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Origin of the inhibitory activity of 4-*O*-substituted sialic derivatives of human parainfluenza virus

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Abstract Human parainfluenza virus (hPIV) is a serious human pathogen causing upper and lower respiratory tract disease, yet there are no effective vaccines or therapies to control parainfluenza virus infections. Recently, we found that 4-*O*-substituted sialic derivatives have potent inhibitory activity against hPIV-1, whereas the anti-influenza inhibitor Zanamivir was less inhibitory. To elucidate the origin of the high potency inhibitory activities of these 4-*O*-substituted derivatives, we performed correlated fragment molecular orbital (FMO)-interfragment interaction energy (IFIE) analysis for hemagglutinin-neuraminidase (HN) glycoprotein complexes of hPIV with the derivatives and compared them with those for Zanamivir. We found key interactions between the inhibitors and the hPIV HN glycoprotein and identified important factors for the inhibitory activity. These theoretical results will be useful for the development of novel anti-hPIV drugs.

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Background

Human parainfluenza virus (hPIV), which belongs to the genus Respirovirus, family *Paramyxoviridae*, is a serious human pathogen that causes upper and lower respiratory tract disease and is a known cause of croup in infants and young children [1, 2]. Currently, there are no effective vaccines or specific therapies to control or treat parainfluenza virus infections [3].

Parainfluenza virus has two spike glycoproteins, the hemagglutinin-neuraminidase (HN) glycoprotein and the fusion (F) glycoprotein, embedded in its envelope. HN proteins recognize sialic acid-containing glycolipids and glycoproteins of target cells, and this recognition allows the virus to bind to those target cells. Furthermore, HN proteins also act as a sialidase, removing sialic acid from virus particles and preventing the self-aggregation of the virus, which promotes the efficient spread of the virus. Thus, the multifunctional roles of HN proteins in the viral life cycle make them an attractive target for the development of chemotherapeutics to treat hPIV infection.

Among the diverse array of compounds related to the sialic acid family [4], 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*galacto*-D-*glycero*-non-2-enonic acid (Neu5Ac2en, **1**) is a known inhibitor of sialidases from both bacterial and viral sources, occupying an important position as a lead compound in modern medicinal chemistry [5–7]. The C-4 position of sialic acid plays an important role in glycoenzyme–substrate interactions, so a variety of Neu5Ac2en derivatives with structural modifications at the C-4 position have been synthesized and

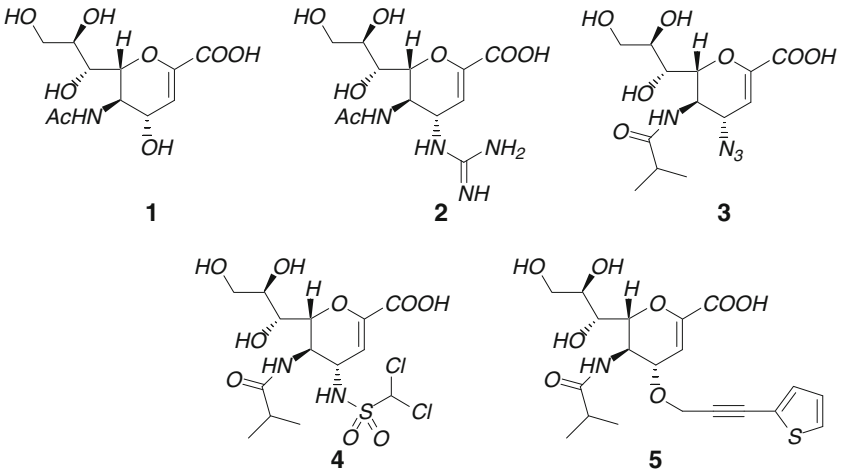

Fig. 1 Structure of hPIV sialidase inhibitors

evaluated as competitive sialidase inhibitors. Many of these derivatives were designed to specifically target paramyxoviruses. For example, von Itzstein *et al.* developed 4-deoxy-4-guanidino-Neu5Ac2en analogs [including Zanamivir (**2**)] as influenza neuraminidase inhibitors by using molecular modeling techniques [8, 9]. In 2004, Portner *et al.* designed the compounds BCX 2798 (**3**) and BCX 2855 (**4**), which are modified at the C-4 and 5 positions of **1**, by referencing the X-ray structure of the HN protein of Newcastle disease virus [10]. Recently, Ikeda *et al.* found that 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en derivative(**5**) has potential inhibitory activities against the sialidase of hPIV [11] (Fig. 1).

