Discovery of Novel NAMPT Inhibitors Based on Pharmacophore Modeling and Virtual Screening Techniques

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Abstract: Nicotinamide phosphoribosyltransferase (NAMPT), an enzyme taking part in main NAD biosynthetic pathway, is an attractive target for anticancer therapy. The purpose of our study is to find novel NAMPT inhibitors based on in silico drug discovery means including the generation of 3D-QSAR models, and virtual screening techniques. Firstly, ten pharmacophore models were generated by Catalyst/HypoGen algorithm. Hypo1 with high correl value (0.96), large Δcost (77.77), and low root mean square deviation (0.81), featured by four chemical features was selected as the best one. Subsequently, Hypo1 was validated through test set prediction and Fischer’s randomization methodologies. Then we screened some public compound libraries (Asinex, Ibscreen and Natural products database) using Hypo1 for a 3D query. The screened hits were further refined by Lipinski’s rule of five, ADMET properties as well as molecular docking studies. Finally, six molecules with diverse scaffolds exhibited the right pharmacophore features and good binding modes between the receptor and ligands, and were selected as possible candidates against NAMPT for further study.

Keywords: 3D QSAR models, Catalyst/HypoGen, molecular docking, NAMPT, virtual screening.

1. INTRODUCTION

Nicotinamide phosphoribosyltransferase (also known as NAMPT, NAMPTase, PBEF or Visfatin) is a crucial enzyme in nicotinamide adenine dinucleotide (NAD) biosynthetic pathway. It can catalyze precursor nicotinamide (NAM) to mononucleotide (NMN) with the aid of 5’-phosphoribosyl-1’-pyrophosphate (PRPP), and NMN will be converted to NAD subsequently [1]. NAD participates in various cellular redox reactions [2], and required in malignant tumor cells much more than normal cells owing to their enhanced ADP-riboseylation activity undoubtedly [3, 4]. Therefore, as a rate-limiting enzyme in main pathway of NAD biosynthesis [5], NAMPT becomes an attractive target for cancer therapy and draws more and more attention in recent years.

APO866 (initially known as FK866, Fig. 1A) is a highly specific inhibitor of NAMPT and has entered Phase II clinical research [6]. Recently, GMX1778 (previously known as CHS828, Fig. 1B) has been identified as another anti-cancer agent targeting NAMPT [7]. GMX1778, the active form of the prodrug GMX1777, has potent antitumor activity against extensive cancer cells [8], and is also in Phase II clinical trials. Nevertheless, low bioavailability, rapid intravenous clearance of APO866 [9], and gastrointestinal side-effects of GMX1778 [10] indicate that they should be further optimized for therapeutic usage or novel inhibitors against NAMPT should be found.

A dozen of NAMPT crystal structures with diverse inhibitors emerged, providing possibility of computer aided drug design. These structures revealed that NAMPT is a dimer, and the tunnel at the interface between chain A and B is the potential binding site for inhibitors. We selected the structure whose PDB code is 4M6P for docking in consideration of some criteria such as homo sapiens, high resolution, new release date, and co-crystallized with NAMPT inhibitor.

Computer-aided drug design technique becomes an important methodology in modern drug design process. Although several kinds of inhibitors have been designed, identification of novel NAMPT inhibitors based on 3D-QSAR pharmacophore study has not been done yet. Hence generating three-dimensional pharmacophore model and further screening library to obtain potential NAMPT inhibitors becomes the main purpose of our work. Moreover, molecular docking approaches with several programs (Glide SP, GOLD and Glide XP) were applied to decrease the quantity of screened hits further and identify binding mode of the receptor and ligands. Finally, several novel molecules with diverse scaffolds were picked out as potential NAMPT inhibitors according to binding energy and binding modes.

2. MATERIALS AND METHODS

2.1. Preparation of Training Set and Test Set Molecules

Discovery Studio Client v2.5.0 was applied to create pharmacophore models and screen library in our work. Prior to create models, we collected a group of 77 existing NAMPT inhibitors from six literatures [5, 11-15] based on certain principles including spanning at least 4 orders of magnitude (0.00055 – 2 μM) and the diversity of scaffolds. Subsequently, these inhibitors were divided into two sets: training set (25 molecules, shown in Fig. 2) and test set...
(another 52 molecules, shown in Supplementary Fig. 1). The 3D structures of all these molecules above were drawn with Chem&Bio 3D 12.0 program.

