60, 127.14, 126.64, 125.27.

3-(thiophen-3-yl)benzaldehyde (7h). 64% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.07 (s, 1H), 8.25 (t, J = 1.6 Hz, 1H), 8.09 – 8.05 (m, 1H), 8.03 (dd, J = 2.9, 1.4 Hz, 1H), 7.83 (dt, J = 7.6, 1.3 Hz, 1H), 7.70 (dd, J = 5.0, 2.9 Hz, 1H), 7.67 – 7.61 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 193.60, 140.61, 137.31, 136.43,

132.40, 130.24, 128.19, 128.00, 127.65, 126.58, 122.65.

4.1.8. General procedure for the synthesis of compounds 8a-8h

The synthetic method was similar to that of compound **1**, and the raw material were compounds **7a-7h**.

Ethyl(*3R,4R,*5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(thiophen-3-yl)benzyl)amino)cyclohex-1-ene-1-carboxylate (8a). 70% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 – 7.78 (m, 2H), 7.66 (d, J = 8.2 Hz, 2H), 7.63 (dd, J = 5.0, 2.9 Hz, 1H), 7.55 (dd, J = 5.0, 1.2 Hz, 1H), 7.34 (d, J = 8.2 Hz, 2H), 6.64 (s, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.01 (d, J = 8.0 Hz, 1H), 3.85 – 3.63 (m, 3H), 3.42 – 3.38 (m, 1H), 2.75 (td, J = 9.7, 5.5 Hz, 1H), 2.67 (dd, J = 17.4, 4.7 Hz, 1H), 2.15 – 2.04 (m, 1H), 1.87 (s, 3H), 1.52 – 1.33 (m, 5H), 1.22 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 14.4, 7.4 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.07, 166.30, 141.82, 138.45, 133.96, 129.12, 128.84, 127.42, 126.57, 126.31, 120.91, 81.34, 75.71, 61.19, 60.82, 54.82, 54.56, 49.86, 29.67, 26.08, 25.64, 23.51, 14.56, 9.92, 9.43.

Ethyl(3*R,4R,*5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(((5-phenylthiophen-2-yl) methyl)amino)cyclohex-1-ene-1-carboxylate (8b). 60% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.80 (d, J = 9.1 Hz, 1H), 7.65 – 7.56 (m, 2H), 7.40 (t, J = 7.7 Hz, 2H), 7.33 (d, J = 3.6 Hz, 1H), 7.28 (t, J = 7.4 Hz, 1H), 6.94 (d, J = 3.5 Hz, 1H), 6.64 (s, 1H), 4.15 (q, J = 7.0 Hz, 2H), 4.03 (d, J = 8.0 Hz, 1H), 4.00 – 3.85 (m, 2H), 3.74 (q, J = 9.0 Hz, 1H), 3.41 – 3.36 (m, 1H), 2.86 – 2.75 (m, 1H), 2.69 (dd, J = 17.6, 4.9 Hz, 1H), 2.17 – 2.08 (m, 1H), 1.88 (s, 3H), 1.52 – 1.33 (m, 4H), 1.22 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 13.4, 7.4 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.07, 166.29, 142.13, 138.40, 134.59, 129.51, 129.15, 127.70, 125.44, 123.48, 81.37, 75.69, 60.82, 54.80, 54.37, 45.35, 30.83, 26.10, 25.68, 23.52, 14.56, 9.89, 9.46.

Ethyl(3*R,4R,*5*S*)-5-(([2,2'-bithiophen]-5-ylmethyl)amino)-4-acetamido-3-(pe ntan-3-yloxy)cyclohex-1-ene-1-carboxylate (8c). 70% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.27 (dd, J = 5.1, 1.1 Hz, 1H), 7.14 (dd, J = 3.6, 1.1 Hz, 1H), 7.04 – 6.97 (m, 2H), 6.86 (d, J = 3.6 Hz, 1H), 6.76 (t, J = 2.0 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 4.05 (dd, J = 11.4, 7.7 Hz, 2H), 3.95 – 3.86 (m, 2H), 3.37 (p, J = 5.7 Hz, 1H), 2.95 – 2.79 (m, 2H), 2.25 – 2.14 (m, 1H), 2.02 (s, 3H), 1.58 – 1.42 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H), 0.93 – 0.89 (m, 3H), 0.89 – 0.85 (m, 3H).^{13}C NMR (100 MHz,

CD_3OD): δ 172.55, 166.46, 142.90, 137.50, 137.40, 136.51, 128.94, 127.42, 125.77, 123.87, 122.97, 122.81, 82.09, 75.69, 60.64, 54.61, 54.00, 48.24, 48.03, 47.89, 47.81, 47.67, 47.60, 47.39, 47.18, 46.96, 44.31, 29.95, 25.80, 25.36, 21.80, 13.10, 8.49, 8.21.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(thiazol-2-yl)benzyl)amino)cyclohex-1-ene-1-carboxylate (8d). 65% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.95 – 7.86 (m, 3H), 7.80 (d, J = 9.1 Hz, 1H), 7.76 (d, J = 3.2 Hz, 1H), 7.44 (d, J = 8.2 Hz, 2H), 6.64 (s, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.02 (d, J = 8.2 Hz, 1H), 3.83 (d, J = 14.1 Hz, 1H), 3.73 (q, J = 8.6 Hz, 2H), 3.37 (q, J = 5.6 Hz, 1H), 2.74 (td, J = 9.5, 5.3 Hz, 1H), 2.66 (dd, J = 17.4, 4.9 Hz, 1H), 2.16 – 1.96 (m, 2H), 1.87 (s, 3H), 1.52 – 1.33 (m, 4H), 1.21 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 13.4, 7.4 Hz, 6H).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.06, 167.63, 166.30, 144.20, 144.12, 138.42, 131.98, 129.16, 129.06, 126.49, 120.52, 81.34, 75.73, 60.80, 54.92, 54.61, 49.86, 30.99, 26.09, 25.66, 23.50, 14.55, 9.90, 9.43.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(((5-phenylthiazol-2-yl)methyl)amino)cyclohex-1-ene-1-carboxylate (8e). 62% yield. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.94 – 7.83 (m, 2H), 7.67 (s, 1H), 7.45 (qd, J = 4.2, 1.7 Hz, 3H), 6.76 (t, J = 2.1 Hz, 1H), 4.20 (q, J = 7.1 Hz, 2H), 4.16 – 4.09 (m, 1H), 4.09 – 3.97 (m, 2H), 3.89 (dd, J = 10.4, 8.5 Hz, 1H), 3.37 (p, J = 5.6 Hz, 1H), 2.96 – 2.77 (m, 2H), 2.28 – 2.14 (m, 1H), 2.02 (s, 3H), 1.57 – 1.43 (m, 4H), 1.26 (s, 3H), 0.89 (q, J = 7.5 Hz, 6H).^{13}C NMR (100 MHz, CD_3OD): δ 172.52, 168.43, 166.43, 140.50, 139.58, 137.49, 133.39, 129.90, 128.94, 128.78, 125.98, 125.93, 82.08, 75.68, 60.64, 54.65, 54.15, 41.66, 30.08, 25.81, 25.37, 21.82, 13.10, 8.50, 8.22.

Ethyl(3*R*,4*R*,5*S*)-5-(([1,1'-biphenyl]-3-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (8f). 75% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.61 (d, J = 8.0 Hz, 3H), 7.51 (d, J = 7.7 Hz, 1H), 7.46 – 7.36 (m, 3H), 7.36 – 7.27 (m, 2H), 6.78 (s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 4.07 (d, J = 8.1 Hz, 1H), 3.95 (dd, J = 11.6, 4.0 Hz, 2H), 3.79 (d, J = 12.9 Hz, 1H), 3.38 (p, J = 5.5 Hz, 1H), 2.94 (td, J = 9.9, 5.3 Hz, 1H), 2.86 (dd, J = 17.5, 5.2 Hz, 1H), 2.36 – 2.22 (m, 1H), 1.98 (s, 3H), 1.59 – 1.42 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H), 0.89 (q, J = 7.5 Hz, 6H).^{13}C NMR (100 MHz, CD_3OD): δ 172.51, 166.43, 141.40, 140.93, 139.97, 137.39, 128.90, 128.65, 128.44, 127.00, 126.72, 126.63, 125.54, 82.03, 75.60, 60.65, 54.46, 54.32, 49.61, 29.62, 25.78, 25.33, 21.76, 13.10, 8.50, 8.18.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(thiophen-2-yl)benzyl)amino)cyclohex-1-ene-1-carboxylate (8g). 66% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.64 (s, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.44 – 7.22 (m, 4H), 7.07 (d, J = 4.8 Hz, 1H), 6.78 (s, 1H), 4.20 (q, J = 7.1 Hz, 2H), 4.15 – 3.93 (m, 3H), 3.84 (d, J = 12.9 Hz, 1H), 3.43 – 3.33 (m, 1H), 3.10 (td, J = 10.0, 5.4 Hz, 1H), 2.88 (dd, J = 17.4, 4.8 Hz, 1H), 2.36 (dd, J = 17.5, 9.7 Hz, 1H), 2.01 (s, 3H), 1.57 – 1.42 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H), 0.88 (q, J = 7.3 Hz, 6H).^{13}C NMR (100 MHz, CD_3OD): δ 172.71, 166.16, 143.66, 138.34, 137.46, 134.83, 129.01, 128.45, 127.81, 127.48, 125.67, 124.72, 123.16, 82.10, 75.41, 60.76, 54.54, 53.73, 48.97, 47.45, 28.82, 25.79, 25.33, 21.95, 13.18, 8.57, 8.24.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(thiophen-3-yl)benzyl)amino)cyclohex-1-ene-1-carboxylate (8h). 53% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.69 – 7.60 (m, 2H), 7.57 (d, J = 7.8 Hz, 1H), 7.47 (d, J = 1.9 Hz, 2H), 7.36 (t, J = 7.6 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 6.78 (s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 4.08 (d, J = 8.2 Hz, 1H), 3.97 (dd, J = 11.7, 4.6 Hz, 2H), 3.80 (d, J = 12.9 Hz, 1H), 3.38 (p, J = 5.6 Hz, 1H), 2.99 (td, J = 10.0, 5.4 Hz, 1H), 2.87 (dd, J = 17.6, 5.2 Hz, 1H), 2.31 (ddt, J = 14.6, 7.0, 2.6 Hz, 1H), 1.99 (s, 3H), 1.57 – 1.44 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.89 (q, J = 7.5 Hz, 6H).^{13}C NMR (100 MHz, CD_3OD): δ 172.63, 166.32, 141.91, 139.10, 137.41, 136.17, 128.77, 128.68, 126.88, 126.13, 125.98, 125.75, 125.08, 120.09, 82.06, 75.48, 60.71, 54.32, 54.18, 49.37, 29.25, 25.77, 25.31, 21.82, 13.13, 8.53, 8.19.

