Biophysical Chemistry 140 (2009) 35–38

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

On the structure-based design of novel inhibitors of H5N1 influenza A virus neuraminidase (NA)

Petar M. Mitrasinovic

Center for Multidisciplinary Studies,[1] University of Belgrade, Kneza Viseslava 1a, 11030 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 3 August 2008
Received in revised form 12 November 2008
Accepted 12 November 2008
Available online 20 November 2008

Keywords:
Influenza A virus
H5N1 neuraminidase
Oseltamivir
Structure-based design
More potent inhibitor

ABSTRACT

The structure-based design of novel H5N1 neuraminidase inhibitors is currently a research topic of vital importance owing to both a recent pandemic threat by the worldwide spread of H5N1 avian influenza and the high resistance of H5N1 virus to the most widely used commercial drug, oseltamivir-OTV (Tamiflu). A specific criterion used in this work for determining fully acceptable conformations of potential inhibitors is a previous experimental proposal of exploiting potential benefits for drug design offered by the '150-cavity' adjacent to the NA active site. Using the crystal structure of H5N1 NA (PDB ID: 2hty) as the starting point, in a set of 54 inhibitors previously proposed by modifying the side chains of oseltamivir, 4 inhibitors were identified using two different computational strategies (ArgusLab4.0.1, FlexX-E3.0.1) both to lower the binding free energy (BFE) of oseltamivir and to have partially acceptable conformations. These 4 oseltamivr structure-based analogues were found to adopt the most promising conformations by identifying the guanidinium side chain of Arg156 as a prospective partner for making polar contacts, but none of the modified 4-amino groups of oseltamivir in the 4 favorable conformations was found to make polar contacts with the guanidinium side chain of Arg156. Hence, the structures of two additional inhibitors were designed and shown to further lower the binding free energy of OTV relative to the previous 54 inhibitors. These two novel structures clearly suggest that it may be possible for a new substituent to be developed by functional modifications at position of the 4-amino group of oseltamivir in order to make polar contacts with the guanidinium side chain of Arg156, and thereby enhance the binding of a more potent inhibitor. Several standpoints of vital importance for designing novel structures of potentially more effective H5N1 NA inhibitors are established.

1. Introduction

Due to different antigenic properties of various glycoprotein molecules, influenza type A viruses are classified into two phylogenetically distinct groups, group-1 (N1, N4, N5, N8) and group-2 (N2, N3, N6, N7, N9), which contain N1 and N2 NAs of viruses that currently circulate in humans. One of such viruses is H5N1 threatening a new pandemic. The high resistance of H5N1 virus to oseltamivir makes the structure-based design of novel anti-viral molecules a priority [1–3]. The crystal structure of H5N1 NA offers a wide spectrum of new opportunities for drug design [4,5]. This can be accounted for by structural differences between group-1 and group-2 NAs. A major consequence of these differences in structure is the presence of a large cavity, known as the '150-cavity', adjacent to the active site in group-1 but not in group-2 neuraminidases. This cavity is accessible from the N1 active site due to the differences in position of Asp151 and Glu119. The combined effect of the difference in position of these two particular residues results in the width increase of the active site cavity by about 5 Å. The conserved Arg156, having the side chain approximately mid-way between Asp151 and Glu119 (Fig. 1) and being at the base of the 150-cavity, adopts almost the same position in the group-1 and group-2 NA structures, thus defining the entrance from the N1 active site into the 150-cavity. Tyr347 is also shown in Fig. 1, because this particular residue in group-1 NAs makes a hydrogen bond interaction with the C1 carboxylate of oseltamivir [4]. N1 neuraminidase initially binds to OTV in this open conformation, but more likely adopts the higher energy or closed conformation of the 150-loop (residues 147–152) via a relatively slow conformational change. Based on an examination of the crystal structure of OTV/H5N1 NA (PDB ID: 2hu0B), it has been proposed [4] that new, more potent inhibitors may be developed from the 4-amino group of oseltamivir into the 150-cavity, while the prominent guanidinium side chain of Arg156 has been hypothesized as a prospective partner for a salt-bridge or

E-mail address: pmitrasinovic@yahoo.ca.
[1] Currently Institute for Multidisciplinary Research.

