Original Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

IDENTIFICATION AND VALIDATION OF LEAD COMPOUNDS AGAINST FURIN

HABIRAMI T. AND RATHI SUGANYA P.*

Department of Bioinformatics, School of Bioengineering, SRM University, Kattankulathur, Tamil Nadu. India 603203

ABSTRACT

Most of the proteins are synthesized as inactive proforms requires a proteolytic process to render them active. Proprotein convertases (PCs) are a family of serine proteases capable of activating substrates. Furin is a family of subtilisin-like proprotein convertases (PCs) which is calcium-dependent serine endoprotease. It is enriched in Golgi apparatus that cleaves protein to active or mature form. It is utilized by many numbers of pathogens (HIV, influenza, dengue fever, Ebola, Marburg virus) to become fully functional. Evolutionary studies of furin have been done to identify the non-conserved sequences across multiple species, which is used as an active site for docking studies. The known furin inhibitors are investigated with virtual screening and the compounds are screened based on QikProp and Lipinski's Rule of 5. More than 19000 unique natural small molecules from ZINC database and marine compounds from SWMD were screened with two-point Pharmacophore, comprising one ring and one hydrogen donor. Binding modes of these molecules were investigated with a multistep molecular docking approach using Glide software. ZINC18087726, ZINC20390250, BS053, GA009 and BS051 may be potential inhibitors of furin. These Screened hits were further analyzed based on their docking score, protein-ligand interaction and validated by their ADME properties and prime/MMGBSA. And stability of ligand – protein complex is estimated using molecular dynamics.

KEYWORDS: Furin, Evolutionary study, Virtual screening, e-Pharmacophore, ADME, Molecular Dynamics

RATHI SUGANYA P
Department of Bioinformatics, School of Bioengineering, SRM University, Kattankulathur, Tamil Nadu. India 603203

* **Corresponding author**

INTRODUCTION

Proteins play the major role of signaling molecules in the cell, which regulate all the functions in our body. These active proteins and peptides are initially formed as larger, inactive precursors. This precursor is then post translationally modified to generate the mature molecule.[1]. Proteolytic processing is one of the important contrivance for the production of mature and active proteins. Proprotein convertases (PC) are a family of serine proteases that cleave proteins at the carboxy terminus of a sequence of basic paired amino acids, RXR/KR.[2] Furin is the member of proprotein convertases family, which plays major role in secretory and endocytic pathways and at the cell surface].[3] It is found in the trans-golgi network and cleaves the precursors of many biologically important proteins, including hormones, growth factors, serum proteins, proteases of the blood-clotting system, matrix metalloproteinases, receptors, viral envelope glycoproteins, and bacterial exotoxins.. Furin activates many pathogens which attack human body like HIV, influenza, dengu fever, ebola and marburg virus.[4,5] Other than these vital roles, furin also plays essential role in embryogenesis, homeostasis. Studies has showed the involvement of furin in tumor metastasis and in neurodegenerative disease's like alzheimer's disease.[6-9] Many clinical trials has been done using furin inhibitors as therapeutic agent for furin-dependent diseases.[10,11] However, a potent drug for furin has not yet been discovered with no sideeffects. Therefore in this study, we focus on identifying a potent drug with all the known pharmacophoric features and unique to the target. Evolutionary conserved sequence of furin were analysed and unique site was identified which was used as the active site for docking.Virtual screening approach was used to identify small molecules with inhibition potential against furin, using computational drug discovery tools. Small molecules from ZINC database and SWMD were screened and filtered with the pharmacophore model developed. Multistep docking of filtered molecules was carried out with Glide and ADME properties of those molecules have been predicted. Binding free energy of docked ligands was calculated using prime/MM-GBSA. The stability of the identified molecule against furin was analyzed by explicit molecular dynamics. A set of commercially available molecules with possible inhibitory activity toward furin was reported.