Here, to elucidate the origin of the potent inhibitory activities of these derivatives, we theoretically analyzed the interactions between hPIV HN and these sialic acid derivatives by using the first-principals calculations based on the fragment molecular orbital (FMO) method, which correctly evaluate not only electrostatic but also van der Waals dispersion interactions.

Computational methodology

We adopted the lead compound **1**, Zanamivir **2**, and the Neu5Ac2en derivative **5** as the hPIV inhibitors to analyze the interactions between the hPIV HN and their inhibitors. The hPIV HN is composed of 432 amino acid residues and 6813 atoms. We carried out the first-principals calculations based on the FMO method at the correlated MP2/cc-pVDZ level for the hPIV HN complexes with **1**, **2**, and **5**. At the level calculations, the hPIV HN/**5** complex is employed by 64,614 basis functions. We also used IFIE analysis to analyze the interaction

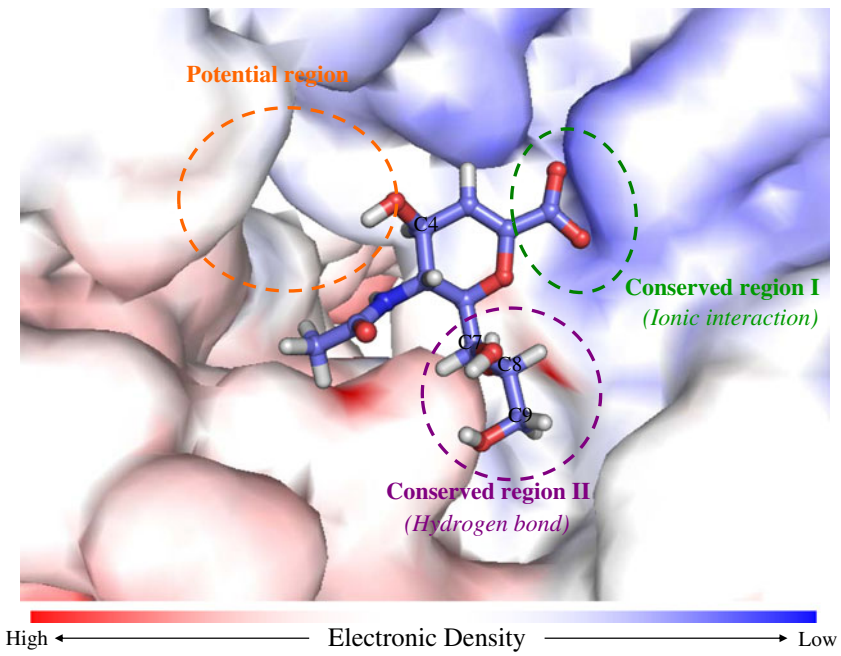

Fig. 2 Pharmacophore of Neu5Ac2en(**1**) bound to hPIV HN. Neu5Ac2en(**1**) has conserved region I (*green*), conserved region II (*purple*), and potential region (*orange*)