4.1.9. General procedure for the synthesis of compounds 9a-9h

The synthetic method was similar to that of compound **2**, and the raw materials were **8a-8h**.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(thiophen-3-yl)benzyl)ami no)cyclohex-1-ene-1-carboxylic acid (9a). White powder, 53% yield, mp: 180.2–182.3°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.18 (d, J = 9.0 Hz, 1H), 8.00 – 7.90 (m, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.66 (dd, J = 4.9, 2.9 Hz, 1H), 7.63 – 7.52 (m, 3H), 6.65 (s, 1H), 4.21 (dt, J = 23.1, 11.0 Hz, 3H), 4.02 (q, J = 9.0 Hz, 1H), 3.42 – 3.37 (m, 3H), 2.93 (dd, J = 17.0, 4.5 Hz, 1H), 2.74 – 2.61 (m, 1H), 1.94 (s, 3H), 1.53 – 1.33 (m, 4H), 0.82 (dt, J = 17.7, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.23, 167.28,

141.22, 138.07, 135.90, 131.13, 128.08, 127.71, 126.60, 121.97, 81.57, 74.96, 54.31, 51.20, 45.84, 26.14, 26.03, 25.47, 23.97, 9.87, 9.32. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 457.2156. Found: m/z 457.2152.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(((5-phenylthiophen-2-yl)methyl)amino)cyclohex-1-ene-1-carboxylic acid (9b). White powder, 57% yield, mp: 178.7–179.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.09 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 7.4 Hz, 2H), 7.48 – 7.38 (m, 3H), 7.33 (t, J = 7.3 Hz, 1H), 7.25 (s, 1H), 6.64 (s, 1H), 4.32 (s, 2H), 4.19 (d, J = 7.1 Hz, 1H), 3.94 (q, J = 9.1 Hz, 1H), 3.64 – 3.43 (m, 1H), 3.34 – 3.08 (m, 2H), 2.89 (d, J = 16.6 Hz, 1H), 2.64 – 2.51 (m, 1H), 1.93 (s, 3H), 1.53 – 1.32 (m, 4H), 0.82 (dt, J = 17.5, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.98, 167.42, 138.07, 133.97, 129.65, 128.39, 128.30, 125.74, 123.96, 90.68, 81.54, 75.07, 54.28, 51.97, 29.70, 26.06, 25.53, 23.87, 9.85, 9.36. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 457.2156. Found: m/z 457.2153.

(3*R*,4*R*,5*S*)-5-(([2,2'-bithiophen]-5-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (9c). White powder, 58% yield, mp: 145.7–157.8°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.37 (d, J = 5.1 Hz, 1H), 7.25 (d, J = 3.5 Hz, 1H), 7.19 (d, J = 3.6 Hz, 1H), 7.16 (d, J = 3.6 Hz, 1H), 7.08 – 7.01 (m, 1H), 6.83 (s, 1H), 4.61 – 4.37 (m, 2H), 4.27 – 4.08 (m, 2H), 3.60 – 3.47 (m, 1H), 3.43 (p, J = 5.6 Hz, 1H), 3.04 (dd, J = 17.3, 5.2 Hz, 1H), 2.58 (dd, J = 17.3, 10.0 Hz, 1H), 2.06 (s, 3H), 1.60 – 1.44 (m, 4H), 0.97 – 0.89 (m, 3H), 0.89 – 0.80 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.41, 167.66, 140.06, 136.95, 136.17, 131.51, 131.22, 127.84, 127.69, 125.05, 124.12, 123.37, 82.30, 74.66, 54.40, 51.98, 42.16, 26.41, 25.75, 25.25, 22.00, 8.41, 8.18. HRMS calcd for $C_{23}H_{30}BrN_2O_4S_2$ $[M + H]^+$: 463.1720. Found: m/z 463.1723.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(thiazol-2-yl)benzyl)amino)cyclohex-1-ene-1-carboxylic acid (9d). White powder, 50% yield, mp: 175.1–176.9°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 8.04 (d, J = 8.1 Hz, 2H), 7.90 (d, J = 3.3 Hz, 1H), 7.65 (d, J = 3.3 Hz, 1H), 7.60 (d, J = 8.1 Hz, 2H), 6.84 (s, 1H), 4.42 (d, J = 13.2 Hz, 1H), 4.34 – 4.13 (m, 3H), 3.57 (td, J = 9.0, 4.6 Hz, 1H), 3.45 (p, J = 5.4 Hz, 1H), 3.12 – 3.00 (m, 1H), 2.71 – 2.58 (m, 1H), 2.06 (s, 3H), 1.64 – 1.45 (m, J = 6.8 Hz, 4H), 1.00 – 0.83 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.45, 167.55, 143.34, 136.53, 134.10, 133.71, 130.25, 126.83, 120.05, 82.25, 74.64, 55.10, 51.89,

47.30, 26.42, 25.74, 25.23, 21.96, 8.42, 8.17. HRMS calcd for $C_{24}H_{31}N_3O_4S$ $[M + H]^+$: 458.2108. Found: m/z 458.2105.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(((5-phenylthiazol-2-yl)methyl)amino)cyclohex-1-ene-1-carboxylic acid (9e). White powder, 56% yield, mp: 175.5–178.7°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.98 (s, 1H), 7.98 – 7.91 (m, 2H), 7.49 (dd, J = 5.1, 1.7 Hz, 3H), 6.87 (s, 1H), 4.75 – 4.59 (m, 2H), 4.26 (d, J = 7.8 Hz, 1H), 4.17 (dd, J = 10.8, 8.3 Hz, 1H), 3.64 (td, J = 10.3, 5.6 Hz, 1H), 3.45 (p, J = 5.6 Hz, 1H), 3.09 (dd, J = 17.2, 5.3 Hz, 1H), 2.71 – 2.57 (m, 1H), 2.07 (s, 3H), 1.61 – 1.45 (m, 4H), 0.96 – 0.90 (m, 3H), 0.90 – 0.85 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.56, 171.49, 167.14, 146.14, 137.38, 132.79, 130.68, 128.94, 127.46, 127.20, 126.23, 82.34, 74.45, 54.85, 51.82, 39.56, 25.74, 25.25, 22.04, 8.38, 8.18. HRMS calcd for $C_{24}H_{31}N_3O_4S$ $[M + H]^+$: 458.2108. Found: m/z 458.2109.

(3*R*,4*R*,5*S*)-5-(([1,1'-biphenyl]-3-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (9f). White powder, 61% yield, mp: 176.0–178.1°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.77 (s, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.66 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.50 – 7.42 (m, 3H), 7.37 (t, J = 7.3 Hz, 1H), 6.83 (s, 1H), 4.45 (d, J = 13.0 Hz, 1H), 4.36 – 4.16 (m, 3H), 3.61 (s, 1H), 3.50 – 3.38 (m, 1H), 3.15 – 2.95 (m, 1H), 2.78 – 2.57 (m, 1H), 2.04 (s, 3H), 1.63 – 1.42 (m, 4H), 0.90 (q, J = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 189.76, 178.34, 142.17, 140.04, 136.32, 131.94, 128.64, 128.21, 128.08, 127.72, 127.50, 126.67, 82.25, 74.60, 55.16, 51.68, 26.25, 25.74, 25.22, 21.98, 8.42, 8.15. HRMS calcd for $C_{27}H_{34}N_2O_4$ $[M + H]^+$: 451.2591. Found: m/z 451.2594.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(thiophen-2-yl)benzyl)amino)cyclohex-1-ene-1-carboxylic acid (9g). White powder, 70% yield, mp: 167.1–168.0°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.79 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.54 – 7.44 (m, 2H), 7.40 (dd, J = 12.0, 6.0 Hz, 2H), 7.11 (dd, J = 5.0, 3.7 Hz, 1H), 6.83 (s, 1H), 4.40 (d, J = 13.0 Hz, 1H), 4.35 – 4.09 (m, 3H), 3.68 – 3.53 (m, 1H), 3.45 (p, J = 5.4 Hz, 1H), 3.15 – 2.92 (m, 1H), 2.67 (dd, J = 15.0, 9.8 Hz, 1H), 2.05 (s, 3H), 1.61 – 1.43 (m, 4H), 0.90 (q, J = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 142.89, 135.47, 132.29, 129.59, 128.29, 127.92, 126.64, 126.31, 125.25, 123.66, 82.26, 74.64, 55.21, 51.64, 26.27, 25.75, 25.23, 22.01, 8.42, 8.16. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 457.2156. Found: m/z 457.2152.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((3-(thiophen-3-yl)benzyl)amino)cyclohex-1-ene-1-carboxylic acid (9h). White powder, 73% yield, mp: 151.0–152.7°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.82 (s, 1H), 7.79 – 7.69 (m, 2H), 7.57 – 7.45 (m, 3H), 7.40 (d, J = 7.5 Hz, 1H), 6.88 (s, 1H), 4.45 (d, J = 12.9 Hz, 1H), 4.37 – 4.16 (m, 3H), 3.72 – 3.60 (m, 1H), 3.45 (p, J = 5.4, 4.9 Hz, 1H), 3.07 (dd, J = 17.2, 4.4 Hz, 1H), 2.78 – 2.62 (m, 1H), 2.05 (s, 3H), 1.62 – 1.46 (m, J = 7.0, 6.5 Hz, 4H), 0.91 (q, J = 7.6 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.51, 167.17, 137.16, 136.92, 131.46, 129.53, 127.94, 127.36, 127.31, 127.09, 126.37, 125.62, 120.84, 82.32, 74.46, 55.06, 51.48, 25.80, 25.72, 25.23, 22.01, 8.40, 8.15. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 457.2156. Found: m/z 457.2151.

