DOI: 10.14529/jsfi220207

Search for Ligands Complementary to the 430-cavity of Influenza Virus Neuraminidase by Virtual Screening

Dmitry K. Nilov[1,2]***, Michaela Schmidtke***[3]***, Vadim A. Makarov***[4]***, Vytas K. Švedas***[2,5]

An anthrapyrazole derivative STK663786 has been identified as a selective ligand of the so-called 430-cavity of influenza virus neuraminidase at virtual screening of a library of low-molecular-weight compounds. It is able to form favorable contacts with hydrophobic residues as well as cation-π interaction and hydrogen bonds with the polar Arg371 residue. The experimentally determined EC_{50} values have been found to be 19 and 30 μM for viruses H1N1 and H3N2, respectively. Complementarity of STK663786 to the 430-cavity adjacent to the sialic acid binding subsite in the active center of neuraminidase makes this compound a valuable structural fragment at construction of bifunctional inhibitors of the enzyme.

Keywords: influenza, neuraminidase, 430-cavity, inhibitor, anthrapyrazole.

Introduction

Seasonal flu affects nearly 10% of the worlds population every year, and the pandemic influenza virus strains pose serious danger worldwide [1–3]. Two glycoproteins on the surface of the viral envelope, hemagglutinin and neuraminidase, are responsible for infectivity. Hemagglutinin binds to terminal sialic acid residues of epithelial receptors, and then virus enters the cell by endocytosis. The neuraminidase enzyme, on the contrary, cleaves sialic acid residues, which promotes the release of newly formed viral particles from the cell surface [4, 5]. The widely used anti-influenza drugs zanamivir and oseltamivir, being structurally similar to the sialic acid residue in natural enzyme substrates, competitively inhibit neuraminidase activity [6, 7]. However, virus strains resistant to these drugs are quickly emerging due to mutations in the sialic acid binding subsite [8, 9].

Another putative binding site of neuraminidase inhibitors, the so-called 430-cavity, is formed by a series of hydrophobic residues [10–13]. The interface between the sialic acid binding subsite and 430-cavity is formed by three arginine residues (Arg118, Arg292, Arg371), with the Arg371 residue making a decisive contribution to the positioning of the carboxyl group of the substrate or competitive inhibitors due to the formation of two hydrogen bonds [14]. In the present study, we have performed virtual screening to identify ligands complementary to the 430-cavity, which can interact with both the hydrophobic residues of the cavity and the guanidine group of Arg371.

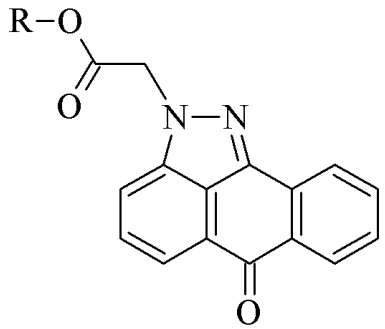
1. Results and Discussion

The molecular model of N1 neuraminidase was constructed based on the 3b7e crystal structure [15] using the Amber 12 package [16]. Hydrogen atoms were added to the protein structure, and then it was solvated by a layer of TIP3P water. The energy minimization of the obtained system included 2500 steps of the steepest descent algorithm followed by 2500 steps of conjugate

[1]Lomonosov Moscow State University, Belozersky Institute of Physicochemical Biology, Moscow, Russia
[2]Lomonosov Moscow State University, Research Computing Center, Moscow, Russia
[3]Department of Virology and Antiviral Therapy, Jena University Hospital, Jena, Germany
[4]Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia
[5]Lomonosov Moscow State University, Faculty of Bioengineering and Bioinformatics, Moscow, Russia

gradient algorithm, and the heavy atoms of the protein were being fixed by positional restraints. The *ff99SB* force field was applied to describe the protein by molecular mechanics [17]. The optimized N1 model was used in docking experiments described below.