MATERIALS AND METHODS

All work was carried using Schrödinger Suite v10.2 (http://www.schrodinger.com) on Windows platform. The following Schrödinger modules were used: Protein Preparation Wizard[12] , LigPrep[13] ,, e-Pharmacophore generation[14,15], , QikProp[16]Desmond[17-19] andGlide[20-22]. All modules were accessed via Maestro graphical interface[23]. MEGA6 (Molecular Evolutionary Genetic Analysis) was used for construction of Phylogenetic tree[24].

Evolutionary relationship

Comparisons of protein sequences among species are used to infer exploratory information about the protein function.[25-27]. Non-conserved amino acid sequence will adopt a new function, become dysfunctional (pseudogene) or adopts a tissue specific expression. Methods that have been used include: Homologous sequence, Visual inspection of aligned amino acid sequence, Phylogenetic analysis]. Amino acid sequence for Furin protein is obtained from UniProt database of id P09958. The database was used to search against protein sequences using BLASTp to identify the homologs of furin. To compare some closely related and some distantly related species, total of 15 furin protein sequence from different species in FASTA format were collected from Blast hits for further analysis. Then multiple sequence alignment was performed with all homologous sequence that is selected by using the ClustalW2 alignment program. The result was analysed by constructing a phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA Version 6) software.

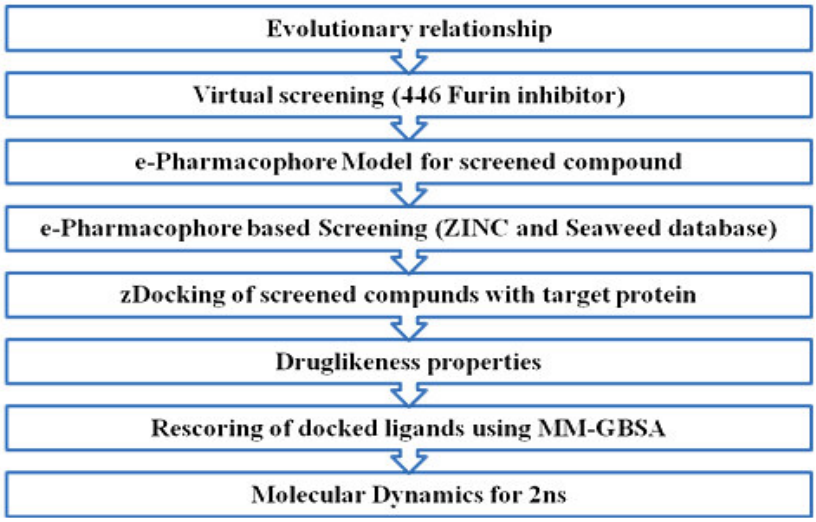

Figure 1
Workflow for identifying potent inhibitors for furin using virtual screening and structure-based pharmacophore modeling

Receptor protein preparation

The target protein furin with PDB id: 4RYD was obtained from RCSB Protein Data Bank (http://www.rcsb.org). The biological assembly shows the protein is a monomer, therefore, from the crystal dimer structure one chain is taken, prepared and refined using the Protein Preparation Wizard. The deposited structure was modified by adding of hydrogen to heavy atoms, charges and bond were assigned and finally all water molecules were deleted for docking calculations. The amino acid flip were assigned and H-Bonds optimised. Using force field OPLS_2005, minimization was carried out. Protein preparation wizard module of Schrödinger was used for protein preparation.

Ligand preparation:

Furin inhibitors of 446 compounds were retrieved from BindingDB (https://www.bindingdb.org/). 2D structures of small molecules from ZINC database were collected. Ligand preparation was performed for the collected molecules via Ligprep module of Schrödinger. Ligprep performs addition of hydrogen, 2D structure is enriched to 3D structure, accepted bond lengths and bond angles are adjusted, low energy structure with correct chirality's, ionization states, tautomers, stereochemistries and ring conformation are checked.

Receptor Grid Generation

After protein preparation, receptor grid was generated with Glide by specifying the active site with a 3D cubic box. This panel defines the receptor structure by excluding any co-crystallized ligand that may be present, determines the size and position of active site and set up the Glide constraints]. The grid map, which was centered at the following residues of the protein furin (ARG 468, LYS 469, THR 470, VAL 471, GLU 472, PRO 473, ASN 474, HIS 475, ILE 476, THR 478, ARG 479) were predicted using Dogsite Server (http://dogsite.zbh.uni-hamburg.de/) and non-conserved sequence information.