4.1.10. General procedure for the synthesis of compounds 10a-10g

The synthetic method was similar to that of compound **1**, the main raw materials were oseltamivir acid and different substituted cinnamaldehyde.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-2-methyl-3-phenylallyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10a). 73% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.35 – 7.12 (m, 5H), 6.79 (s, 1H), 6.46 (s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 4.11 (d, J = 8.4 Hz, 1H), 3.93 – 3.80 (m, 1H), 3.46 – 3.35 (m, 2H), 3.00 – 2.80 (m, 2H), 2.80 – 2.63 (m, 1H), 2.56 – 2.32 (m, 1H), 2.21 (ddt, J = 15.1, 9.1, 2.5 Hz, 1H), 2.01 (s, 3H), 1.88 (s, 3H), 1.61 – 1.44 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.91 (q, J = 7.5 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.56, 166.45, 137.71, 137.40, 135.81, 128.89, 128.81, 128.50, 127.86, 127.71, 126.71, 126.03, 82.03, 75.52, 60.65, 54.63, 54.05, 54.00, 29.70, 25.79, 25.34, 21.75, 15.24, 13.11, 8.52, 8.19.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-(dimethylamino)phenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10b). 65% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.82 (d, J = 9.1 Hz, 1H), 7.11 (dd, J = 92.5, 8.7 Hz, 2H), 6.73 – 6.58 (m, 3H), 6.35 (d, J = 15.9 Hz, 1H), 6.00 (dt, J = 15.8, 6.3 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.01 (d, J = 7.9 Hz, 1H), 3.67 (q, J = 9.0 Hz, 1H), 3.34 – 3.29 (m, 1H), 3.22 (dd, J = 14.1, 6.5 Hz, 1H), 2.89 (s, 6H), 2.77 (td, J = 9.6, 5.5 Hz, 1H), 2.67 (dd, J = 17.8, 4.9 Hz, 1H), 2.09 – 1.96 (m, 1H), 1.85 (s, 3H), 1.69 – 1.54 (m, 1H), 1.50 – 1.35 (m, 4H), 1.22 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 15.1, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.03, 166.33, 150.13, 138.41, 130.54, 129.14,

127.38, 125.49, 125.11, 112.70, 81.29, 75.69, 60.81, 54.70, 54.56, 48.86, 40.89, 40.52, 32.56, 31.03, 26.05, 25.62, 23.50, 14.56, 9.92, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(2-methoxyphenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10c). 62% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.82 (d, J = 9.1 Hz, 1H), 7.45 (dd, J = 7.6, 1.5 Hz, 1H), 7.25 – 7.17 (m, 1H), 6.97 (d, J = 7.7 Hz, 1H), 6.95 – 6.88 (m, 1H), 6.73 (d, J = 16.1 Hz, 1H), 6.64 (s, 1H), 6.23 (dt, J = 16.0, 6.1 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.01 (d, J = 8.1 Hz, 1H), 3.79 (s, 3H), 3.68 (q, J = 8.9 Hz, 1H), 3.45 – 3.37 (m, 1H), 3.31 – 3.22 (m, 1H), 2.77 (td, J = 9.7, 5.2 Hz, 1H), 2.67 (dd, J = 17.5, 4.9 Hz, 1H), 2.04 (ddt, J = 17.5, 9.1, 2.6 Hz, 1H), 1.85 (s, 3H), 1.72 – 1.56 (m, 1H), 1.50 – 1.35 (m, 4H), 1.22 (t, J = 7.1 Hz, 3H), 0.82 (dt, J = 14.8, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.05, 166.33, 156.44, 138.42, 130.53, 129.15, 128.85, 126.64, 125.90, 124.77, 120.94, 111.66, 81.31, 75.71, 60.81, 55.78, 54.84, 54.54, 49.04, 31.04, 26.05, 25.63, 23.46, 14.54, 9.92, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((E)-3-(4-methoxyphenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10d). 70% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (d, J = 9.1 Hz, 1H), 7.24 (d, J = 8.7 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 6.54 (s, 1H), 6.33 (d, J = 15.9 Hz, 1H), 6.02 (dt, J = 15.9, 6.1 Hz, 1H), 4.04 (q, J = 7.0 Hz, 2H), 3.91 (d, J = 7.9 Hz, 1H), 3.64 (s, 3H), 3.60 – 3.48 (m, 1H), 3.15 (dd, J = 14.4, 6.5 Hz, 1H), 2.77 – 2.63 (m, 1H), 2.57 (dd, J = 17.6, 5.0 Hz, 1H), 1.99 – 1.86 (m, 1H), 1.75 (s, 3H), 1.40 – 1.24 (m, 4H), 1.12 (t, J = 7.1 Hz, 3H), 0.72 (dt, J = 15.2, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.06, 166.32, 159.01, 138.42, 130.03, 129.91, 129.61, 129.11, 127.73, 114.42, 114.08, 104.73, 81.30, 75.68, 60.82, 55.53, 54.69, 54.53, 48.60, 30.97, 26.05, 25.62, 23.51, 14.55, 9.92, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-fluorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10e). 68% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.42 (dd, J = 8.6, 5.5 Hz, 2H), 7.17 – 6.95 (m, 2H), 6.76 (d, J = 29.0 Hz, 1H), 6.66 – 6.40 (m, 1H), 6.28 – 6.02 (m, 1H), 4.30 – 4.16 (m, 2H), 4.11 (d, J = 7.5 Hz, 1H), 4.02 – 3.84 (m, 1H), 3.57 – 3.35 (m, 2H), 3.07 (qd, J = 11.8, 10.2, 7.1 Hz, 1H), 2.90 – 2.75 (m, 1H), 2.65 (ddt, J = 18.4, 11.2, 6.4 Hz, 1H), 2.34 – 2.16 (m, 1H), 2.01 (s, 3H), 1.62 – 1.42 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.90 (q, J = 7.5 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 172.67, 166.29, 162.39 (d, $^{1}J_{CF}$ = 242.4 Hz), 137.34,

131.66, 129.64, 128.58, 127.79 (2C, d, $^{3}J_{CF}$ = 8.0 Hz), 125.73, 122.09, 118.23, 114.91 (2C, d, $^{2}J_{CF}$ = 21.5 Hz), 82.02, 75.33, 60.73, 60.69, 54.10, 53.93, 29.13, 25.73, 25.29, 21.78, 13.08, 8.48, 8.16.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-chlorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10f). 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, J = 9.1 Hz, 1H), 7.43 (d, J = 8.6 Hz, 2H), 7.37 (d, J = 8.6 Hz, 2H), 6.64 (s, 1H), 6.49 (d, J = 16.0 Hz, 1H), 6.32 (dt, J = 16.0, 5.9 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 4.06 – 3.95 (m, 1H), 3.67 (q, J = 8.9 Hz, 1H), 3.42 – 3.37 (m, 1H), 3.28 (dd, J = 15.0, 6.1 Hz, 1H), 2.77 (td, J = 9.7, 5.2 Hz, 1H), 2.67 (dd, J = 17.6, 4.9 Hz, 1H), 2.62 – 2.52 (m, 1H), 2.09 – 1.97 (m, 1H), 1.85 (s, 3H), 1.81 – 1.65 (m, 1H), 1.52 – 1.32 (m, 4H), 1.22 (td, J = 7.1, 2.7 Hz, 3H), 0.82 (dt, J = 14.9, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.05, 166.32, 138.41, 136.40, 131.93, 131.42, 130.62, 129.12, 128.98, 128.86, 128.58, 128.21, 81.30, 75.69, 60.81, 54.75, 54.58, 48.39, 31.01, 26.05, 25.62, 23.51, 14.55, 9.91, 9.41.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(3-chlorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (10g). 70% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.47 (s, 1H), 7.42 – 7.15 (m, 3H), 6.83 (s, 1H), 6.77 – 6.63 (m, 1H), 6.38 – 6.19 (m, 1H), 4.23 (q, J = 7.1 Hz, 2H), 4.19 – 4.11 (m, 1H), 3.99 (dt, J = 13.5, 6.9 Hz, 1H), 3.75 – 3.58 (m, 1H), 3.41 (dq, J = 10.6, 5.2 Hz, 1H), 2.94 (dd, J = 17.7, 4.9 Hz, 1H), 2.80 – 2.56 (m, 1H), 2.45 – 2.27 (m, 1H), 2.03 (s, 3H), 1.62 – 1.44 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.97 – 0.80 (m, 6H).^{13}C NMR (100 MHz, MeOD-d_6): δ 173.04, 165.98, 138.42, 137.31, 134.29, 129.82, 127.95, 127.60, 125.98, 124.68, 82.13, 74.89, 60.83, 54.11, 53.32, 27.92, 25.72, 25.26, 21.84, 13.09, 8.44, 8.16.

