Virtual Screening on Neuraminidase Inhibitors activity of plant- derived natural products by using Pharmacophore Modelling and Docking

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ABSTRACT

Neuraminidase (NA) plays an important role in the replication and release of new avian influenza virion. Due to this event, NA had been considered as a valid target in drug design against influenza virus. The aim of this study is to identify the new neuraminidase inhibitors, using pharmacophore modeling and docking based virtual screening from natural compounds of Malaysia. A variety of natural compounds from plant sources of Malaysia which collected in NADI database has been screened to possess substantial neuraminidase inhibitors properties. Pharmacophore model was developed using Catalyst software embedded in Discovery Studio 2.1 by using sialic acid derivatives which act as N1 inhibitors. The pharmacophore hypothesis selected had five features (one hydrogen bond donor (D), one negative ionizable (N), one positive ionizable (P), and two hydrophobic moiety (Hy), also included two excluded volume. Best pharmacophore was validated by Hyporefine in DS 2.1 which has a lowest total cost value (92.055), the highest cost difference (107.807), the lowest RMSD (1.197), and the best correlation coefficient (0.944651)..The X-ray crystal structure of Neuraminidase N1 (1F8B) was used in the docking studies, using Autodock 3.0.5 software, and the free energy of binding was used to rank the hits, whereas oseltamivir and DANA have been used as the ligands in controlled docking. *In silico* screening had been carried out on the compounds of our laboratories database (NADI). The mapping of NADI database natural compounds shows that 46 compounds of 2350 NADI compounds map to three feature of common pharmacophore training set. MSC458 (*Morinda citrifolia*) have the highest fit value with free energy docking -9.48 kcal/mol (Ki 1.12x 10^{-7}) into neuraminidase, whereas MSC605 have the most negative free energy of bind (-12.10) and Ki 1.35x 10^{-9}.

Keywords: Neuraminidase, Pharmacophore, Docking, Virtual Screening

Abbreviation : NADI : Natural-Based Drug Discovery Intelligent, N1 : Neuraminidase 1, DANA: 2-deoxy-2,3-dehydro-Neu5Ac

1. INTRODUCTION

Influenza virus has impacted the world since long time ago. It infects nearly 20% of the human population of the world [1]. This virus consists of a membrane-enveloped, segmented, negative-strand RNA viruses, and two glycoproteins on the surface of the hemagglutinin (HA) and neuraminidase (NA). HA and NA have a major role in viral replication. HA is a trimer macromolecules responsible for attachment to the surface of the cell receptors that are associated with terminal sialic acid [2] whereas NA is an important viral enzyme that play a role in virus proliferation and infectivity. Therefore, blocking its activity generates antivirus effects, making it attractive to target this viral glycoprotein for the design and development of anti influenza drugs. In influenza virus, it was found that the enzyme active site of viral NA of both virus A and B is highly conserved in amino acid sequence variation.

In the recent time, the analog NA inhibitors was developed from chemical reagent starting as lead compound, but little case which using by natural plants bioactive compounds as starting materials for be developed lead compounds of NA Inhibitors, because Malaysia or Indonesia has great potential to develop her abundant natural resources to increase the market based on herbal products.

NADI (Natural Based Drug Discovery) [3] is a database of Malaysian medicinal plants which aims to be a one-stop centre for in silico drug discovery from natural products. NADI was developed with the aim to assist the research in performing drug discovery at Universiti Sains Malaysia. It provides structural information on 3000 different compounds along with the information of the botanical sources of plants species that could be used in virtual screening.

2. COMPUTATIONAL DETAILS

Materials

Database of Bioactive Compounds in NADI (USM)

The bioactive compounds of diversity Laboratories collection in NADI, was used as a test set in this virtual screening. The database, at present, contains 3000 compounds of Malaysia natural product which geometry have been built and optimized using MOPAC semi-empirical programme.

Training Set : 24 representative structures obtained from the literature (Table 1) was taken for training set in this study.