Virtual screening

The purpose of virtual screening is to find potential leads with different scaffolds and high inhibitory activity to furin. Virtual screening techniques are commonly classified into the structure and the ligand based virtual screening. Furin inhibitors were downloaded from BindingDB database and it was used for virtual screening based on lipinski's rule of 5. .

e-Pharmacophore generation

Schrödinger module of energy based pharmacophore (e-Pharmacophore) was used for the structure based screening of ligands. e-Pharmacophores were constructed by mapping the energetic terms from Glide XP scoring function onto atom centres. e-Pharmacophore generation using default set of five chemical features: hydrogen bond acceptor (A), hydrogen bond donor (D), Positive Ionizable Region (P), aromatic rings(R), hydrophobic groups(H). The known compounds active pharmacophoric features will be identified and used for screening novel compounds.

e-Pharmacophore based screening

The constructed e-Pharmacophore was used as query for screening of compounds from Zinc database and SWMD (Seaweed metabolite database) . Screening molecules should match at least 3 sites for a hypothesis with 4 sites. The fitness score is a measured by aligning the ligand conformer with the hypothesis based on site matching, vector alignments and volume terms. Database hits were ranked based on this fitness score. The fitness scoring function is an equally weighted combination for three terms and the acceptable range is between 0 to 3, as implemented in the default database screening of Phase. The ligands were short listedconsidering the fitness score. The ligands with the best fitness scores were docked into the binding sites of target protein.

ADME prediction

The QikProp program was used to obtain ADME properties of the short listed compounds. ADME predicts physically important descriptors and pharmaceutically applicable properties. All the compounds were neutralized before being used by QikProp. The neutralizing step helps in generating structure and properties.. The program was processed in normal mode, and predicted 44 propertiesIt also evaluated the acceptability of the compounds based on Lipinski's rule of five, which is essential for rational drug design.

Rescoring value of docked ligands

Prime/MMGBSA (Molecular Mechanics with Generalized Born and Surface Area salvation) is the rescoring value of docked ligand which calculates the binding free energy ΔGbind between the receptor and a ligand in its complex. To obtain the re-rank score of the docked conformations of each ligand atttained from the Glide XP method. OPLS_2005 force field was used for energy minimization for the complex within Macro Model was performed.

ΔGbind = Gcomplex - (Gprotein + Gligand)

(x) Molecular Dynamics simulation

Molecular dynamics (MD) simulation was performed to check the stability and conformational changes of the ligands in the active site of receptor on different time scales. OPLS 2005 force field was used for protein interaction and TIP3P was used for salvation of the model. Protein and ligand was packed in orthorhombic box and the system was neutralized with Na^+ ions. Energy minimization using steepest descent algorithm is carried out for the solvated system. Prepared protein–ligand complexes were simulated for a 2-ns time period to investigate the stability of the docked ligands. Energy, root mean square deviation (RMSD) and root mean square fluctuations (RMSF), radius of gyration (ROG) and protein ligand interactions were analyzed for the simulated system with respect to simulation time.

RESULTS

Evolutionary analysis

Furin protein sequence was downloaded in FASTA format and homolog sequence of target protein is identified using BLASTp. Based on identity score, most closely related and distantly related species were selected (*Homo sapiens, pan paniscus, Gorilla gorilla,*