4.1.11. General procedure for the synthesis of compounds 11a-11g

The synthetic method was similar to that of compound **2**, and the raw materials of the reactions were **10a-10g**.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-2-methyl-3-phenylallyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11a). White powder, 53% yield, mp: 129.4–130.0°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.32 (s, 1H), 8.15 (dd, J = 18.6, 9.0 Hz, 1H), 7.43 – 7.18 (m, 5H), 6.65 (s, 2H), 4.29 (s, 1H), 3.95 (q, J = 8.9 Hz, 1H), 3.67 (s, 1H), 3.60 –3.20(m, 2H), 3.01 – 2.77 (m, 2H), 2.67 – 2.52 (m, 1H), 1.92 (d, J =

18.6 Hz, 6H), 1.44 (tt, *J* = 10.9, 6.3 Hz, 4H), 0.85 (t, *J* = 7.4 Hz, 3H), 0.80 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.00, 170.88, 167.34, 140.08, 137.99, 137.84, 136.91, 129.54, 129.19, 128.77, 128.67, 128.32, 127.50, 126.46, 81.60, 81.52, 75.06, 54.70, 54.08, 51.46, 51.06, 48.50, 32.95, 26.03, 25.51, 23.87, 23.81, 17.30, 17.06, 9.86, 9.83, 9.31. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 415.2591. Found: m/z 415.2592.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-(dimethylamino)phenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11b). White powder, 59% yield, mp: 143.2–147.0°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.30 (d, *J* = 8.8 Hz, 2H), 6.77 – 6.65 (m, 3H), 6.63 (s, 1H), 5.99 (dt, *J* = 15.3, 7.3 Hz, 1H), 4.18 – 4.01 (m, 2H), 3.84 (dd, *J* = 13.2, 6.7 Hz, 1H), 3.71 (dd, *J* = 13.2, 7.9 Hz, 1H), 3.45 (dp, *J* = 17.0, 5.6 Hz, 2H), 2.94 (s, 7H), 2.54 – 2.42 (m, 1H), 2.03 (s, 3H), 1.59 – 1.47 (m, 4H), 0.90 (q, *J* = 7.5 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 173.27, 172.01, 151.02, 138.64, 132.67, 132.02, 128.54, 127.61, 123.73, 113.32, 112.82, 112.00, 81.90, 74.98, 54.23, 52.26, 46.82, 39.93, 39.17, 27.20, 25.76, 25.12, 21.90, 8.45, 8.18. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 444.2857. Found: m/z 444.2857.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(2-methoxyphenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11c). White powder, 56% yield, mp: 126.7–127.5°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.48 (dd, *J* = 7.7, 1.5 Hz, 1H), 7.34 – 7.26 (m, 1H), 7.19 – 7.13 (m, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.94 (t, *J* = 7.5 Hz, 1H), 6.85 (s, 1H), 6.35 – 6.23 (m, 1H), 4.21 (t, *J* = 8.8 Hz, 1H), 4.10 (dd, *J* = 10.5, 8.0 Hz, 1H), 3.95 (dd, *J* = 13.0, 6.6 Hz, 1H), 3.90 – 3.78 (m, 4H), 3.62 – 3.54 (m, 1H), 3.45 (p, *J* = 5.5 Hz, 1H), 3.07 – 2.98 (m, 1H), 2.58 – 2.48 (m, 1H), 2.05 (s, 3H), 1.61 – 1.47 (m, 4H), 0.96 – 0.85 (m, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.94, 157.42, 156.77, 137.76, 129.98, 129.88, 129.08, 127.94, 127.16, 124.86, 121.05, 120.75, 111.89, 111.12, 81.48, 75.09, 55.89, 54.13, 52.42, 46.70, 43.54, 27.54, 27.19, 26.03, 25.50, 23.85, 9.88, 9.34. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 431.2540. Found: m/z 431.2541.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-methoxyphenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11d). White powder, 50% yield, mp: 135.2–136.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15 (d, *J* = 8.7 Hz, 1H), 7.26 (dd, *J* = 98.3, 8.5 Hz, 2H), 6.89 (dd, *J* = 28.3, 8.5 Hz, 2H), 6.74 – 6.54 (m, 2H), 6.16 (dt, *J*

= 14.8, 6.4 Hz, 1H), 4.16 (d, *J* = 6.6 Hz, 1H), 3.86 (d, *J* = 10.2 Hz, 1H), 3.76 (s, 3H), 3.70 – 3.53 (m, 2H), 3.45 – 3.32 (m, 2H), 2.99 – 2.68 (m, 2H), 2.63 – 2.51 (m, 1H), 2.44 – 2.32 (m, 1H), 1.90 (s, 3H), 1.41 (th, *J* = 13.9, 7.2 Hz, 4H), 0.81 (dt, *J* = 18.3, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.98, 159.69, 137.83, 133.22, 129.67, 128.99, 128.63, 128.26, 114.59, 114.24, 81.49, 75.06, 55.61, 53.94, 52.33, 46.37, 31.70, 26.03, 25.48, 23.87, 9.87, 9.34. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 431.2540 Found: m/z 431.2559.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-fluorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11e). White powder, 51% yield, mp: 185.1–187.1°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.51 (dd, *J* = 8.6, 5.4 Hz, 2H), 7.10 (t, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 15.8 Hz, 1H), 6.83 (s, 1H), 6.21 (dt, *J* = 15.3, 6.9 Hz, 1H), 4.29 – 4.18 (m, 1H), 4.14 – 4.02 (m, 1H), 4.01 – 3.90 (m, 1H), 3.89 – 3.78 (m, 1H), 3.65 – 3.53 (m, 1H), 3.50 – 3.41 (m, 1H), 3.26 – 2.97 (m, 2H), 2.97 – 2.80 (m, 1H), 2.57 – 2.39 (m, 1H), 2.05 (s, 3H), 1.59 – 1.47 (m, 4H), 0.96 – 0.84 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 174.69, 167.04, 163.03 (d, $^1J_{CF}$ = 244.8 Hz), 137.36, 131.92 (d, $^4J_{CF}$ = 3.4 Hz), 129.74, 129.66, 128.51 (2C, d, $^3J_{CF}$ = 8.1 Hz), 117.67, 117.65, 115.22 (2C, d, $^2J_{CF}$ = 21.7 Hz), 114.94, 114.73, 82.23, 74.49, 54.50, 52.11, 48.29, 46.30, 31.36, 25.72, 25.21, 22.02, 8.39, 8.16. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 419.2339. Found: m/z 419.2339.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(4-chlorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11f). White powder, 59% yield, mp: 165.4–167.1°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 8.69 (d, *J* = 8.6 Hz, 2H), 8.59 (d, *J* = 8.5 Hz, 2H), 8.16 – 7.99 (m, 2H), 7.55 – 7.42 (m, 1H), 5.51 (d, *J* = 8.5 Hz, 1H), 5.31 (dd, *J* = 11.3, 8.6 Hz, 1H), 5.20 (dd, *J* = 13.7, 6.3 Hz, 1H), 5.08 (dd, *J* = 13.6, 7.8 Hz, 1H), 4.86 (td, *J* = 10.6, 5.6 Hz, 1H), 4.74 – 4.64 (m, 1H), 4.23 (dd, *J* = 17.0, 5.4 Hz, 1H), 3.83 – 3.70 (m, 1H), 3.30 (s, 3H), 2.82 – 2.63 (m, 4H), 2.08 (dt, *J* = 10.7, 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.24, 167.30, 138.10, 135.36, 133.22, 130.65, 129.26, 128.75, 128.01, 121.90, 81.56, 74.78, 53.77, 51.69, 45.42, 26.31, 26.01, 25.45, 24.00, 9.86, 9.31. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 435.2045. Found: m/z 435.2043.

(3*R*,4*R*,5*S*)-4-acetamido-5-(((*E*)-3-(3-chlorophenyl)allyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (11g). White powder, 55% yield, mp:

179.1–181.2°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.50 (d, J = 16.1 Hz, 1H), 7.46 – 7.15 (m, 4H), 6.87 (d, J = 15.7 Hz, 1H), 6.82 – 6.67 (m, 1H), 6.36 – 6.25 (m, 1H), 4.30 – 4.18 (m, 1H), 4.08 (dd, J = 9.5, 6.8 Hz, 1H), 4.01 – 3.90 (m, 1H), 3.90 – 3.79 (m, 1H), 3.61 – 3.50 (m, 1H), 3.49 – 3.41 (m, 1H), 3.21 – 3.10 (m, 1H), 3.10 – 2.81 (m, 2H), 2.80 – 2.63 (m, 1H), 2.56 – 2.41 (m, 1H), 2.05 (s, 3H), 1.62 – 1.42 (m, 4H), 0.91 (q, J = 7.3 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.06, 167.54, 144.02, 138.72, 137.97, 133.98, 131.05, 128.61, 128.28, 127.54, 126.49, 125.61, 81.50, 75.09, 54.00, 52.42, 45.97, 43.00, 27.59, 26.03, 25.50, 23.90, 9.87, 9.33. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 435.2045. Found: m/z 435.2042.

4.1.12. General procedure for the synthesis of compounds 12a-12b

1.2 equiv. of acid (benzoic acid or bibenzoic acid) was added to the solution of 10 mL triethylamine and 30 mL acetonitrile or dichloromethane of oseltamivir phosphate (0.82 g, 2.0 equiv), HBTU or TBTU (2.4 mmol). The mixture was stirred at room temperature for 5 hours. After TLC detection, the solvent was removed under reduced pressure. Sodium chloride solution (30 mL) was added to the residue and extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed twice with saturated sodium chloride (30 mL). And dried by anhydrous $MgSO_4$, the solvent was removed under reduced pressure after filtering and removing $MgSO_4$, and the crude product was purified by column chromatography to obtain the corresponding intermediates **12a** and **12b**.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-benzamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (12a). 73% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.77 (d, J = 7.3 Hz, 2H), 7.53 (t, J = 7.3 Hz, 1H), 7.45 (t, J = 7.5 Hz, 2H), 6.83 (s, 1H), 4.31 (td, J = 10.7, 5.5 Hz, 1H), 4.27 – 4.15 (m, 3H), 4.07 (dd, J = 11.2, 8.7 Hz, 1H), 3.45 (p, J = 5.6 Hz, 1H), 2.81 – 2.73 (m, 1H), 2.46 (ddt, J = 17.4, 10.4, 2.9 Hz, 1H), 1.86 (s, 3H), 1.62 – 1.45 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.91 (dt, J = 21.3, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.61, 168.81, 166.11, 137.69, 134.20, 131.39, 129.05, 128.20, 126.92, 82.52, 75.60, 60.73, 54.47, 48.61, 29.85, 25.98, 25.47, 21.43, 13.08, 8.47, 8.28.