Software for Virtual Screening : Hyperchem 7.0. (*Hyperchem, Inc*), Autodock 3.0.5 (*Molecular Graphics Laboratory, The Scripps Research Institute*), Discovery Studio 2.5. (Accelryl Inc.).

Hardware : The computational molecular modeling studies were carried out using Catalyst in Discovery Studio 2.5. (Accelrys, San Diego, CA) running on Windows XP in a Dual Core processor 2 GHz (Intel, Santa Clara, CA), whereas Autodock 3.0.5 running on LINUX Fedora in a Dual Core Processor 3 GHz.

Methods

Generation of Conformation Library of Bioactive Compounds: For the training and test sets molecules, conformational models representing their available conformational space were calculated. All molecules were built using the 2D and 3D sketcher of Hyperchem 7.0, and optimized using MM2 in Hyperchem 7.0. A conformational set was generated for each molecule using the poling algorithm and the best energy option, based on CHARMm force field from Discovery Studio 2.5 [4]. The molecules associated with their conformational models were mapped onto the pharmacophore model using the "best fit" option to obtain the bioactive conformation of each molecule. Generation of **Pharmacophore Hypothesis:** All the pharmacophore modeling calculations were carried out by using the Discovery Studio 2.1 software package (Accelrys, San Diego, USA). The HipHop modules within Catalyst in D.S 2.1 were used for the constructions of qualitative and quantitative models, respectively. The features considered were H-bond donor (D), hydrophobic (Hy), H-bond acceptor (A), and positive ionizable (P), and negative ionizable (N), and also excluded volume included (E). **Validation of Pharmacophore Models:** Based on the information of the qualitative models, the quantitative pharmacophore models were created by Hypogen within Catalyst in DS 2.1 packages. 255 Conformers was chosen to be minimized as best conformation, and 20 kcal/mol was set as energy threshold as global energy minimum for conformation searching [5], this protocol is available in DS 2.1 packages. The best pharmacophore models was validated according to Deng et al. [6] in terms of cost functions and other statistical parameters which were calculated by HypoRefine module during hypothesis generation. A good pharmacophore model should have a high correlation coefficient, lowest total cost and RMSD values, and the total cost should be close to the fixed cost and away from the null cost. The best pharmacophore model was further validated by test set method and Fischer's randomization test [7]. **Pharmacophore-Based Virtual Screening of NADI database:** Out of the 3000 molecules contained in NADI database, 2350 molecules were filtered as drug-like molecules which were then converted into separate Catalyst libraries. Using the Ligand Pharmacophore Mapping protocol, the 'Best Mapping' was performed with the 'rigid fitting method' and maximum omitted features were set to zero, and one [8]. **Molecular Docking:** Linux operating system version Fedora 6 Redhat on dual core processor was used, to screen the potential bioactive compounds from NADI database. Ligands dataset was already available in pdb file. The neuraminidase protein of subtype N1 binding with DANA complex (PDB code : 1F8B)[9] and oseltamivir (2HUO) [10] was used as the target. Docking simulations were performed with AutoDock [11]. The AutoDockTools (ADT) script was used to convert the ligand PDB to the pdbq format by adding Gasteiger charges, checking polar hydrogens and assigning ligand flexibility. In addition, the ADT was also performed to prepare the protein targets for the simulations. Using ADT interface, the Kollman charges were added for the macromolecule and a grid box of 60 x 60 x 60 points, with a spacing of 0.375 Å, centered on the binding site for the co-crystallized ligand (26.507; 17.972; 57.828) was setup for AutoGrid and AutoDock calculations.

3. RESULTS AND DISCUSSION

The common feature pharmacophore model was generated by HipHop from four most active compounds. The results of HipHop model identified five feature (one hydrogen-bond donor (D), two hydrophobic moiety (Hy), one negatively ionizable (N), and one positive ionizable (P)), as can be shown in Figure 2. After that, these features were chosen as the initial chemical features in the quantitative pharmacophore modeling which generated by Hypogen. To consider the steric effect, the value for excluded volume was set to 2 [5].

