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Nilotinib based pharmacophore models for BCR-ABL

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Abstract:
Tyrosine kinase inhibitors have revolutionized the treatment of several malignancies, converting lethal diseases in a manageable aspect. Imitanib, a small molecule ABL kinase inhibitor is a highly effective therapy for early phase chronic myeloid leukemia (CML), which has constitutively active ABL kinase activity owing to the over expression of the BCR-ABL fusion protein. But some patients develop imatinib resistance, particularly in the advanced phases of CML.The discovery of resistance mechanisms of imitanib; urge forward the development of second generation drugs. Nilotinib, a second generation drug is more potent inhibitor of BCR-ABL than imatinib. But nilotinib also develops dermatologic events and headache in patients. Large information about BCR-ABL structure and its inhibitors are now available. Based on the pharmacophore modeling approaches, it is possible to decipher the molecular determinants to inhibit BCR-ABL. We conducted a structure based and ligand based study to identify potent natural compounds as BCR-ABL inhibitor. First kinase inhibitors were docked with the receptor (BCR-ABL) and nilotinib was selected as a pharmacophore due its high binding efficiency. Eleven compounds were selected out of 1457 substances which have mutual pharmacopohre features with nilotinib. These eleven compounds were validated and used for docking study to find the drug like molecules. The best molecules from the final set of screening candidates can be evaluated in cell lines and may represent a novel class of BCR-ABL inhibitors.

Keywords: Ligand docking, BCR-ABL, Nilotinib, Glide score, Pharmacophore modeling

Abbreviations: CML –Chronic myeloid leukemia, PDGFR -Platelet derived growth factor receptor, TKI - Tyrosine kinase inhibitors

Background:
Chronic myeloid leukemia (CML) is a cancer of blood cells, characterized by replacement of the bone marrow with malignant, leukemic cells. Many of these leukemic cells can be found circulating in the blood and can cause enlargement of the spleen, liver, and other organs. The BCR-ABL oncogene, which is the product of Philadelphia chromosome (Ph) 22q, encodes a chimeric BCR-ABL protein that has constitutively activated ABL tyrosine kinase activity and it is basic cause of chronic myeloid leukemia **[1-3]**. Imitanib, a small molecule ABL kinase inhibitor is a highly effective therapy for early phase of CML **[4].** It also inhibits platelet derived growth factor receptor (PDGFR) at physiologically relevant concentrations on the field of cancer therapy has been dramatic **[5].** However, there is a high relapse rate among advanced and blast crisis phase patients owing to the development of mutations in the ABL kinase domain that cause drug resistance .Several approaches to overcoming resistance have been studied both in vitro and in vivo. They include dose escalation of imatinib, the combination of imatinib with chemotherapeutic drugs, alternative BCR-ABL inhibitors, and inhibitors of kinases acting downstream of BCR-ABL such as Src kinases. Various novel tyrosine kinase inhibitors (TKI) have been synthesized and have now reached the pre-clinical or clinical phase **[6]**. Classes of these new inhibitors include selective ABL inhibitors, inhibitors of ABL and Src family kinases, Aurora kinase inhibitors and non ATP

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competitive inhibitors of BCR-ABL. But these drugs inevitably damage and debilitate too many normal cells and organs. They undermine and destroy patient's immunity and patients' abilities to resist disease, their health and natural healing abilities. It is ideal for a chemopreventive drug to be nontoxic, effective at lower doses, economical and easily available. So in recent years natural products have drawn a great deal of attention both from researchers because of its potential effects to suppress cancer and also reduce the risk of cancer development. Natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy and biology. Natural products have taken a secondary role in drug discovery and drug development, after molecular biology.

Computational chemistry has been playing a more and more important role in drug discovery. Computational chemistry made rational design of chemical compounds to target specific molecules. In particular, computational high-throughput docking has become a powerful tool for screening and identifying novel lead compounds. Computational approaches could not only save time and costs spent during in vitro screening by providing a candidate list of potential off-targets but also provide insight into understanding the molecular mechanisms of protein–drug interactions. It has been shown that potential off-targets can be identified in silico by establishing the structure–activity relationship of small molecules **[7-14]**. Pharmacophore modeling is a computer-aided drug design tool used in the discovery of new classes of compounds for a given therapeutic category **[15].** Pharmacophores generally are fragments or functional groups of a chemical compound **[16].** It has to describe the nature of functional groups involved in ligand–target interactions, as well as type of the non covalent bonding and distances. The compound nilotinib has previously shown high binding affinity with BCR-ABL when compared with other kinase inhibitors. Therefore, modeling studies can be intensively used to decipher the molecular determinants of BCR-ABL. This knowledge can be used to design new compounds with the help of natural compound database of Supercomputing Facility for Bioinformatics and computational Biology, IIT, Delhi **[17]** and develop more effective therapeutic drugs. The objective of the current study was to evaluate the binding affinity of BCR-ABL second generation inhibitors with the help of GLIDE and design effective drugs with the help of pharmacophore modeling.