0301-4622/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2008.11.004

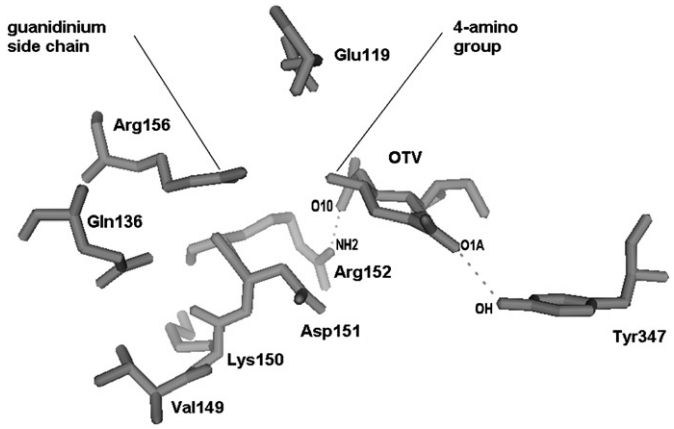

Fig. 1. Experimental proposal of the possibility of exploiting the 150-cavity of H5N1 NA (PDB ID: 2hu0B) by developing a new substituent through modifications of the 4-amino group of oseltamivir (OTV) to make polar contacts with the guanidinium side chain of Arg156 [4].

hydrogen bond with a new inhibitor (Fig. 1). In this paper this proposal is explored and rationalized.

2. Methods

Flexible docking calculations were performed using two different methods: (I) the AScore/ShapeDock protocol from the ArgusLab4.0.1 suite of programs [6] and (II) the FlexX-E3.0.1 program [7].

I. AScore is based on the decomposition of the total protein–ligand binding free energy, taking into account the following contributions: the van der Waals interaction between the ligand and the protein, the hydrophobic effect, the hydrogen bonding between the ligand and the protein, the hydrogen bonding involving charged donor and/or acceptor groups, the deformation effect, and the effects of the translational and rotational entropy loss in the binding process, respectively. The ShapeDock docking engine approximates a complicated search problem. Flexible ligand docking is available by describing the ligand as a torsion tree. Groups of bonded atoms that do not have rotatable bonds are nodes, while torsions are connections between the nodes. Topology of a torsion tree is a determinative factor influencing efficient docking. The AScore/ShapeDock protocol is fast, reproducible, and formally explores all energy minima [6]. This particular protocol was shown to be very consistent for docking OTV into the crystal structures of H5N1 NAs [1].

II. The FlexX scoring function [8] is an estimate of the free binding energy for an ideal hydrogen bond, ionic, aromatic, or lipophilic protein–ligand interaction, adjusted by a penalty function depending on deviation from the ideal interatomic radius for the two interacting elements [9]. The computational algorithm underlying FlexX is based on the decomposition of a ligand into pieces, which are then flexibly built up in the active site using a variety of placement strategies. To take into account receptor flexibility, the docking experiment also consists of an ensemble-based soft docking procedure using FlexX-Ensemble [10]. At the very end, an effective flexible receptor–ligand complex optimization is accomplished by means of the Yasara program [11]. All the calculations were done using default parameters.

Figures shown in this paper were generated by PyMol [12].

3. Results and discussion

The binding of the previously proposed [3] structures of 54 oseltamivir analogues (Fig. 2) to H5N1 NA (PDB ID: 2hty) was herein investigated using FlexX-E (Table S1, Supplementary material) and ArgusLab (Table S2, Supplementary material). Besides considering the binding free energies of the complexes for identifying the promising candidates for more potent inhibitors, conformational requirement, based on the experimental proposal for exploiting the 150-cavity of H5N1 NA (Fig. 1), was considered too. A most favorable conformation was thus viewed to have a modified 4-amino group of oseltamivir making polar contacts with the guanidinium side chain of Arg156.

Four inhibitors (11 and 12 with X=NHC(=NH_2^+)NH_2; 13 and 17 with X=NHC(=N-CH_4^+)NH_2) were identified both to lower the binding free energy of oseltamivir (Tables S1 and S2, Supplementary material) and to have partially acceptable conformations. All the spatial orientations of the particular inhibitors (Fig. 3) properly make polar contacts with the guanidinium side chain of Arg156, but none of them has its X side chain (modified 4-amino group of OTV) involved in the interactions with Arg156, as experimentally proposed (Fig. 1). While the carboxylic group of ligand 11 makes a polar contact with Arg156 (Fig. 3a), the –O-Y side chain of ligand 12 makes 2 polar contacts with Arg156 (Fig. 3b). Note that the X side chain of ligand 11 (Fig. 3a) is not involved in any polar contact, while the X side chain of ligand 12 makes 1 intraligand polar contact with the carboxylic group (Fig. 3b), thus accounting for the difference in position of the X group of ligand 11 relative to that of ligand 12. The X side chain of ligand 13 makes 2 polar contacts with Asp151 (Fig. 3c), while that of ligand 17 makes 1 polar contact with Asp151 (Fig. 3d). At the first look, this might indicate a similar spatial orientation of the ligands 13 and 17. However, it is important to note 1 electrostatic interaction between Tyr347 and the C1 carboxylate of ligand 17 (Fig. 3d), which also exists in the case of ligand 12 (Fig. 3b). The specific Tyr347-carboxylic group contact is not present in the cases of ligands 11 and 13 (Fig. 3a and c), thus showing different spatial orientations of the –COOH group in these ligands relative to that in ligands 12 and 17 (Fig. 3b and d).