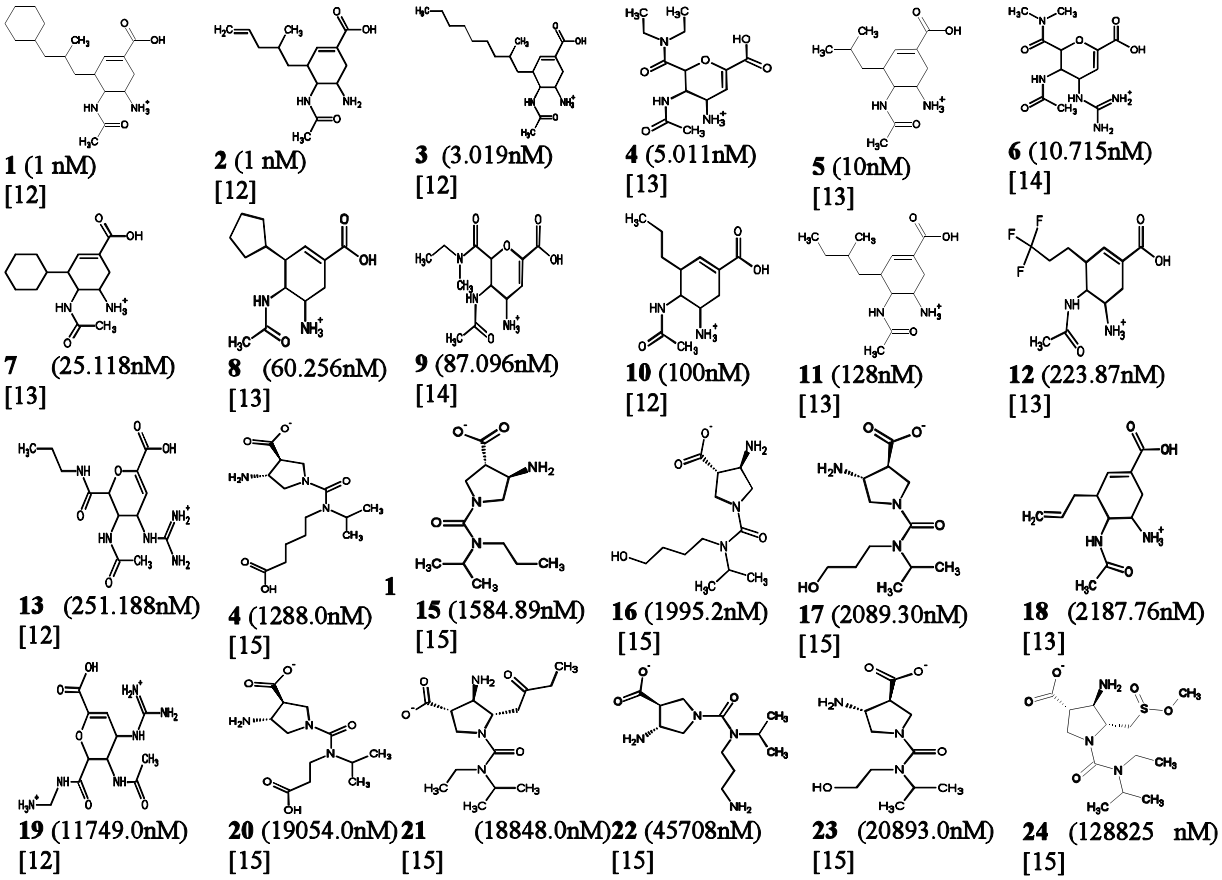


Figure 1. Sialic Acid Derivatives Structure, Activity (IC50), and Reference Data of Training Set

