Structure-based discovery of new influenza neuraminidase inhibitors

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Introduction

Influenza virus infections are a continuous threat to humans. The death toll every year in the USA amounts to about 30,000. Antiviral compounds against influenza viruses are limited in number. Two substances directed against the activity of the neuraminidase, oseltamivir (OTV, Tamiflu®) and zanamivir (Relenza®), reduce the severity of the course of infection. Mainly OTV has been applied as a therapy in human cases infected with H5N1, but virus strains resistant to OTV have been isolated from patient material, underscoring the need for alternative inhibitors.
HK103 has been selected previously by a dynamic combinatorial chemistry method. It displays a K_i value of 16 nM for the neuraminidase [1]. Cell assay and animal experiments, as well as docking results, show that HK103 may be worth developing into a drug against influenza virus infection.

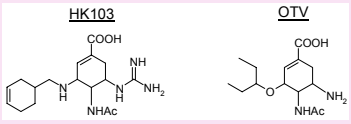


Fig. 1 schematic structures of Hk103 and OTV

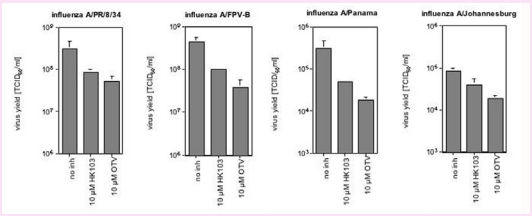
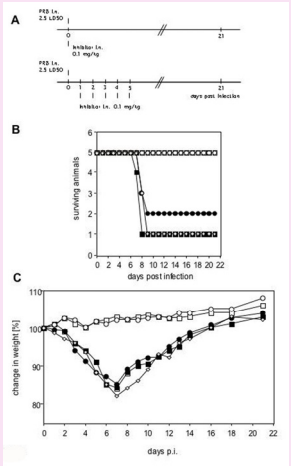


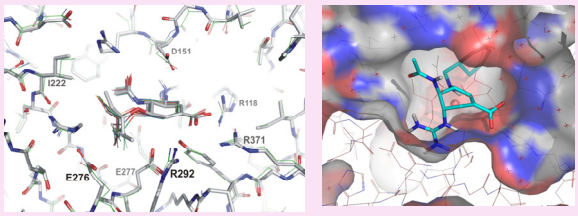
Fig. 2 Inhibition of human and avian influenza A strains by HK103 in cell culture.

The infection was carried out with influenza A/PR/8/34, A/FPV-B, A/Panama, and A/Johannesburg. The data show that all strains show a similar 3- to 10-fold reduction of virus titer when treated with 10 µM HK103 and a 5- to 12-fold reduction in the presence of 10 µM OTV.



Groups of 5 mice were infected by the intranasal route with 200 $TCID_{50}$ of influenza A/PR/8/34 virus (20 µl) (Fig. 3). Two groups of mice received once 0.1 mg/kg of either HK103 (empty circles) or OTV (empty squares) immediately after infection by the intranasal route. Three groups of infected mice received 0.1 mg/kg of either HK103 (filled circles), OTV (filled squares), or PBS (empty diamonds) at 24 h post infection by the intranasal route. For the latter 3 groups, application of inhibitor or PBS was repeated 4 times at 24-h intervals by the intranasal route. The results reveal that HK103 but also OTV are effective when given at the time of viral challenge.

Fig. 3 Inhibition of influenza A virus *in vivo*. (A) treatment schemes; (B) Percentage of mice surviving infection; (C) weight changes of mice during the course of infection. The weight of each animal was determined every 24 h to 48 h as indicated.



(a) (b)

Fig. 4 (a) Docking results for OTV into the active site of the N9 enzyme (1L7F, green) compared to the crystal structure of the complex between the closed form of N1 with the drug (2HU4 [2], sticks).

(b) Binding mode of HK103 with the N1 enzyme using the validated docking parameters.

Table 1 Docking energies for three inhibitors with N1 and N9 neuraminidase structures

	BCX-1812			oseltamivir			HK103	
	docking energy [kcal/mol]	Ki [nM]	RMSD [Å]	docking energy [kcal/mol]	Ki [nM]	RMSD [Å]	docking energy [kcal/mol]	Ki [nM]
1L7F (wt)	-13.01	22	0.85	-13.20	19	0.92	-13.56	20
1L7F + HOH35	-14.18	3	0.80	-13.53	14	0.99	-13.59	12
1L7G (E119G)	-14.57	2	0.53	-12.70	67	1.13	-14.28	4
1L7G + HOH265	-14.76	2	0.71	-13.03	40	1.14	-13.57	19
1L7H (R292K)	-14.07	5	0.67	-12.40	106	1.31	-13.14	29
1L7H + HOH213	-14.44	2	0.64	-12.85	50	1.38	-13.47	15
2HTY (free)	n.d.	n.d.	n.d.	-11.38	706	1.24	-13.01	48
2HU0 (OTV, open)	n.d.	n.d.	n.d.	-11.29	784	1.39	-11.78	169
2HU4 (OTV, closed)	n.d.	n.d.	n.d.	-12.79	67	0.92	-13.53	14

n.d. – not determined

From the binding energy, we were able to explain why the R292K mutant of N9 confers resistance to OTV: the loss of the interaction betwwen R292 and the carboxylate of OTV causes a substantial decrease in binding energy. On the other hand, the mutation E119G may still be susceptible to HK103.

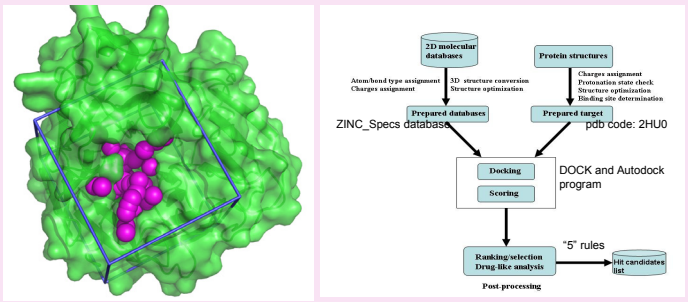


Fig. 5 Docking model (left) and procedure (right) for virtual screening

Conclusions

- HK103 was shown to be effective against influenza virus in a mouse model.
- HK103 was docked into the active site of the N1 and N9 neuriaminidases.
- Docking to the Relenza-resistant E119G mutation suggests that HK103 will be active against this mutation.
- Based on the X-ray structure of H5N1 neuraminidase, 20 inhibitor candidates from virtual screening were selected; activities assay is in progress.
- In order to overcome drug resistance, a double or triple therapy, as is now common standard in chemotherapy of HIV/AIDS, may have to be envisaged.

References

[1] M. Hochgürtel, et al., *PNAS* **99**, 3382-3387 (2002)

[2] R.J. Russell, *et al*., *Nature* **443**, 45-49 (2006).