The same set of 54 potential inhibitors (Table S1, Supplementary material) was previously investigated using two different scoring functions, the Amber and Grid scores, implemented in the Dock6.01 program [3]. Of these 54 ligands, the identified inhibitors, having both

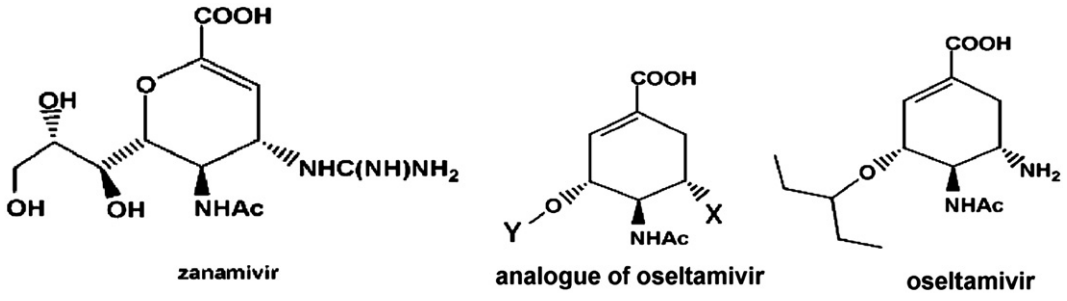

Fig. 2. General skeleton of oseltamivir structure-based analogues [3] that were considered in Tables S1 and S2 (Supplementary material).