Pongo abelii, Cercocebus atys, Chlorocebus sabas, Echinops telfairi, Erinaceus europaeus, Sorex araneus, Ursus maritimus, Calypte anna, Melopsittacus undulates, Pseudopodoces humilis, Ficedula albicollis, Zonotrichia albicollis, Camelus ferus). Then the selected hits were aligned using ClustalW program which shows that *pan paniscus, Gorilla gorilla, Pongo abelii, Cercocebus atys, Ursus maritimus , Echinops telfairi, Sorex araneus* have alignment score <90. The result suggests that there must be strong conservation and relationship between the species and function of furin protein. Figure 2 shows Sequence alignment of furin among various species is aligned using MEGA tool. To elucidate the Phylogenetic relationship among the furin protein of various species and infer the evolutionary history of this protein, a phylogenetic tree was constructed using MEGA software. Here we used Neighbour joining method (NJ method) for tree construction and distance was calculated for each node. Figure 3 shows the Phylogenetic tree of furin protein among various species. Distance is shown on top of each branch and different labels are used to indicate closely related species. And alignment score which is generated in ClustalW was shown in tabular column.

Figure 2
Sequence alignment of furin among various species using MEGA tool. The sequence that is shown inside the box is non-conserved sequences

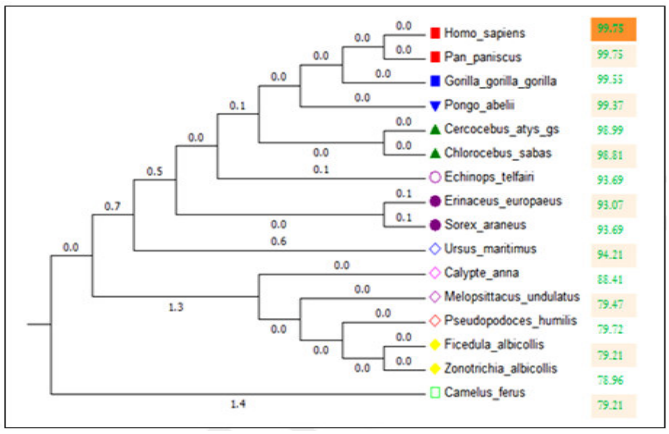

Figure 3
Phylogenetic tree of furin protein among various species with alignment score

Screening of inhibitors

Furin inhibitors of 446 compounds from BindingDB was downloaded and screened based on QikProp and Lipinski rule of 5. Out of 446 compound, the inhibitor 46232267 which has satisfactory drug-like properties. And the resulted compound had shown to have IC50 value as 9.4 µm and docking score as -3.748 Kcal/mol for the target protein.

e-Pharmacophore Modeling

The e-Pharmacophore combines the aspect of structure-based and ligand-based techniques. Incorporating protein–ligand contacts into ligand-based pharmacophore model will be more specific in using ligand information alone. This method enriches the contact scoring by incorporating structural and energetic information using the scoring function in Glide XP. Two pharmacophore features were predicted for the screened hit and their hypothesis consists of an aromatic ring (R), and a H-bond donors (D) and their distance are shown in Figure 4.

Docking with screened compounds

The natural compounds from ZINC database and marine compounds from SWMD were downloaded and screened based on hypothesis that is generated by e-

Pharmacophore. The molecules obtained by filtering with hypothesis DR were docked to the receptor furin with Glide software to predict the binding affinity of molecules to the target and to investigate the different bonds and their strength.. The binding coarse and features of each database molecule relative to the receptor protein were identified and graded by score's with internal scoring function Glide Score. Docking scores varied between −10.72 and +4.5 Kcal/mol in Glide XP mode. The docking results of the selected hits are given in Table I and Table II. The compounds ZINC59227949, ZINC79210088, ZINC72320143, RRO17, BL005 and BS053 are the compounds that have high docking score. The 2D representation of the selected hits and their receptor hydrogen bond and hydrophobic interactions is shown in Figure 5 Hydrogen bond interactions and their atomic distances (in Å) are shown in dashed lines. THR 472, GLU 477, LEU475 are commonly known residues to interact with selected hits and target protein.

