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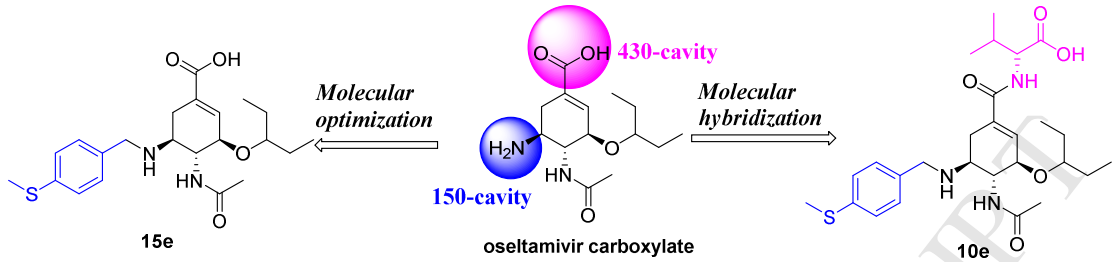
Graphical abstract



IC_{50} (μM) = 0.044 ± 0.0049 (N1, H5N1)
8.34 ± 0.69 (N2, H5N2)
1.40 ± 0.061 (N1, H5N1-H274Y)
EC_{50} (μM) = 0.66 ± 0.13 (H5N1)
0.48 ± 0.10 (H5N2)

IC_{50} (μM) = 0.067 ± 0.0095 (N1, H5N1)
0.0045 ± 0.31 (N2, H5N2)
2.45 ± 0.31 (N1, H5N1-H274Y)
EC_{50} (μM) = 0.82 ± 0.07 (H5N1)
0.17 ± 0.07 (H5N2)

IC_{50} (μM) = 0.21 ± 0.0012 (N1, H5N1)
> 100 (N2, H5N2)
> 100 (N1, H5N1-H274Y)
EC_{50} (μM) = > 100 (H5N1)
26.56 ± 2.40 (H5N2)

Design, Synthesis and Biological Evaluation of "Multi-Site"-Binding Influenza Virus Neuraminidase Inhibitors

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Abstract:

Encouraged by our earlier discovery of neuraminidase inhibitors targeting 150-cavity or 430-cavity, herein, to yield more potent inhibitors, we designed, synthesized, and biologically evaluated a series of novel oseltamivir derivatives via modification of C-1 and C5-NH_2 of oseltamivir by exploiting 150-cavity and/or 430-cavity. Among the synthesized compounds, compound **15e**, the most potent N1-selective inhibitor targeting 150-cavity, showed 1.5 and 1.8 times greater activity than oseltamivir carboxylate (OSC) against N1 (H5N1) and N1 (H5N1-H274Y). In cellular assays, **15e** also exhibited greater potency than OSC against H5N1 with EC_{50}

of 0.66 μM. In addition, **15e** demonstrated low cytotoxicity in vitro and low acute toxicity in mice. Molecular docking studies provided insights into the high potency of **15e** against N1 and N1-H274Y mutant NA. Overall, we envisioned that the significant breakthrough in the discovery of potent group-1-specific neuraminidase inhibitors may lead to further investigation of more potent anti-influenza agents.

Keywords: Influenza virus, Neuraminidase inhibitors, 430-cavity, 150-cavity, Oseltamivir derivatives.

1. Introduction

In the recent years, the Influenza A and B virus infection has become a serious threat for human health with the potential to cause epidemics or pandemics with mass casualties. These viruses belong to the Orthomyxoviridae family of negative sense, single stranded, and segmented RNA viruses [1]. In April 2009, a new type Swine flu, also known as influenza A (H1N1) rapidly spread worldwide through human-to-human transmission, giving rise to a serious public panic [2]. H7N9, a novel and highly virulent avian-origin influenza A virus, occurred to infect human in eastern China in the spring 2013. By the end of January 2017, 1161 people were infected with H7N9, with 433 deaths, and the mortality rate was as high as 37.3% [3]. In addition, the highly pathogenic avian influenza A (H5N1) virus has resulted in a fatality rate over 60%, which is a great threat to human [4,5]. Though there is no clear evidence of efficient human-to-human transmission [6,7], the worrying possibility

remains that further adaptation of avian flu for person-to-person transmission may lead to a global influenza pandemic.

Influenza A virus is enveloped by a lipid membrane containing two important surface antigenic glycoproteins namely hemagglutinin (HA) and neuraminidase (NA) [8], which play crucial roles in the life cycle of viruses [9]. There are different subtypes according to the distinct antigenic properties of each protein: eighteen for HA (H1–H18) and eleven for NA (N1–N11) [10,11]. NA cleaves the connection between the hemagglutinin and the host cell to release newly formed virion from infected cells and facilitate propagation of the virus [12], making this enzyme a promising target for anti-influenza drugs [13]. Currently, four neuraminidases inhibitors (NAIs) have been developed for the prophylaxis and treatment of patients infected with influenza A or B viruses [14,15], including oral oseltamivir (Tamiflu) [16], inhaled zanamivir (Relenza) [17], intravenous peramivir (Rapivab) [18] and inhaled laninamivir octanoate (Inavir) [19] (**Figure 1**). Among the approved drugs, orally administered oseltamivir (a prodrug of oseltamivir carboxylate) (OSC) has been a first-line therapy since its approval in 1999. However, resistance to oseltamivir has constantly been reported due to its widely used in clinic such as the most frequent NA substitutions reported in H5N1 (H274Y) [20-22]. In addition, various mutants with resistance to the others NA inhibitors have also appeared [20,21,23,24]. Therefore, there is an urgent and continuing need for the next generation neuraminidase inhibitors.

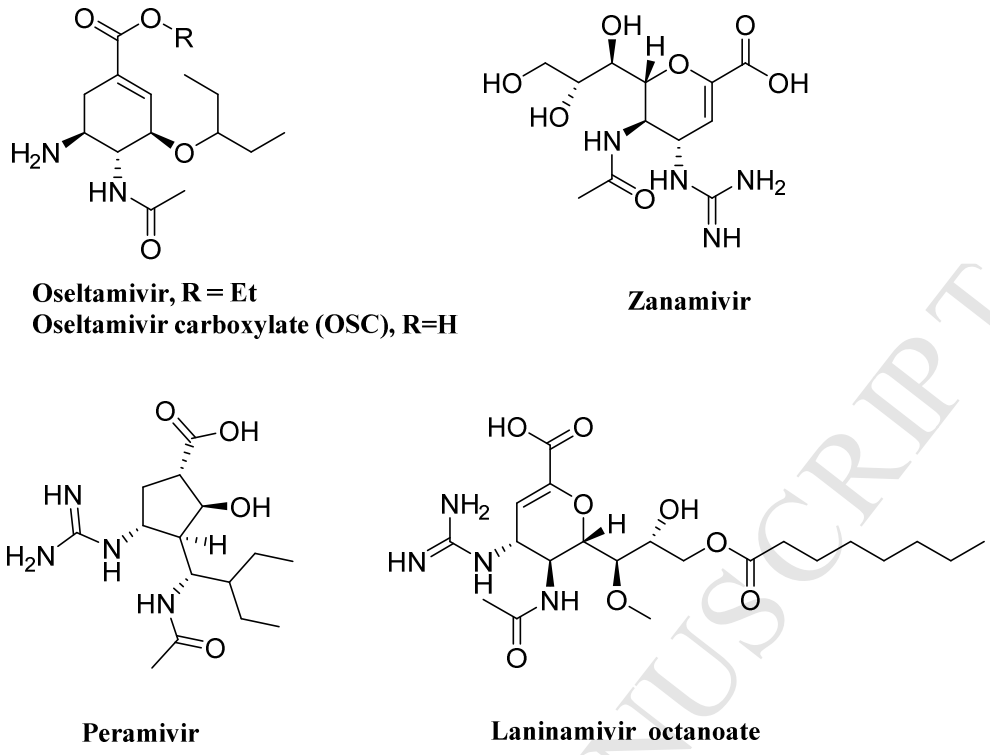


Figure 1. Structures of approved NA inhibitors for the treatment of influenza virus infection.

With the exception of N10, recently identified in a bat influenza A virus genome [25], the N1−N9 subtypes can be categorized phylogenetically into two groups: group-1 (N1, N4, N5, and N8 subtypes), and group-2 (N2, N3, N6, N7, and N9 subtypes) [26]. The X-ray crystallographic structures show that in group-1 NAs [except N1 of 2009pdmH1N1 (09N1)] [27], there is a large cavity termed the 150-cavity (formed by an open conformation of the 150-loop containing residues 147–151) adjacent to the active site, which is absent in group-2 NAs [28] (**Figure 2**). Based on this finding, several C-5 amino-substituted oseltamivir derivatives **JMC32**, **JMC20I** [29], and **JMC21h** [30] (**Figure 3**) have been identified to inhibit H5N1 (group-1 NA) at low-nanomolar level (approximately 8-fold, 9-fold and 28-fold more potent than OSC, respectively) through targeting the 150-cavity in our lab. In 2013,

Wu and co-workers demonstrated for the first time that oseltamivir carboxylate could induce the rigid closed N2 150-loop into a half-open one [25]. Thus, there was another significant discovery in our lab that a series of N-substituted oseltamivir derivatives such as **JMC15b** and **JMC15c** (**Figure 3**) showed exceptionally active against both group-1 and -2 NAs, especially for 09N1, N2, N6, and N9 subtypes (6.8−12.5 and 1.2−3.9 times greater activity than OSC). They also showed an increase in inhibitory activity of about 4.2-fold and 2.5-fold relative to OSC toward H274Y and E119V variant, respectively [31].

Moreover, there has also been evidenced the existence of another auxiliary binding site adjacent to sialic acid binding site called “430-cavity” (formed by the 430-loop comprising residues Arg430-Thr439 [32]) (**Figure 2**) which could be exploited for the design of new antivirals has been revealed [33]. It was worth noting that, 430-cavity adopted a more open conformation in various group-1 and -2 subtypes, and could provide greater chemical space for further modification [34,35]. In 2018, our lab reported a panel of C-1 modified oseltamivir derivatives via targeting 430-cavity. Among them, compound **EJMC8b** (**Figure 3**) exhibited the best inhibitory activity with IC_{50} values of 0.088 μM and 0.097 μM against H5N1 and H5N6 NAs, respectively. Besides this, **EJMC5c** (**Figure 3**) also showed a moderate activity against H5N1 and H5N6 (IC_{50} = 0.94 and 2.78 μM, respectively) [36].

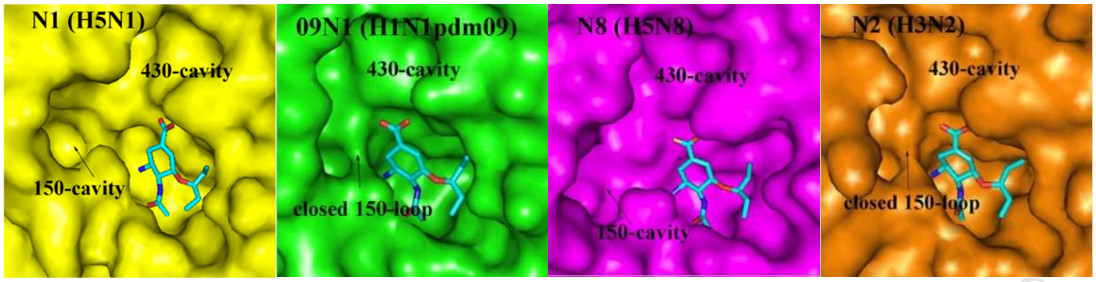


Figure 2. Comparison of the crystal structures of N1 (H5N1, PDB ID: 2HU0), 09N1 (H1N1pdm09, PDB ID: 3TI6), N8 (H5N8, PDB ID: 2HT7) and N2 (H3N2, PDB ID: 4GZP) bound with OSC. It can be seen that 09N1, along with N2 have a closed 150-loop, but N1 and N8 have an open 150-cavity. Moreover, 430-cavity widely exists in a variety of subtypes including group-1 (N1, 09N1 and N8) as well as group-2 (N2).

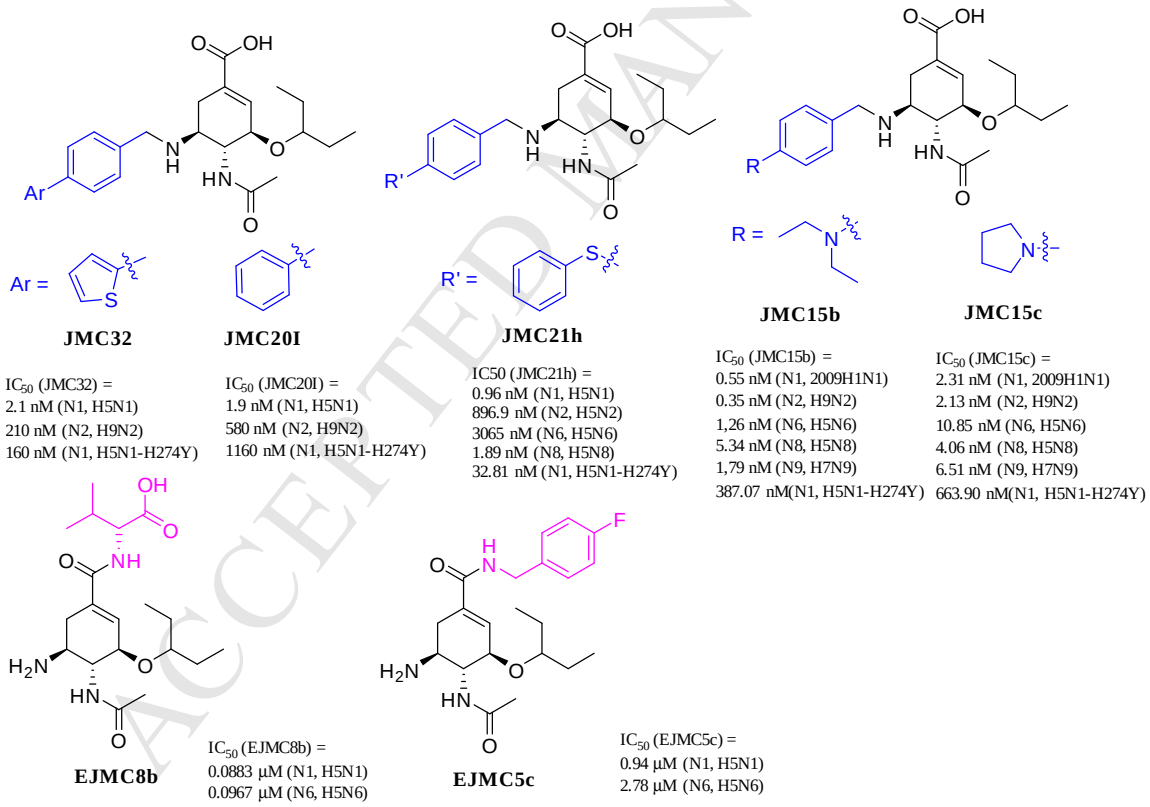


Figure 3. Structures of the group-1 selective NA inhibitors (**JMC32**, **JMC20I** and **JMC21h**) and nonspecific group-1 and group-2 NA inhibitors (**JMC15b** and **JMC15c**) targeting 150-cavity, as well as oseltamivir derivatives (**EJMC8b** and **EJMC5c**) targeting 430-cavity.

The main objective of our research was to discover potent group-1 selective NA inhibitors functioning as anti-influenza agents via exploiting the 150-cavity and/or 430-cavity adjacent to the NAs active site of influenza A. Recently, several group-1 selective NA inhibitors were discovered by structure-based design targeting the activity site and 150-cavity simultaneously. Notably, **JMC20I** and **JMC21h**, containing hydrophobic *p*-phenyl and *p*-phenylthiobenzyl groups, respectively, showed more potent N1-selective inhibitory activity than OSC [29,30]. Besides, another report revealed that a series of C-5 amino-substituted oseltamivir derivatives such as **JMC15b** and **JMC15c** bearing *N,N*-diethylamino and 1-pyrrolidine groups, respectively, exhibited excellent inhibitory activity against both group-1 and -2 NAs [31]. On the other hand, the exploration of 430-cavity resulted in the discovery of **EJMC8b** (IC_{50}: 0.088 and 0.097 μM) and **EJMC5c** (IC_{50}: 0.94 and 2.78μM) exerted the greatest and moderate inhibition against H5N1 and H5N6, respectively [36].

Encouraged by these results, in this manuscript, using molecular hybridization strategy, the well-matched privileged moieties of 150-cavity and 430-cavity were introduced into C-5 and C-1 positions of oseltamivir core structure simultaneously to fill these two cavities (**Figure 4**). Finally, 21 novel oseltamivir analogues were designed, synthesized, and evaluated their biological activity.