2.2. Pharmacophore Modeling and Hypotheses Generation

After the 3D structures of all molecules were drawn, they were simulated under CHARMM Forcefield [16] in DS. All compounds were utilized to predict possible pharmacophore features with Feature Mapping protocol. Then Generate Conformations protocol was used to generate conformations for all compounds with BEST method considering the importance of molecular flexibility. The 25 training set molecules described above were used to generate pharmacophore hypotheses on the basis of structure-activity relationship by HypoGen algorithm. Hydrogen bond donor (HBD), hydrogen bond acceptor (HBA), hydrophobic (HY) and ring aromatic (RA) were selected as possible chemical features considering feature mapping results. Meanwhile the minimum and maximum feature counts were changed to one and three for every pharmacophore feature, respectively. The uncertainty value was set to 2. Left the default values for other parameters. Maximum of ten predictive models (hypotheses) were generated and ranked by scores. Hypo1, the top ranked hypothesis, was considered as the best one.

2.3. Validation of Pharmacophore Model

The purpose of this section is to judge the quality of our pharmacophore model applying test set prediction and Fischer’s randomization approaches.

Test set prediction method is one of the most common methods to evaluate the quality of pharmacophore models. Test set molecules were also needed to be prepared such as stimulating with CHARMM Forcefield and adding activity values. Hypo1 was acted as validation object. Then Ligand Pharmacophore Mapping protocol was launched again also with Flexible Fitting Method, FAST Conformation Generation, and “zero” Maximum Omitted Features option of CATALYST. In consideration of predicted activity, we only kept those molecules whose fit value was greater than 6. Moreover, Lipinski’s rule of five and ADMET properties [18, 19] were applied in further filtration of the screened molecules to narrow the number of hits. The molecules satisfied all above criteria were subjected to molecular docking.

2.4. Virtual Screening

This economical and accurate technology plays a more and more important part in drug discovery acted as a supplementary means. Pharmacophore based virtual screening was carried out to find novel scaffolds against NAMPT in this section. Firstly, the authenticated Hypo1 was served as a query to screen several public compound databases (Asinex, Ibscreen and Natural products database) possessing 970,991 small molecules for potential leads. Ligand Pharmacophore Mapping protocol was launched again also with Flexible Fitting Method, FAST Conformation Generation, and “zero” Maximum Omitted Features option of CATALYST. In consideration of predicted activity, we only kept those molecules whose fit value was greater than 6. Moreover, Lipinski’s rule of five and ADMET properties [18, 19] were applied in further filtration of the screened molecules to narrow the number of hits. The molecules satisfied all above criteria were subjected to molecular docking.

2.5. Molecular Docking Techniques with Multiple Programs

The compounds passed all drug-likeness filters were then refined via docking techniques to further decrease molecules in quantity and predict binding modes between the protein and small molecules. Different docking programs use their respective scoring functions, and it probably leads to the diversity of the outputs. In an effort to eliminate this bias, three docking methodologies (Glide SP, GOLD and Glide XP) were applied to reduce the uncertainty and make our results more convincing. Firstly, standard precision (SP) mode of Glide 5.7 and GOLD v5.0 software were applied. Both top ranked molecules in two procedures, glidescore < -8 in SP docking mode and ChemScore > 33 in GOLD software, would be picked out for advanced docking. Subsequently, extra precision (XP) mode of Glide 5.7 was used to obtain potential inhibitors for NAMPT based on glide score and binding mode. The crystal structure of NAMPT with a resolution of 1.75 Å (PDB code: 4M6P) was downloaded directly from the Protein Data Bank (www.rcsb.org). We retained two water molecules in the cavity of the receptor which can link ligand with OH-group of Ser275 and removed other waters before docking.
Fig. (2). 2D structures of the 25 training set molecules and their IC₅₀ (μM).
Prior to dock screened molecules, the docking methodologies were verified by redocking the co-crystallized inhibitor and docking the test set molecules.