Virtual screening was carried out using a subset of low-molecular-weight compounds retrieved from the Vitas-M commercial library (https://vitasmlab.biz). Molecules containing a carboxyl group and obeying the rule of three (molecular weight < 300, $\log P \leq 3$, hydrogen bond donors ≤ 3, hydrogen bond acceptors ≤ 3, rotatable bonds ≤ 3) [18] were retrieved by a substructure search in ACD/ChemFolder (http://www.acdlabs.com): in total, 3734 compounds. 3D structures of the compounds were generated with the CORINA software [19]. Each compound was docked into the N1 active site using Lead Finder 1.1.15 [20, 21], and then the modeled positions were subjected to structural filtration [22] to select molecules capable of forming hydrophobic contacts with 430-cavity residues as well as hydrogen bonds with the Arg371 guanidinium group. For this purpose, the vsFilt software [23] integrated into a high-throughput virtual screening platform of the Lomonosov Moscow State University supercomputer was used. Visual inspection of the selected molecules allowed us to identify the anthrapyrazole derivative STK663786 (Fig. 1; $\Delta G^{calc} = -5.9$ kcal/mol) as a promising ligand of the 430-cavity.



STK663786 *R*=H
STK734244 *R*=CH_3

Figure 1. Chemical structure of anthrapyrazole derivatives

In the obtained model, the anthrapyrazole scaffold of STK663786 forms favorable hydrophobic contacts with the Trp403, Ile427, and Pro431 residues (Fig. 2a), while the carboxyl group is involved in a network of hydrogen bonds with the arginine triad residues Arg118, Arg292, and Arg371 (Fig. 2b). In addition, effective binding of the STK663786 scaffold is facilitated by a cation-π interaction with the guanidine group of Arg371. The positioning of the ligand in the 430-cavity makes it possible to elongate its structure towards the adjacent sialic acid binding subsite. The carboxyl groups of zanamivir and STK663786 occupy nearly the same position (Fig. 2c), and therefore we believe that prototypes of bifunctional inhibitors can be constructed by combining two scaffolds, zanamivir (or its structural analogue) and STK663786, into a single chimeric molecule. An appropriate ester linker or isosteric analogues of the carboxyl linker (sulfo, phosphono, etc.) capable of interacting with the arginine triad may be of interest.

A preliminary *in vitro* study of STK663786 has confirmed its inhibitory effect against influenza viruses. In cytopathic effect (CPE) assay [25], the EC_{50} values were found to be 19 and 30 μM for viruses H1N1 (8178/09) and H3N2 (HK/68), respectively. We have also tested a methyl ester of STK663786, compound STK734244 (Fig. 1). It showed an inhibitory effect (EC_{50} equal to 53 and 85 μM for viruses H1N1 and H3N2, respectively), though less pronounced compared