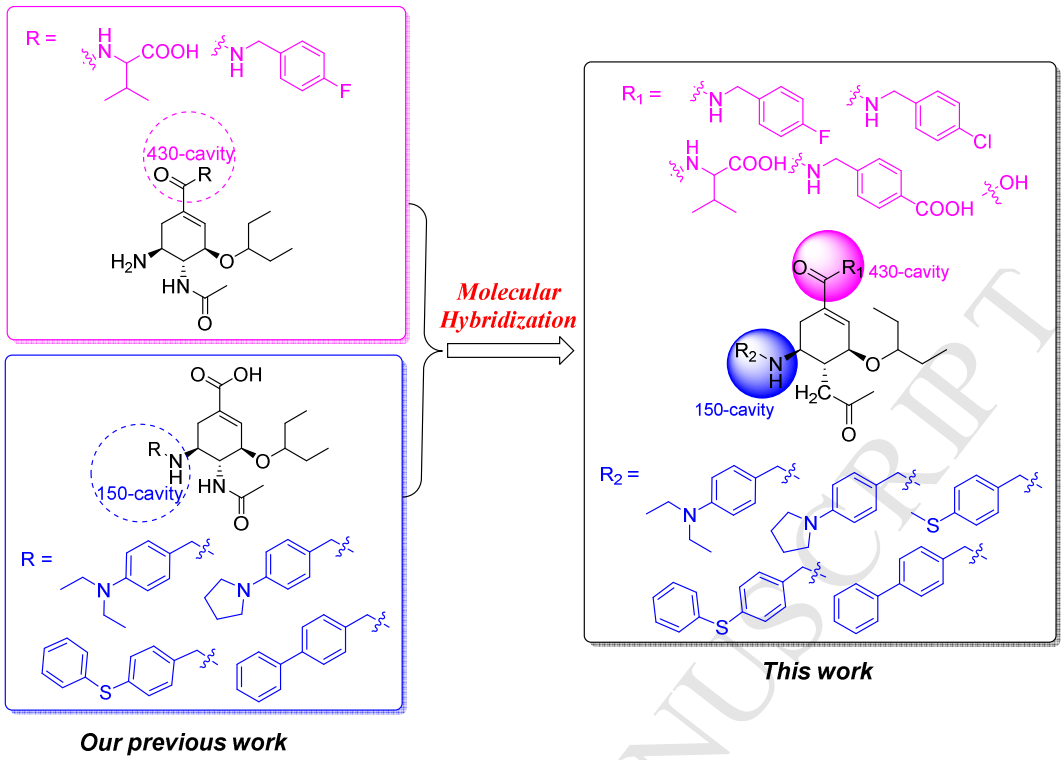


Figure 4. Strategy for structure optimization of oseltamivir analogues via targeting 150-cavity and/or 430-cavity.

2. Results and discussion

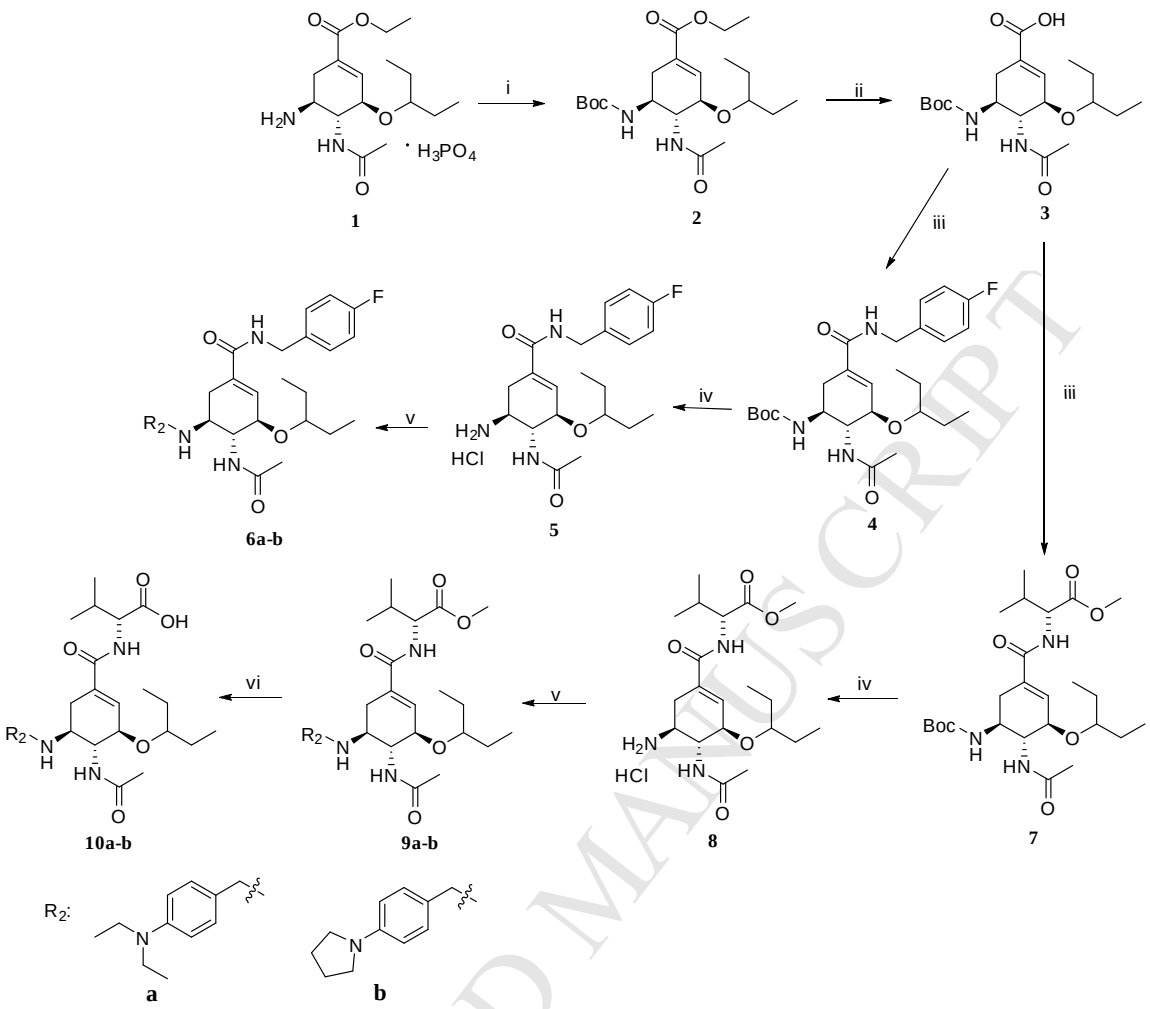
2.1. Chemistry

The synthetic route of C-5 and/or C-1 modified oseltamivir derivatives (**6a–e**, **10a–e**, **16a–e**, **18a–e**) and intermediate (**13**) were conducted following reactions depicted in **Scheme 1, 2** and **3**. All derivatives were synthesized by well-established methods using commercially available oseltamivir phosphate **1** as the primary starting material. In **Scheme 1,** oseltamivir phosphate **1** was treated with Boc-anhydride and DMAP in CH_2Cl_2 and Et_3N to afford **2**, which was hydrolyzed in the presence of 4 M NaOH aqueous solution and acidified with 3 M HCl aqueous solution to give the key intermediate **3**. Subsequently, the intermediate **3** reacted with corresponding aminesin

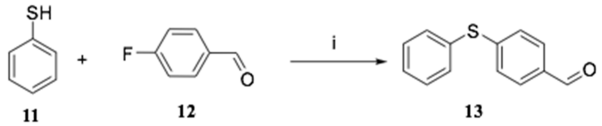
the presence of HBTU and DMAP to afford **4** and **7**, and then prepared as hydrochlorides **5**and **8**, respectively, with ethyl acetate solution of hydrogen chloride. Subsequently, the target compounds **6a–b** and **9a–b** were prepared via reacting with corresponding aldehydes in the presence of $NaBH_3CN$, and eventually **9a–b** were hydrolyzed by NaOH to give target compounds **10a–b**.

As shown in **Scheme 2**, the 4-(phenylthio) benzaldehyde (**13**) were formed by reaction of benzenethiol (**11**) with 4-fluorobenzaldehyde (**12**) in the presence of potassium carbonate.

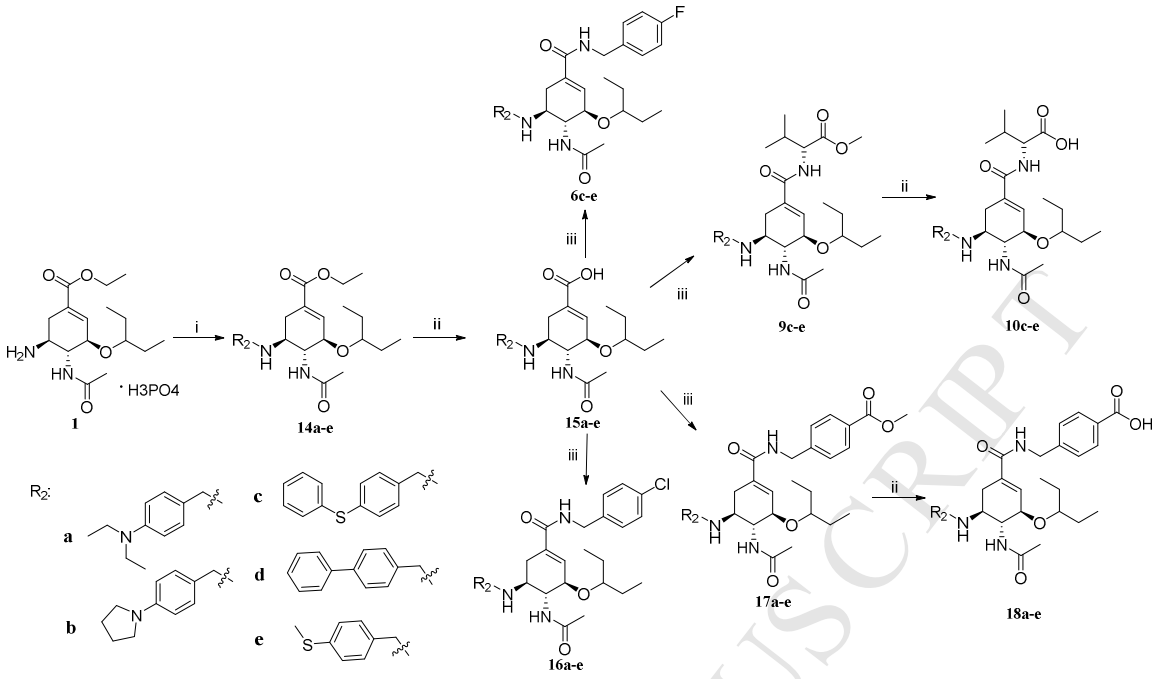
As outlined in **Scheme 3**, the synthesis of compounds **6c–e**, **10c–e**, **16a–e** and **18a–e** were carried out starting with oseltamivir phosphate **1**. **1** was treated with a series of different aldehydes in the presence of $NaBH_3CN$ to afford **14a–e**, which followed by hydrolyzation in the presence of 4 M NaOH to give the key intermediates **15a–e**. The target compounds (**6c–e**, **16a–e**) and intermediates (**9c–e** and **17a–e**) were prepared via similar procedures (different corresponding amines) as compounds **4** and **7**, and finally the intermediates (**9c–e** and **17a–e**) were hydrolyzed in the presence of NaOH to result in the corresponding acids **10c–e** and **18a–e**.



Scheme 1. Reagents and conditions: (i) $(Boc)_2O$, Et_3N, CH_2Cl_2, DMAP, rt; (ii) 4M NaOH, CH_3OH, rt, then 3M HCl; (iii) corresponding amines, HBTU, DMAP, Et3N, CH_3CN, rt; (iv) HCl/ethyl acetate, rt; (v) corresponding aldehydes, $NaBH_3CN$, CH_3OH, rt; (vi) 4M NaOH, CH_3OH, rt, then 3M HCl.



Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, 120° C.



Scheme 3. Reagents and conditions: (i) corresponding aldehydes, $NaBH_3CN$, CH_3OH, rt; (ii) 4M NaOH, CH_3OH, rt, then 3M HCl; (iii) corresponding amines, HBTU, DMAP, Et_3N, CH_3CN, rt.

2.2. Biological activity

2.2.1. In vitro inhibitory activities of Influenza Neuraminidases

For NA inhibition screening against N1 (H5N1) from group-1 and N2 (H5N2) from group-2, and all the newly synthesized 21 oseltamivir derivatives and oseltamivir carboxylate (OSC) were evaluated using a chemiluminescence-based assay with 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) as the substrate [29]. The measured inhibition potencies of the synthesized compounds toward N1 (H5N1), N2(H5N2) as well as N1 (H5N1-H274Y) were summarized in **Table 1**. It was clear that OSC showed more potency against N2 (IC_{50} = 0.0045 μM) than N1 (IC_{50} = 0.067 μM), meanwhile, it exhibited less potency toward the H5N1-H274Y mutant (IC_{50} = 2.45 μM), which was consistent with reported data

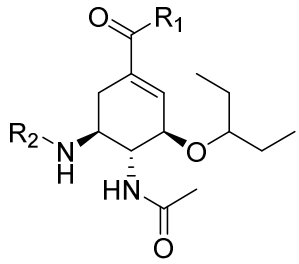
[29,30,31,36].

As shown in **Table 1**, the series of oseltamivir derivatives (**6a–e, 10a–e, 16a–e, 18a–e**) exhibited significantly decreased activities compared with the positive drug OSC and "dual-site"-binding NA inhibitors, confirming that "three-site"-binding oseltamivir analogues were not suitable for binding with NAs. Compared with OSC, **6a–e** and **10a–e** demonstrated less potent or no activities against H5N1, H5N2 and H5N1-H274Y. Among **6a–e**, compounds **6c–e** with R_2 group of 4-phenylthio benzyl, 4-phenyl benzyl and 4-methylsulfanyl benzyl, respectively, showed better inhibitory activities against N1 (H5N1) (IC_{50} = 8.33 μM, 22.14 μM and 34.64 μM, respectively) than N2 (H5N2) (IC_{50} > 100 μM). In contrast, **6b** replaced by 4-(pyrrolidin-1-yl) benzyl at the R_2 position resulted in similar activities for both H5N1 and H5N2. The inhibitory effects of **10a–e** bearing *D*-valine of R_1 group toward the three NA subtypes performed unfavorable activities, and similarly, **10c–e** were N1-selective inhibitors but **10b** was 23-fold more potent against H5N2 compared to H5N1. Impressively, compound **10e** exerted robust inhibitory activity against H5N1 with IC_{50} value of 0.21 μM. As for compounds **16a–e** with 4-chloro benzyl at the R_1 position, all the five inhibitor molecules were showing much lower potency than OSC. Among them, compounds **16b** and **16e** bearing 4-(pyrrolidin-1-yl) benzyl and 4-methylsulfanyl benzyl groups, respectively, exhibited selective inhibition against H5N2 (with IC_{50} value of 0.94 μM) and H5N1 (with IC_{50} value of 27.39 μM), respectively. In addition, when the R_1 group was substituted by 4-carboxy benzyl, the

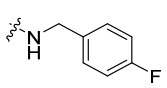
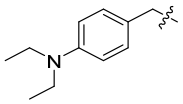
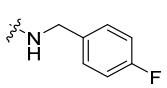
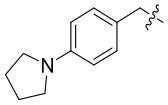
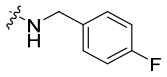
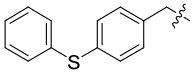
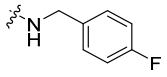
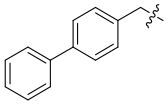
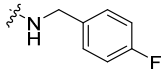
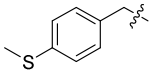
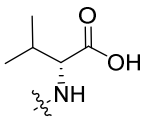
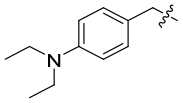
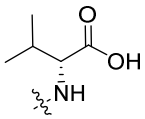
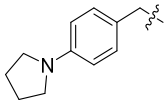
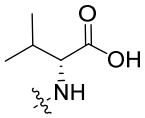
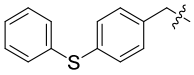
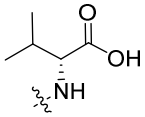
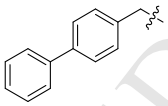
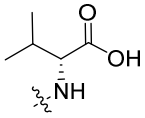
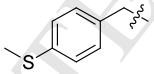
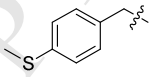
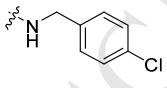
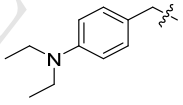
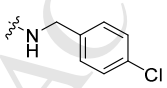
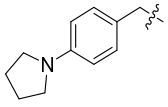
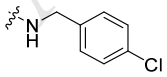
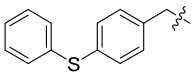
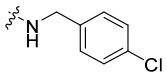
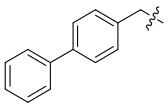
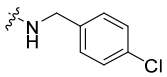
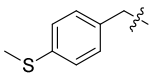
resulting compounds **18a–e** appeared to have a similar effect on the potency as **6a–e** and **10a–e**. It was worth noting that IC_{50} values of **18a** and **18b** were lower against N2 (H5N2) than against N1 (H5N1) of group-1. Indeed, the IC_{50} value of **18c** against NAs of H5N1 and H5N1-H274Y were approximately 46 and 3 times lower than OSC for the same NAs, respectively.