Ethyl(3*R*,4*R*,5*S*)-5-([1,1'-biphenyl]-4-carboxamido)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (12b). 65% yield, ^{1}H NMR (400 MHz,

DMSO-d_6): δ 8.31 (d, J = 8.9 Hz, 1H), 8.00 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 7.75 – 7.69 (m, 2H), 7.50 (t, J = 7.5 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 6.70 (s, 1H), 4.26 – 4.08 (m, 4H), 4.02 – 3.87 (m, 1H), 3.45 (p, J = 5.6 Hz, 1H), 2.60 (dd, J = 17.5, 5.5 Hz, 1H), 2.50 – 2.40 (m, 1H), 1.72 (s, 3H), 1.55 – 1.32 (m, 4H), 1.23 (t, J = 7.1 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.18, 165.36, 164.92, 142.05, 138.60, 137.75, 132.98, 128.41, 128.07, 127.41, 127.24, 126.27, 125.87, 80.61, 74.40, 59.89, 53.41, 47.90, 29.32, 25.26, 24.70, 22.14, 13.49, 8.85, 8.47.

4.1.13. General procedure for the synthesis of compounds 13a and 13b

The synthetic method was similar to that of compound **2**, and the raw materials of the reaction were compounds **12a** and **12b**

(3*R*,4*R*,5*S*)-4-acetamido-5-benzamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (13a). White powder, 77% yield, mp: 280.0–281.4°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.60 (s, 1H), 8.21 (d, J = 8.9 Hz, 1H), 7.96 (d, J = 9.2 Hz, 1H), 7.79 – 7.70 (m, 2H), 7.58 – 7.49 (m, 1H), 7.45 (t, J = 7.3 Hz, 2H), 6.66 (s, 1H), 4.21 – 4.09 (m, 2H), 3.98 – 3.85 (m, 1H), 3.43 (p, J = 5.6 Hz, 1H), 2.56 (dd, J = 17.6, 5.4 Hz, 1H), 2.48 – 2.34 (m, 1H), 1.70 (s, 3H), 1.54 – 1.32 (m, 4H), 0.85 (t, J = 7.4 Hz, 3H), 0.78 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.24, 167.73, 166.77, 138.21, 135.33, 131.51, 129.81, 128.68, 127.58, 81.61, 75.55, 54.49, 49.05, 30.48, 26.32, 25.73, 23.18, 9.91, 9.52. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 389.2071. Found: m/z 389.2072.

(3*R*,4*R*,5*S*)-5-([1,1'-biphenyl]-4-carboxamido)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (13b). White powder, 70% yield, mp: 187.2–189.7°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.63 (s, 1H), 8.30 (d, J = 8.9 Hz, 1H), 7.99 (d, J = 9.2 Hz, 1H), 7.86 (d, J = 8.4 Hz, 2H), 7.79 – 7.71 (m, 4H), 7.50 (t, J = 7.5 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 6.68 (s, 1H), 4.18 (dt, J = 14.4, 7.9 Hz, 2H), 4.00 – 3.89 (m, 1H), 3.44 (p, J = 5.6 Hz, 1H), 2.58 (dd, J = 17.5, 5.3 Hz, 1H), 2.46 (dd, J = 17.6, 10.5 Hz, 1H), 1.72 (s, 3H), 1.55 – 1.33 (m, 4H), 0.86 (t, J = 7.4 Hz, 3H), 0.79 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.20, 166.65, 165.36, 142.04, 138.61, 137.24, 133.04, 128.73, 128.41, 127.40, 127.24, 126.27, 125.88, 80.57, 74.53, 53.51, 48.02, 38.69, 29.45, 25.28, 24.69, 22.15, 8.86, 8.47. HRMS calcd for

$C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 465.2384. Found: m/z 465.2389.

4.1.14. General procedure for the synthesis of compounds 14a-14e

The synthetic method was similar to that of compounds **12**, and the main raw materials were oseltamivir phosphate and substituted cinnamic acid.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-cinnamamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (14a). 74% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (d, J = 8.9 Hz, 1H, NH), 7.88 (d, J = 9.2 Hz, 1H, NH), 7.56 (d, J = 7.0 Hz, 2H, Ph-H), 7.48 – 7.29 (m, 4H, Ph-H, CH), 6.68 (s, 1H, CH), 6.60 (d, J = 15.8 Hz, 1H, CH), 4.16 (ddd, J = 14.1, 6.7, 4.4 Hz, 3H, CH_2, CH), 4.06 (td, J = 9.9, 5.8 Hz, 1H, CH), 3.85 (q, J = 9.1 Hz, 1H, CH), 3.35 (s, 1H, CH), 2.59 (dd, J = 17.5, 4.9 Hz, 1H, CH), 2.27 (dd, J = 17.4, 10.3 Hz, 1H, CH), 1.74 (s, 3H, CH_3), 1.42 (dtq, J = 20.7, 13.7, 7.0 Hz, 4H, CH_2×2), 1.23 (t, J = 7.1 Hz, 3H, CH_3), 0.85 (t, J = 7.3 Hz, 3H, CH_3), 0.77 (t, J = 7.3 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.92, 165.93, 165.28, 139.07, 138.88, 135.31, 129.90, 129.39, 128.95, 127.96, 122.71, 81.59, 75.52, 60.92, 54.25, 48.04, 30.75, 26.19, 25.71, 23.24, 14.53, 9.86, 9.48.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((*E*)-3-(p-tolyl)acrylamido)cyclohex-1-ene-1-carboxylate (14b). 70% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.96 (d, J = 8.9 Hz, 1H), 7.86 (d, J = 9.2 Hz, 1H), 7.45 (d, J = 7.9 Hz, 2H), 7.37 (d, J = 15.8 Hz, 1H), 7.23 (d, J = 7.8 Hz, 2H), 6.68 (s, 1H), 6.53 (d, J = 15.8 Hz, 1H), 4.15 (tt, J = 6.8, 2.9 Hz, 3H), 4.10 – 4.00 (m, 1H), 3.83 (q, J = 9.1 Hz, 1H), 3.44 – 3.40 (m, 1H), 2.59 (dd, J = 17.6, 4.9 Hz, 1H), 2.41 – 2.18 (m, 4H), 1.74 (s, 3H), 1.53 – 1.31 (m, 4H), 1.23 (t, J = 7.1 Hz, 3H), 0.85 (t, J = 7.3 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.95, 165.95, 165.44, 139.67, 139.05, 138.90, 132.54, 130.00, 128.95, 127.96, 121.63, 81.59, 75.52, 60.95, 54.23, 48.00, 30.77, 26.18, 25.69, 23.25, 21.41, 14.54, 9.88, 9.48.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((*E*)-3-(m-tolyl)acrylamido)cyclohex-1-ene-1-carboxylate (14c). 59% yield, ^{1}H NMR (400 MHz, DMSO-*d*6): δ 12.59 (s, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.43 – 7.33 (m, 3H), 7.30 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7.3 Hz, 1H), 6.65 (s, 1H), 6.59 (d, J = 15.8 Hz, 1H), 4.12 (d, J = 8.0 Hz, 1H), 4.05 (ddd, J = 15.5, 10.3, 5.3 Hz, 1H), 3.83 (q, J = 9.1 Hz, 1H), 3.41 (p, J = 5.4 Hz, 1H), 2.57 (dd, J = 17.6, 5.0 Hz, 1H), 2.33 (s, 3H), 2.28 –

2.18 (m, 1H), 1.74 (s, 3H), 1.41 (dtq, *J* = 20.7, 13.7, 7.1 Hz, 4H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). 13C NMR (100 MHz, DMSO-d_6): δ 169.93, 167.65, 165.31, 139.08, 138.54, 138.37, 135.26, 130.58, 129.60, 129.28, 128.48, 125.18, 122.60, 81.55, 75.65, 54.39, 48.16, 30.89, 26.21, 25.69, 23.24, 21.36, 9.87, 9.48.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((*E*)-3-(4-methoxyphenyl)acrylamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (14d). 53% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88 (dd, *J* = 16.5, 9.1 Hz, 2H), 7.51 (d, *J* = 8.7 Hz, 2H), 7.36 (d, *J* = 15.7 Hz, 1H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.67 (s, 1H), 6.45 (d, *J* = 15.8 Hz, 1H), 4.16 (qd, *J* = 7.0, 2.3 Hz, 3H), 4.05 (tt, *J* = 10.1, 5.1 Hz, 1H), 3.79 (s, 4H), 3.43 (q, *J* = 5.5 Hz, 1H), 2.58 (dd, *J* = 17.7, 5.1 Hz, 1H), 2.37 – 2.16 (m, 1H), 1.74 (s, 3H), 1.54 – 1.31 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 3H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.93, 165.95, 165.58, 160.77, 138.90, 138.80, 129.56, 128.97, 127.86, 120.18, 114.85, 81.59, 75.54, 60.94, 55.72, 54.25, 47.98, 30.81, 26.19, 25.69, 23.26, 14.54, 9.88, 9.49.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((*E*)-3-(4-fluorophenyl)acrylamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (14e). 57% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.00 (d, *J* = 8.9 Hz, 1H), 7.88 (d, *J* = 9.3 Hz, 1H), 7.63 (dd, *J* = 8.7, 5.6 Hz, 2H), 7.41 (d, *J* = 15.8 Hz, 1H), 7.25 (t, *J* = 8.8 Hz, 2H), 6.68 (s, 1H), 6.55 (d, *J* = 15.8 Hz, 1H), 4.23 – 4.11 (m, 3H), 4.11 – 4.00 (m, 1H), 3.90 – 3.79 (m, 1H), 3.42 (p, *J* = 5.6 Hz, 1H), 2.59 (dd, *J* = 17.5, 5.1 Hz, 1H), 2.33 – 2.19 (m, 1H), 1.74 (s, 3H), 1.52 – 1.31 (m, 4H), 1.23 (t, *J* = 7.1 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 3H), 0.77 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.93, 165.93, 165.21, 163.12 (d, $^1J_{CF}$ = 244.6 Hz), 138.87, 137.89, 131.94 (d, $^4J_{CF}$ = 3.2 Hz), 130.12 ((2C, d, $^3J_{CF}$ = 8.4 Hz), 128.94, 122.60, 122.58, 116.35 (2C, d, $^2J_{CF}$ = 21.5 Hz), 81.60, 75.51, 60.92, 54.22, 48.06, 30.75, 26.19, 25.70, 23.24, 14.52, 9.86, 9.48.