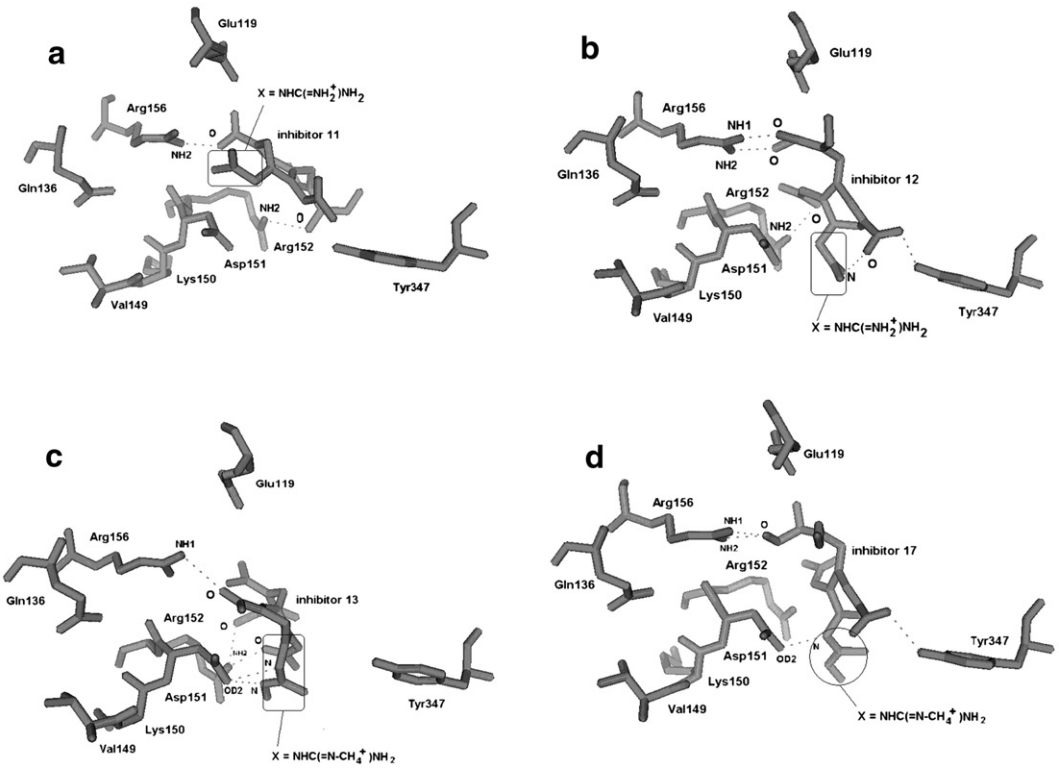

Fig. 3. The conformations of (a) ligand 11 (X=NHC(=NH_2^+)NH_2), (b) ligand 12 (X=NHC(=NH_2^+)NH_2), (c) ligand 13 (X=NHC(=N-CH_4^+)NH_2), and (d) ligand 17 (X=NHC(=N-CH_4+)NH_2) considered in Table S2 (Supplementary material) using AScore/ShapeDock. All the conformations properly identify the guanidinium side chain of Arg156 as a prospective partner for making polar contacts, but none of the contacts is with the X side chain (modified 4-amino group of OTV) of the inhibitors, as experimentally proposed in Fig. 1.