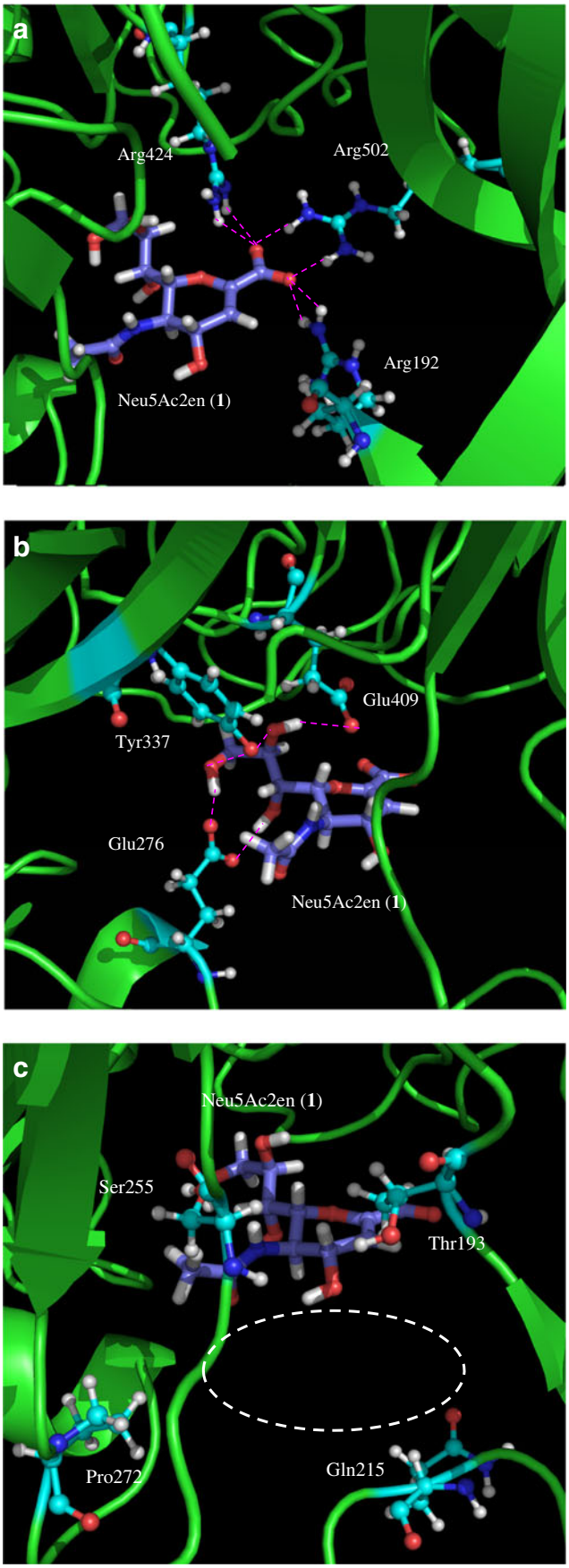

Fig. 3 **a** Interaction between Arg192, Arg424, and Arg502 of hPIV HN and conserved region I of Neu5Ac2en(**1**). Neu5Ac2en(**1**) has ionic interaction and hydrogen bonds between Arg192, Arg424, and Arg502. **b** Interaction between Glu276, Tyr337, Glu409 of hPIV HN and conserved region II of Neu5Ac2en(**2**). These residues bind to the hydroxyl group of glycerol portion of Neu5Ac2en(**1**). **c** Amino acid residues around 4 position of Neu5Ac2en(**1**) (potential region). The white dotted circle shows no amino acid residues interact Neu5Ac2en (**1**) effectively

energies between the inhibitors and the residues in the hPIV HN. In the FMO calculations, the hPIV HN was divided into one-residue fragments, with cut-off points at Cα of each residue; and inhibitors were treated as a fragment. The structures of the hPIV HN complexes with **1** and **2** were constructed on the basis of the X-ray crystal structures of the complexes (Protein Data Bank code number 1V3D [12] and 1V3E [12]), respectively. All hydrogen atoms and missing side chains were automatically amended using the molecular graphic software SYBYL 7.3 (Tripos Associates, St. Louis, MO) and refined with the Tripos force field incorporated in the package. The positions of the hydrogen atoms forming the hydrogen bonds between the hPIV HN and the inhibitors were optimized at the traditional HF/6-31G** level for partial model systems with the GAUSSIAN 09 program package [13]. In general, the interaction of the water molecules with the ligand may affect the binding energy, when there seems to be a possible space for water molecules, which will interact with the ligand at the ligand-binding site. We have evaluated the difference of the binding energy of Neu5Ac2en with/without crystal waters at the small binding-site model. The difference is only −4.0 kcal/mol, because only one water exists in the space around C4 in the complex. Therefore, all water molecules in the crystal structure of the complexes were deleted in the present study. No experimental structural data on the hPIV HN complex with 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en derivative **5** existed, so that the structures of the complex was built as follows: (1) for isolated system, the positions of hydrogen and heavy atoms of 4-substituent group in **5** were optimized at the HF/6-31G** level, with Neu5Ac2en part fixed, using the partial energy minimization; (2) internal rotation potential energy surface for 4-substituent was searched at the partial model including the derivative and amino acid residues within 5 Å of the derivative; (3) the coordinates of all atoms of 4-substituent were reoptimized from five local minimum geometries at the potential surface; (4) each optimized geometry of the derivative **5** superimposed onto Neu5Ac2en in the crystal structure (1V3D) and Neu5Ac2en was then removed. And we have evaluated the binding affinities at the all local minimum geometries; these were −21.50, −23.66, −36.90, −15.27, and −13.82 kcal/mol, respectively. We have adopted the most stable conformation of C4-subsituent for the derivative **5**. This was used for the