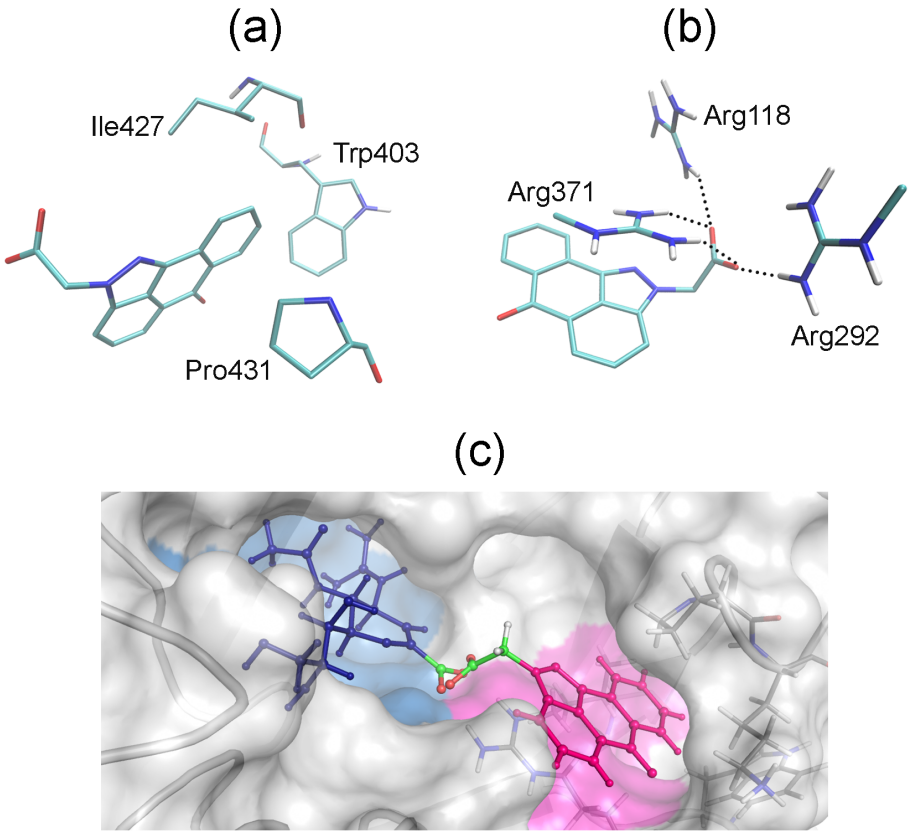


Figure 2. Binding mode of STK663786 to the N1 neuraminidase: (a) Interactions with hydrophobic residues of the 430-cavity, for clarity, non-polar hydrogen atoms are omitted; (b) Interactions with the arginine triad, guanidinium groups of arginine residues are shown; (c) Comparison of zanamivir and STK663786 positions in the neuraminidase active center: the sialic acid binding subsite and zanamivir molecule are colored blue, the 430-cavity and STK663786 molecule are colored magenta. Carboxyl substituents forming hydrogen bonds with Arg371 are shown in green. The figure was prepared using VMD and PyMOL [24]

to STK663786. Apparently, this is due to the absence of a negative charge on the esterified carboxyl group and the weakening of the interaction with the positively charged arginine triad. Thus, virtual screening made it possible to identify the ligand STK663786, complementary to the 430-cavity, which by itself inhibits neuraminidase, disrupting the interaction between the substrate and the arginine triad, and can be used as a structural fragment of a chimeric molecule of bifunctional inhibitors capable of occupying both the cavity-430 and sialic acid binding subsite in the neuraminidase active center.

Acknowledgements

This work was supported by the Russian Science Foundation (grant No. 21-71-30003). The research was carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.

References

1. Zambon, M.C.: Epidemiology and pathogenesis of influenza. J. Antimicrob. Chemother. 44, 3–9 (1999). https://www.doi.org/10.1093/jac/44.suppl_2.3

2. Layne, S.P., Monto, A.S., Taubenberger, J.K.: Pandemic influenza: an inconvenient mutation. Science. 323(5921), 1560–1561 (2009). https://www.doi.org/10.1126/science.323.5921.1560

3. Hussain, M., Galvin, H.D., Haw, T.Y., *et al.*: Drug resistance in influenza A virus: the epidemiology and management. Infect. Drug Resist. 10, 121–134 (2017). https://www.doi.org/10.2147/IDR.S105473

4. Bouvier, N.M., Palese, P.: The biology of influenza viruses. (2008) Vaccine. 26, D49–D53 (2008). https://www.doi.org/10.1016/j.vaccine.2008.07.039

5. Herold, S., Becker, C., Ridge, K.M., Budinger, G.R.: Influenza virus-induced lung injury: pathogenesis and implications for treatment. Eur. Respir. J. 45(5), 1463–1478 (2015). https://www.doi.org/10.1183/09031936.00186214

6. von Itzstein, M., Wu, W.Y., Kok, G.B., *et al.*: Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature. 363(6428), 418–423 (1993). https://www.doi.org/10.1038/363418a0

7. Lew, W., Chen, X., Kim, C.U.: Discovery and development of GS 4104 (oseltamivir): an orally active influenza neuraminidase inhibitor. Curr. Med. Chem. 7(6), 663–672 (2000). https://www.doi.org/10.2174/0929867003374886

8. Thorlund, K., Awad, T., Boivin, G., Thabane, L.: Systematic review of influenza resistance to the neuraminidase inhibitors. BMC Infect. Dis. 11, 134 (2011). https://www.doi.org/10.1186/1471-2334-11-134

9. Hurt A.C., Besselaar T.G., Daniels R.S., *et al.* Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014-2015. Antiviral Res. 132, 178–185 (2016). https://www.doi.org/10.1016/j.antiviral.2016.06.001

10. Amaro, R.E., Minh, D.D., Cheng, L.S., *et al.*: Remarkable loop flexibility in avian influenza N1 and its implications for antiviral drug design. J. Am. Chem. Soc. 129(25), 7764–7765 (2007). https://www.doi.org/10.1021/ja0723535