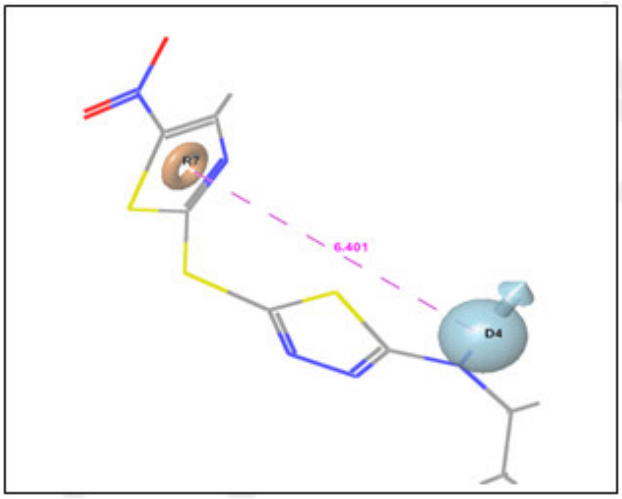

Figure 4
e-Pharmacophore site points and site-site distances on compound 46232267. Geometry of the Pharmacophore with the distance between the pharmacophore sites

Predicted ADME properties

Pharmacokinetic properties, druglikeness as well as other significant descriptors, such as molecular weight, H-bond donors, H-bond acceptors, solvent accessible surface area (SASA), log HERG (blockage of K+ channels), log S (aqueous solubility), log P (octanol/water), and human oral absorption, for the selected hits were determined by QikProp. Druglikeness, as predicted by the Lipinski rule, was investigated along with the predicted ADME and molecular properties. According to this rule, in order for a compound to be drug-like and orally active, it should have a molecular weight less than 500 Da, hydrogen bond donor equal to or less than five, hydrogen bond acceptor equal to or less than 10, and partition coefficient (QP log P o/w) less than five. Molecular weight, donor and acceptor atom numbers of the selected molecules were within the allowed values. Table I and II shows ADME and druglikeness properties of the selected hits by QikProp. ZINC18087726, ZINC20390250, BSO53, GA009 and BS051 are the compounds known to have good oral absorption when compared to other compounds.

Table 1
Docking results and ADME properties of selected hits of ZINC database

S. No	Compound id	Docking Score (Kcal/mol)	% of Human oral absorption
1	ZINC59227949	-10.742	27.805
2	ZINC79210088	-9.886	10.994
3	ZINC72320143	-9.684	47.862
4	ZINC70672768	-9.627	2.587
5	ZINC68591780	-9.258	63.03
6	ZINC03874317	-9.613	25.235
7	ZINC18087726	-8.88	94.155
8	ZINC68604232	-8.497	80.55
9	ZINC20390250	-8.391	100
10	ZINC68591787	-8.391	88.861
11	ZINC68606315	-8.252	66.1

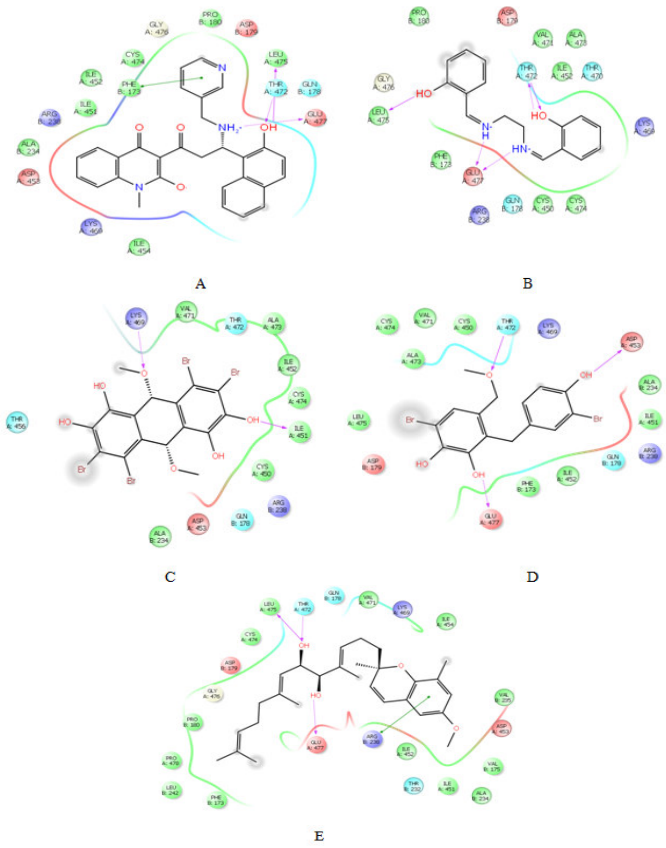

Figure 5

Interaction of selected compounds with the active site residues of furin protein A)ZINC18087726; B)ZINC20390250; C) BSO53 D) GA009 and E) BS051. THR 472, GLU 477 and LEU 475 are commonly known residues that interact with selected hits and target protein