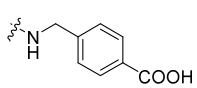
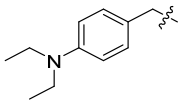
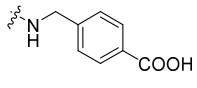
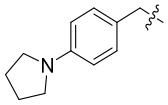
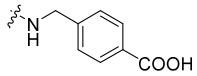
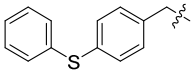
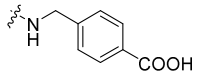
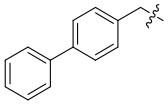
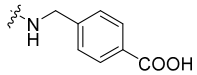
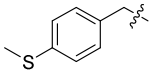
Fortunately, the inhibitory effects of **15e** bearing hydroxyl and 4-methylsulfanyl benzyl at the R_1 and R_2-position, respectively, had equal inhibitory potency to OSC. In particular, **15e** showed a high degree of selectivity for N1 over N2. Compound **15e** was approximately 1.5- and 1.8-fold more potent against N1 (H5N1) and N1 (H5N1-H274Y) than the OSC and was deemed a suitable lead compound for further investigation.

Table 1. NA Inhibitory Activities of Target Compounds **6a–e, 10a–e, 15e,16a–e** and **18a–e.**



			NA Enzyme-Inhibitory Assay, IC_{50} (μM)[a]		
compds	R_1	R_2	H5N1[b]	H5N2[c]	H5N1-H274Y[d]
			group-1	group-2	group-1

6a			> 100	> 100	> 100
6b			20.17 ± 1.87	48.43 ± 2.92	> 100
6c			8.33 ± 0.76	> 100	> 100
6d			22.14 ± 1.50	> 100	> 100
6e			34.64 ± 5.64	> 100	> 100
10a			> 100	> 100	> 100
10b			56.58 ± 7.35	2.41 ± 0.23	> 100
10c			9.34 ± 0.77	> 100	48.82 ± 3.30
10d			70.03 ± 8.52	> 100	> 100
10e			0.21 ± 0.0012	> 100	> 100
15e			0.044 ± 0.0049	8.34 ± 0.69	1.40 ± 0.061
16a			> 100	> 100	> 100
16b			31.81 ± 2.59	0.94 ± 0.09	> 100
16c			> 100	> 100	> 100
16d			> 100	> 100	> 100
16e			27.39 ± 0.61	> 100	> 100

18a			41.02 ± 6.44	13.28 ± 0.73	> 100
18b			15.53 ± 2.83	4.70 ± 0.31	> 100
18c			3.07 ± 0.53	> 100	8.49 ± 0.57
18d			> 100	> 100	> 100
18e			8.38 ± 1.19	> 100	> 100
OSC			0.067 ± 0.0095	0.0045 ± 0.31	2.45 ± 0.31

[a]Concentration required to reduce NA activity to 50% of control NA activity (IC_{50}) and values are shown as mean ± SD of three experiments.

[b]A/goose/Guangdong/SH7/2013. [c] A/Chicken/Hebei/LZF/2014. [d]A/Anhui/1/2005.

Next, we investigated the potency of some representative compounds (**10c**, **10e**,**15e**, **16e**) against influenza B neuraminidase by the same assay, and data was shown in **Table 2**. Compound **15e** exhibited weaker activity against influenza B NA, approximately 215-fold lower than OSC. The other compounds **10c**, **10e** and **16e** showed no activities toward influenza B **NA**. The X-ray crystallographic structural information supports the conclusion that influenza B neuraminidases are similar to the group-2 enzymes [26].

Table 2. The Influenza Virus B[a] NA Inhibitory Activities of Selected Compounds.

compds	**10c**	**10e**	**15e**	**16e**	**OSC**
IC_{50} (μM)[b]	> 100	> 100	7.97 ± 1.27	> 100	0.037 ± 0.0014

[a]B/PHUKET/3073/2013.

[b]Concentration required to reduce NA activity to 50% of control NA activity (IC_{50}). Values are shown as mean ± SD of three experiments.

2.2.2. In vitro anti-influenza virus activity

Anti-influenza virus potency of representative compounds **10e, 15e** and **16b** was evaluated in Chicken Embryo Fibroblast cells infected with A/goose/Guangdong/SH7/2013 (H5N1) as well as A/Chicken/Hebei/LZF/2014 (H5N2), and oseltamivir carboxylate (OSC) were selected as reference compounds in parallel. The values of EC_{50} (antiavian influenza A virus potency) and CC_{50} (cytotoxicity) of the selective compounds were summarized in **Table 3**. Notably, all the tested compounds with the exception of **16b** exhibited no appreciable cytotoxicity at the highest tested concentrations (CC_{50} > 200 μM) in chicken embryo fibroblasts (CEFs).

In case of the H5N1 viruses, the activity of **15e** (EC_{50} = 0.66μM) was superior to that of OSC (EC_{50} = 0.82 μM). As for H5N2 virus, **15e** performed poor potent inhibitory activity than OSC, which is in accordance with their lower potency for NA of H5N2. In contrast, **10e** and **16b** displayed no or little antiviral activity against both H5N1 and H5N2. Overall, the anti-H5N1 and -H5N2 activities of the novel oseltamivir derivatives were consistent with the results of the NAs (H5N1 and H5N2)-inhibitory activities, suggesting these compounds inhibited influenza virus replication by binding to NAs in cellular level.

Table 3. Anti-Influenza Virus Activity and Cytotoxicity of Oseltamivir Derivatives.

Compds	EC_{50}[a] values (μM) towards influenza viruses		CC_{50}[b]
	H5N1[c]	H5N2[d]	
	group-1	group-2	
10e	> 100	26.56 ± 2.40	> 200
15e	0.66 ± 0.13	0.48 ± 0.10	> 200
16b	> 100	> 100	77.13 ± 20.83
OSC	0.82 ± 0.07	0.17 ± 0.07	> 200

[a]EC_{50}: concentration of compound required to achieve 50% protection of CEF cultures against influenza virus-induced cytotoxicity, presented as the mean ± standard deviation (SD) and determined by the CCK-8 method.

[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.

2.3. Molecular modeling

To postulate the interactions of the newly synthesized compounds with the target and to account for the experimental results, molecular docking studies of representative compounds **15e** and **10e** to the N1 NA (PDB 2HU0) were conducted using SurflexeDock SYBYL-X 2.0 software. PyMOL was used to visualize the results. Firstly, by comparing the amino acid sequences of A/goose/Guangdong/SH7/2013 (H5N1)-N1 with the protein as used for the docking studies, we found that amino acid sequence of A/goose/Guangdong/SH7/2013

(H5N1)-N1 showed a high degree (> 95%) of similarity to 2HU0. Only 19 of the 387 residues were different between the two proteins, which might make a great contribution to the credibility of the docking studies. The docking protocol was described in the molecular docking section.

As shown in **Figure 5**, the 4-methylthiobenzyl group of compound **15e** (Figure **5A**) occupied the 150-cavity while the other structural elements of this compound interacted with the active site in a manner similar to the binding pattern of oseltamivir carboxylate, and the "three-site"-binding compound **10e** (Figure **5C**) bounded with active site, 150-cavity and 430-cavity simultaneously. This result was in good agreement with the purpose of our design. As can be seen in **Figure 5B**, compound **15e** showed key interactions in both the active site and the 150-cavity as expected. These key interactions include the electrostatic interaction between the carboxylate group of **15e** and Arg292, Tyr347 and Tyr406, as observed in the X-ray crystal structure of N1 bound to OSC. Moreover, **15e** formed a strong hydrogen-bonding interaction between the 5-amino linker and Asp151, which was not observed in OSC. Furthermore, the 4-methylthiobenzyl group of **15e** was well adapted to the 150-cavity of NA, which was surrounded mostly by polar and non-polar residues. It was clearly shown in **Figure 5D** that the carboxyl on valine of compound **10e** formed four hydrogen-bonds with Arg292, Tyr347, Tyr406 and Arg18 around 430-cavity region, which had less hydrogen-bonds than C1-carboxyl of compound **15e**. Meanwhile, the hydrogen-bond with Asp151 at C5-NH- of **10e** was not observed, which might be another reason for the decreased enzyme inhibitory activity. Thus, the molecular

modeling helped to interpret the binding mode of the newly designed compounds with NA, and explained the reason why compound **15e** exhibited high potency but **10e** showed less potency against NA.

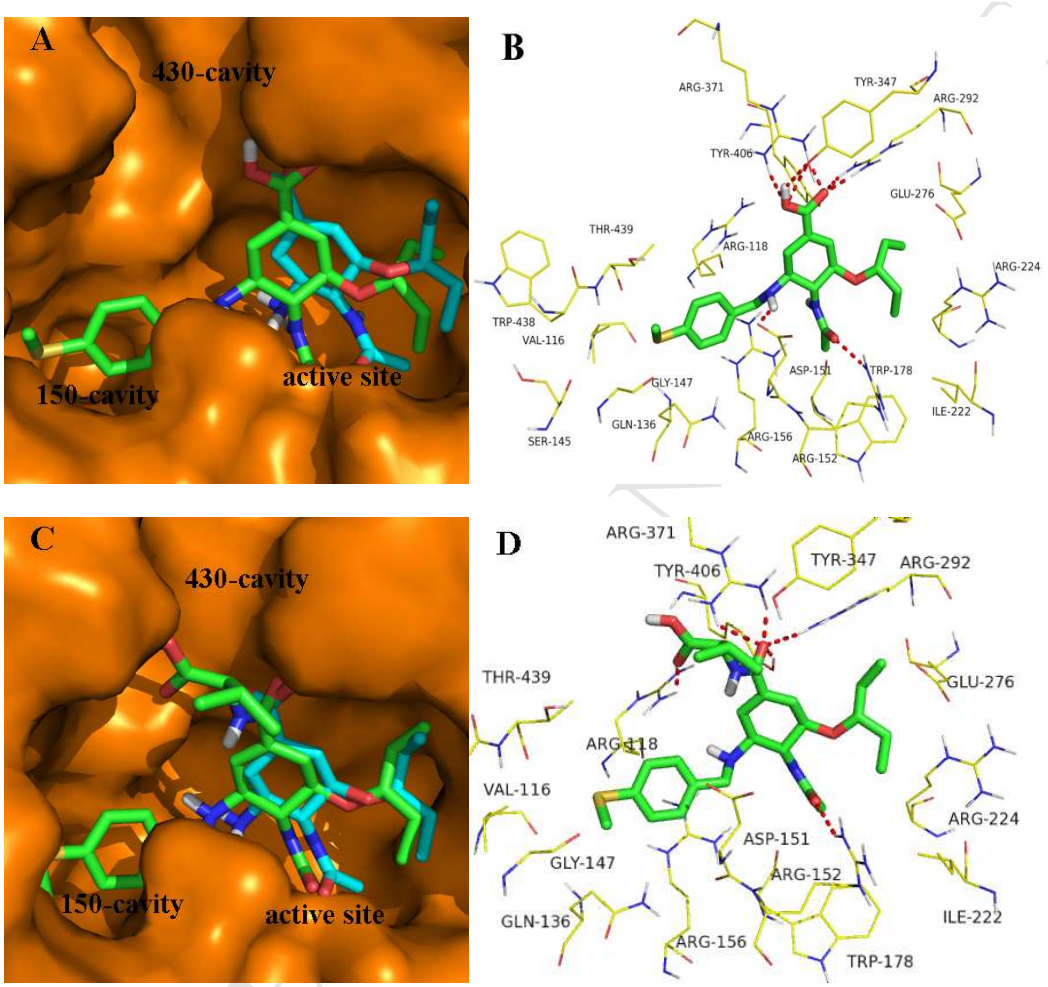


Figure 5. Binding modes of compounds **15e** (green, **B**) and **10e** (green, **D**) with N1 (PDB code: 2HU0) and superposition with the binding mode of OSC (cyan, **A** and **C**).

2.4. Safety assessment

A single-dose toxicity test of compound **15e** was conducted in Kunming mice. After intragastric administration of **15e** at a dose of 2 g/kg, no death occurred and

there was no abnormity of body weight increase over the subsequent week as shown in **Figure 6** and **Table 4**.

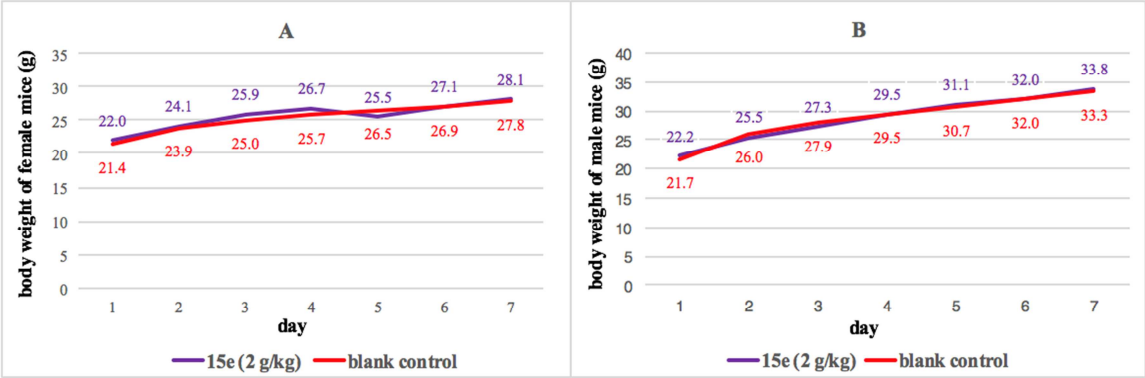


Figure 6. There were no obvious differences in the weight gain from the two different treatment groups. A: body weight of female mice (g)-time (day); B: body weight of male mice (g)-time (day).

Table 4. Clinical Behaviors after Administration of **15e** in Mice

		Day, number of mice										
		1					2	3	4	5	6	7
Dose of **15e** (g/kg)	Clinical behaviors	10min	30min	1h	3h	6h						
Female mice												
Blank 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
2	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												
Blank 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
2	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

In conclusion, to explore the chemical space of 430-cavity and 150-cavity in NAs, a series of novel oseltamivir derivatives were designed, synthesized and evaluated by modifying C1-COOH and C5-NH_2 of OSC. Among 20 "three-site"-binding inhibitors, we found that the NA-inhibitory potency were sharply decreased in comparison with OSC and "dual-site"-binding compounds, suggesting that it was unreasonable to introduce large groups to bind with 150-cavity and 430-cavity simultaneously. On the other hand, **15e**, group-1-specific NA inhibitor targeting 150-cavity, exhibited similar or greater inhibitory potency against N1 (H5N1) and N1 (H5N1-H274Y), with IC_{50} value of 0.044 μM and 1.40 μM, respectively, compared to the activities against N2 (H5N2) and influenza B virus NA (IC_{50} value of 8.34 μM and 7.97 μM, respectively). In cellular assay, **15e** displayed better activity than OSC against H5N1, in accordance with the results of the NA-inhibitory activities. Moreover, **15e** demonstrated favorable pre-clinical safety profile in Kunming mice by intragastric administration. Considering these in vitro and in vivo results, we envision that the further optimization of oseltamivir targeting 150- and 430-cavities via introducing suitable size groups will afford more potent compounds with improved drug-resistance profile.

