In vitro Validation of Virtual Screening Method Using Health GRID for the Development of Neuraminidase Inhibitor

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The neuraminidase (NA) of influenza virus is a surface glycoprotein that catalyzes the hydrolysis of glycosidic linkages between sialic acids and adjacent sugar moieties. Neuraminidase of H5N1 avian influenza is principle protein for the development of the new anti-influenza molecules to block the worldwide spread of virus. *In silico* drug discovery is one of the attractive strategies to speed-up the drug discovery process and to reduce the cost. Fortunately, the crystal structure of H5N1 avian influenza neuraminidase (NA) suggests new opportunities for drug design [1]. In the present work, we developed potential new inhibitor compounds using international Grid and following *in vitro* experiment to evaluate the potential usefulness of chemicals as the NA inhibitor. In April 2006, data challenge for influenza neuraminidase (H5N1) was carried out by Grid-enabled high throughput *in-silico* screening based on AutoDock and Python programs against 308,585 compounds [2]. Then, the recombinant NA from H5N1 influenza virus strain A/Vietnam/1203/04 was successfully expressed and purified in this experiment. Neuraminidase activity was determined using (MU-Neu5Ac) as a fluorogenic substrate. Inhibition activity of NA was determined by incubating enzyme solution with 40 mM sodium phosphate buffer (pH 7.2), MU-Neu5Ac [2'-(4-methylumberlliferyl)-α-D-*N*-acetylneuraminic acid], and with or without target compounds as following the measuring the fluorescence using excitation at 365 nm and emission at 450 nm. Compared with Grid score, target compounds were ranked by the degree of inhibition of NA. These results are helping biomedical area to reduce the cost and time of the structure-based drug design.

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