4.1.15. General procedure for the synthesis of compounds 15a-15e

The synthetic method was similar to that of compound **2**, the raw materials of the reaction were compounds **14a-14e**.

(3*R*,4*R*,5*S*)-4-acetamido-5-cinnamamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (15a). White powder, 47% yield, mp: 283.3–284.1°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.59 (s, 1H), 7.98 (d, *J* = 8.9 Hz, 1H), 7.84 (d, *J* = 9.2 Hz, 1H),

7.56 (d, *J* = 7.2 Hz, 2H), 7.47 – 7.32 (m, 4H,), 6.65 (s, 1H), 6.60 (d, *J* = 15.8 Hz, 1H), 4.13 (d, *J* = 8.1 Hz, 1H), 4.06 (tt, *J* = 9.9, 5.2 Hz, 1H), 3.83 (t, *J* = 9.0 Hz, 1H), 3.41 (p, *J* = 5.5 Hz, 1H), 2.57 (dd, *J* = 17.6, 5.1 Hz, 1H), 2.29 – 2.18 (m, 1H), 1.74 (s, 3H), 1.42 (th, *J* = 13.8, 7.0 Hz, 4H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.93, 167.65, 165.26, 139.01, 138.37, 135.32, 129.89, 129.61, 129.39, 127.96, 122.76, 81.56, 75.64, 54.38, 48.16, 30.88, 26.21, 25.70, 23.24, 9.87, 9.48. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 415.2227. Found: m/z 415.2227.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((*E*)-3-(p-tolyl)acrylamido)cyclohex-1-ene-1-carboxylic acid (15b). White powder, 49% yield, mp: 281.0–281.5°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.58 (s, 1H), 7.93 (d, *J* = 8.9 Hz, 1H), 7.83 (d, *J* = 9.2 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 15.8 Hz, 1H), 7.22 (d, *J* = 7.9 Hz, 2H), 6.65 (s, 1H), 6.54 (d, *J* = 15.8 Hz, 1H), 4.12 (d, *J* = 7.9 Hz, 1H), 4.05 (ddd, *J* = 15.6, 10.2, 5.2 Hz, 1H), 3.83 (q, *J* = 9.1 Hz, 1H), 3.41 (p, *J* = 5.6 Hz, 1H), 2.56 (dd, *J* = 17.8, 5.1 Hz, 1H), 2.32 (s, 3H), 2.28 – 2.17 (m, 1H), 1.73 (s, 3H), 1.52 – 1.31 (m, 4H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.92, 167.65, 165.41, 139.62, 138.97, 138.38, 132.57, 129.98, 129.60, 127.94, 121.72, 81.55, 75.65, 54.38, 48.13, 39.57, 30.90, 26.21, 25.69, 23.25, 21.39, 9.87, 9.48. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 429.2384. Found: m/z 429.2386.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-((*E*)-3-(m-tolyl)acrylamido)cyclohex-1-ene-1-carboxylic acid (15c). White powder, 51% yield, mp: 267.0–268.1°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.59 (s, 1H), 7.90 (dd, *J* = 45.6, 9.1 Hz, 2H), 7.48 – 7.23 (m, 4H), 7.19 (d, *J* = 7.3 Hz, 1H), 6.77 – 6.47 (m, 2H), 4.12 (d, *J* = 8.0 Hz, 1H), 4.09 – 3.97 (m, 1H), 3.83 (q, *J* = 9.1 Hz, 1H), 3.41 (p, *J* = 5.4 Hz, 1H), 2.57 (dd, *J* = 17.6, 5.0 Hz, 1H), 2.29 – 2.18 (m, 1H), 1.74 (s, 3H), 1.52 – 1.31 (m, 4H), 0.85 (t, *J* = 7.3 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.93, 167.65, 165.31, 139.08, 138.54, 138.37, 135.26, 130.58, 129.60, 129.28, 128.48, 125.18, 122.60, 81.55, 75.65, 54.38, 48.16, 30.89, 26.21, 25.69, 23.24, 21.36, 9.87, 9.48. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 429.2384. Found: m/z 429.2385.

(3*R*,4*R*,5*S*)-4-acetamido-5-((*E*)-3-(4-methoxyphenyl)acrylamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (15d). White powder, 55% yield, mp:

275.1–277.6°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.58 (s, 1H), 7.85 (dd, J = 16.6, 9.1 Hz, 2H), 7.51 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 15.7 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 6.65 (s, 1H), 6.45 (d, J = 15.8 Hz, 1H), 4.12 (d, J = 8.2 Hz, 1H), 4.05 (ddd, J = 15.6, 10.4, 5.3 Hz, 1H), 3.89 – 3.80 (m, 1H), 3.79 (s, 3H), 3.41 (p, J = 5.5 Hz, 1H), 2.56 (dd, J = 17.8, 5.3 Hz, 1H), 2.23 (dd, J = 17.5, 10.3 Hz, 1H), 1.74 (s, 3H), 1.41 (dtq, J = 20.9, 13.9, 7.2 Hz, 4H), 0.85 (t, J = 7.4 Hz, 3H), 0.77 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.93, 167.66, 165.57, 160.76, 138.73, 138.37, 129.62, 129.54, 127.88, 120.26, 114.84, 81.55, 75.67, 55.71, 54.39, 48.11, 30.93, 26.21, 25.69, 23.25, 9.87, 9.48. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 445.2333. Found: m/z 445.2332.

(3*R*,4*R*,5*S*)-4-acetamido-5-((*E*)-3-(4-fluorophenyl)acrylamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (15e). White powder, 55% yield, mp: 279.4–280.7°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.59 (s, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.84 (d, J = 9.2 Hz, 1H), 7.63 (dd, J = 8.5, 5.6 Hz, 2H), 7.41 (d, J = 15.8 Hz, 1H), 7.25 (t, J = 8.8 Hz, 2H), 6.65 (t, J = 2.4 Hz, 1H), 6.55 (d, J = 15.8 Hz, 1H), 4.13 (d, J = 7.8 Hz, 1H), 4.09 – 4.01 (m, 1H), 3.83 (q, J = 9.1 Hz, 1H), 3.41 (p, J = 5.1 Hz, 1H), 2.56 (dd, J = 17.8, 5.0 Hz, 1H), 2.28 – 2.19 (m, 1H), 1.74 (s, 3H), 1.42 (ddp, J = 21.4, 13.8, 7.0 Hz, 4H), 0.85 (t, J = 7.3 Hz, 3H), 0.77 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.93, 167.64, 165.19, 163.12 (d, $^{1}J_{CF}$ = 244.8 Hz), 138.37, 137.84, 131.95 (d, $^{4}J_{CF}$ = 3.1 Hz), 130.12 (2C, d, $^{3}J_{CF}$ = 8.4 Hz), 129.58, 122.65, 116.35 (2C, d, $^{2}J_{CF}$ = 21.5 Hz)., 81.56, 75.63, 54.35, 48.17, 30.87, 26.21, 25.69, 23.24, 9.86, 9.48. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 433.2133. Found: m/z 433.2138.

4.1.16. General procedure for the synthesis of compounds 16a-16g

To a solution of oseltamivir phosphate (0.82 g, 2.0 mmol), 10 mL of triethylamine and 30 mL of acetonitrile or dichloromethane, arylsulfonyl chloride was added (1.2 equiv.) The mixture was stirred at room temperature for 6 hours. After confirming the completion of the reaction by TLC, the solvent was evaporated under reduced pressure. Sodium chloride solution (30 mL) was added to the residue and extracted with ethyl acetate (3 × 40 mL). The combined organic layer was washed twice with saturated sodium chloride (30 mL). And dried with anhydrous $MgSO_4$, Then, filtered and the solvent was removed, the crude product was obtained, and purified by column chromatography to obtain the corresponding intermediates

16a-16g.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(phenylsulfonamido)cyclohex-1-ene-1-carboxylate (16a). 79% yield, 1H NMR (400 MHz, Methanol-d_4): δ 7.86 (d, J = 7.3 Hz, 2H), 7.65 – 7.52 (m, 3H), 6.71 (s, 1H), 4.20 – 4.05 (m, 3H), 3.79 (dd, J = 11.0, 8.8 Hz, 1H), 3.48 (td, J = 10.6, 5.6 Hz, 1H), 3.38 (p, J = 5.6 Hz, 1H), 2.42 (dd, J = 17.7, 5.3 Hz, 1H), 2.17 (ddt, J = 17.2, 10.1, 2.7 Hz, 1H), 1.84 (s, 3H), 1.57 – 1.40 (m, 4H), 1.23 (t, J = 7.1 Hz, 3H), 0.87 (dt, J = 15.2, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.62, 165.82, 142.29, 137.58, 132.14, 128.89, 128.45, 126.30, 82.36, 75.42, 60.64, 54.39, 52.26, 31.35, 25.85, 25.39, 21.63, 13.03, 8.39, 8.23.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-methylphenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (16b). 80% yield, ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.73 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 6.71 (s, 1H), 4.14 (dtt, J = 10.9, 6.9, 3.8 Hz, 3H), 3.78 (dd, J = 11.1, 8.6 Hz, 1H), 3.51 – 3.41 (m, 1H), 3.38 (p, J = 5.7 Hz, 1H), 2.50 – 2.36 (m, 4H), 2.16 (ddt, J = 17.5, 10.1, 2.9 Hz, 1H), 1.85 (s, 3H), 1.55 – 1.40 (m, 4H), 1.24 (t, J = 7.1 Hz, 3H), 0.87 (dt, J = 15.1, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.63, 165.84, 143.15, 139.30, 137.55, 129.39, 128.48, 126.40, 82.36, 75.41, 60.64, 54.37, 52.19, 31.30, 25.85, 25.40, 21.62, 20.04, 13.03, 8.38, 8.23.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-(tert-butyl)phenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (16c). 73% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.69 (t, J = 9.2 Hz, 3H), 7.60 (dd, J = 13.3, 8.8 Hz, 3H), 6.57 (s, 1H), 4.18 – 3.97 (m, 3H), 3.72 – 3.55 (m, 1H), 3.36 – 3.23 (m, 2H), 2.30 – 2.05 (m, 2H), 1.63 (s, 3H), 1.46 – 1.32 (m, 4H), 1.30 (s, 9H), 1.15 (t, J = 7.1 Hz, 3H), 0.81 (t, J = 7.4 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.92, 165.65, 155.43, 140.03, 138.73, 128.29, 126.52, 126.36, 81.50, 75.22, 60.88, 54.00, 52.75, 35.25, 31.61, 31.26, 26.14, 25.61, 23.26, 14.48, 9.86, 9.43.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-chlorophenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (16d). 67% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.82 (d, J = 8.9 Hz, 1H), 7.80 – 7.76 (m, 2H), 7.71 (d, J = 9.1 Hz, 1H), 7.69 – 7.62 (m, 2H), 6.57 (s, 1H), 4.17 – 3.99 (m, 3H), 3.72 – 3.58 (m, 1H), 3.35 –