4. Experimental section

4.1. Chemistry

The key chemical reactant oseltamivir phosphate was provided by Shandong Qidu Pharmaceutical Co., Ltd. The other common chemicals and reagents such as

HBTU, DMAP, corresponding amines and amino acids, etc. were purchased from Aladdin, TCI, J&K, ENERGY CHEMICAL, and Sino pharm Chemical Reagent Co., Ltd. with purities of at least 97%. Solvents were obtained from commercial suppliers and were purified and dried by means of standard methods when necessary. Thin layer chromatography (TLC) was performed using plates coated with Silica Gel GF254 for TLC (Merck), and spots were detected under UV light (254 nm). Flash column chromatography was conducted on Silica Gel (200-300 mesh), purchased from Qingdao Haiyang Chemical Company. Melting points (mp) were measured on a micro melting point apparatus (RY-1G, Tianjin TianGuang Optical Instruments) and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a Bruker AV-400 spectrometer in CD_3OD or DMSO-d_6 with TMS as the internal standard. Coupling constants (J) were reported in hertz (Hz), and chemical shifts were given in parts per million (ppm) from TMS. ESI-MS was carried out using an API 4000 LC/MS spectrometer (Applied Biosystems, USA). High resolution mass spectrometry (HRMS) was obtained on an Agilent 6520 Q-TOF LC/MS spectrometer (Agilent, Germany).

4.1.1. General procedure for the synthesis of ethyl (3R, 4R, 5S)-4-acetamido-5-((*tert*-butoxycarbonyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate (**2)**

To a solution of commercially available oseltamivir phosphate **1** (8.2 g, 0.02 mol, 1 equiv) in 50 mL dichloromethane was added di-*tert*-butyl dicarbonate

($(Boc)_2O$, 8.72 g, 2 equiv), DMAP (0.2 g) and triethylamine (TEA, 30 mL) at room temperature, and the mixture was stirred for 2 h. Subsequently, the solution was evaporated under reduced pressure to remove dichloromethane and TEA and then saturated citric acid solution was added. After that, the mixture was extracted with ethyl acetate, and the organic phase was dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give the crude product, which was purified by recrystallization form isopropyl ether to afford **2** as a white powder, yield 87.2%. mp: 149.1–150.2°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 6.76 (s, 1H, CH), 4.20 (q, *J* = 7.1 Hz, 2H, CH_2), 4.10 (d, *J* = 8.3 Hz, 1H, CH), 3.85 (dd, *J* = 11.0, 8.7 Hz, 1H, CH), 3.76 – 3.66 (m, 1H, CH), 3.40 (p, *J* = 5.6 Hz, 1H, CH), 2.68 (dd, *J* = 17.7, 5.1 Hz, 1H, CH), 2.30 – 2.18 (m, 1H, CH), 1.95 (s, 3H, CH_3), 1.56 – 1.41 (m, 13H, $2CH_2$, $3CH_3$), 1.29 (t, *J* = 7.1 Hz, 3H, CH_3), 0.95 – 0.83 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.34, 166.13, 156.67, 137.67, 129.05, 82.33, 78.85, 75.71, 60.68, 54.77, 48.95, 30.48, 27.33, 25.87, 25.40, 21.60, 13.09, 8.46, 8.26. ESI-MS: m/z 413.5 $[M + H]^+$, $C_{21}H_{36}N_2O_6$ (412.52).

4.1.2. General procedure for the synthesis of (3R,4R,5S)-4-acetamido-5-((*tert*-butoxycarbonyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylic acid (**3)**

To a solution of intermediate **2** (5 g, 12.1 mmol) was dissolved in 70 mL methanol, and 4N NaOH aqueous solution was added until the pH to 12, and then the solution was stirred at room temperature for 2 h. The reaction solution was evaporated

under reduced pressure to remove methanol, then the residue was taken up in water (30 mL), and the pH was adjusted to 2 with 3 M HCl aqueous solution. This solution was extracted with ethyl acetate and tetrahydrofuran (V:V = 2:1, 4 × 30 mL). The combined organic phase was washed with saturated sodium chloride (2 × 30 mL) and water (30 mL), then dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to afford **3** as white powder, yield 80.37%. mp: 208.9–209.8°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 6.78 (s, 1H, CH), 4.11 (d, *J* = 8.3 Hz, 1H, CH), 3.85 (dd, *J* = 11.1, 8.7 Hz, 1H, CH), 3.71 (td, *J* = 10.7, 5.4 Hz, 1H, CH), 3.46 – 3.36 (m, 1H, CH), 2.68 (dd, *J* = 17.7, 5.1 Hz, 1H, CH), 2.30 – 2.17 (m, 1H, CH), 1.96 (s, 3H, CH_3), 1.56 – 1.40 (m, 13H, $2CH_2$, $3CH_3$), 0.96 – 0.84 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.42, 168.00, 156.73, 137.71, 129.30, 82.37, 78.89, 75.81, 54.84, 49.01, 30.56, 27.33, 25.88, 25.40, 21.61, 8.37. ESI-MS: m/z 385.4 $[M + H]^+$, $C_{19}H_{32}N_2O_6$ (384.47).

4.1.3. General procedure for the synthesis of compounds **4** and **7**.

To a solution of acid **3** (1.25 g, 3.1 mmol), HBTU (1.6 g, 3.7 mmol), and Et_3N (10 mL) in 20 mL acetonitrile was added 1.2 equimolar amount of the appropriate amine. The mixture was kept stirring at room temperature for 5 h. TLC detection found that the reaction was complete, then the mixture was evaporated under reduced pressure to remove solvent. The residue was taken up saturated sodium chloride and 1 mL 3N HCl and extracted with ethyl acetate (30 mL × 3), and the combined organic layers were washed two times with saturated sodium chloride and 1 mL 3N HCl. The

organic layers were dried ($MgSO_4$), filtered, and solvent was removed under reduced pressure at 40°C to give the crude product, which was purified by column chromatography to produce the corresponding intermediate, **4** and **7**.

Tert-butyl((1S,5R,6R)-6-acetamido-3-((4-fluorobenzyl)carbamoyl)-5-(pentan-3-yloxy)cyclohex-3-en-1-yl)carbamate (**4**). White powder, 75.4% yield, mp: 186.1–188.2°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.30 (dd, *J* = 8.4, 5.5 Hz, 2H, 2Ph-H), 7.03 (t, *J* = 8.8 Hz, 2H, 2Ph-H), 6.47 (s, 1H, CH), 4.45 – 4.34 (m, 2H, CH_2), 4.10 (d, *J* = 8.2 Hz, 1H, CH), 3.86 (dd, *J* = 11.0, 8.6 Hz, 1H, CH), 3.73 (td, *J* = 10.5, 5.3 Hz, 1H, CH), 3.46 – 3.36 (m, 1H, CH), 2.64 (dd, *J* = 17.3, 5.0 Hz, 1H, CH), 2.38 – 2.25 (m, 1H, CH), 1.96 (s, 3H, CH_3), 1.57 – 1.39 (m, 13H, overlapped, $2CH_2$, $3CH_3$), 0.89 (dt, *J* = 12.4, 7.4 Hz, 6H, $2CH_3$).^{13}C NMR (100 MHz, CD_3OD) δ: 172.40, 168.05, 163.25, 160.82, 156.64, 134.78, 132.57, 132.07, 129.02, 82.33, 78.87, 75.79, 54.83, 49.05, 42.12, 30.75, 27.32, 25.85, 25.34, 21.58, 8.36. ESI-MS: m/z 492.4 $[M + H]^+$, $C_{26}H_{38}FN_3O_5$ (491.60).

Methyl((3R,4R,5S)-4-acetamido-5-((*tert*-butoxycarbonyl)amino)-3-(pentan-3yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**7**). White powder, 42.8% yield, mp: 195.1–196.2°C.^{1}H NMR (400 MHz, CD_3OD) δ: 6.42 (s, 1H), 4.32 (d, *J* = 6.8 Hz, 1H), 4.11 (d, *J*= 8.2 Hz, 1H), 3.87 (dd, *J* = 10.9, 8.6 Hz, 1H), 3.80 – 3.68 (m, 4H), 3.47 – 3.39 (m, 1H), 2.63 (dd, *J* = 17.4, 5.1 Hz, 1H), 2.39 – 2.26 (m, 1H), 2.18 (dq, *J* = 13.6, 6.8 Hz, 1H), 1.96 (s, 3H), 1.59 – 1.39 (m, 13H), 1.01 – 0.82 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.30, 168.83, 156.65, 132.52, 132.13, 82.34, 78.85,

75.76, 58.35, 54.84, 51.10, 48.98, 30.76, 30.20, 27.32, 25.82, 25.40, 21.58, 18.16, 17.60, 8.37. ESI-MS: m/z 498.5 $[M + H]^+$, $C_{25}H_{43}N_3O_7$ (497.62).

4.1.4. General procedure for the synthesis of compounds **5** and **8**.

The compound **4** (0.6 g) was dissolved in 30.0 mL ethyl acetate solution of hydrogen chloride and the mixture was stirred at room temperature for 2 h. When the reaction was complete, the mixture was evaporated under reduced pressure to remove solvent and was recrystallized from isopropyl ether to afford **5**. Compound **8** was prepared using the same method as **5**.

(3R,4R,5S)-4-acetamido-5-amino-N-(4-fluorobenzyl)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**5**). White powder, 72.1% yield, mp: 83.1–84.2°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.32 (dd, *J* = 8.4, 5.5 Hz, 2H, 2Ph-H), 7.03 (dd, *J* = 8.7 Hz, 2H, 2Ph-H), 6.50 (s, 1H, CH), 4.41 (s, 2H, CH_2), 4.26 (d, *J* = 8.6 Hz, 1H, CH), 3.98 (dd, *J* = 11.3, 8.5 Hz, 1H, CH), 3.54 (td, *J* = 10.6, 5.6 Hz, 1H, CH), 3.46 (p, *J* = 5.6 Hz, 1H, CH), 2.86 (dd, *J* = 17.6, 5.1 Hz, 1H, CH), 2.58 – 2.44 (m, 1H, CH), 2.05 (s, 3H, CH_3), 1.52 (qd, *J* = 14.1, 7.0 Hz, 4H, $2CH_2$), 0.93 – 0.85 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.39, 167.53, 160.85, 134.70, 132.09, 130.34, 129.12, 114.83, 114.61, 82.33, 74.31, 53.03, 49.49, 42.15, 28.43, 25.75, 25.16, 21.84, 8.40, 8.18. ESI-MS: m/z 392.4 $[M + H]^+$, $C_{21}H_{30}FN_3O_3$ (391.48).

Methyl((3R,4R,5S)-4-acetamido-5-amino-3-(pentan-3-yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**8**). Pale yellow sticky substance, 70.1% yield. ^{1}H NMR (400 MHz, CD_3OD) δ: 6.51 (s, 1H, CH), 4.33 (d, *J* = 6.8 Hz, 1H, CH), 4.27 (d, *J* = 8.3 Hz,

1H, CH), 3.99 (dd, *J* = 14.0, 5.4 Hz, 1H, CH), 3.72 (s, 3H, CH_3), 3.59 – 3.42 (m, 2H, overlapped, 2CH), 2.83 (dd, *J* = 17.3, 5.3 Hz, 1H, CH), 2.58 – 2.44 (m, 1H, CH), 2.19 (dq, *J* = 13.6, 6.8 Hz, 1H, CH), 2.05 (s, 3H, CH_3), 1.63 – 1.45 (m, 4H, $2CH_2$), 1.01 – 0.86 (m, 12H, overlapped, $4CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.38, 172.17, 168.29, 132.32, 130.16, 82.37, 74.27, 58.42, 53.06, 51.13, 49.49, 30.18, 28.47, 25.72, 25.22, 21.82, 18.15, 17.62, 8.30. ESI-MS: m/z 398.5 $[M + H]^+$, $C_{20}H_{35}N_3O_5$ (397.51).

4.1.5. General procedure for the synthesis of compounds **6a–b, 9a–b**.

To a solution of compound **5** or **8** (2.0 mmol, 1 equiv) and aldehydes (2.4 mmol, 1.2 equiv) in 30 mL ethanol, $NaBH_3CN$ (4.0 mmol, 2 equiv) was slowly added. The mixture was stirred at room temperature for 6 h and then concentrated. To the residue, 20 mL saturated NaCl and 10 mL saturated Na_2CO_3 solution was added, and the mixture was extracted with EtOAc. The combined extracts were dried over anhydrous $MgSO_4$ and concentrated to give the crude product, which was purified by column chromatography to produce the corresponding target compounds **6a–b** and intermediates **9a–b**.

(3R,4R,5S)-4-acetamido-5-((4-(diethylamino)benzyl)amino)-N-(4-fluorobenzyl)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**6a**). Yellow powder, 75.3% yield, mp: 82.6–84.3°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.31 (dd, *J* = 8.2, 5.6 Hz, 2H, 2Ph-H), 7.10 (d, *J* = 8.5 Hz, 2H, 2Ph-H), 7.03 (t, *J* = 8.7 Hz, 2H, 2Ph-H), 6.66 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.48 (s, 1H, CH), 4.41 (s, 2H, CH_2), 4.03 (d, *J* = 8.1 Hz, 1H, CH), 3.96 – 3.87 (m, 1H, CH), 3.74 (d, *J* = 12.5 Hz, 1H, CH), 3.55 (d, *J* = 12.5 Hz,

1H, CH), 3.45 – 3.31 (m, 5H, overlapped, $2CH_2$, CH), 2.90 (td, *J* = 10.0, 5.3 Hz, 1H, CH), 2.78 (dd, *J* = 17.2, 5.0 Hz, 1H, CH), 2.37 – 2.22 (m, 1H, CH), 1.99 (s, 3H, CH_3), 1.49 (dt, *J* = 14.5, 7.2 Hz, 4H, $2CH_2$), 1.11 (t, *J* = 7.0 Hz, 6H, $2CH_3$), 0.96 – 0.80 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.51, 168.31, 163.25, 160.83, 147.11, 134.84, 132.59, 131.77, 129.31 – 128.92, 125.83, 114.82, 114.60, 112.20, 82.02, 75.77, 54.54, 53.94, 49.00, 44.10, 42.17, 29.73, 25.73, 25.19, 21.75, 11.43, 8.49, 8.18. HRMS calcd for $C_{32}H_{45}FN_4O_3$ $[M + H]^+$: 553.3548. Found: m/z 553.3546.

(3R,4R,5S)-4-acetamido-N-(4-fluorobenzyl)-3-(pentan-3-yloxy)-5-((4-(pyrrolidi n-1-yl)benzyl)amino)cyclohex-1-ene-1-carboxamide (**6b**). White powder, 75.9% yield, mp: 173.1–174.5°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.31 (dd, *J* = 8.3, 5.5 Hz, 2H, 2Ph-H), 7.11 (d, *J* = 8.4 Hz, 2H, 2Ph-H), 7.03 (t, *J* = 8.7 Hz, 2H, 2Ph-H), 6.52 (d, *J* = 8.4 Hz, 2H, 2Ph-H), 6.48 (s, 1H, CH), 4.41 (s, 2H, CH_2), 4.03 (d, *J* = 8.3 Hz, 1H, CH), 3.96 – 3.87 (m, 1, CH), 3.74 (d, *J* = 12.4 Hz, 1H, CH), 3.55 (d, *J* = 12.4 Hz, 1H, CH), 3.40 – 3.33 (m, 1H, CH), 3.23 (t, *J* = 6.3 Hz, 4H, $2CH_2$), 2.90 (td, *J* = 10.0, 5.4 Hz, 1H, CH), 2.77 (dd, *J* = 17.2, 5.0 Hz, 1H, CH), 2.29 (ddd, *J* = 13.7, 8.5, 2.5 Hz, 1H, CH), 2.05 – 1.92 (m, 7H, overlapped, $2CH_2$, CH_3), 1.56 – 1.42 (m, 4H, $2CH_2$), 0.94 – 0.83 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.49, 168.29, 163.25, 160.83, 147.56, 134.82, 132.60, 131.79, 129.05, 125.57, 114.82, 114.60, 111.55, 82.02, 75.78, 54.56, 54.00, 49.21, 47.34, 42.16, 29.76, 25.73, 24.97, 21.75, 8.49, 8.18. HRMS calcd for $C_{32}H_{43}FN_4O_3$ $[M + H]^+$: 551.3392. Found: m/z 551.3392.

Methyl((3R,4R,5S)-4-acetamido-5-((4-(diethylamino)benzyl)amino)-3-(pentan-3 -yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**9a**). White powder, 74.2% yield, mp:

123.1–128.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.12 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.67 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.43 (s, 1H, CH), 4.34 (d, *J* = 6.8 Hz, 1H, CH), 4.05 (d, *J* = 8.2 Hz, 1H, CH), 3.94 (dd, *J* = 10.4, 8.6 Hz, 1H, CH), 3.77 (d, *J* = 12.5 Hz, 1H, CH), 3.73 (s, 3H, CH_3), 3.59 (d, *J* = 12.5 Hz, 1H, CH), 3.44 – 3.26 (m, 5H, overlapped, $2CH_2$, CH), 2.94 (td, *J* = 10.0, 5.3 Hz, 1H, CH), 2.79 (dd, *J* = 17.2, 5.1 Hz, 1H, CH), 2.37 – 2.26 (m, 1H, CH), 2.25 – 2.11 (m, 1H, CH), 2.00 (s, 3H, CH_3), 1.61 – 1.41 (m, 4H, $2CH_2$), 1.12 (t, *J* = 7.0 Hz, 6H, $2CH_3$), 0.97 (dd, *J* = 6.7, 4.6 Hz, 6H, $2CH_3$), 0.90 (dt, *J* = 11.4, 7.4 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.55, 172.25, 169.07, 147.22, 132.39, 131.85, 129.24, 125.46, 112.18, 82.07, 75.66, 58.39, 54.38, 53.90, 51.11, 48.97, 44.09, 30.22, 29.62, 25.69, 25.26, 21.75, 18.18, 17.64, 11.43, 8.48, 8.21. ESI-MS: m/z 559.5 $[M + H]^+$, $C_{31}H_{50}N_4O_5$ (558.75).