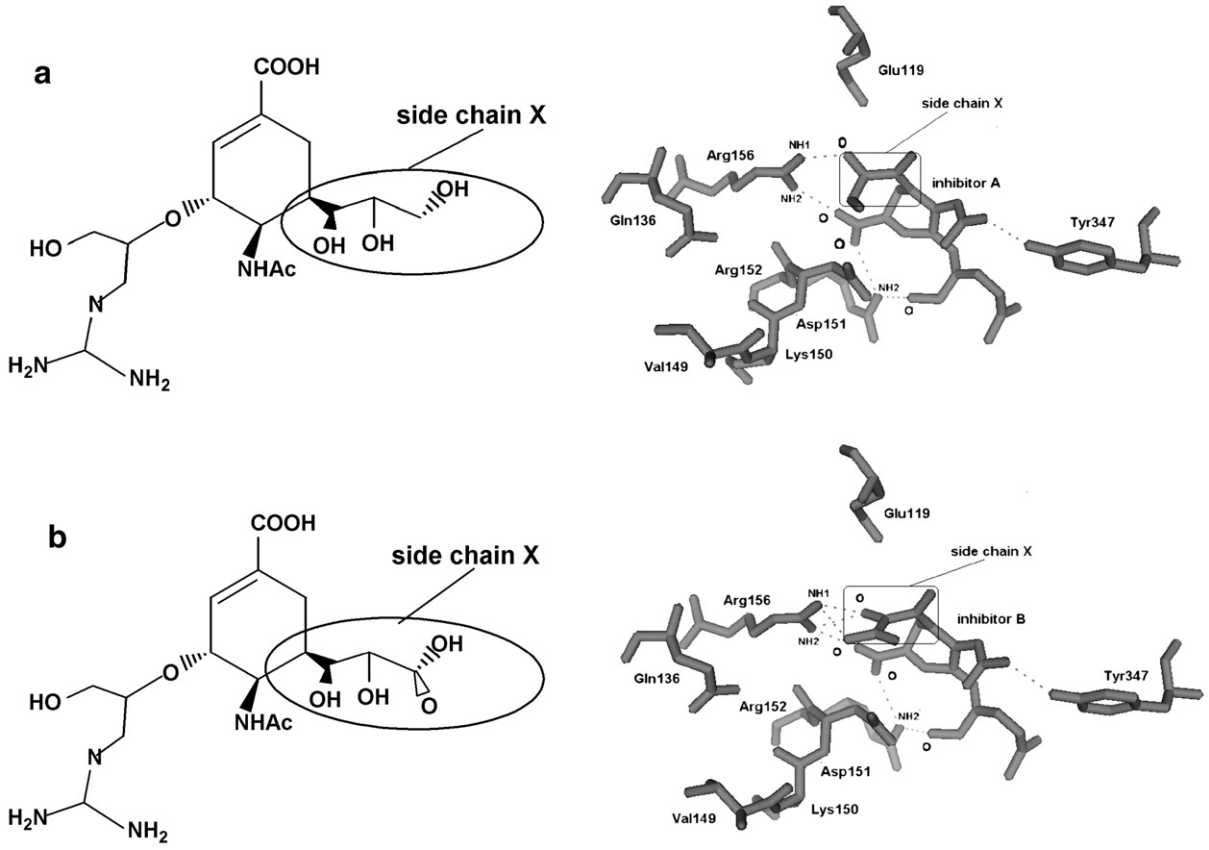

Fig. 4. (a) Proposal of chemical structure of new inhibitor A designed by AScore/ShapeDock. The X side chain makes 1 polar contact with Arg156. The BFEs of the H5N1 NA-inhibitor A complex are −9.17 kcal/mol (ArgusLab) and −51.56 kcal/mol (FlexX-E). (b) Proposal of chemical structure of new inhibitor B designed by AScore/ShapeDock. The X side chain makes 3 polar contacts with Arg156. The BFEs of the H5N1 NA-inhibitor B complex are −9.37 kcal/mol (ArgusLab) and −58.78 kcal/mol (FlexX-E). Note that the BFEs of the H5N1 NA-oseltamivir complex are −5.89 kcal/mol (ArgusLab) and −41.06 kcal/mol (FlexX-E).

the better binding affinity with respect to oseltamivir and the most favorable conformations, were: inhibitors 4, 7 and 13 with X=NHC(=NH_2^+)NH_2, inhibitor 14 with X=NH_3^+, and inhibitor 14 with X=NHC(=NH_2^+)NH_2 [3]. Because the conformational requirement for selecting the most favorable conformations in the previous study [3] was substantially different from the experimental proposal shown in Fig. 1, these 5 previously identified favorable conformations [3] are distinct from the 4 most promising ones shown in Fig. 3.

Since the spatial orientation of the –COOH group is different in the ligands 11, 12, 13, and 17 (Fig. 3) as discussed above, and none of the particular conformations was found to fit the '150-cavity' as experimentally proposed (Fig. 1), it is therefore difficult to say which one of the 4 favorable conformations would be the most rational choice leading to the structure-based design of more potent inhibitors. It is well established that two or three Arg residues surrounding the carboxylic group of NA inhibitors play a key role in orienting and stabilizing various inhibitors [13,14]. The fact that Arg156 makes 1 polar contact with the –COOH group in the inhibitors 11 and 13 (Fig. 3a and c) was the first crucial idea for us to attempt to exploit two nearby Arg residues, Arg152 and Arg156, as a predominant factor for orienting and stabilizing novel inhibitors by making electrostatic interactions with the carboxylic group. The second crucial idea was that the X side chain of novel inhibitors needs to be involved in electrostatic contacts with the guanidinium side chain of Arg156, as experimentally proposed (Fig. 1). Taking into account the high resistance of H5N1 virus both to oseltamivir and most likely to the OTV analogues [3], the third crucial idea was to increase the probability of escaping viable drug-resistant H5N1 mutants by maintaining a clear resemblance of the structures of more potent inhibitors to sialic acid, a natural substrate from which zanamivir is directly derived with minimal functional modifications (Fig. 2), as more recently recommended experimentally [5] and computationally [1]. To reconcile the three standpoints of vital importance for developing novel structures of potentially more effective inhibitors, chemical intuition was employed in the present study.

Oseltamivir, having the –O-Y group instead of the glycerol side chain, is quite a different inhibitor from zanamivir (Fig. 2). The key difference is the –O-Y group of OTV that is capable of rotating around the single bond between oxygen and alkyl chain Y, thus adapting itself to a comfortable position relative to its environment. Hence, various modifications (Table S1, Supplementary material) of the 4-amino group of OTV did not establish a consistent spatial orientation of the carboxylic group, which could stabilize the oseltamivir analogues by providing an effective fit of the '150-cavity'. Shortly after this indication in our search for more potent inhibitor structures, it became interesting that the nature of the X side chain must be quite different from that of the 4-amino group of OTV in order to have the –COOH group stabilized by the Arg152 and Arg156 amino acid residues. The fact that the X side chains of the oseltamivir derivatives (Table S1, Supplementary material) were not involved in electrostatic interactions with Arg156 indicated that substantial modifications of the 4-amino group are expected to be followed by substantial modifications of the Y side chain of OTV. After examining many different substituents for the alkyl chain Y, it was quite indicative that the particular side chain needs to be primarily based on oxygen and nitrogen atoms that are able to make additional electrostatic interactions. In this context, the first encouraging proposal of a new inhibitor structure was that given in Fig. 4a. The binding free energies of the H5N1 NA-inhibitor A complex, determined by FlexX-E and ArgusLab, are −51.56 kcal/mol and −9.17 kcal/mol respectively. The computed energies are lower than those reported in Tables S1 and S2 (Supplementary material). The carboxylic group of inhibitor A makes 2 polar contacts, the first with Arg152 and the second with Arg156. In agreement with the experimental proposal (Fig. 1), the X side chain of inhibitor A makes 1 polar contact with Arg156 (Fig. 4a). A slight modification, reflected by introducing a –COOH group at the very end of the X side chain of inhibitor A, led to the most encouraging chemical structure of inhibitor B shown in Fig. 4b. The binding free energies of the H5N1 NA-inhibitor B complex, determined by FlexX-E and ArgusLab, are −58.78 kcal/mol and −9.37 kcal/mol respectively. The first of the computed energies is significantly lower (by about 7 kcal/mol) than that reported above for inhibitor A. Besides, the carboxylic group of inhibitor B makes 3 polar contacts with Arg residues, 1 with Arg152 and 2 with Arg156. Most importantly, the X side chain of inhibitor B properly identifies the guanidinium side chain of Arg156 as a prospective partner by making 3 polar contacts with Arg156 (Fig. 4b). Because the experimental structure of H5N1 avian influenza neuraminidase, in the same way, previously suggested new opportunities for the design of more potent anti-viral molecules [4], the proposed inhibitor structures (Fig. 4) are believed to confer specificity for avian flu strain of influenza.