3.20 (m, 2H), 2.25 (dd, *J* = 17.6, 5.6 Hz, 1H), 2.19 – 2.04 (m, 1H), 1.68 (s, 3H), 1.37 (ddh, *J* = 20.9, 14.0, 7.2 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR: (100 MHz, DMSO-d_6) δ 170.00, 165.62, 141.60, 138.63, 137.44, 129.72, 128.71, 128.24, 81.55, 75.21, 60.90, 53.95, 52.75, 31.56, 26.14, 25.62, 23.29, 14.43, 9.84, 9.43.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-((4-fluorophenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (16e). 68% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.85 (ddd, *J* = 8.8, 5.0, 2.4 Hz, 2H), 7.74 (t, *J* = 9.1 Hz, 2H), 7.49 – 7.36 (m, 2H), 6.57 (s, 1H), 4.20 – 3.98 (m, 3H), 3.73 – 3.55 (m, 1H), 3.32 – 3.21 (m, 1H), 3.08 (q, *J* = 7.2 Hz, 1H), 2.29 – 2.02 (m, 2H), 1.69 (s, 3H), 1.48 – 1.27 (m, 4H), 1.16 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.75 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.02, 165.63, 164.41 (d, $^{1}J_{CF}$ = 248.5 Hz), 139.10 (d, $^{4}J_{CF}$ = 3.0 Hz), 138.64, 129.74 (2C, d, $^{3}J_{CF}$ = 9.4 Hz), 128.23, 116.71 (2C, d, $^{2}J_{CF}$ = 22.5 Hz), 81.54, 75.20, 60.91, 53.93, 52.70, 46.03, 31.51, 26.14, 25.61, 23.32, 14.43, 9.86, 9.44.

Ethyl(3*R*,4*R*,5*S*)-4-acetamido-5-(naphthalene-2-sulfonamido)-3-(pentan-3-yl oxy)cyclohex-1-ene-1-carboxylate (16f). 63% yield, ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 – 8.37 (m, 1H), 8.18 – 8.09 (m, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.85 – 7.74 (m, 2H), 7.74 – 7.61 (m, 3H), 6.55 (s, 1H), 4.07 (d, *J* = 9.4 Hz, 1H), 4.05 – 3.93 (m, 2H), 3.73 – 3.61 (m, 1H), 3.35 – 3.25 (m, 2H), 2.29 (dd, *J* = 17.6, 5.4 Hz, 1H), 2.22 – 2.06 (m, 1H), 1.47 – 1.25 (m, 4H), 1.05 (t, *J* = 7.1 Hz, 3H), 0.80 (t, *J* = 7.4 Hz, 3H), 0.74 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.10, 165.60, 139.55, 138.58, 134.54, 132.16, 129.74, 129.65, 129.03, 128.27, 127.93, 127.31, 122.81, 81.52, 75.19, 60.82, 53.98, 52.80, 31.65, 26.13, 25.61, 23.28, 14.30, 9.82, 9.43.

Ethyl(3*R*,4*R*,5*S*)-5-([1,1'-biphenyl]-4-sulfonamido)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (16g). 85% yield, ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92 – 7.82 (m, 4H), 7.79 – 7.67 (m, 4H), 7.52 (t, *J* = 7.5 Hz, 2H), 7.44 (dd, *J* = 8.2, 6.4 Hz, 1H), 6.58 (s, 1H), 4.10 (dd, *J* = 7.7, 3.6 Hz, 1H), 4.09 – 3.98 (m, 2H), 3.74 – 3.62 (m, 1H), 3.40 – 3.36 (m, 1H), 2.31 (dd, *J* = 17.6, 5.3 Hz, 1H), 2.23 – 2.10 (m, 1H), 1.69 (s, 3H), 1.37 (qp, *J* = 13.9, 7.2 Hz, 4H), 1.11 (t, *J* = 7.1 Hz, 3H), 0.81 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.04, 165.65, 144.05, 141.51, 139.00, 138.68, 129.59, 128.91, 128.30, 127.73, 127.44, 127.40, 81.53, 75.23, 60.87, 54.03, 52.79, 31.64, 26.15, 25.63, 23.32, 14.41,

9.85, 9.44.

4.1.17. General procedure for the synthesis of compounds 17a-17g

The synthetic method was similar to that of compound **2**, and the raw materials of the reaction were compounds **16a-16g**.

(3*R*,4*R*,5*S*)-4-acetamido-3-(pentan-3-yloxy)-5-(phenylsulfonamido)cyclohex-1-ene-1-carboxylic acid (17a). White powder, 53% yield, mp: 214.1–215.9°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (s, 1H), 7.79 (dt, J = 6.8, 1.4 Hz, 2H), 7.69 (dd, J = 8.9, 5.2 Hz, 2H), 7.66 – 7.53 (m, 3H), 6.54 (s, 1H), 4.08 (d, J = 8.5 Hz, 1H), 3.70 – 3.59 (m, 1H), 3.34 – 3.15 (m, 2H), 2.27 – 2.04 (m, 2H), 1.68 (s, 3H), 1.49 – 1.25 (m, 4H), 0.81 (t, J = 7.4 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.05, 167.39, 142.73, 138.18, 132.61, 129.59, 128.95, 126.63, 81.49, 75.30, 54.13, 52.82, 31.66, 26.17, 25.60, 23.33, 9.87, 9.44. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 425.1741. Found: m/z 425.1742.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-methylphenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (17b). White powder, 55% yield, mp: 238.1–239.4°C. ^{1}H NMR (400 MHz, Methanol-d_4): δ 7.73 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 6.72 (s, 1H), 4.11 (d, J = 8.4 Hz, 1H), 3.79 (dd, J = 11.1, 8.7 Hz, 1H), 3.45 (td, J = 10.6, 5.6 Hz, 1H), 3.38 (p, J = 5.7 Hz, 1H), 2.51 – 2.37 (m, 4H), 2.16 (ddt, J = 17.4, 10.2, 2.8 Hz, 1H), 1.86 (s, 3H), 1.56 – 1.39 (m, 4H), 0.87 (dt, J = 15.2, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 172.66, 167.63, 143.16, 139.27, 137.58, 129.38, 128.74, 126.37, 82.35, 75.50, 54.47, 52.26, 31.40, 25.86, 25.40, 21.64, 20.03, 8.38, 8.24. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 439.1897. Found: m/z 439.1901.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-(tert-butyl)phenyl)sulfonamido)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (17c). White powder, 61% yield. mp: 210.5–210.9°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (s, 1H), 7.70 (d, J = 8.6 Hz, 2H), 7.66 (d, J = 9.2 Hz, 1H), 7.58 (d, J = 8.5 Hz, 3H), 6.53 (s, 1H), 4.09 (d, J = 8.4 Hz, 1H), 3.74 – 3.56 (m, 1H), 3.35 – 3.31 (m, 1H), 3.31 – 3.20 (m, 1H), 2.26 – 2.01 (m, 2H), 1.64 (s, 3H), 1.48 – 1.31 (m, 4H), 1.30 (s, 9H), 0.81 (t, J = 7.4 Hz, 3H), 0.75 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.94, 167.40, 155.44, 139.98, 138.13, 128.99, 126.51, 126.38, 81.46, 75.35, 54.13, 52.87, 35.25, 31.73, 31.26, 26.16, 25.60, 23.28, 9.87, 9.42. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 481.2367. Found:

m/z 481.2396.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-chlorophenyl)sulfonamido)-3-(pentan-3-yloxy) cyclohex-1-ene-1-carboxylic acid (17d). White powder, 67% yield, mp: 222.1–223.6°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.58 (s, 1H), 7.86 – 7.75 (m, 3H), 7.73 – 7.62 (m, 3H), 6.54 (s, 1H), 4.07 (d, J = 8.5 Hz, 1H), 3.70 – 3.57 (m, 1H), 3.34 – 3.13 (m, 2H), 2.26 (dd, J = 17.6, 5.5 Hz, 1H), 2.20 – 2.03 (m, 1H), 1.68 (s, 3H), 1.48 – 1.27 (m, 4H), 0.78 (dt, J = 22.9, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.04, 167.38, 141.59, 138.16, 137.39, 129.72, 128.90, 128.66, 81.50, 75.30, 54.10, 52.85, 31.77, 26.16, 25.60, 23.30, 9.86, 9.43. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 459.1351. Found: m/z 459.1348.

(3*R*,4*R*,5*S*)-4-acetamido-5-((4-fluorophenyl)sulfonamido)-3-(pentan-3-yloxy) cyclohex-1-ene-1-carboxylic acid (17e). White powder, 65% yield, mp: 180.7–181.0°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.56 (s, 1H), 7.89 – 7.80 (m, 2H), 7.71 (dd, J = 13.2, 9.0 Hz, 2H), 7.48 – 7.38 (m, 2H), 6.54 (s, 1H), 4.07 (d, J = 8.4 Hz, 1H), 3.73 – 3.55 (m, 1H), 3.35 – 3.13 (m, 2H), 2.24 (dd, J = 17.6, 5.5 Hz, 1H), 2.18 – 2.04 (m, 1H), 1.70 (s, 3H), 1.50 – 1.27 (m, 4H), 0.78 (dt, J = 22.2, 7.4 Hz, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.06, 167.37, 164.38 (d, $^{1}J_{CF}$ = 247.7 Hz), 139.10 (d, $^{4}J_{CF}$ = 3.0 Hz), 138.14, 129.69 (2C, d, $^{3}J_{CF}$ = 9.4 Hz), 128.93, 116.70 (2C, d, $^{2}J_{CF}$ = 22.4 Hz), 81.50, 75.29, 54.10, 52.81, 31.71, 26.17, 25.61, 23.32, 9.85, 9.44. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 443.1647. Found: m/z 443.1666.