Methyl((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(pyrrolidin-1-yl)benz yl) amino)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**9b**). Light yellow powder, 70.5% yield, mp: 116.8–119.3°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.17 (dd, *J* = 18.1, 8.5 Hz, 2H, 2Ph-H), 6.60 – 6.50 (m, 2H, 2Ph-H), 6.45 (s, 1H, CH), 4.34 (d, *J* = 6.8 Hz, 1H, CH), 4.07 (d, *J* = 8.2 Hz, 1H, CH), 3.98 (dd, *J* = 10.5, 8.5 Hz, 1H, CH), 3.87 (d, *J* = 12.6 Hz, 1H, CH), 3.73 (s, 3H, CH_3), 3.69 (d, *J* = 12.6 Hz, 1H, CH), 3.44 – 3.36 (m, 1H, CH), 3.27 – 3.22 (m, 4H, $2CH_2$), 3.05 (td, *J* = 10.1, 5.4 Hz, 1H, CH), 2.81 (dd, *J* = 17.3, 5.2 Hz, 1H, CH), 2.45 – 2.32 (m, 1H, CH), 2.19 (dq, *J* = 13.6, 6.8 Hz, 1H, CH), 2.11 – 1.93 (m, 7H, $2CH_2$, CH_3), 1.59 – 1.44 (m, 4H, $2CH_2$), 0.97 (dd, *J* = 6.7, 4.3 Hz, 6H, $2CH_3$), 0.88 (dq, *J* = 12.0, 7.6 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz,

CD_3OD) δ: 172.72, 172.24, 168.85, 147.87, 132.31, 131.48, 129.40, 111.58, 82.12, 75.39, 58.41, 53.89, 51.12, 48.95, 47.30, 46.51, 30.21, 28.95, 25.68, 25.23, 24.99, 21.79, 18.18, 17.64, 8.46, 8.20, 7.94. ESI-MS: m/z 557.6 $[M + H]^+$, $C_{31}H_{48}N_4O_5$ (556.74).

4.1.6. General procedure for the synthesis of compounds **10a–b**.

The compounds **10a–b** were synthesized with the same procedure reported above as 4.1.2.

((3R,4R,5S)-4-acetamido-5-((4-(diethylamino)benzyl)amino)-3-(pentan-3-yloxy) cyclohex-1-ene-1-carbonyl)-*D*-valine (**10a**). White powder, 68.5% yield, mp: 131.5–136.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.24 (dd, *J* = 20.8, 8.6 Hz, 2H, 2Ph-H), 6.77 (dd, *J* = 43.1, 8.6 Hz, 2H, 2Ph-H), 6.54 (s, 1H, CH), 4.43 – 3.93 (m, 5H, overlapped, 3CH, CH_2), 3.62 – 3.33 (m, 6H, overlapped, $2CH_2$, 2CH), 3.05 – 2.64 (m, 2H, CH_2), 2.31 – 2.13 (m, 1H, CH), 2.05 (s, 3H, CH_3), 1.64 – 1.44 (m, 4H, $2CH_2$), 1.13 (td, *J* = 7.0, 3.7 Hz, 6H, $2CH_3$), 1.06 – 0.95 (m, 6H, $2CH_3$), 0.95 – 0.85 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 148.49, 131.84, 130.91, 130.27, 111.62, 82.28, 74.40, 53.91, 51.52, 47.54, 43.96, 30.42, 26.11, 25.62, 25.11, 21.98, 18.38, 17.41, 11.34, 8.39, 8.16. HRMS calcd for $C_{30}H_{48}N_4O_5$ $[M + H]^+$: 545.3697. Found: m/z 545.3692.

((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(pyrrolidin-1-yl)benzyl)amin o)cyclohex-1-ene-1-carbonyl)-*D*-valine (**10b**). White powder, 69.5% yield, mp: 138.5–141.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.27

(d, *J* = 8.3 Hz, 2H, 2Ph-H), 6.59 (d, *J* = 8.4 Hz, 2H, 2Ph-H), 6.54 (s, 1H, CH), 4.41 – 4.07 (m, 5H, overlapped, 3CH, CH_2), 3.59 – 3.49 (m, 1H, CH), 3.49 – 3.43 (m, 1H, CH), 3.27 (t, *J* = 6.1 Hz, 4H, $2CH_2$), 2.96 (dd, *J* = 13.4, 4.3 Hz, 1H, CH), 2.76 – 2.63 (m, 1H, CH), 2.29 – 2.15 (m, 1H, CH), 2.05 (s, 3H, CH_3), 2.02 (t, *J* = 6.4 Hz, 4H, $2CH_2$), 1.60 – 1.49 (m, 4H, $2CH_2$), 1.04 – 0.94 (m, 6H, $2CH_3$), 0.93 – 0.87 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.66, 173.59, 173.33, 148.79, 131.61, 130.69, 129.38, 116.09, 111.62, 82.23, 74.29, 53.87, 52.99, 51.45, 48.11, 47.50, 47.29, 30.78, 26.03, 25.63, 25.10, 25.04, 24.99, 21.94, 18.54, 17.35, 8.42, 8.15. HRMS calcd for $C_{30}H_{46}N_4O_5$ $[M + H]^+$: 543.3541. Found: m/z 543.3546.

4.1.7. General procedure for the synthesis of compounds **14a–e**.

The compounds **14a–e** were synthesized with the same procedure reported above as 4.1.5.

Ethyl(3R,4R,5S)-4-acetamido-3-(*sec*-butoxy)-5-((4(methylthio)benzyl)amino)cy clohex -1-ene-1-carboxylate (**14e**). White powder, 77.3% yield, mp: 122.5–124.3°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.23 – 7.12 (m, 4H, 4Ph-H), 6.70 (s, 1H, CH), 4.14 (q, *J* = 7.1 Hz, 2H, CH_2), 3.98 (d, *J* = 8.4 Hz, 1H, CH), 3.84 (dd, *J* = 10.2, 8.6 Hz, 1H, CH), 3.77 (d, *J* = 13.0 Hz, 1H, CH), 3.60 (d, *J* = 13.0 Hz, 1H, CH), 3.30 (p, *J* = 5.6 Hz, 1H, CH), 2.88 – 2.70 (m, 2H, 2CH), 2.38 (s, 3H, CH_3), 2.16 (ddt, *J* = 17.2, 9.4, 2.8 Hz, 1H, CH), 1.93 (s, 3H, CH_3), 1.51 – 1.36 (m, 4H, $2CH_2$), 1.22 (t, *J* = 7.1 Hz, 3H, CH_3), 0.82 (dt, *J* = 10.0, 7.4 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.53, 166.42, 137.47, 136.15, 128.87, 128.64, 126.43, 82.03, 75.61, 60.65, 54.47,

54.07, 49.05, 29.61, 25.78, 25.33, 21.74, 14.44, 13.11, 8.51, 8.18.ESI-MS: m/z 450.3 $[M + H]^+$, $C_{24}H_{36}N_2O_4S$ (448.62).

4.1.8. General procedure for the synthesis of compounds **15a–e**.

The compounds **15a–e** were synthesized with the same procedure reported above as Section 4.1.2.

(3R,4R,5S)-4-acetamido-3-(*sec*-butoxy)-5-((4-(methylthio) benzyl) amino) cyclohex-1-ene-1-carboxylic acid (**15e**). White powder, 70.1% yield, mp: 167.3–175.1°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.43 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 7.34 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 6.83 (s, 1H, CH), 4.35 (d, *J* = 13.1 Hz, 1H, CH), 4.28 – 4.15 (m, 3H, overlapped, CH, CH_2), 3.56 (td, *J* = 10.0, 5.5 Hz, 1H, CH), 3.47 (p, *J* = 5.6 Hz, 1H, CH), 3.03 (dd, *J* = 17.4, 5.1 Hz, 1H, CH), 2.65 (dd, *J* = 17.1, 9.7 Hz, 1H, CH), 2.51 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 1.63 – 1.49 (m, 4H, $2CH_2$), 0.98 – 0.88 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.40, 141.08, 136.03, 129.98, 127.50, 126.21, 82.25, 74.65, 54.80, 51.76, 47.28, 26.34, 25.75, 25.23, 21.97, 13.81, 8.41, 8.16. HRMS calcd for $C_{22}H_{32}N2O_4S$ $[M + H]^+$: 421.2156. Found: m/z 421.2153.

4.1.9. General procedure for the synthesis of compound **6c–e, 9c–e, 16a–e** and **17a–e**.

The compounds **6c–e, 9c–e, 16a–e** and **17a–e** were synthesized with the same procedure reported above as Section 4.1.3.

(3R,4R,5S)-4-acetamido-*N*-(4-fluorobenzyl)-3-(pentan-3-yloxy)-5-((4-(phenylthio)benzyl)amino)cyclohex-1-ene-1-carboxamide (**6c**). White powder, 75.2% yield, mp:

138.1–143.3°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.34 – 7.09 (m, 11H, 11Ph-H), 7.01 – 6.89 (m, 2H, 2Ph-H), 6.40 (s, 1H, CH), 4.33 (s, 2H, CH_2), 3.98 (d, *J* = 8.2 Hz, 1H, CH), 3.92 – 3.80 (m, 2H, overlapped, 2CH), 3.69 (d, *J* = 13.2 Hz, 1H, CH), 3.35 – 3.27 (m, 1H, CH), 2.90 (td, *J* = 10.1, 5.4 Hz, 1H, CH), 2.71 (dd, *J* = 16.6, 5.6 Hz, 1H, CH), 2.31 – 2.20 (m, 1H, CH), 1.91 (s, 3H, CH_3), 1.51 – 1.33 (m, 4H, $2CH_2$), 0.86 – 0.74 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.71, 168.23, 163.26, 160.84, 137.41, 135.48, 135.12, 134.78, 132.33, 131.61, 130.82, 129.07, 127.00, 114.82, 114.60, 82.09, 75.53, 54.29, 48.72, 42.16, 29.37, 25.73, 25.19, 21.77, 8.45, 8.19. HRMS calcd for $C_{34}H_{40}FN_3O_3S$ $[M + H]^+$: 590.2847. Found: m/z 590.2852.

(3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-*N*-(4-fluorobenzyl)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**6d**). White powder, 74.2% yield, mp: 198.5–200.1°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.66 – 7.55 (m, 4H, 4Ph-H), 7.48 – 7.39 (m, 4H, 4Ph-H), 7.38 – 7.29 (m, 3H, 3Ph-H), 7.10 – 7.00 (m, 2H, 2Ph-H), 6.51 (s, 1H, CH), 4.44 (s, 2H, CH_2), 4.07 (d, *J* = 8.3 Hz, 1H, CH), 4.02 – 3.91 (m, 2H, 2CH), 3.78 (d, *J* = 13.0 Hz, 1H, CH), 3.41 (p, *J* = 5.6 Hz, 1H, CH), 2.96 (td, *J* = 10.0, 5.3 Hz, 1H, CH), 2.84 (dd, *J* = 17.2, 5.1 Hz, 1H, CH), 2.35 (ddt, *J* = 15.5, 9.4, 2.5 Hz, 1H, CH), 2.04 (s, 3H, CH_3), 1.61 – 1.45 (m, 4H, $2CH_2$), 0.97 – 0.85 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.59, 168.33, 163.26, 160.83, 140.70, 140.12, 138.30, 134.81, 132.51, 131.79, 129.06, 128.54, 126.84, 126.49, 114.82, 114.61, 82.06, 75.72, 54.64, 54.15, 49.10, 42.17,

29.83, 25.74, 25.21, 21.77, 8.48, 8.20. HRMS calcd for $C_{34}H_{40}FN_3O_3$ $[M + H]^+$: 558.3126. Found: m/z 558.3131.

(3R,4R,5S)-4-acetamido-*N*-(4-fluorobenzyl)-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**6e**). White powder, 68.5% yield, mp: 153.5–155.1°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.38 – 7.22 (m, 6H, 6Ph-H), 7.12 – 7.01 (m, 2H, 2Ph-H), 6.51 (s, 1H, CH), 4.44 (s, 2H, CH_2), 4.08 (d, *J* = 8.1 Hz, 1H, CH), 3.97 (dd, *J* = 10.6, 8.6 Hz, 1H, CH), 3.92 (d, *J* = 13.0 Hz, 1H, CH), 3.75 (d, *J* = 13.0 Hz, 1H, CH), 3.45 – 3.37 (m, 1H, CH), 2.99 (td, *J* = 10.0, 5.3 Hz, 1H, CH), 2.82 (dd, *J* = 17.1, 5.3 Hz, 1H, CH), 2.47 (s, 3H, CH3), 2.42 – 2.31 (m, 1H, CH), 2.02 (s, 3H, CH_3), 1.61 – 1.46 (m, 4H, 2CH2), 0.97 – 0.85 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.64, 168.26, 160.85, 137.96, 134.95, 134.77, 132.29, 131.70, 129.06, 128.79, 126.42, 82.07, 75.53, 54.29, 48.86, 42.18, 29.42, 25.74, 25.22, 21.75, 14.37, 8.43, 8.18. HRMS calcd for $C_{29}H_{38}FN_3O_3S$ $[M + H]^+$: 528.2691. Found: m/z 528.2686.

Methyl((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(phenylthio)benzyl)amino)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**9c**). White powder, 70.6% yield, mp: 78.2–83.5°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.44 – 7.25 (m, 9H, overlapped, 9Ph-H), 6.50 (s, 1H, CH), 4.36 (d, *J* = 6.8 Hz, 1H, CH), 4.18 – 4.09 (m, 2H, overlapped, 2CH), 4.05 (dd, *J* = 10.5, 8.3 Hz, 1H, CH), 3.96 (d, *J* = 13.2 Hz, 1H, CH), 3.74 (s, 3H, CH_3), 3.45 (p, *J* = 5.6 Hz, 1H, CH), 3.22 (td, *J* = 10.0, 5.5 Hz, 1H, CH), 2.87 (dd, *J* = 17.3, 5.3 Hz, 1H, CH), 2.56 – 2.44 (m, 1H, CH), 2.28 – 2.14 (m, 1H, CH), 2.03 (s, 3H, CH_3), 1.62 – 1.48 (m, 4H, $2CH_2$), 1.00 (dd, *J* = 6.7,

4.6 Hz, 6H, $2CH_3$), 0.93 (dt, *J* = 10.7, 7.4 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.02, 172.24, 168.67, 138.47, 136.73, 134.76, 132.12, 131.50, 131.06, 130.29, 129.61, 129.11, 127.37, 82.19, 75.03, 58.42, 54.52, 53.33, 51.13, 30.21, 28.19, 25.67, 25.21, 21.81, 18.16, 17.63, 8.41, 8.20. ESI-MS: m/z 596.5 $[M + H]^+$, $C_{33}H_{45}N_3O_5S$ (595.79).

Methyl((3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-3-(penta n-3-yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**9d**). White powder, 65.7% yield. ^1H NMR (400 MHz, CD_3OD) δ: 7.66 – 7.56 (m, 4H, 4Ph-H), 7.48 – 7.39 (m, 4H, 4Ph-H), 7.37 – 7.30 (m, 1H, Ph-H), 6.45 (s, 1H, CH), 4.37 (d, *J* = 6.7 Hz, 1H, CH), 4.09 (d, *J* = 7.8 Hz, 1H, CH), 4.02 – 3.92 (m, 2H, 2CH), 3.80 (d, *J* = 13.1 Hz, 1H, CH), 3.75 (s, 3H, CH_3), 3.47 – 3.38 (m, 1H, CH), 2.98 (td, *J* = 9.8, 5.3 Hz, 1H, CH), 2.83 (dd, *J* = 17.3, 5.1 Hz, 1H, CH), 2.42 – 2.30 (m, 1H, CH), 2.27 – 2.15 (m, 1H, CH), 2.04 (s, 3H, CH_3), 1.62 – 1.46 (m, 4H, $2CH_2$), 0.99 (dd, *J* = 6.7, 3.8 Hz, 6H, $2CH_3$), 0.97 – 0.88 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.58, 172.26, 169.13, 140.73, 140.07, 138.50, 132.40, 131.94, 128.53, 126.82, 126.49, 82.10, 75.70, 58.39, 54.64, 54.12, 51.11, 49.14, 30.22, 29.93, 25.71, 25.29, 21.77, 18.17, 17.63, 8.48, 8.24. ESI-MS: m/z 565.3 $[M + H]^+$, $C_{33}H_{45}N_3O_5$ (563.73).