structural analysis of the hPIV HN and the derivative complex structure. All FMO [14] calculations were performed on 2.93 GHz Nehalem 8Core 7 CPUs (56CPUs) cluster system using the Parallelized *ab initio* Calculation System based on FMO (PAICS) program (available from http://www.paics.net) [15].

Results and discussion

We performed the FMO-IFIE analysis based on the first-principals calculations for the hPIV HN complexes with **1**, **2**, and **5** at the FMO-RI-MP2/cc-pVDZ level. FMO-IFIE analysis revealed that the lead compound **1**, Neu5Ac2en, effectively interacted with the binding pocket in hPIV HN *via* regions I, II, and III (Fig. 2). Neu5Ac2en **1** was stabilized by hydrogen bonding and ionic interactions with hPIV HN *via* a carboxyl group and Arg192, Arg424, and Arg502 with interaction energies of −84.5 kcal/mol, −94.8 kcal/mol, and −141.4 kcal/mol, respectively (Figs. 3a and 4a). Moreover, in the glycerol region, we found hydrogen-bonding interactions with Glu276, Tyr337, and Glu409 with interaction energies of −15.0 kcal/mol, −14.4 kcal/mol, and +29.7 kcal/mol, respectively (Figs. 3b and 4b). The large ionic instabilities of Glu409 was slightly stabled than Glu549 (+49.0 kcal/mol) by the hydrogen bonding with Neu5Acen **1**. The terminal methyl group is an acetyl group, and Tyr319 worked with stabilization energy of −3.8 kcal/mol due to the CH-π interaction. On the other hand, there are few amino acid residues which interact effectively around the 4 position of Neu5Ac2en **1** which bound to hPIV HN (Figs. 3c and 4c). In addition, the binding affinity of Neu5Ac2en **1** was −344.2 kcal/mol.

The interaction between hPIV HN and the anti-influenza drug Zanamivir **2** was not very effective (Fig. 5a). FMO-IFIE analysis also revealed that Zanamivir **2** does not effectively interact with hPIV HN. Compared with Neu5Ac2en **1**, Zanamivir **2** was strongly repelled from hPIV HN *via* ionic interactions. In other words, the interaction energies between Zanamivir 2 and Asp216, Glu409, and Glu549 were −12.8 kcal/mol, −23.5 kcal/mol, and +3.9 kcal/mol, respectively. There was, therefore, a decrease in the interaction energies for Zanamivir **2** compared with Neu5Ac2en **1**, the interaction energies of which were −34.2 kcal/mol, −53.2 kcal/mol, and −45.1 kcal/mol, respectively (Fig. 6a). On the other hand, the interaction energies between Zanamivir **2** and Arg192, Lys254, and Arg502 were −13.8 kcal/mol, +17.6 kcal/mol, and −97.2 kcal/mol, respectively (Fig. 6a). These interaction energies thus represent a destabilization in interaction energy compared with Neu5Ac2en **1**, the interaction energies of which were +70.8 kcal/mol, +44.2 kcal/mol, and +44.2 kcal/mol, respectively, for Arg192, Lys254, and Arg502. The guanidino group of Zanamivir **2** formed stable hydrogen bonds with Tyr530 and Thr193 with stabilizing interactions of −17.4 kcal/mol, and −14.4 kcal/mol, respectively, compared with −12.8 kcal/mol and −14.2 kcal/mol for Neu5Ac2en **1**. This result is consistent with the experimental finding of a Zanamivir-resistant T193I mutant of hPIV HN [16, 17]. In addition, the binding affinity of Zanamivir **2** was more unstable +40.3 kcal/mol for Neu5Ac2en. Therefore, Zanamivir is binding to hPIV HN in a weak, low activity factors have been identified theoretically.