3. RESULTS AND DISCUSSION

3.1. Pharmacophore Modeling

Ten pharmacophore hypotheses with a mass of statistical data (summarized in Table 1) were generated by HypoGen algorithm utilizing the structure-activity relationship of the 25 training set molecules (Fig. 2). One HBA, one HBD, one HY, and one RA appearing in all hypotheses simultaneously reflects the identity and stability of our hypotheses. The cost value analysis is the most fundamental means to assess the quality of 3D-QSAR pharmacophore models. Our best pharmacophore model (Hypo1) owns high $\text{corr}$ value (0.96), high $\Delta\text{cost}$ value (77.77), low RMS (0.81), and good configuration value (14.58). The fixed cost and null cost are 88.28 and 174.79 for all hypotheses, respectively. $\text{corr}$ value indicates the correlation of model and structure-activity relationship with respect to training set molecules. The closer it gets to 1, the more statistically significant the hypothesis is believed to be. RMS value should not greater than 2.0.

![Fig. (3).](image)

**Fig. (3).** Pharmacophore model of NAMPT inhibitors generated by HypoGen. (A) The 3D space and chemical features of the best hypothesis Hypo1. (B) The distance constraints between each chemical features of Hypo1. (C) Hypo1 aligning with the most active compound 1 ($IC_{50}$ 0.000055 μM) in training set. (D) Hypo1 aligning with the least active compound 25 ($IC_{50}$ 2 μM) in training set. Pharmacophore features including HBA, HBD, HY and RA are represented by green, magenta, cyan and orange color spheres, respectively.

<table>
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<tr>
<th>Hypothesis</th>
<th>Correl Value</th>
<th>RMS$^a$</th>
<th>Total Cost</th>
<th>$\Delta\text{Cost}^b$</th>
<th>Chemical Features</th>
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$^a$RMS represents root mean square deviation.

$^b$Configuration value equals to 14.58, fixed cost equals to 88.28, and null cost equals to 174.79.

$\Delta\text{Cost}$ is the difference between null cost and total cost.
than 1. In this study, our correlation value and RMS value are 0.96 and 0.81, respectively, indicating a good correlation of our model. Ideal total cost should lie somewhere between fixed cost and null cost, and get close to the former and escape from the latter. In general, if the difference between null cost and total cost is greater than 60, there is an excellent significance (>90% probability) the hypothesis represents a true correlation. As listed in Table 1, the cost difference between fixed cost and total cost is 8.75, along with a large cost difference between null cost and total cost (77.77) in Hypo1, which shows that Hypo1 has more than 90% credibility. As another parameter to assess the quality of hypothesis, configuration value should not be greater than 17.0 in standard HypoGen mode and it is 14.58 for Hypo1. Therefore, these fantastic values above indicated that Hypo1 was not obtained by chance. The 3D spatial arrangement and distance constraints of the chemical features of Hypo1 are shown in Fig. (3A, B).

The experimental and estimated activities of the 25 training set molecules are listed in Table 2. We classified

<table>
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<tr>
<th>Compound No.</th>
<th>Exp. IC₅₀ (μM)</th>
<th>Esti. IC₅₀ (μM)</th>
<th>Fit Value</th>
<th>Error</th>
<th>Exp. Scale</th>
<th>Esti. Scale</th>
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</table>

Exp. represents experimental activity.
Esti. represents estimated activity calculated by Hypo1.
Fit value stands for the matching degree between pharmacophore features and molecules, and the greater, the better.
set, respectively. Obviously, as the least active molecule, compound 25 only mapped three features with HBA was not mapped, whereas, all the four pharmacophore features were perfectly mapped on the most active compound 1. Therefore, we concluded that Hypo1 has the capacity to predict activity values of training set molecules.

Fig. (4). Correlation curve between experimental and predicted activity values pIC50 for training and test set molecules.