(3*R*,4*R*,5*S*)-4-acetamido-5-(naphthalene-2-sulfonamido)-3-(pentan-3-yloxy)c yclohex-1-ene-1-carboxylic acid (17f). White powder, 63% yield. mp: 225.2–226.7°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.54 (s, 1H), 8.45 – 8.37 (m, 1H), 8.19 – 8.09 (m, 2H), 8.04 (d, J = 7.8 Hz, 1H), 7.85 – 7.73 (m, 2H), 7.73 – 7.60 (m, 3H), 6.53 (s, 1H), 4.06 (d, J = 8.4 Hz, 1H), 3.74 – 3.58 (m, 1H), 3.34 – 3.22 (m, 2H), 2.32 (dd, J = 17.7, 5.4 Hz, 1H), 2.24 – 2.09 (m, 1H), 1.61 (s, 3H), 1.47 – 1.25 (m, 4H), 0.80 (t, J = 7.4 Hz, 3H), 0.73 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.16, 167.37, 139.50, 138.12, 134.51, 132.15, 129.74, 129.66, 129.04, 128.95, 128.27, 127.94, 127.29, 122.78, 81.48, 75.28, 54.14, 52.91, 31.92, 26.16, 25.60, 23.29, 9.84, 9.43. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 475.1897. Found: m/z 475.1894.

(3*R*,4*R*,5*S*)-5-([1,1'-biphenyl]-4-sulfonamido)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (17g). White powder, 58% yield, mp: 252.5–254.9°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.56 (s, 1H), 7.97 – 7.81 (m, 4H), 7.80 – 7.61 (m, 4H), 7.52 (t, J = 7.5 Hz, 2H), 7.44 (t, J = 7.3 Hz, 1H), 6.55 (s, 1H), 4.10 (d, J= 8.2 Hz, 1H), 3.67 (q, J = 9.0 Hz, 1H), 3.37 – 3.34 (m, 2H), 2.31 (dd, J = 17.6, 5.3 Hz, 1H), 2.23 – 2.07 (m, 1H), 1.69 (s, 3H), 1.49 – 1.27 (m, 4H), 0.81 (t, J = 7.4 Hz, 3H), 0.76 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.08, 167.41, 144.04, 141.51, 139.04, 138.15, 129.59, 128.99, 128.90, 127.76, 127.48, 127.36, 81.49, 75.33, 54.19, 52.90, 31.83, 26.17, 25.62, 23.33, 9.86, 9.44. HRMS calcd for $C_{25}H_{32}N_2O_4S$ $[M + H]^+$: 501.2054. Found: m/z 501.2053.

4.2. *In Vitro* Anti-Influenza Virus Assay and Cytotoxicity Assay in CEFs

The anti-influenza activity of the newly synthesized oseltamivir derivatives were evaluated with H5N1, H5N2, H5N6 and H5N8 strains in Chicken Embryo Fibroblast cells using hemagglutination titer method. Results were expressed as IC_{50} values, which meant the concentration of compounds needed to inhibit 50% proliferate of H5N1, H5N2, H5N6 and H5N8 viruses in CEFs. The prepared CEFs solution (1×10^5 cells / mL) was added to 96-well cell plate at a volume of 100 μL / well. The culture medium was removed after incubation in a cell incubator (37°C, 5.0% CO_2) for 24 hours, and washed with serum-free DMEM. A 100 μL dilution of an equal volume of 100 $TCID_{50}$ of H5N1, H5N2, H5N6 and H5N8 strains was mixed with an equal volume of a continuous one-half dilution of the compound solution (1% FBS in DMEM) and incubated for 1 h. The mixture was inoculated into prepared 96-well cell plates to infect CEFs. The cell plates inoculated with avian influenza virus were incubated in a cell culture incubator (37°C, 5.0% CO_2) for 72 hours. The virus titer assay was performed on each of the virus proliferation solutions in each well according to the standard method [28]. The titer of the virus growth solution under the corresponding concentration of the compound, and the inhibition rate at the corresponding concentration were obtained according to the titer data. The IC_{50} value of the inhibitor was determined by fitting the curve between virus proliferation and NAIs concentration. At the same time, the virus control group and the normal cell control group were detected.

We used the CCK-8 method to detect the 50% cytotoxic concentration values

(CC_{50}). CEFs solution (1×10^5 cells / mL) was prepared to add to 96-well cell plate at a volume of 100 μL/well. The culture medium was removed after incubation in a cell incubator (37°C, 5.0% CO_2) for 24 hours, and washed with serum-free DMEM. The equal volume of 2-fold continuously diluted new compound solution (1% FBS DMEM) was inoculated into the prepared 96-well cell plate and incubated in the cell incubator (37°C, 5.0% CO_2) for 48 hours. Then 100 μL culture medium (10 μL CCK-8 and 90 μL 1% FBS DMEM medium) was added to each well. After incubation in the cell incubator (37°C, 5.0% CO_2) for 90 minutes, the absorbance at 450 nm was read on the microplate reader. The CC_{50} values of inhibitors were determined by fitting the curve of cytopathic effect (CPE) and NA inhibitor concentration. At the same time, blank control and cell control were set up in each experiment.

4.3. *In Vitro* Neuraminidase Enzyme Inhibitory Assay

The NA inhibition assay was carried out according to the standard method [29]. Dilute suspensions of influenza viruses (H5N1, H5N2, H5N6 and H5N8) were harvested from the allantoic fluid of influenza virus-infected embryonated chicken eggs. A/Anhui/1/2005 (H5N1-H274Y mutation) was obtained from Sino Biological Inc. 2'-4-(methylumbelliferyl)-α-*D*-acetylneuraminic acid sodium salt hydrate (MUNAN; Sigma) as a substrate was cleaved by NA to afford a quantifiable fluorescent product. The 96-well fluorescent plate was incubated at 37°C for 10 minutes by adding 10 μL diluted virus supernatant or NA assay diluent, 70 μL of MES buffer and 10 μL of test compound at different concentrations. Then 10 μL fluorescent substrate was added to each well to start the reaction and incubated at 37°C for 40 to 60 minutes. After incubation, 150 μL of termination solution (6.01 g glycine and 3.20 g NaOH dissolved in 400 mL Milli-Q water) was added to each well to terminate the reaction. The fluorescence was measured with an Envision plate reader (PerkinElmer, Wellesley, MA) using excitation and emission wavelengths of 365 and 460 nm, respectively. Substrate blanks were subtracted from the sample readings. The inhibitor IC_{50} values were determined from the dose-response curves by plotting the percent inhibition of NA activity versus inhibitor concentration.

4.4. Molecular Docking

Molecular modeling of compounds **9a** and **11e** were performed using the Tripos

molecular modeling package Sybyl-X 2.0 [30, 31]. All compounds used for docking are constructed using standard bond lengths and angles from Sybyl-X 2.0/Base Builder and are optimized for 10000 generations until the maximum derivative of energy becomes 0.005 kcal/(mol * A). The flexible docking method (Surflex-Dock) docked the ligand automatically into the ligand-binding site of the receptor by the use of protocol-based approach and an empirically derived scoring function. Before docking, the protein was prepared by removing the ligand, water molecules, and other unnecessary small molecules from the crystal structure of the ligand-protein complex (PDB code: 2HU0 and 2HT7); then polar hydrogen atoms and charges were added to the protein. Surflex-Dock default settings were used for other parameters, such as the maximum number of rotatable bonds per molecule (set to 100) and the maximum number of poses per ligand (set to 20). During docking, all single bonds in the residue side chain in the defined NA binding pocket are considered rotatable or flexible, and the ligand was allowed to rotate at all single bonds and to move flexibly within the tentative binding pocket [31]. The atomic charges were recalculated using the Kollman all-atom approach for the protein and the Gasteig-Hückel approach for the ligand. The binding interaction energy was calculated, containing van der Waals, electrostatic, and torsional energy terms defined in the Tripos force field. The structure optimization was performed for 10000 generations using a genetic algorithm, and the 20-best-scoring ligand−protein complexes were kept for further analysis. After the protocol was generated, the optimized **9a** and **11e** were docked into the binding pockets.

4.5. Acute Toxicity Experiment

Kunming mice (15-17 g) were purchased from the Animal Experimental Center of Shandong University. The animal feeding procedure was carried out according to the guidelines of the Animal Care and use Committee of Shandong University, and was approved by the Animal Ethics and Welfare Committee (AEWC). The mice were fed at 25 ± 1°C and humidity of 60 ± 10%, 12 hours of light and 12 hours of darkness every day, the mice were given free access to food and water, changed the cushion every two days, and fed for one week to evaluate the acute toxicity of compound **11e**. We purchased 20 healthy Kunming mice, half male and half female, and randomly divided them into two groups (5 female mice and 5 male mice per group). Each group

was fed separately with 5 male mice and 5 female mice. Compound **11e** was prepared with 5% DMSO, 50% PEG-400 and 45% water at a concentration of 0.15 g /mL, After fasting for 12 h, a group of mice (5 male and 5 female mice) was given a dose of 1.5 g/kg by intragastric administration [32]. At the same time, the blank group was given the same solution without **11e**. Death, body weight, and behavior (death, lethargy, clonic convulsion, anorexia, ruffled fur, and no abnormality) were monitored every day. At the end of the experiment, all animals were sacrificed for subsequent experimental studies.

Conflicts of Interest

The authors declare no conflict of interest.

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Highlights

1. By exploiting 150-cavity of influenza virus neuraminidase, novel oseltamivir derivatives were reported.
2. Compounds **9a** and **11e** showed similar or greater activity than OSC in both NA inhibitory activity and cellular assay.
3. Compound **11e** demonstrated low cytotoxicity in vitro and low acute toxicity in mice.