Rescoring value of protein-ligand complex using prime/MM-GBSA

The rescoring of docked ligand complex for furin protein based on the binding affinities was performed using Prime MMGBSA method. The compounds were computed based on their free energy estimates. The compounds ZINC68604232, BS051 showed least ligand binding energy of -63.541Kcal/mol and -96.89Kcal/mol (Table III) which was analysed by MM-GBSA. The overall complex binding energy was also significantly very low with -124310Kcal/mol and -124225Kcal/mol respectively.

Table 2

Docking results and ADME properties of selected hits of SWMD

S. No	Compound_id	Docking Score (Kcal/mol)	% of Human Oral Absorption
1	RR017	-8.479	62.041
2	BL005	-7.496	78.823
3	BS053	-7.378	100
4	GA009	-6.124	93.163
5	BS051	-5.697	100
6	RR010	-5.949	80.845
7	RP003	-5.646	81.047

Table 3

Free binding energy for the pharmacophore hits of furin protein complex

Compound_id	MM-GBSA bind (Kcal/mol)	MM-GBSA E-complex (Kcal/mol)	MM-GBSA E-protein (Kcal/mol)
ZINC18087726	-50.107	-124119	-124105
ZINC68604232	-63.541	-124310	-124105
ZINC68591865	-55.247	-124233	-124105
ZINC20390250	-67.643	-124169	-124105
BS053	-94.29	-124216	-124105
RR017	-79.79	-124211	-124105
GA009	-68.07	-124185	-124105
BS051	-96.89	-124225	-124105

MD stimulation of active compounds with furin

Molecular dynamics simulation was performed for the two different docked complex structures of furin-ZINC68604232 and furin-BS051for 2000ps. The stability of the active compounds with the receptor, furin was measured in terms of deviations and fluctuations. The molecular dynamics simulation shows the conformational change which takes place in the protein-ligand complex.The root mean square deviation (RMSD) of the trajectory for the ligands in the active site of furin protein is represented in Fig. 6A & 6B. The RMSDs of the trajectory with respect to their initial structure range varied from 0.2 to 2.2 Å for 4RYD complex with ZINC68604232 and 0.7 to 1.3 Å for 4RYD complex with BS051. The overall molecular dynamics results suggest that the binding pocket and the conformations of two ligands are unstable for 2ns, even though the complexes have been undergone several movements.

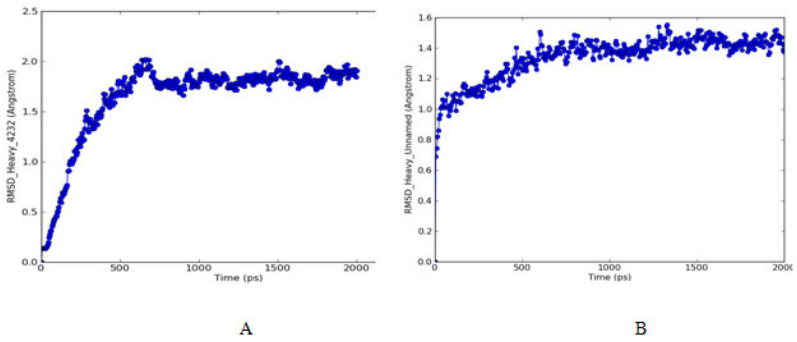

Figure 6
RMSD trajectory analyses (A) Furin- ZINC68604232 complex and (B) Furin-BS051 complex