3.2. Validation of Pharmacophore Hypothesis

3.2.1. Test Set Method

To understand whether Hypo1 is capable of predicting the activity of other small molecules, an independent test set which contains 52 external compounds (shown in Supplementary Fig. 1) was used. Supplementary Table 1 exhibited the predicted IC50 values of all test set molecules derived from Hypo1 and their experimental values. In order to assess the predictive ability of Hypo1, test set molecules were also classified into high active, moderate active and low active molecules in the same way as training set ones. In detail, 10 of 19 high active, 19 of 27 moderate active and 4 of 6 less active molecules were estimated correctly. 9 high active molecules were mistaken for moderate active ones with the activity values less than one order of magnitude; 8 moderate active molecules were mistaken for high active ones; 2 low active molecules were misestimated as moderate active ones. Moreover, all error values of test set molecules were less than 10, indicating excellent predictive ability of Hypo1. From Fig. (4), a good correlation coefficient ($r^2$) of 0.72 between experimental and predicted activity values for 52 test set molecules is observed, which proved the good predictive capability of Hypo1 further.

3.2.2. Fischer Validation

In order to further prove the reliability of pharmacophore models, Fishcher’s randomization method was employed. In comparison with the nineteen pharmacophore hypotheses generated randomly, the original hypotheses possess the lowest total cost (Fig. 5), forecasting a 95% confidence level of Hypo1.

3.3. Virtual Screening Based on Pharmacophore and Drug-Likeness

After validation, Hypo1 was used as a three-dimensional query for screening novel and potential inhibitors against NAMPT from various databases (970,991 compounds). A number of 140,888 hits were obtained in the initial screening. Of these, totally 10,738 compounds were selected that had fit values over 6 (predicted IC50 < 0.05 μM) for further study. Subsequently, these molecules were screened again based on their drug-likeness (Lipinski’s rule of five and ADMET properties). Finally, 994 compounds were retrieved and would suffer from further development such as docking studies.

Fig. (5). The difference in total costs between original hypothesis (Hypo1) spread sheet and nineteen hypotheses generated randomly spread sheets.
3.4. Molecular Docking Studies

3.4.1. Validation of Reliability of Docking Results

Firstly, we redocked the co-crystallized inhibitor of NAMPT (PDB code: 4M6P) into the cavity of the receptor using GOLD, Glide SP, and Glide XP with RMSD values between crystallized and docked conformations by superpositioning the heavy atoms was calculated are 1.14, 0.49, and 1.11, respectively. The low RMSD showed high reproducibility level which indicated our docking parameters and scoring functions were appropriate initially. The good superimposition cases of crystallized and docked conformations in three docking programs reflected this conclusion (Fig. 6).

Subsequently, test set molecules were docked into NAMPT using GOLD software. Compound 1, 42, and 50 in test set (Supplementary Fig. 1) were selected as typical molecules to analyze the rationality of docking method. Because they have absolute higher activity, similar activity, and obvious lower activity (IC\textsubscript{50} are 0.0003, 0.059, and 1.4 μM, respectively) than co-crystallized inhibitor (namely reference ligand, IC\textsubscript{50} is 0.054μM). The binding modes between three test set compounds and the protein are shown in Fig. (7). The aromatic ring of these compounds were all stuck in the interspace of two benzenes in Tyr18 (B) and Phe193 (A) and formed typical face-to-face π-π interactions. Meanwhile, they all formed an H-bond with one water molecule which can develop another H-bond with OH-group of Ser275. In addition, compound 1 formed two strong H-bonds with Val242 and Phe193 and hydrophobic interactions with Ala379 and Ala245. So compound 1 exhibited high inhibitory activity and high ChemScore (37.10). However, compounds 42 and 50 only developed several H-bonds with Asp219, Ser241, and Arg311. Besides, the differences of structure and conformation between compound 42 and 50 led to different activities. Their ChemScore are 34.04 and 31.41, respectively compared with reference ligand whose ChemScore is 33.72. The consistency between experimental activity values and docking scores suggested rationality of docking method.

3.4.2. Molecular Docking

First of all, 994 hits obtained from virtual screening were docked into active cavity of NAMPT with the aid of Glide.
SP protocol and GOLD 5.0 software. According to the docking scores, only the molecules with glide score < -8.0 and ChemScore > 33.0 were identified as candidates with a total number of 49. For the purpose of refining the retrieved hits further as well as determining favorable interactions within the active site, an additional docking study was

Fig. (8). The 2D structures of six potential inhibitors of NAMPT.