Methyl((3R,4R,5S)-4-acetamido-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-y loxy)cyclohex-1-ene-1-carbonyl)-*D*-valinate (**9e**). Pale yellow sticky substance, 65.4% yield. ^1H NMR (400 MHz, CD_3OD) δ: 7.39 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 7.32 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 6.54 (s, 1H, CH), 4.36 (d, *J* = 2.8 Hz, 1H, CH), 4.33 (d, *J* = 3.3 Hz, 1H, CH), 4.23 – 4.12 (m, 3H, overlapped, 3CH), 3.73 (s, 3H, CH_3), 3.58 –

3.50 (m, 1H, CH), 3.50 – 3.44 (m, 1H, CH), 2.95 (dd, *J* = 17.1, 5.5 Hz, 1H, CH), 2.73 – 2.63 (m, 1H, CH), 2.49 (s, 3H, CH_3), 2.23 – 2.17 (m, 1H, CH), 2.04 (s, 3H, CH_3), 1.62 – 1.48 (m, 4H, $2CH_2$), 1.01 – 0.95 (m, 6H, $2CH_3$), 0.91 (dt, *J* = 9.9, 7.4 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.60, 172.23, 168.17, 141.36, 131.98, 130.01, 129.96, 126.74, 126.13, 82.34, 74.37, 58.49, 54.80, 51.76, 51.16, 30.19, 29.49, 25.62, 25.11, 21.89, 18.14, 17.63, 13.68, 8.34, 8.17. ESI-MS: m/z 534.3 $[M + H]^+$, $C_{28}H_{43}N_3O_5S$ (533.72).

(3R,4R,5S)-4-acetamido-*N*-(4-chlorobenzyl)-5-((4-(diethylamino)benzyl)amino) -3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**16a**). Light yellow powder, 76.1% yield, mp: 142.8–148.5°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.38 – 7.23 (m, 4H, 4Ph-H), 7.11 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.66 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.49 (s, 1H, CH), 4.42 (s, 2H, CH_2), 4.04 (d, *J* = 8.3 Hz, 1H, CH), 3.93 (dd, *J* = 10.5, 8.6 Hz, 1H, CH), 3.75 (d, *J* = 12.5 Hz, 1H, CH), 3.56 (d, *J* = 12.5 Hz, 1H, CH), 3.41 – 3.31 (m, 5H, overlapped, $2CH_2$, CH), 2.92 (td, *J* = 10.0, 5.4 Hz, 1H, CH), 2.79 (dd, *J* = 17.2, 5.1 Hz, 1H, CH), 2.30 (ddt, *J* = 15.6, 9.6, 2.6 Hz, 1H, CH), 2.00 (s, 3H, CH_3), 1.63 – 1.39 (m, 4H, $2CH_2$), 1.11 (t, *J* = 7.0 Hz, 6H, $2CH_3$), 0.95 – 0.82 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.54, 168.34, 147.19, 137.65, 132.57, 131.70, 129.20, 128.75, 128.16, 125.64, 112.20, 82.03, 75.74, 54.49, 53.94, 48.99, 44.10, 42.20, 29.66, 25.73, 25.19, 21.75, 11.43, 8.49, 8.18. HRMS calcd for $C_{32}H_{45}ClN_4O_3$ $[M + H]^+$: 569.3253. Found: m/z 569.3253.

(3R,4R,5S)-4-acetamido-*N*-(4-chlorobenzyl)-3-(pentan-3-yloxy)-5-((4-(pyrrolidi n-1-yl)benzyl)amino)cyclohex-1-ene-1-carboxamide (**16b**). White powder, 78.9%

yield, mp: 180.0–181.5°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.27 – 7.15 (m, 4H, 4Ph-H), 7.02 (d, *J* = 8.4 Hz, 2H, 2Ph-H), 6.44 (d, *J* = 8.5 Hz, 2H, 2 Ph-H), 6.41 (s, 1H, CH), 4.39 – 4.27 (m, 2H, CH_2), 3.95 (d, *J* = 8.3 Hz, 1H, CH), 3.83 (dd, *J* = 10.5, 8.6 Hz, 1H, CH), 3.66 (d, *J* = 12.4 Hz, 1H, CH), 3.47 (d, *J* = 12.4 Hz, 1H, CH), 3.29 (p, *J* = 5.6 Hz, 1H, CH), 3.20 – 3.09 (m, 4H, $2CH_2$), 2.86 – 2.78 (m, 1H, CH), 2.69 (dd, *J* = 17.2, 5.1 Hz, 1H, CH), 2.21 (ddt, *J* = 9.6, 5.4, 2.4 Hz, 1H, CH), 1.96 – 1.87 (m, 7H, overlapped, $2CH_2$, CH_3), 1.50 – 1.34 (m, 4H, $2CH_2$), 0.86 – 0.74 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.49, 168.33, 147.57, 137.66, 132.58, 131.74, 129.00, 128.74, 128.15, 125.58, 111.55, 82.02, 75.77, 54.56, 54.00, 49.22, 47.35, 42.19, 29.76, 25.73, 25.20, 24.97, 21.74, 8.49, 8.18. HRMS calcd for $C_{32}H_{43}ClN_4O_3$ $[M + H]^+$: 567.3096. Found: m/z 567.3098.

(3R,4R,5S)-4-acetamido-*N*-(4-chlorobenzyl)-3-(pentan-3-yloxy)-5-((4-(phenylthio)benzyl)amino)cyclohex-1-ene-1-carboxamide (**16c**).White powder, 77.6% yield, mp: 166.1–167.3°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.43 – 7.08 (m, 13H, overlapped, 13Ph-H), 6.46 (s, 1H, CH), 4.40 (s, 2H, CH_2), 4.03 (d, *J* = 8.0 Hz, 1H, CH), 3.94 – 3.80 (m, 2H, overlapped, 2CH), 3.68 (d, *J* = 13.2 Hz, 1H, CH), 3.40 – 3.33 (m, 1H, CH), 2.87 (td, *J* = 9.9, 5.8 Hz, 1H, CH), 2.75 (dd, *J* = 17.3, 4.5 Hz, 1H, CH), 2.34 – 2.21 (m, 1H, CH), 1.97 (s, 3H, CH_3), 1.57 – 1.41 (m, 4H, $2CH_2$), 0.94 – 0.79 (m, 6H, $2CH_3$).^{13}C NMR (100 MHz, CD_3OD) δ: 172.54, 168.41, 138.97, 137.64, 135.84, 134.34, 132.52, 131.82, 130.94, 130.62, 128.98, 128.93, 128.73, 128.16, 126.82, 82.05, 75.73, 54.72, 54.18, 48.96, 42.19, 29.96,

25.74, 25.23, 21.75, 8.48, 8.21. HRMS calcd for $C_{34}H_{40}ClN_3O_3S$ $[M + H]_+$: 606.2552. Found: m/z 606.2548.

(3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-*N*-(4-chlorobenzyl)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**16d**). White powder, 75.4% yield, mp: 190.1–192.5°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.64 – 7.55 (m, 4H, 4Ph-H), 7.46 – 7.38 (m, 4H, 4Ph-H), 7.37 – 7.24 (m, 5H, 5Ph-H), 6.50 (s, 1H, CH), 4.42 (s, 2H, CH_2), 4.07 (d, *J* = 8.1 Hz, 1H, CH), 4.04 – 3.94 (m, 2H, 2CH), 3.83 (d, *J* = 13.1 Hz, 1H, CH), 3.43 – 3.36 (m, 1H, CH), 3.02 (td, *J* = 10.0, 5.4 Hz, 1H, CH), 2.84 (dd, *J* = 17.2, 5.2 Hz, 1H, CH), 2.43 – 2.32 (m, 1H, CH), 2.02 (s, 3H, CH_3), 1.58 – 1.44 (m, 4H, $2CH_2$), 0.94 – 0.84 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.74, 168.25, 140.58, 140.45, 137.62, 137.08, 132.52, 132.47, 131.52, 128.82, 128.76, 128.50, 128.16, 127.03, 126.85, 126.51, 82.11, 75.54, 54.26, 48.90, 46.54, 42.21, 29.32, 25.73, 25.19, 21.80, 8.46, 8.19. HRMS calcd for $C_{34}H_{40}ClN_3O_3$ $[M + H]^+$: 574.2831. Found: m/z 574.2835.

(3R,4R,5S)-4-acetamido-*N*-(4-chlorobenzyl)-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamide (**16e**). White powder, 73.7% yield, mp: 160.2–164.1°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.38 – 7.17 (m, 8H, 8Ph-H), 6.49 (s, 1H, CH), 4.42 (s, 2H, CH_2), 4.06 (d, *J* = 8.4 Hz, 1H, CH), 3.94 (dd, *J* = 10.5, 8.6 Hz, 1H, CH), 3.89 (d, *J* = 13.0 Hz, 1H, CH), 3.72 (d, *J* = 13.0 Hz, 1H, CH), 3.42 – 3.35 (m, 1H, CH), 2.96 (td, *J* = 10.0, 5.4 Hz, 1H, CH), 2.79 (dd, *J* = 17.0, 5.4 Hz, 1H, CH), 2.45 (s, 3H, CH_3), 2.39 – 2.29 (m, 1H, CH), 2.00 (s, 3H, CH_3), 1.58 – 1.45 (m, 4H, $2CH_2$), 0.95 – 0.83 (m, 6H, $2CH_3$). ^{13}C NMR (100

MHz, CD_3OD) δ: 172.66, 168.26, 137.92, 137.63, 135.13, 132.51, 131.57, 128.80, 128.75, 128.16, 126.33, 82.09, 75.59, 54.33, 54.17, 48.75, 42.20, 29.45, 25.73, 25.19, 21.78, 14.32, 8.48, 8.20. HRMS calcd for $C_{29}H_{38}ClN_3O_3S$ $[M + H]^+$: 544.2395. Found: m/z 544.2394.

Methyl4-(((3R,4R,5S)-4-acetamido-5-((4-(diethylamino)benzyl)amino)-3-(penta n-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoate (**17a**). White powder, 68.5% yield, mp: 147.9–149.2°C. ^1H NMR (400 MHz, CD_3OD) δ: 7.97 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.40 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.11 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.66 (d, *J* = 8.6 Hz, 2H, 2Ph-H), 6.51 (s, 1H, CH), 4.59 – 4.45 (m, 2H, CH_2), 4.04 (d, *J* = 8.4 Hz, 1H, CH), 3.93 (dd, *J* = 10.5, 8.7 Hz, 1H, CH), 3.89 (s, 3H, CH_3), 3.75 (d, *J* = 12.5 Hz, 1H, CH), 3.56 (d, *J* = 12.5 Hz, 1H, CH), 3.42 – 3.31 (m, 5H, overlapped, $2CH_2$, CH), 2.92 (td, *J* = 10.0, 5.3 Hz, 1H, CH), 2.80 (dd, *J* = 17.2, 5.2 Hz, 1H, CH), 2.31 (ddt, *J* = 15.6, 9.6, 2.6 Hz, 1H, CH), 2.00 (s, 3H, CH_3), 1.58 – 1.42 (m, 4H, $2CH_2$), 1.11 (t, *J* = 7.0 Hz, 6H, $2CH_3$), 0.89 (dd, *J* = 16.3, 7.5 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.53, 168.43, 166.96, 147.17, 144.47, 132.75, 131.68, 129.26, 128.76, 127.05, 125.81, 112.21, 82.03, 75.76, 54.54, 53.92, 51.18, 49.01, 44.10, 42.57, 29.72, 25.73, 25.20, 21.74, 11.43, 8.49, 8.18. ESI-MS: m/z 593.6 $[M + H]^+$, $C_{34}H_{48}N_4O_5$ (592.77).

Methyl4-(((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(pyrrolidin-1-yl)be n-zyl)amino)cyclohex-1-ene-1-carboxamido)methyl)benzoate (**17b**). Light yellow powder, 71.7% yield, mp: 176.5–178.3°C (along with the decomposition). ^1H NMR (400 MHz, CD_3OD) δ: 7.99 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.42 (d, *J* = 8.2 Hz, 2H,

2Ph-H), 7.14 (d, *J* = 8.5 Hz, 2H, 2Ph-H), 6.66 – 6.45 (m, 3H, 2Ph-H, CH), 4.60 – 4.46 (m, 2H, CH_2), 4.07 (d, *J* = 8.4 Hz, 1H, CH), 3.96 (dd, *J* = 10.5, 8.6 Hz, 1H, CH), 3.91 (s, 3H, CH_3), 3.80 (d, *J* = 12.5 Hz, 1H, CH), 3.62 (d, *J* = 12.5 Hz, 1H, CH), 3.45 – 3.36 (m, 1H, CH), 3.31 – 3.18 (m, 4H, $2CH_2$), 2.96 (ddd, *J* = 19.4, 12.4, 7.4 Hz, 1H, CH), 2.83 (dd, *J* = 17.2, 5.2 Hz, 1H, CH), 2.42 – 2.29 (m, 1H, CH), 2.13 – 1.92 (m, 7H, overlapped, $2CH_2$, CH_3), 1.64 – 1.44 (m, 4H, $2CH_2$), 0.98 – 0.83 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.55, 168.34, 166.94, 147.65, 144.46, 132.71, 131.59, 129.34, 129.12, 128.76, 127.06, 124.91, 111.56, 82.04, 75.68, 54.37, 53.98, 51.19, 49.14, 47.33, 42.57, 29.51, 25.73, 25.19, 24.97, 21.76, 8.49, 8.18. ESI-MS: m/z 591.5 $[M + H]^+$, $C_{34}H_{46}N_4O_5$ (590.75).

Methyl4-(((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(phenylthio)benzyl) amino)cyclohex-1-ene-1-carboxamido)methyl)benzoate (**17c**). White powder, 69.8% yield, mp: 167.3–170.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.99 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.41 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.38 – 7.20 (m, 9H, 9Ph-H), 6.53 (s, 1H, CH), 4.52 (s, 2H, CH_2), 4.08 (d, *J* = 8.3 Hz, 1H, CH), 3.99 – 3.86 (m, 5H, overlapped, 2CH, CH_3), 3.73 (d, *J* = 13.2 Hz, 1H, CH), 3.45 – 3.37 (m, 1H, CH), 2.93 (td, *J* = 9.9, 5.3 Hz, 1H, CH), 2.81 (dd, *J* = 17.2, 5.0 Hz, 1H, CH), 2.39 – 2.27 (m, 1H, CH), 2.02 (s, 3H, CH_3), 1.59 – 1.46 (m, 4H, $2CH_2$), 0.96 – 0.86 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.57, 168.45, 166.95, 144.45, 138.79, 135.80, 134.43, 132.64, 131.74, 130.92, 130.65, 129.35, 128.90, 127.05, 126.83, 82.06, 75.71, 54.67, 54.19, 51.19, 48.93, 42.57, 29.90, 25.74, 25.22, 21.76, 8.49, 8.21. ESI-MS: m/z 630.4 $[M + H]^+$, $C_{36}H_{43}N_3O_5S$ (629.81).

Methyl4-(((3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoate (**17d**). White powder, 66.7% yield, mp: 179.8–183.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.99 (d, *J* = 8.1 Hz, 2H, 2Ph-H), 7.65 – 7.56 (m, 4H, 4Ph-H), 7.50 – 7.38 (m, 6H, 6Ph-H), 7.37 – 7.30 (m, 1H, Ph-H), 6.54 (s, 1H, CH), 4.60 – 4.46 (m, 2H, CH_2), 4.10 (d, *J* = 8.0 Hz, 1H, CH), 4.03 – 3.94 (m, 2H, 2CH), 3.91 (s, 3H, CH_3), 3.81 (d, *J* = 13.0 Hz, 1H, CH), 3.45 – 3.38 (m, 1H, CH), 3.00 (td, *J* = 9.9, 5.4 Hz, 1H, CH), 2.86 (dd, *J* = 17.3, 5.0 Hz, 1H, CH), 2.45 – 2.33 (m, 1H, CH), 2.04 (s, 3H, CH_3), 1.62 – 1.46 (m, 4H, $2CH_2$), 0.97 – 0.86 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.63, 168.41, 166.96, 144.45, 140.67, 140.20, 138.02, 132.67, 131.65, 129.35, 128.77, 128.66, 128.48, 127.06, 126.95, 126.77, 126.50, 82.08, 75.68, 54.55, 54.18, 51.19, 49.06, 42.58, 29.72, 25.74, 25.22, 21.79, 8.49, 8.20. ESI-MS: m/z 598.5 $[M + H]^+$, $C_{36}H_{43}N_3O_5$ (597.74).