4. Summary

Our results (Fig. 4) suggest that successful modifications of both the –O-Y and 4-amino side chains of oseltamivir are possible in order to exploit experimentally identified potential benefits (Fig. 1) offered by the '150-cavity' adjacent to the H5N1 NA active site. Interestingly, a slightly modified Y side chain of zanamivir (Fig. 2) may be a promising candidate for the X side chain of novel inhibitors. Two Arg residues, Arg152 from the 150-loop and Arg156, are presumably needed to make electrostatic interactions with the carboxylic group of potential inhibitors, thus being a prevailing factor for orienting and stabilizing more potent candidates.

Acknowledgment

The Ministry of Science of the Republic of Serbia.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bpc.2008.11.004.

References

[1] M.L. Mihajlovic, P.M. Mitrasinovic, Another look at the molecular mechanism of the resistance of H5N1 influenza A virus neuraminidase (NA) to oseltamivir (OTV), Biophys. Chemist. 136 (2008) 152–158.
[2] M. Von Itzstein, The war against influenza: discovery and development of sialidase inhibitors, Nat. Rev. Drug Discov. 6 (2007) 967–974.
[3] Q.-S. Du, S.-Q. Wang, K.-C. Chou, Analog inhibitors by modifying oseltamivir based on the crystal neuraminidase structure for treating drug-resistant H5N1 virus, Biochem. Biophys. Res. Commun. 362 (2007) 525–531.
[4] 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.
[5] P.J. Collins, L.F. Haire, Y.P. Lin, J. 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.
[6] M.A. Thompson, ArgusLab 4.0.1. Planaria Software, LLC, Seatle, WA, 2004 http://www.arguslab.com.
[7] H. Claussen, C. Buning, M. Rarey, T. Lengauer, FlexE: efficient molecular docking considering protein structure variation, J. Mol. Biol. 308 (2001) 377–395. M. Rarey, FlexX 3.0.1. BioSolveIT GmbH, St. Augustin, Germany, 2008, http://www.biosolveit.de.
[8] M. Gastreich, M. Lilienthal, H. Briem, H. Claussen, Ultrafast de novo docking combining pharmacophores and combinatorics, J. Comput.-Aided Mol. Des. 20 (2006) 717–734.
[9] H.-J. Böhm, The development of a simple empirical scoring function to estimate the binding constant for a protein–ligand complex of known three-dimensional structure, J. Comput.-Aided Mol. Des. 8 (1994) 243–256.
[10] M. Rarey, B. Kramer, T. Lengauer, G. Klebe, A fast flexible docking method using an incremental construction algorithm, J. Mol. Biol. 261 (1996) 470–489.
[11] E. Krieger, YASARA Biosciences, Graz, Austria, http://www.yasara.org/.
[12] W.L. DeLano, PyMol™. Release 0.97. DeLano Scientific, LLC, San Carlos, CA, 2004.
[13] T. Wang, R.C. Wade, Comparative binding energy (COMBINE) analysis of influenza neuraminidase–inhibitor complexes, J. Med. Chem. 44 (2001) 961–971.
[14] A.R. Ortiz, M.T. Pisabarro, F. Gago, R.C. Wade, Prediction of drug binding affinities by comparative binding energy analysis, J. Med. Chem. 38 (1995) 2681–2691.