24 Compounds (Fig. 1) selected were used as the training set compounds in the Hypogen run in 3D-QSAR Pharmacophore DS 2.1 packages. The 10 hypotheses were produced and the results of statistical parameters are given in Table 1. The best hypothesis (Hypo1), shown in Fig. 3(i), is characterized by the lowest total cost value (92.055), the highest cost difference (84.395), the lowest RMSD (1.197), and the best correlation coefficient (0.944651). The fixed cost and null cost are 97.2168 and 197.498 bits, respectively. Hypo1 contains five features: one hydrogen-bond donor (D), two Hydrophobic aliphatic moiety (Hy), one negatively ionizable (N), and one positive ionizable (P). Two excluded volumes are also involved in Hypo1. The 3D space and distance constraints of these pharmacophore features are shown in Fig. 3(ii).

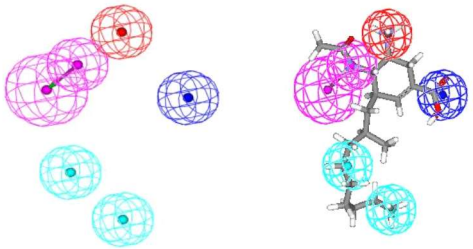


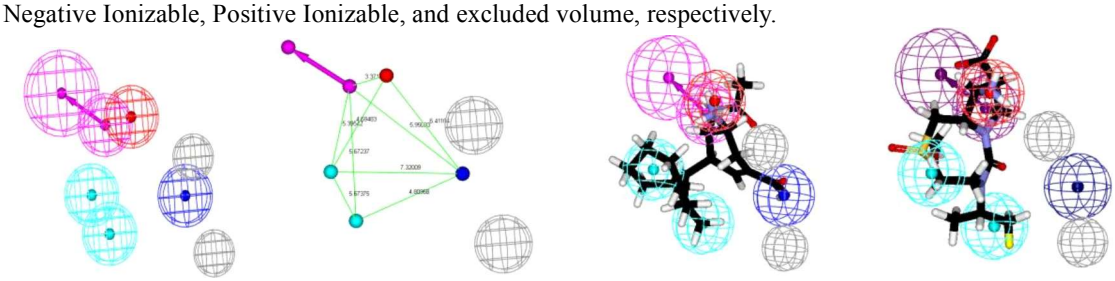
Figure 2. HipHop pharmacophore model for NA inhibitors. (i) The HipHop pharmacophore model. (ii) The HipHop model mapped with the most active compound 1 in the training set. Pharmacophore features are color coded; magenta: hydrogen-bond donor (D), blue – hydrophobic feature (Hy), dark blue – negative ionizable(N), red – positive ionizable (P), and grey – excluded volume.

Table 1. Statistical parameters of the top 10 hypotheses of Neuraminidase inhibitors generated by HypoRefine program

Hypo no.	Total cost	Cost diff.[1]	RMSD (Å)	Correlation (r)	Features[2]
1.	113.103	84.395	1.03907	0.944357	DHyHyNPEE
2.	114.131	83.367	0.98385	0.952229	ADHyPEE
3.	116.433	81.065	1.10535	0.937879	ADHyHyPE
4.	120.136	77.362	1.31857	0.907315	ADHyHyPEE
5.	121.363	76.135	1.29581	0.912158	DHyNPE
6.	121.742	75.756	1.42300	0.889973	ADHyHyPEE
7.	122.472	75.026	1.44230	0.886802	ADHyHyPEE
8.	123.657	73.841	1.41536	0.892491	DHyHyNPE
9.	123.752	73.746	1.32968	0.908464	ADHyPE
10.	123.917	73.581	1.32585	0.909342	DHyNPEE

[1](Null cost-total cost), null cost =197.498, fixed cost =97.2168, configuration cost =15.3653.

[2] A, D, Hy, N, P, and E represent hydrogen-bond acceptor, hydrogen-bond donor, hydrophobic feature, Negative Ionizable, Positive Ionizable, and excluded volume, respectively.