Methyl4-(((3R,4R,5S)-4-acetamido-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoate (**17e**). White powder, 69.3% yield. ^{1}H NMR (400 MHz, CD_3OD) δ: 7.87 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 7.30 (d, *J* = 8.2 Hz, 2H, 2Ph-H), 7.21 – 7.10 (m, 4H, 4Ph-H), 6.42 (s, 1H, CH), 4.41 (s, 2H, CH_2), 3.97 (d, *J* = 8.2 Hz, 1H, CH), 3.90 – 3.77 (m, 5H, overlapped, 2CH, CH_3), 3.65 (d, *J* = 13.0 Hz, 1H, CH), 3.32 – 3.26 (m, 1H, CH), 2.90 (td, *J* = 10.0, 5.4 Hz, 1H, CH), 2.73 (dd, *J* = 17.2, 5.2 Hz, 1H, CH), 2.35 (s, 3H, CH_3), 2.32 – 2.22 (m, 1H, CH), 1.91 (s, 3H, CH_3), 1.48 – 1.34 (m, 4H, $2CH_2$), 0.84 – 0.75 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 172.72, 168.29, 166.96, 144.43, 138.14, 134.63, 132.54,

131.45, 129.35, 128.88, 128.77, 127.07, 126.32, 82.10, 75.50, 54.19, 51.20, 48.72, 42.58, 29.24, 25.73, 25.19, 21.78, 14.28, 8.47, 8.19. ESI-MS: m/z 568.5 $[M + H]^+$, $C_{31}H_{41}N_3O_5S$ (567.74).

4.1.10. General procedure for the synthesis of compound **10c–e** and **18a–e**.

The compounds **10c–e** and **18a–e** were synthesized with the same procedure reported above as Section 4.1.2.

((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(phenylthio)benzyl)amino)cyclohex-1-ene-1-carbonyl)-*D*-valine (**10c**). White powder, 79.2% yield, mp: 120.1–125.1°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.35 – 7.23 (m, 7H, 7Ph-H), 7.23 – 7.16 (m, 2H, 2Ph-H), 6.44 (s, 1H, CH), 4.31 – 4.22 (m, 2H, 2CH), 4.16 – 4.04 (m, 3H, overlapped, CH, CH_2), 3.49 (td, *J* = 9.9, 5.7 Hz, 1H, CH), 3.38 (p, *J*= 5.5 Hz, 1H, CH), 2.86 (dd, *J* = 18.1, 6.2 Hz, 1H, CH), 2.67 – 2.55 (m, 1H, CH), 2.12 (dq, *J* = 13.5, 6.7 Hz, 1H, CH), 1.94 (s, 3H, CH_3), 1.51 – 1.37 (m, 4H, $2CH_2$), 0.90 (dd, *J* = 6.7, 1.5 Hz, 6H, $2CH_3$), 0.81 (dd, *J* = 16.1, 7.6 Hz, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.53, 168.02, 139.41, 133.56, 132.50, 131.81, 130.37, 130.13, 129.54, 129.30, 128.88, 128.00, 82.30, 74.32, 58.32, 54.89, 51.69, 30.23, 26.11, 25.63, 25.10, 21.93, 18.29, 17.41, 8.26. HRMS calcd for $C_{32}H_{43}N_3O_5S$ $[M + H]^+$: 582.2996. Found: m/z 582.2995.

((3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valine (**10d**). White powder, 72.1% yield, mp: 178.5–183.5°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.75

(d, *J* = 8.0 Hz, 2H, 2Ph-H), 7.65 (d, *J* = 7.5 Hz, 2H, 2Ph-H), 7.60 (d, *J* = 8.0 Hz, 2H, 2Ph-H), 7.48 (t, *J* = 7.6 Hz, 2H, 2Ph-H), 7.39 (t, *J* = 7.3 Hz, 1H, Ph-H), 6.58 (s, 1H, CH), 4.48 (d, *J* = 13.0 Hz, 1H, CH), 4.42 – 4.18 (m, 4H, overlapped, 2CH, CH_2), 3.68 (dt, *J* = 13.9, 6.4 Hz, 1H, CH), 3.51 (p, *J* = 5.4 Hz, 1H, CH), 3.09 – 2.98 (m, 1H, CH), 2.83 – 2.74 (m, 1H, CH), 2.31 – 2.20 (m, 1H, CH), 2.09 (s, 3H, CH_3), 1.66 – 1.49 (m, 4H, $2CH_2$), 1.03 (d, *J* = 5.3 Hz, 6H, $2CH_3$), 0.97 – 0.89 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.54, 139.87, 131.93, 130.13, 129.68, 128.64, 127.50, 126.62, 82.31, 74.40, 54.80, 51.71, 47.31, 30.28, 26.15, 25.64, 25.12, 22.00, 18.31, 17.43, 8.38, 8.18. HRMS calcd for $C_{32}H_{43}N_3O_5$ $[M + H]^+$: 550.3275. Found: m/z 550.3280.

((3R,4R,5S)-4-acetamido-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carbonyl)-*D*-valine (**10e**). Light pink powder, 75.7% yield, mp: 161.2–166.3°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.39 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 7.32 (d, *J* = 8.3 Hz, 2H, 2Ph-H), 6.54 (s, 1H, CH), 4.40 – 4.30 (m, 2H, 2CH), 4.25 – 4.13 (m, 3H, 3CH), 3.60 – 3.51 (m, 1H, CH), 3.51 – 3.44 (m, 1H, CH), 2.96 (dd, *J* = 17.4, 5.6 Hz, 1H, CH), 2.75 – 2.65 (m, 1H, CH), 2.49 (s, 3H, CH_3), 2.27 – 2.17 (m, 1H, CH), 2.04 (s, 3H, CH_3), 1.62 – 1.48 (m, 4H, $2CH_2$), 1.04 – 0.97 (m, 6H, $2CH_3$), 0.97 – 0.85 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.49, 141.27, 131.70, 130.28, 130.00, 127.03, 126.15, 82.29, 74.32, 54.71, 51.71, 30.39, 26.16, 25.63, 25.11, 21.89, 18.35, 17.37, 13.71, 8.36, 8.16. HRMS calcd for $C_{27}H_{41}N_3O_5S$ $[M + H]^+$: 520.284. Found: m/z 520.2838.

4-(((3R,4R,5S)-4-acetamido-5-((4-(diethylamino)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoic acid (**18a**). Light yellow powder,

72.1% yield, mp: 141.2–146.5°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.00 (d, J = 7.6 Hz, 2H, 2Ph-H), 7.43 (d, J = 7.6 Hz, 2H, 2Ph-H), 7.28 (d, J = 8.3 Hz, 2H, 2Ph-H), 6.74 (d, J = 8.4 Hz, 2H, 2Ph-H), 6.60 (s, 1H, CH), 4.55 (s, 2H, CH_2), 4.39 – 4.01 (m, 4H, overlapped, 2CH, CH_2), 3.64 – 3.35 (m, 6H, overlapped, $2CH_2$, 2CH), 3.08 – 2.98 (m, 1H, CH), 2.78 – 2.64 (m, 1H, CH), 2.07 (s, 3H, CH_3), 1.67 – 1.45 (m, 4H, $2CH_2$), 1.16 (t, J = 6.9 Hz, 6H, $2CH_3$), 1.02 – 0.82 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.44, 167.50, 148.53, 143.86, 131.98, 130.87, 130.16, 129.87, 127.02, 115.91, 111.59, 82.30, 74.46, 53.95, 53.06, 51.66, 43.93, 42.64, 26.15, 25.66, 25.07, 21.96, 11.34, 8.39, 8.13. HRMS calcd for $C_{33}H_{46}N_4O_5$ $[M + H]^+$: 579.3541. Found: m/z 579.3545.

4-(((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(pyrrolidin-1-yl)benzyl)amino)cyclohex-1-ene-1-carboxamido)methyl)benzoic acid (**18b**). Light yellow powder, 69.8% yield, mp: 148.5–152.9°C. ^{1}H NMR (400 MHz, CD_3OD) δ: 8.05 – 7.97 (m, 2H, 2Ph-H), 7.48 – 7.39 (m, 2H, 2Ph-H), 7.37 – 7.06 (m, 2H, 2Ph-H), 6.71 – 6.49 (m, 3H, 2Ph-H, CH), 4.54 (s, 2H, CH_2), 4.37 – 4.07 (m, 4H, overlapped, 2CH, CH_2), 3.55 (td, *J* = 10.1, 5.6 Hz, 1H, CH), 3.50 – 3.43 (m, 1H, CH), 3.32 – 3.24 (m, 4H, $2CH_2$), 3.02 (dd, *J* = 16.5, 6.0 Hz, 1H, CH), 2.80 – 2.65 (m, 1H, CH), 2.07 (s, 3H, CH_3), 2.06 – 1.95 (m, 4H, $2CH_2$), 1.66 – 1.47 (m, 4H, $2CH_2$), 1.00 – 0.84 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.42, 168.26, 167.47, 148.82, 144.01, 131.99, 130.70, 130.11, 129.49, 127.05, 115.85, 111.62, 82.30, 74.43, 53.98, 51.63, 47.75, 42.64, 26.10, 25.66, 25.03, 21.98, 8.40, 8.13. HRMS calcd for $C_{33}H_{44}N_4O_5$ $[M + H]^+$: 577.3384. Found: m/z 577.3388.

4-(((3R,4R,5S)-4-acetamido-3-(pentan-3-yloxy)-5-((4-(phenylthio) benzyl) amino)cyclohex-1-ene-1-carboxamido)methyl)benzoic acid (**18c**). White powder, 71.3% yield, mp: 190.1–195.1°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.99 (d, J = 7.8 Hz, 2H, 2Ph-H), 7.55 – 7.23 (m, 11H, 11Ph-H), 6.60 (s, 1H, CH), 4.54 (s, 2H, CH_2), 4.37 (d, J = 13.1 Hz, 1H, CH), 4.30 – 4.14 (m, 3H, overlapped, CH, CH_2), 3.62 (td, J = 9.9, 5.8 Hz, 1H, CH), 3.52 – 3.44 (m, 1H, CH), 3.01 (dd, J = 17.0, 5.1 Hz, 1H, CH), 2.78 – 2.67 (m, 1H, CH), 2.06 (s, 3H, CH_3), 1.65 – 1.49 (m, 4H, $2CH_2$), 1.00 – 0.84 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 173.50, 167.48, 143.98, 139.21, 133.65, 132.43, 132.00, 130.34, 130.12, 129.59, 129.28, 127.95, 127.04, 82.30, 74.47, 54.90, 51.84, 42.65, 26.26, 25.68, 25.09, 21.98, 8.40, 8.15. HRMS calcd for $C_{35}H_{41}N_3O_5S$ $[M + H]^+$: 616.284. Found: m/z 616.2843.

4-(((3R,4R,5S)-5-(([1,1'-biphenyl]-4-ylmethyl)amino)-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoic acid (**18d**). White powder, 75.2% yield, mp: 224.1–229.2°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 8.00 (d, J = 3.8 Hz, 2H, 2Ph-H), 7.72 (d, J = 8.0 Hz, 2H, 2Ph-H), 7.64 (d, J = 7.7 Hz, 2H, 2Ph-H), 7.58 (d, 2H, 2Ph-H), 7.50 – 7.35 (m, 5H, 5Ph-H), 6.60 (s, 1H, CH), 4.55 (s, 2H, CH_2), 4.42 (d, J = 13.0 Hz, 1H, CH), 4.32 – 4.16 (m, 3H, overlapped, CH, CH_2), 3.59 (td, J = 9.8, 5.7 Hz, 1H, CH), 3.48 (p, J = 5.5 Hz, 1H, CH), 3.05 (dd, J = 17.1, 5.1 Hz, 1H, CH), 2.80 – 2.68 (m, 1H, CH), 2.08 (s, 3H, CH_3), 1.65 – 1.47 (m, 4H, $2CH_2$), 1.00 – 0.84 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, CD_3OD) δ: 167.57, 143.79, 142.21, 139.94, 132.11, 130.56, 130.25, 129.96, 128.63, 127.50,

127.35, 127.02, 126.60, 82.30, 74.62, 54.77, 52.06, 42.65, 26.55, 25.69, 25.11, 22.00, 8.42, 8.17. HRMS calcd for $C_{35}H_{41}N_3O_5$ $[M + H]^+$: 584.3119. Found: m/z 584.3116.

4-(((3R,4R,5S)-4-acetamido-5-((4-(methylthio)benzyl)amino)-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxamido)methyl)benzoic acid (**18e**). White powder, 73.9% yield, mp: 166.1–172.0°C (along with the decomposition). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.87 (d, *J* = 7.3 Hz, 2H, 2Ph-H), 7.33 – 7.25 (m, 4H, 4Ph-H), 7.23 – 7.17 (m, 2H, 2Ph-H), 6.47 (s, 1H, CH), 4.42 (s, 2H, CH_2), 4.19 (d, *J* = 13.1 Hz, 1H, CH), 4.14 – 3.99 (m, 3H, overlapped, 3CH), 3.44 – 3.31 (m, 2H, overlapped, 2CH), 2.88 (dd, *J* = 17.1, 5.3 Hz, 1H, CH), 2.62 – 2.52 (m, 1H, CH), 2.38 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 1.50 – 1.36 (m, 4H, $2CH_2$), 0.84 – 0.76 (m, 6H, $2CH_3$).^{13}C NMR (100 MHz, CD_3OD) δ: 173.39, 167.60, 143.72, 140.83, 132.05, 130.30, 129.87, 129.63, 129.61, 128.05, 126.98, 126.13, 82.28, 74.62, 54.64, 52.10, 42.65, 26.60, 25.68, 25.09, 21.94, 13.79, 8.41, 8.15. HRMS calcd for $C_{30}H_{39}N_3O_5S$ $[M + H]^+$: 554.2683. Found: m/z 544.2680.

4.2. In vitro neuraminidase enzyme inhibitory assay

The NA inhibition assay was carried out according to the standard method [29,37]. Influenza virus suspensions were harvested from the allantoic fluid of influenza virus-infected chicken embryo layer. In addition, A/Anhui/1/2005 (H5N1-H274Y mutation) and B/PHUKET/3073/2013 (B virus NA) were obtained from Sino Biological Inc. Influenza neuraminidase activity was determined by using 2'-(4-methylumbelliferyl)-α-D-acetylneuraminic acid sodium salt hydrate

(4-MUNANA) (Sigma, M8639) as a substrate, which was purchased from Sigma. The tested compounds were dissolved in DMSO in advance and then diluted to the required concentrations in MES buffer (3.54 g 2-(N-morpholino)-ethanesulfonic acid and 0.185 g $CaCl_2$ in 400 mL Milli-Q water, pH 6.5). To a 96-well plate, 10 μL of the diluted virus supernatant or NA diluent, 70 μL of MES buffer, and 10 μL of compounds diluent at certain concentration gradient were added in order and incubated at 37°C for 10 min. Then the reaction was started by the addition 10 μL of the substrate. Incubating for 40 min at 37°C, the reaction was terminated by adding 150 μL of 0.2 M glycine-NaOH (pH 10.2) in Milli-Q water. Fluorescence was measured (excitation at 365nm and emission at 455 nm), and substrate blanks were subtracted from the sample readings. The 50%-inhibitory concentration (IC_{50}) values were determined from the dose-response curves conducted by plotting the percent inhibition of NA activity relative to inhibitor concentrations.

4.3. In vitro anti-influenza virus assay and cytotoxicity assay in Chicken Embryo Fibroblast (CEFs)

The anti-influenza activity and cytotoxicity of the newly synthesized oseltamivir derivatives were evaluated with H5N1 and H5N2 strains in Chicken Embryo Fibroblast cells using Cell Counting Kit-8 (CCK-8) method as described previously [30,31,36]. Results were expressed as EC_{50} values, which are the concentrations of compounds required for 50% protection of the influenza virus infection-mediated cytopathic effects (CPE). The tested compounds dissolved in DMSO were serially

diluted by 2-fold in assay media (1% FBS in DMEM). Aliquots of 50 μL of diluted influenza virus (H5N1, H5N2) at 100 $TCID_{50}$ were mixed with equal volumes of solutions of the newly synthesized compounds at different concentrations. After incubation for 48h at 37°C under 5% CO_2, the 100 μL of Cell Counting Kit-8 (CCK-8, Dojindo Laboratories) reagent solution (10 μL CCK-8 and 90 μL media) was added according to the manufacturer's manual. Incubating at 37°C for 90 min, the absorbance at 450 nm was read on a microplate reader. The 50% effective antiviral concentration (EC_{50}) was defined as the concentration of the test compound, affording 50% protection from viral cytopathogenicity, and was determined by fitting the curve of percent cytopathic effect (CPE) versus concentrations of NA inhibitor. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration that reduced the absorbance of Chicken Embryo Fibroblast cells by 50%, and was determined in the same manner as EC_{50} but without virus infection.