CONCLUSION

Furin is a important proprotein convertase, which cleaves inactive proteins an make them functional proteins. Many Secretory pathway and diverse types of functional proteins are activated by furin. The known proprotein substrates that are activated by furin include both host proteins and pathogen proteins. In this study, evolutionary relationship of furin among various species has been studied and non-conserved sequence has been determined. This unique site is used as active site for docking studies as this will be unique for furin. Known furin inhibitor has been screened and e-Pharmacophore has been generated. The screened pharmacophoric features were used to generate pharmacophoric hypothesis. The Hypothesis was screened with natural compounds from ZINC database and marine compounds from SWMD. Binding orientation and protein-ligand interaction has been analyzed and found that THR 472 , GLU 477 and LEU 475 commonly known to interact with selected ligands. ADME properties of selected hits were found. Based on the docking results, ADME properties and MM-GBSA, we report that chemical compounds ZINC18087726, ZINC20390250, BS053, GA009 and BS051 may be potential inhibitors of furin. Molecular dynamics simulation (MD) further indicated that the conformation derived from docked complex are stable.

ACKNOWLEDGEMENT

The authors thank SRM University for their constant support and funding.

CONFLICT OF INTEREST

Conflict of interest declared none

REFERENCES

1. Duckert P, Brunak S, Blom N. Prediction of proprotein convertase cleavage sites. Protein Engineering Design and Selection. 2004 Jan 1;17(1):107-12.
2. Bassi DE, Mahloogi H, De Cicco RL, Klein-Szanto A. Increased furin activity enhances the malignant phenotype of human head and neck cancer cells. The American journal of pathology. 2003 Feb 28;162(2):439-47.
3. Thomas G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. Nature reviews Molecular cell biology. 2002 Oct 1;3(10):753-66.
4. Denault JB, Leduc R. Furin/PACE/SPC1: a convertase involved in exocytic and endocytic processing of precursor proteins. FEBS letters. 1996 Jan 29;379(2):113-6.
5. Omotuyi IO. Ebola virus envelope glycoprotein derived peptide in human Furin-bound state: computational studies. Journal of Biomolecular Structure and Dynamics. 2015 Mar 4;33(3):461-70.
6. Bassi DE, De Cicco RL, Mahloogi H, Zucker S, Thomas G, Klein-Szanto AJ. Furin inhibition results in absent or decreased invasiveness and tumorigenicity of human cancer cells. Proceedings of the National Academy of Sciences. 2001 Aug 28;98(18):10326-31.
7. Bassi DE, Mahloogi H, De Cicco RL, Klein-Szanto A. Increased furin activity enhances the malignant phenotype of human head and neck cancer cells.