(i) (ii) (iv) (v)

Figure 3. Best Pharmacophore with Validation by Hyporefine Run in DS 2.1. (i) The best HypoRefine pharmacophore model, Hypo1. (ii) 3D spatial relationship and geometric parameters of Hypo1. (iii) Hypo1 aligned with the most-active compound 1 (IC_{50}: 1 nM). (iv) Hypo1 aligned with the least active compound, Compound 24 (IC_{50}: 128825 nM). Pharmacophore features are color coded; magenta: hydrogen-bond donor (D), blue – hydrophobic feature (Hy), dark blue – negative ionizable(N), red – positive ionizable (P), and grey – excluded volume.

Validation of Pharmacophore Model

1. Fischer Randomization test

Fischer Randomization is provided in the DS 2.1 package to evaluate models of best pharmacophore. The confidence level was set to 95 % and produced a total 19 random spreadsheets (Figure 4). From the figure, we can see that the correlation (r^2) of all pharmacophore models generated using the 19 random spreadsheet are much lowest than the correlation of corresponding original pharmacophore (blue line) which have r^2 value greater than 0.9. These results provide confidence on our pharmacophore.

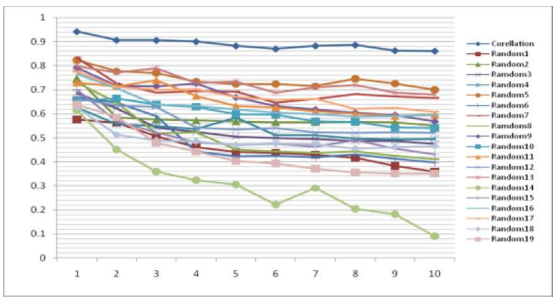


Figure 4. The difference correlation of hypotheses between the initial spreadsheet and 19 random spreadsheets after CatScramble run.

2. Test set

Hypo1 was applied against the 96 (www.bindingDB.org) test set compounds which gave a correlation coefficient of between experimental and estimated activities as shown in Fig 5.

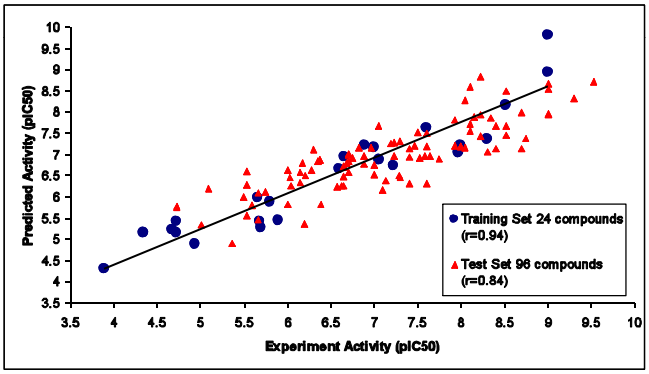


Figure 5. Plot of the correlation (r) between the experimental activity and the predicted activity by Hypo1 for the test set molecules (red) and the training set molecules (blue).

Figure 5 showed that there is a significant correlation between the experimental values and estimated values, where the correlation value of the test set is 0.84, which means that 84% of the total variation in test set can be explained by the linear relationship between experimental activity and predicted activity. This observation further proves that our pharamacophore model is a good model.

Virtual Screening of NADI database based on Feature Pharmacophore of Training Set

In this study, 45 compounds from NADI database were successfully captured by using four features with set maximum omitted 1 (allowed one missing feature). There are no compounds of NADI which have high affinity to the five feature into D, Hy, N and P by set maximum omitted zero (no missing feature).

All compounds captured into feature pharmacopore model, have carboxylic acid and hydroxyl groups. Carboxylic groups of NADI compounds mapped into the positive ionizable feature, such as shown by sialic acid derivatives. The hydroxyl group satisfactorily mapped into H-bond donor feature. On the other hand, the carbonyl or the enol groups, and aromatic moiety and alkyl chain mapped into the hydrogen bond acceptor and hydrophobic features, respectively.