Fig. (9). Superimposition of six potential inhibitors of NAMPT and Hypo1. A-F represent ZINC08627153, ZINC20744986, ZINC13084862, ZINC19131362, ZINC19346186, and ZINC19502349, respectively.
launched with Glide XP protocol. Finally, we selected six molecules as potential inhibitors of NAMPT based on binding energy, binding modes as well as diversity of chemical scaffolds. Fig. (8) exhibit 2D structures of the six candidates. From the figure we can see that these six compounds pertain to four scaffolds. ZINC08627153 and ZINC20744986 share common scaffold. ZINC19131362 and ZINC19502349 share one scaffold. ZINC13084862 and ZINC19346186 belong to different scaffolds, respectively. The information of six candidates aligned with Hypo1 is described in Fig. (9). Obviously, all these molecules mapped four pharmacophore features perfectly.

Fig. (10) shows the docked structure of ZINC08627153 in the active site of NAMPT. From this figure, we can identify that NAMPT was a dimer and the two subunits both contributed to the active site. ZINC08627153 was located close to the interface between the subunits. We just selected four molecules (ZINC08627153, ZINC13084862, ZINC19346186, and ZINC19502349) with different

Fig. (10). Docked structure of ZINC08627153 (space-filling model) in the active site of NAMPT (cartoon). Chain A is shown in blue, chain B in green, ZINC08627153 in pink (colored illustration is available online).

Fig. (11). Docked conformation of ZINC08627153 (A), ZINC13084862 (B), ZINC19346186 (C), and ZINC19502349 (D) in the active site of NAMPT. Ligands are described in yellow sticks. Hydrogen bonds are indicated with dashed yellow lines. Crucial residues are shown in cyan and green sticks (colored illustration is available online).
chemical scaffolds to analyze the binding modes with receptor after Glide XP docking considering the situation of common scaffolds in six hits (Fig. 11). Obviously, the aromatic rings of all these molecules were sandwiched in the middle of two benzenes in Phe193 (A) and Tyr18 (B), forming typical face-to-face $\pi-\pi$ interactions. Besides, ZINC08627153 formed one H-bond with the right water molecule which interacted with Ala245, Arg311, and Ser275, and it was the same as ZINC13084862. However, the oxygen atom of carbonyl group of ZINC19346186 formed an H-bond with the left water molecule in the figure which was linked to OH-group of Ser275. While two H-bonds between ZINC19502349 and different two water molecules appeared, contributing to the stability of the ligand-receptor complex. All four potential inhibitors can form other H-bonds with surrounding amino acid residue, such as Ser241, Val242, His191, Asp219, and Ala245. The hydrophobic interactions were observed in the phenyl group of ZINC13084862 and the pyridine ring of ZINC19346186 with Ala379. ZINC19502349 located in the hydrophobic pocket with some residues such as Tyr188 (A), His191 (A), Val242 (A), Phe193 (A), Ala245 (A), and Tyr18 (B), enabling a tighter interaction with NAMPT. Moreover, ZINC08627153 scored a GlideScore of -9.577 which is lower than that of reference ligand (-8.619) markedly, indicating a higher binding energy. Therefore, the well-docked binding modes and high binding energy indicated that these six molecules have potential inhibitory against NAMPT.

CONCLUSION

In this study, a 3D pharmacophore model was generated using 25 structurally diverse training set molecules. Hypo1 with the highest cost difference (77.77), the lowest RMS (0.81), the highest correl value (0.96) along with four chemical features (one HBA, one HBD, one HY and one RA) was selected as the best pharmacophore model. Then Hypo1 suffered from test set validation and Fischer verification in order to conform its reliability. Subsequently, pharmacophore-based and drug-likeness-based virtual screening was launched, and obtained 994 potential leads from the database with a total number of 970,991 compounds. The screened molecules were docked into the active cavity of NAMPT with the aid of Glide SP, GOLD 5.0, and Glide XP programs after verifying the rationality of docking parameters. According to the docking scores and binding modes, six compounds (ZINC08627153, ZINC20744986, ZINC13084862, ZINC19131362, ZINC19346186, and ZINC19502349) with novel scaffolds were selected as potential inhibitors against NAMPT. As a consequence, we concluded that our pharmacophore model, Hypo1, is capable of identifying novel potential inhibitors against NAMPT from small molecules databases, and the six compounds above can have potent inhibitory activities and could be used for further investigation.

CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher’s web site along with the published article.

REFERENCES


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