11. Landon, M.R., Amaro, R.E., Baron, R., *et al.*: Novel druggable hot spots in avian influenza neuraminidase H5N1 revealed by computational solvent mapping of a reduced and representative receptor ensemble. Chem. Biol. Drug Des. 71(2), 106–116 (2008). https://www.doi.org/10.1111/j.1747-0285.2007.00614.x

12. Cheng, L.S., Amaro, R.E., Xu, D., *et al.*: Ensemble-based virtual screening reveals potential novel antiviral compounds for avian influenza neuraminidase. J. Med. Chem. 51(13), 3878–3894 (2008). https://www.doi.org/10.1021/jm8001197

13. Feng, E., Shin, W.J., Zhu, X., *et al.*: Structure-based design and synthesis of C-1- and C-4-modified analogs of zanamivir as neuraminidase inhibitors. J. Med. Chem. 56(3), 671–684 (2013). https://www.doi.org/10.1021/jm3009713

14. Taylor, N.R., von Itzstein, M.: Molecular modeling studies on ligand binding to sialidase from influenza virus and the mechanism of catalysis. J. Med. Chem. 37(5), 616624 (1994). https://www.doi.org/10.1021/jm00031a011

15. Xu, X., Zhu, X., Dwek, R.A., *et al.*: Structural characterization of the 1918 influenza virus H1N1 neuraminidase. J. Virol. 82(21), 10493–10501 (2008). https://www.doi.org/10.1128/JVI.00959-08

16. Case, D.A., Darden, T.A., Cheatham III, T.E., *et al.*: AMBER 12. University of California, San Francisco (2012). http://ambermd.org/doc12/Amber12.pdf

17. Hornak, V., Abel, R., Okur, A., *et al.*: Comparison of multiple Amber force fields and development of improved protein backbone parameters. Proteins. 65(3), 712–725 (2006). https://www.doi.org/10.1002/prot.21123

18. Congreve, M., Carr, R., Murray, C., Jhoti H.: A 'rule of three' for fragment-based lead discovery? Drug Discov. Today. 8(19), 876–877 (2003). https://www.doi.org/10.1016/s1359-6446(03)02831-9

19. Schwab, C.H.: Conformations and 3D pharmacophore searching. Drug Discov. Today Technol. 7(4), e245–e253 (2010). https://www.doi.org/10.1016/j.ddtec.2010.10.003

20. Stroganov, O.V., Novikov F.N., Stroylov, V.S., *et al.*: Lead finder: an approach to improve accuracy of protein-ligand docking, binding energy estimation, and virtual screening. J. Chem. Inf. Model. 48(12), 2371–2385 (2008). https://www.doi.org/10.1021/ci800166p

21. Novikov, F.N., Stroylov, V.S., Zeifman, A.A., *et al.*: Lead Finder docking and virtual screening evaluation with Astex and DUD test sets. J. Comput. Aided Mol. Des. 26(6), 725–735 (2012). https://www.doi.org/10.1007/s10822-012-9549-y

22. Novikov, F.N., Stroylov, V.S., Stroganov, O.V., Chilov, G.G.: Improving performance of docking-based virtual screening by structural filtration. J. Mol. Model. 16(7), 1223–1230 (2010). https://www.doi.org/10.1007/s00894-009-0633-8

23. Gushchina, I.V., Polenova, A.M., Suplatov, D.A., *et al.*: vsFilt: a tool to improve virtual screening by structural filtration of docking poses. J. Chem. Inf. Model. 60(8), 3692–3696 (2020). https://www.doi.org/10.1021/acs.jcim.0c00303

24. Martinez, X., Krone, M., Alharbi, N., *et al.*: Molecular graphics: bridging structural biologists and computer scientists. Structure. 27(11), 1617–1623 (2019). https://www.doi.org/10.1016/j.str.2019.09.001

25. Schmidtke, M., Schnittler, U., Jahn, B., *et al.*: A rapid assay for evaluation of antiviral activity against coxsackie virus B3, influenza virus A, and herpes simplex virus type 1. J. Virol. Methods. 95(1-2), 133–143 (2001). https://www.doi.org/10.1016/s0166-0934(01)00305-6