The American journal of pathology. 2003 Feb 28;162(2):439-47.
8. Bennett BD, Denis P, Haniu M, Teplow DB, Kahn S, Louis JC, Citron M, Vassar R. A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's β-secretase. Journal of Biological Chemistry. 2000 Dec 1;275(48):37712-7.
9. Thomas G. Furin at the cutting edge: from protein traffic to embryogenesis and disease. Nature reviews Molecular cell biology. 2002 Oct 1;3(10):753-66.
10. Kibirev VK, Osadchuk TV, Radavskiĭ I. [Furin and its biological role]. Ukrains' kyi biokhimichnyi zhurnal (1999). 2006 Dec;79(6):5-18.
11. Coppola JM, Bhojani MS, Ross BD, Rehemtulla A. A small-molecule furin inhibitor inhibits cancer cell motility and invasiveness. Neoplasia. 2008 Apr 30;10(4):363-70.
12. Sastry, G.M.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W., "Protein and ligand preparation: Parameters, protocols, and influence on virtual screening enrichments," ‼ HYPERLINK "http://link.springer.com/article/10.1007/s10822-013-9644-8" \t "_blank" ¶J. Comput. Aid. Mol. Des., 2013, 27(3), 221-234⊥
13. Schrödinger Release 2016-3: LigPrep, version 3.9, Schrödinger, LLC, New York, NY, 2016.
14. Salam, N.K.; Nuti, R.; Sherman, W., "Novel Method for Generating Structure-Based Pharmacophores Using Energetic Analysis," ‼ HYPERLINK "http://pubs.acs.org/doi/abs/10.1021/ci900212v" \t "_blank" ¶J. Chem. Inf. Model., 2009, 49, 2356–2368⊥
15. Loving, K.; Salam, N.K.; Sherman, W., "Energetic analysis of fragment docking and application to structure-based pharmacophore hypothesis generation," ‼ HYPERLINK "http://www.springerlink.com/content/07747281v175403g/" \t "_blank" ¶J. Comput. Aided Mol. Des., 2009, 23, 541–554⊥
16. Small-Molecule Drug Discovery Suite 2016-3: QikProp, version 4.9, Schrödinger, LLC, New York, NY, 2016.
17. Shivakumar, D.; Williams, J.; Wu, Y.; Damm, W.; Shelley, J.; Sherman, W., "Prediction of Absolute Solvation Free Energies using Molecular Dynamics Free Energy Perturbation and the OPLS Force Field," ‼ HYPERLINK "http://pubs.acs.org/doi/abs/10.1021/ct900587b" \t "_blank" ¶J. Chem. Theory Comput., 2010, 6, 1509–1519⊥
18. Guo, Z.; Mohanty, U.; Noehre, J.; Sawyer, T. K.; Sherman, W.; Krilov, G., "Probing the α-Helical Structural Stability of Stapled p53 Peptides: Molecular Dynamics Simulations and Analysis," ‼ HYPERLINK "http://www3.interscience.wiley.com/journal/123299874/abstract" \t "_blank" ¶Chem. Biol. Drug Des., 2010, 75, 348-359⊥
19. Kevin J. Bowers, Edmond Chow, Huafeng Xu, Ron O. Dror, Michael P. Eastwood, Brent A. Gregersen, John L. Klepeis, Istvan Kolossvary, Mark A. Moraes, Federico D. Sacerdoti, John K. Salmon, Yibing Shan, and David E. Shaw, "Scalable Algorithms for Molecular Dynamics Simulations on Commodity Clusters," ‼ HYPERLINK "http://dl.acm.org/citation.cfm?id=1188455" \t "_blank" ¶Proceedings of the ACM/IEEE Conference on Supercomputing (SC06), Tampa, Florida, 2006, November 11-17⊥
20. Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren,T. A.; Sanschagrin, P. C.; Mainz, D. T., "Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes," ‼ HYPERLINK "http://dx.doi.org/10.1021/jm051256o" \t "_blank" ¶J. Med. Chem., 2006, 49, 6177–6196⊥
21. Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L., "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening," ‼ HYPERLINK "http://pubs.acs.org/cgi-bin/abstract.cgi/jmcmar/2004/47/i07/abs/jm030644s.html" \t "_blank" ¶J. Med. Chem., 2004, 47, 1750–1759⊥
22. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Francis, P.; Shenkin, P. S., "Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy," ‼ HYPERLINK "http://pubs.acs.org/cgi-bin/abstract.cgi/jmcmar/2004/47/i07/abs/jm030643 0.html" \t "_blank" ¶J. Med. Chem., 2004, 47, 1739–1749⊥
23. Schrödinger Release 2016-3: Maestro, version 10.7, Schrödinger, LLC, New York, NY, 2016.
24. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution. 2013 Dec 1;30(12):2725-9.
25. Greenblatt MS, Beaudet JG, Gump JR, Godin KS, Trombley L, Koh J, Bond JP. Detailed computational study of p53 and p16: using evolutionary sequence analysis and disease-associated mutations to predict the functional consequences of allelic variants. Oncogene. 2003 Feb 27;22(8):1150-63.
26. Karthik G, Duraivelu R, Santhalingam K, NAGARAJAN D. Phylogenetic analysis and homology based inhibitor design for short neurotoxins of forest cobra. Int J Pharma and Bio Sci. 2011;2:84-95.
27. Abraham JA, Chauhan RI. Bioactivity and in silico analysis of R-alpine borane produced by Streptomyces toxytricini JAR4. Int J Pharm Biol Sci. 2015;6(4):265-76.