Our analysis revealed the key interactions that cause the high inhibitory activity of the 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en derivative **5** against hPIV HN (Fig. 6b). Compared with Neu5Ac2en **1**, 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en **5** had a stronger binding affinity for hPIV HN.

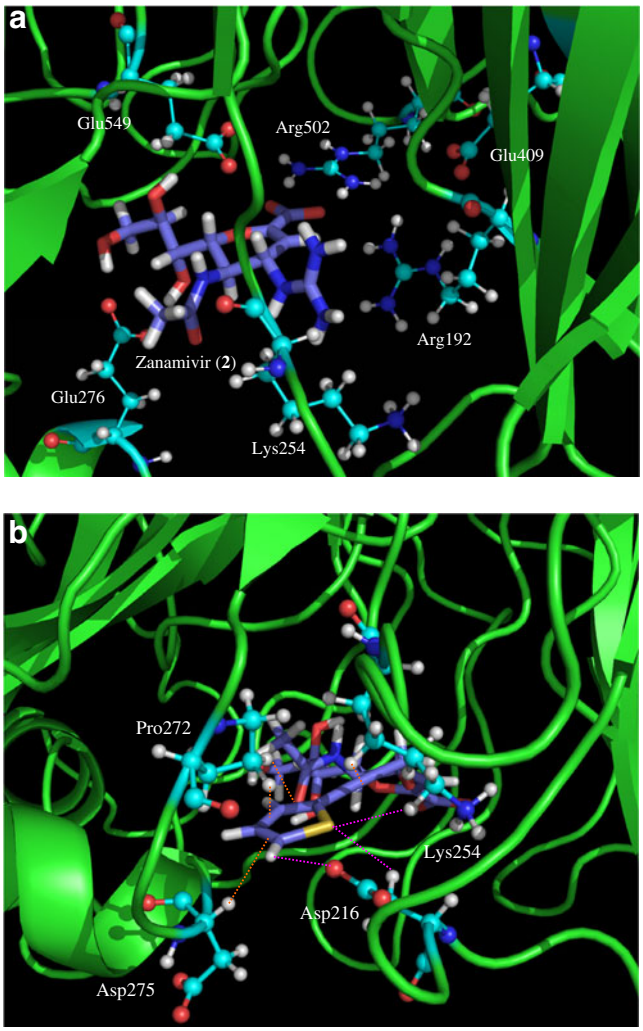

Fig. 4 **a** Amino acid residues of binding pocket of hPIV HN around Zanamivir(**2**). That the presence of basic residues such as Arg192 and Lys254 near the guanidino group of Zanamivir (**2**). **b** Amino acid residues of binding pocket of hPIV HN around Neu5Ac2en derivative **5**. 4 position of substituted group penetrate well into the hydrophobic pocket surrounded by Asp216, Lys254, Pro272, and Asp275