Docking Study

All 45 compounds that mapped into pharmacophore model were docked into neuraminidase enzyme (PDBcode: 1F8B) using Autodock 3.0.5. The docking orientation of NADI compounds were compared to oseltamivir (PDBcode: 2HUO)[10].

From molecular docking study, MSC605 has shown the most negative free energy of binding (-12.10 kcal/mol). In Figure 5, -COOH of oseltamivir (grey carbon) and MSC607 (black carbon) gave similar charge and H-bond interaction with Arg371, Arg118 and Arg292, and N-acetyl of oseltamivir and OH-C24 of CGA interact into Asp151, and Glu227 residue, while MSC605 didn't have interaction into Glu119 and Asp151 as shown at NH_3^+-OSTM. Isopentyl groups of oseltamivir and aromatic moiety of MSC605 given strong hydrophobic interaction into Glu 276, Glu 277, Ala 246 and Arg 224.

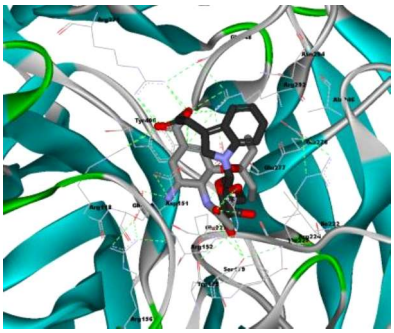
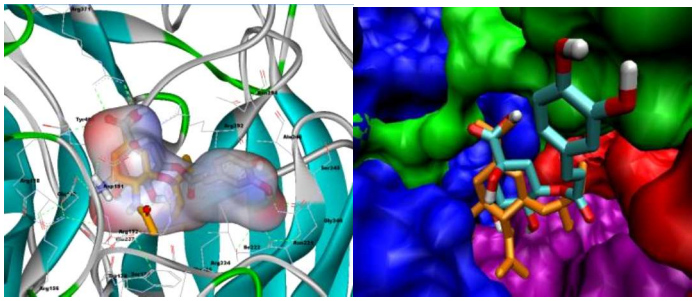


Figure 5. Conformation of MSC605 (black carbon) and OSTM X ray (pink carbon) bonded N1, surface visualizased with DS 2.1.

In addition, MSC927 was found that binding interaction into N1 (1F8B) [9] have similarity with DANA X-ray, although have fit value (mapping) and free energy docking less than better if be compared with Oseltamivir (2HUO)[10] and DANA X-ray (1F8B)[9]. MSC927 also have mapping similar with OSTM, but MSC927 doesn't has positive ionizable feature. Figure 6b and 6c show CGA and OSTM interaction at N1.



(i) (ii)

Figure 6. (i) Conformation of MSC927 (green) and OSTM X ray (orange) bonded N1, surface visualizased with DS 2.1. Non-polar hydrogen atom at ligand did not seems in order to clear. B. (ii) Pocket area of site active of neuraminidase A (green : NI, red : strong hydrophobic, purple : PI, blue : weak hydrophobic) with visualizased VMD 1.8.5 by Linux

4. CONCLUSION

The best quantitative pharmacophore model, Hypo1 showed the lowest total cost value (92.055), the highest cost difference (107.807), the lowest RMSD (0.966317), and the best correlation coefficient (0.941732) compared to other models. Hypo1 contains four features: one hydrogen-bond donor (D), one Hydrophobic aliphatic moiety (Hy), one negatively ionizable (N), and one positive ionizable (P). There were 45 compounds of 3000 NADI database that showed high predictive affinity when matched into Hypo1's HBD, Hy, NI and PI by optimizing the minimum predicted activity to 1 mM. This is also further predicted by docking results showed that MSC605 and MSC927 possessed the best binding interaction into site active of neuraminidase A (1F8B).

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