4.4. Molecular docking

Molecular simulations of molecules **15e** and **10e** were performed using the Tripos molecular modeling package Sybyl-X 2.0 [36,38]. All the molecules for docking were built using standard bond lengths and angles from Sybyl-X 2.0/Base Builder and were optimized for 10000-generations till the maximum derivative of energy became 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. Prior to

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); 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 the docking procedure, all of the single bonds in residue side chains inside the defined NA binding pocket were regarded as rotatable or flexible, and the ligand was allowed to rotate at all single bonds and to move flexibly within the tentative binding pocket. 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 **15e** and **10e** were docked into the binding pockets and to define the binding interactions.

Amino acid sequences of N1 (A/goose/Guangdong/SH7/2013 (H5N1)) compared with that of 2HU0 (H5N1), the different residues were marked in red.

(A/goose/Guangdong/SH7/2013 (H5N1)-N1

ITLTGSSSLCPIRGWAVHSKDNSIRIGSKGDVFVIREPFISCSHIECRTFFLTHGAL

LNDKHSNGTVKDRSPHRTLMSCPVGEAPSPYNSRFESVAWSASACHDGTSWL

TIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFTVMTDG

PSNGQASYKIFKIEKGKVVKSVELNAPNYHYEECSCYPDSGEIMCVCRDNWH

GSNRPWVTFNQNLEYQIGYICSGVFGDNPRPNDGTGSCGPMSLNGAYGIKGF

SFKYGNGVWIGRTKSTNSRSGFEMIWDPNGWTGTDSEFSVKQDIVAITDWSG

YSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTIWTSGSSISFCGVNSDTVSW

SWPDGAELPFTIDK

2HU0 (H5N1)-N1:

VKLAGNSSLCPINGWAVYSKDNSIRIGSKGDVFVIREPFISCSHLECRTFFLTQG

ALLNDKHSNGTVKDRSPHRTLMSCPVGEAPSPYNSRFESVAWSASACHDGTS

WLTIGISGPDNGAVAVLKYNGIITDTIKSWRNNILRTQESECACVNGSCFTVMT

DGPSNGQASYKIFKMEKGKVVKSVELDAPNYHYEECSCYPNAGEITCVCRDN

WHGSNRPWVSFNQNLEYQIGYICSGVFGDNPRPNDGTGSCGPVSSNGAYGVK

GFSFKYGNGVWIGRTKSTNSRSGFEMIWDPNGWTETDSSFSVKQDIVAITDWS

GYSGSFVQHPELTGLDCIRPCFWVELIRGRPKESTIWTSGSSISFCGVNSDTVG

WSWPDGAELPFTIDK

4.5. Acute toxicity experiment

Kunming mice (18−22 g) were purchased from the animal experimental center of Shandong University. Procedures involving animals were conducted in conformity with the institutional guidelines of Animal Care and Use Committee at Shandong University and was approved by the Animal Ethical and Welfare Committee (AEWC). Animals were housed at 25 ± 1°C, and relative humidity was 60 ± 10%. Two days

were maintained and mice were given free access to food and water. To investigate the acute toxicity of compound **15e** in mice, we used 20 healthy Kunming mice (5 males and 5 females per group) and divided them into two groups of five mice each. The agent **15e** was suspended in 5% DMSO, 20% PEG-400 and 75% water at concentrations of d 0.1 g/mL, and administered intragastrically by gavage after the mice had been fasted for 12 h. Dosage of 2 g/kg were administered to 10 mice (5 males and 5 females) [38]. Control group (without **15b**) was employed at the same time. 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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References

[1] K. Rohini, V. Shanthi, Hyphenated 3D-QSAR statistical model-drug repurposing analysis for the identification of potent neuraminidase inhibitor, Cell Biochem. Biophys. 76 (2018) 357-376.

[2] M.P. Girard, J.S. Tam, O.M. Assossou, M.P. Kieny, The 2009 A (H1N1) influenza virus pandemic: A review, Vaccine. 28 (2010) 4895-4902.

[3] S. Zheng, L. Tang, H. Gao, Y. Wang, F. Yu, D. Cui, G. Xie, X. Yang, W. Zhang, X. Ye, Z. Zhang, X. Wang, L. Yu, Y. Zhang, S. Yang, W. Liang, Y. Chen, L. Li, Benefit of early initiation of neuraminidase inhibitor treatment to hospitalized patients with avian influenza A(H7N9) virus, Clin. Infect. Dis. 66 (2018) 1054-1060.

[4] S. Mohan, P.S. Kerry, N. Bance, M. Niikura, B.M. Pinto, Serendipitous discovery of a potent influenza virus A neuraminidase inhibitor, Angew. Chem. Int. Edit. 53 (2014) 1076-1080.

[5] C.L. Xu, L.B. Dong, L. Xin, Y. Lan, Y.K. Chen, L.M. Yang, Y.L. Shu, Human avian influenza A (H5N1) virus infection in China, Sci. China Ser. C-Life Sci. 52 (2009) 407-411.

[6] Y.H. Bi, J.Y. Liu, H.F. Xiong, Y. Zhang, D. Liu, Y.X. Liu, G.F. Gao, B.B. Wang, A new reassortment of influenza A (H7N9) virus causing human infection in Beijing, 2014, Sci. Rep. 6 (2016) 26624.

[7] J. Stevens, O. Blixt, L.M. Chen, R.O. Donis, J.C. Paulson, I.A. Wilson, Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity, J. Mol. Biol. 381 (2008) 1382-1394.

[8] D.K. Behera, P.M. Behera, L. Acharya, A. Dixit, P. Padhi, In silico biology of H1N1: molecular modelling of novel receptors and docking studies of inhibitors to reveal new insight in flu treatment, J. Biomed. Biotechnol. 2012 (2012) 714623.

[9] M. Sahoo, L. Jena, S.N. Rath, S. Kumar, Identification of suitable natural inhibitor against influenza A (H1N1) neuraminidase protein by molecular docking, Genomics Inform. 14 (2016) 96-103.

[10] Y. Wu, Y. Wu, B. Tefsen, Y. Shi, G.F. Gao, Bat-derived influenza-like viruses H17N10 and H18N11, Trends Microbiol. 22 (2014) 183-191.

[11] N. Spanakis, V. Pitiriga, V. Gennimata, A. Tsakris, A review of neuraminidase inhibitor susceptibility in influenza strains, Expert Rev. Anti. Infect. Ther. 12 (2014) 1325-1336.

[12] J. Yang, S.W. Liu, L.Y. Du, S.B. Jiang, A new role of neuraminidase (NA) in the influenza virus life cycle: implication for developing NA inhibitors with novel mechanism of action, Rev. Med. Virol. 26 (2016) 242-250.

[13] A. Loregian, B. Mercorelli, G. Nannetti, C. Compagnin, G. Palu, Antiviral strategies against influenza virus: towards new therapeutic approaches, Cell Mol. Life Sci. 71 (2014) 3659-3683.

[14] N. Tewawong, B.M. Marathe, Y. Poovorawan, S. Vongpunsawad, R.J. Webby, E.A. Govorkova, Neuraminidase inhibitor susceptibility and neuraminidase enzyme kinetics of human influenza A and B viruses circulating in Thailand in 2010-2015, PLoS One. 13 (2018) e0190877.

[15] S. Kashiwagi, A. Watanabe, H. Ikematsu, M. Uemori, S. Awamura, Laninamivir Prophylaxis Study Group,. Long-acting neuraminidase inhibitor laninamivir octanoate as post-exposure prophylaxis for influenza, Clin. Infect. Dis. 63 (2016) 330-337.

[16] K. McClellan, C.M. Perry, Oseltamivir: A review of its use in influenza, Drugs. 61 (2001) 263-283.

[17] M. von Itzstein, W.Y. Wu, G.B. Kok, M.S. Pegg, J.C. Dyason, B. Jin, T.V. Phan, M.L. Smythe, H.F. White, S.W. Oliver, P.M. Colman, J.N. Varghese, D.M. Ryan, J.M. Woods, R.C. Bethell, V.J. Hotham, J.M. Cameron, C.R. Penn, Rational design of potent sialidase-based inhibitors of influenza-virus replication, Nature. 363 (1993) 418-423.

[18] N. Anuwongcharoen, W. Shoombuatong, T. Tantimongcolwat, V. Prachayasittikul, C. Nantasenamat, Exploring the chemical space of influenza neuraminidase inhibitors, Peerj. 4 (2016) e1958.

[19] S. Kubo, T. Tomozawa, M. Kakuta, A. Tokumitsu, M. Yamashita, Laninamivir prodrug CS-8958, a long-acting neuraminidase inhibitor, shows superior anti-influenza virus activity after a single administration, Antimicrob. Agents Chemother. 54 (2010) 1256-1264.

[20] M. Samson, A. Pizzorno, Y. Abed, G. Boivin, Influenza virus resistance to neuraminidase inhibitors, Antiviral Res. 98 (2013) 174-185.

[21] H.L. Yen, J.L. McKimm-Breschkin, K.T. Choy, D.D.Y. Wong, P.P.H. Cheung, J. Zhou, I.H. Ng, H. Zhu, R.J. Webby, Y. Guan, R.G. Webster, J.S.M. Peiris, Resistance to neuraminidase inhibitors conferred by an R292K mutation in a human influenza virus H7N9 isolate can be masked by a mixed R/K viral population, Mbio. 4 (2013) e00396-13.

[22] P.J. Collins, L.F. Haire, Y.P. Lin, J.F. Liu, R.J. Russell, P.A. Walker, J.J. Skehel, S.R. Martin, A.J. Hay, S.J. Gamblin, Crystal structures of oseltamivir-resistant influenza virus neuraminidase mutants, Nature. 453 (2008) 1258-1261.

[23] C. Dapat, H. Kondo, I.C. Dapat, T. Baranovich, Y. Suzuki, Y. Shobugawa, K. Saito, R. Saito, H. Suzuki, Neuraminidase inhibitor susceptibility profile of pandemic and seasonal influenza viruses during the 2009-2010 and 2010-2011 influenza seasons in Japan, Antiviral Res. 99 (2013) 261-269.

[24] M.J. Memoli, R.J. Hrabal, A. Hassantoufighi, M.C. Eichelberger, J.K. Taubenberger, Rapid selection of oseltamivir- and peramivir-resistant pandemic H1N1 virus during therapy in 2 immunocompromised hosts, Clin. Infect. Dis. 50 (2010) 1252-1255.

[25] Y. Wu, G.R. Qin, F. Gao, Y. Liu, C.J. Vavricka, J.X. Qi, H.L. Jiang, K.Q. Yu, G.F. Gao, Induced opening of influenza virus neuraminidase N2 150-loop suggests an important role in inhibitor binding, Sci. Rep. 3 (2013) 1551.

[26] R.J. Russell, L.F. Haire, D.J. Stevens, P.J. Collins, Y.P. Lin, G.M. Blackburn, A.J. Hay, S.J. Gamblin, J.J. Skehel, The structure of H5N1 avian influenza neuraminidase suggests new opportunities for drug design, Nature. 443 (2006) 45-49.

[27] Q. Li, J.X. Qi, W. Zhang, C.J. Vavricka, Y. Shi, J.H. Wei, E.G. Feng, J.S. Shen, J.L. Chen, D. Liu, J.H. He, J.H. Yan, H. Liu, H.L. Jiang, M.K. Teng, X.B. Li, G.F. Gao, The 2009 pandemic H1N1 neuraminidase N1 lacks the 150-cavity in its active site, Nat. Struct. Mol. Biol. 17 (2010) 1266-1268.

[28] G.M. Air, Influenza neuraminidase, Influenza Other Resp. 6 (2012) 245-256.

[29] Y. Xie, D. Xu, B. Huang, X. Ma, W. Qi, F. Shi, X. Liu, Y. Zhang, W. Xu, Discovery of N-substituted oseltamivir derivatives as potent and selective inhibitors of H5N1 influenza neuraminidase, J. Med. Chem. 57 (2014) 8445-8458.

[30] J. Zhang, N.A. Murugan, Y. Tian, C. Bertagnin, Z.J. Fang, D.W. Kang, X.J. Kong, H.Y. Jia, Z.S. Sun, R.F. Jia, P. Gao, V. Poongavanam, A. Loregian, W.F. Xu, X.L. Ma, X. Ding, B. Huang, P. Zhan, X.Y. Liu, Structure-based optimization of n-substituted oseltamivir derivatives as potent anti-influenza A virus agents with significantly improved potency against oseltamivir-resistant N1-H274Y variant, J. Med. Chem. 61 (2018) 9976-9999.

[31] J. Zhang, V. Poongavanam, D.W. Kang, C. Bertagnin, H.M. Lu, X.J. Kong, H. Ju, X.Y. Lu, P. Gao, Y. Tian, H.Y. Jia, S. Desta, X. Ding, L. Sun, Z.J. Fang, B.S. Huang, X.W. Liang, R.F. Jia, X.L. Ma, W.F. Xu, N.A. Murugan, A. Loregian, B. Huang, P. Zhan, X.Y. Liu, Optimization of N‐substituted oseltamivir derivatives as potent inhibitors of group‐1 and -2 influenza A neuraminidases, including a drug-resistant variant, J. Med. Chem. 61 (2018) 6379-6397.

[32] R.E. Amaro, D.D.L. Minh, L.S. Cheng, W.M. Lindstrom, A.J. Olson, J.H. Lin, W.W. Li, J.A. McCammon, Remarkable loop flexibility in avian influenza N1 and its implications for antiviral drug design, J. Am. Chem. Soc. 129 (2007) 7764-7765.

[33] K. Swaminathan, J.C. Dyason, A. Maggioni, M. von Itzstein, K.M. Downard, Binding of a natural anthocyanin inhibitor to influenza neuraminidase by mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 6563-6572.

[34] R.E. Amaro, X.L. Cheng, I. Ivanov, D. Xu, J.A. McCammon, Characterizing loop dynamics and ligand recognition in human- and avian-type influenza neuraminidases via generalized born

molecular dynamics and end-point free energy calculations, J. Am. Chem. Soc. 131 (2009) 4702-4709.

[35] E. Feng, W.J. Shin, X.L. Zhu, J. Li, D.J. Ye, J. Wang, M.Y. Zheng, J.P. Zuo, K.T. No, X. Liu, W.L. Zhu, W. Tang, B.L. Seong, H.L. Jiang, H. Liu, Structure-based design and synthesis of C-1- and C-4-modified analogs of zanamivir as neuraminidase inhibitors, J. Med. Chem. 56 (2013) 671-684.

[36] H. Ju, J. Zhang, Z.S. Sun, Z. Huang, W.B. Qi, B. Huang, P. Zhan, X.Y. Liu, Discovery of C-1 modified oseltamivir derivatives as potent influenza neuraminidase inhibitors, Eur. J. Med. Chem. 146 (2018) 220-231.

[37] A.L. Liu, H.D. Wang, S.M.Y. Lee, Y.T. Wang, G.H. Du, Structure-activity relationship of flavonoids as influenza virus neuraminidase inhibitors and their in vitro anti-viral activities, Bioorg. Med. Chem. 16 (2008) 7141-7147.

[38] D.W. Kang, Z.J. Fang, B.S. Huang, X.Y. Lu, H. Zhang, H.R. Xu, Z.P. Huo, Z.X. Zhou, Z. Yu, Q. Meng, G.C. Wu, X. Ding, Y. Tian, D. Daelemans, E. De Clercq, C. Pannecouque, P. Zhan, X.Y. Liu, Structure-based optimization of thiophene[3,2-d]pyrimidine derivatives as potent HIV-1 non-nucleoside reverse transcriptase inhibitors with improved potency against resistance-associated variants, J. Med. Chem. 60 (2017) 4424-4443.

Highlights

- By exploiting 150-cavity and/or 430-cavity of influenza virus neuraminidase, novel oseltamivir derivatives were reported as potent antiviral agents.
- Compound **15e** showed similar or greater activity than OSC in both NA inhibitory activity and cellular assay.
- Compound **15e** demonstrated low cytotoxicity in vitro and low acute toxicity in mice.