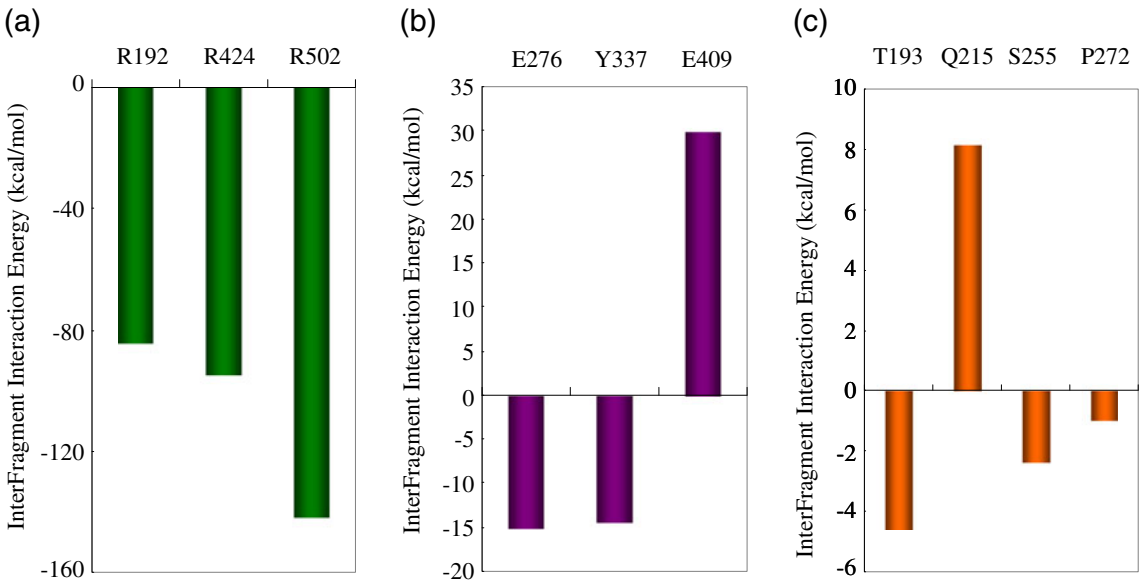

Fig. 5 **a** Interfragment Interaction Energy (IFIE) of Neu5Ac2en(**1**) and amino acid residues of hPIV HN around carboxyl group of Neu5Ac2en(**1**). **b** Interfragment Interaction Energy (IFIE) of Neu5Ac2en(**1**) and amino acid residues of hPIV HN around glycerol group of Neu5Ac2en(**1**). **c** Interfragment Interaction Energy (IFIE) of Neu5Ac2en(**1**) and amino acid residues of hPIV HN around 4 position of Neu5Ac2en(**1**)

In other words, the thiophene ring penetrated well into the hydrophobic pocket surrounded by Asp216, Lys254, Pro272, and Asp275, with interaction energies of +14.0 kcal/mol, −36.4 kcal/mol, −3.5 kcal/mol, and +14.0 kcal/mol, respectively. Note that the Neu5Ac2en derivative **5** forms the stable CH-π interaction between π-electrons of propargyl groups and the hydrogen atoms of C_β-H in Lys254, and CH-π interaction between π-electrons of thiophene ring and hydrogen atoms in side chains in Asp216, Pro272, and Asp275, and that the sulfur of thiophene ring forms the effective hydrogen bond with the C_ε-H of Lys254. These interactions reflect greater stabilization compared with Neu5Ac2en **1**, the interaction energies of which were −7.4 kcal/mol, −9.8 kcal/mol, −2.6 kcal/mol, and −9.5 kcal/mol, respectively. In addition, the binding affinity of the inhibitor **5** was more stable −36.9 kcal/mol for Neu5Ac2en This is consistent with the finding that Zanamivir **2** did not show good inhibitory activity against hPIV HN.

The carboxyl group of Neu5Ac2en interacts extensively with Arg192, Arg424, and Arg502 by forming hydrogen bonds. The hydroxyl group of glycerol also forms stable hydrogen bonds with Glu276, Tyr337, and Glu409. These theoretical results are in good agreement with the published data that the hydroxyl groups at positions 7, 8, and 9 of sialic acid derivatives are conserved among hPIV sialidase inhibitors [18, 19]. Although Zanamivir can form stable hydrogen bonds with Tyr530 and Thr193, its binding affinity for hPIV HN is weak compared with that of Neu5Ac2en **1** due to large ionic repulsions of Arg192, Lys254, and Arg502.

The interaction of the 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en derivative **5** with hPIV was very similar to that of Neu5Ac2en **1** except for at the 4-substituent position. On the other hand, the 4-substituted thiophene ring penetrated well into the hydrophobic pocket surrounded by Asp216, Lys254, Pro272, and Asp275 with improved binding affinity and additional weak interactions, such as CH-π interactions. This finding suggests that these weak interactions are important to the inhibitory activity of this hPIV sialidase.

Conclusions

We theoretically analyzed the FMO-IFIEs between sialic derivatives and amino acid residues in hPIV HN, comparing our findings with those for the anti-influenza inhibitor Zanamivir, to elucidate the origins of the high potency inhibitory activities of these sialic acid derivatives to hPIV. We found that the sialic acid derivative 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en, which had the best inhibitory activity against hPIV, formed important stable interactions between its 4-substituent and Asp216, Lys254, Pro272, and Asp275 around the hydrophobic pocket of the HN of hPIV, whereas Zanamivir exhibited large ionic (electrostatic) repulsions. These theoretical results provide useful information for the development of novel inhibitors to hPIV. Our future studies will include the rational design of a novel inhibitor to hPIV based on the predicted pharmacophore.

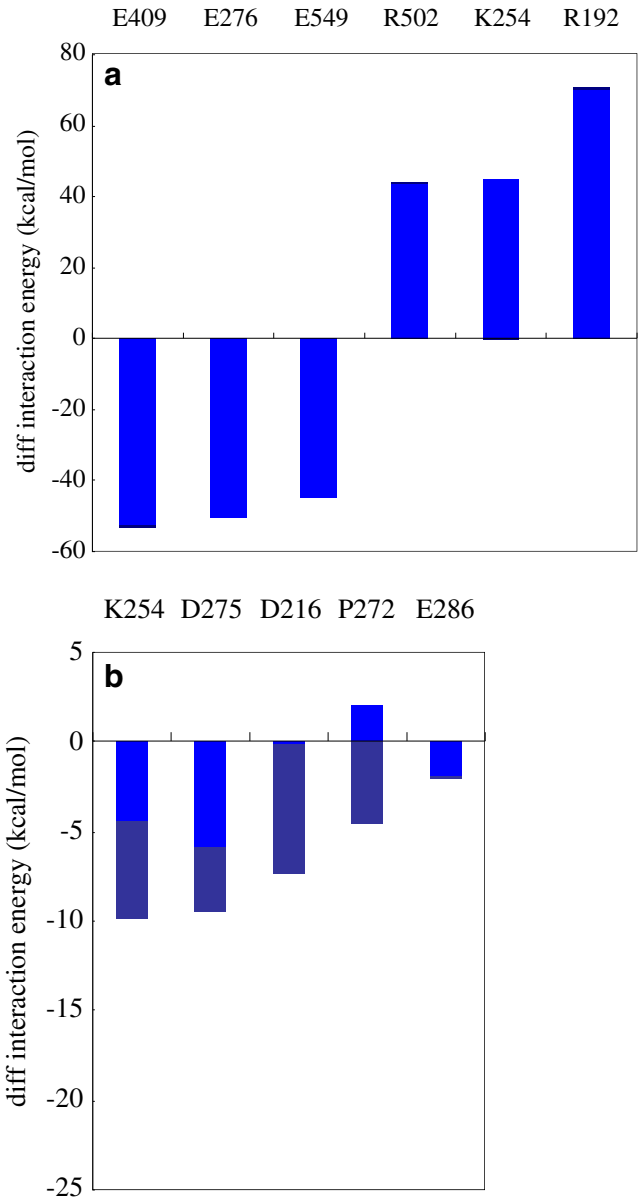

Fig. 6 **a** ΔIFIE of Zanamivir(**2**) and amino acid residues of hPIV HN. ΔIFIE was derived difference between IFIE of Zanamiivir(**2**) and amino acid residues of hPIV HN and IFIE of Neu5Ac2en(**1**) and amino acid residues of hPIV HN. **b** ΔIFIE of 4-*O*-(2-thienyl-2-propynyl) Neu5Ac2en derivative(**5**) and amino acid residues of hPIV HN. ΔIFIE was derived difference between IFIE of the derivate(**5**) and amino acid residues of hPIV HN and IFIE of Neu5Ac2en(**1**) and amino acid residues of hPIV HN

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