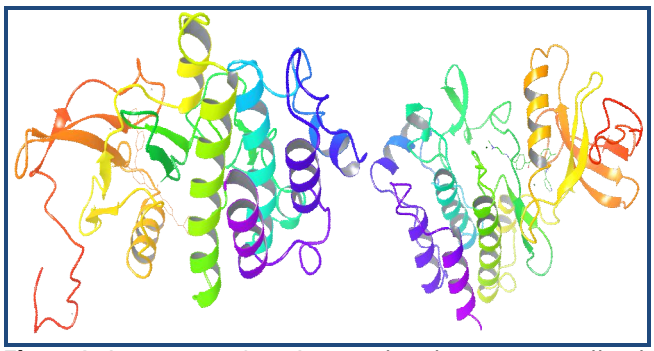

Figure 1: C-ABL KINASE DOMAIN (1IEP) structure predicted by X-ray crystallography , Hydrogen bonds are added, protonation states of residues are corrected and energy minimized by Schrodinger Protein preparation wizard.

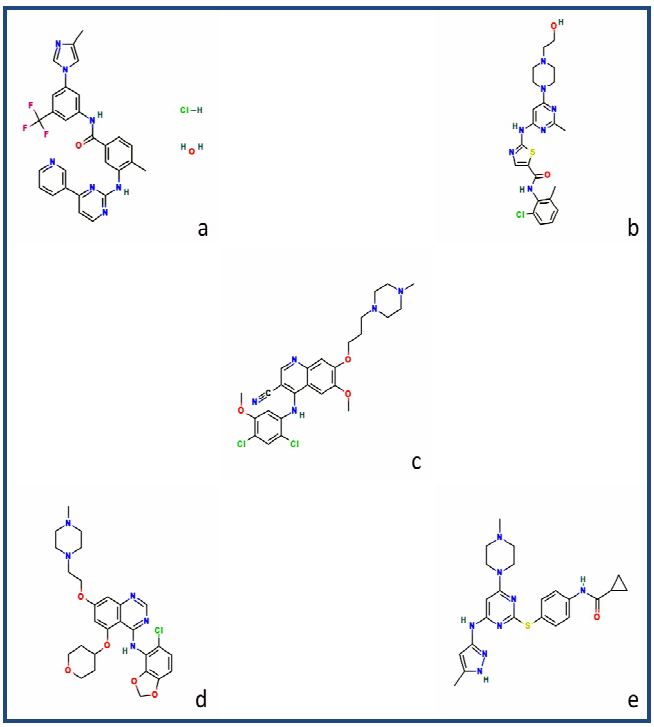

Figure 2: a) Nilotinib CID 16757572; **b)** Dasatinib CID: 3062316; **c)** Bosutinib CID: 5328940; **d)** AZD0530 CID: 10302451; **e)** MK-0457 CID: 5494449

Methodology:

C-ABL KINASE DOMAIN IN COMPLEX WITH STI-571 was downloaded (1IEP) from PDB database **[18].**

Protein preparation wizard

Using Schrödinger's Protein Preparation Wizard, full PDB file (1IEB) was imported from PDB website and we added missing hydrogen atoms, corrected metal ionization states to ensure proper formal charge and force field treatment to enumerate bond orders to HET groups. Co-crystallized water molecules were removed. Optimal protonation states for histidine residues were determined and potentially transposed heavy atoms in arginine, glutamine, and histidine side chains were corrected. Optimize the protein's hydrogen bond network by means of a systematic, cluster-based approach, which greatly decreases preparation times. Perform a restrained minimization that allows hydrogen atoms to be freely minimized, while allowing for sufficient heavy-atom movement to relax strained bonds and angles **(Figure 1).**

Ligand preparation

LigPrep goes beyond simple 2D to 3D conversions by including tautomeric, stereochemical and ionization variations as well as energy minimized 3D molecular structures. It also applies sophisticated rules to correct Lewis structures and to eliminate mistakes in order to reduce downstream computational errors **[9].** The following 5 inhibitors of BCR-ABL kinase were downloaded from Pubchem **[20] (Figure 2a, b, c, d & e)**. We did ligPrep using Schrodinger tool for these inhibitors. LigPrep optionally expands tautomeric and ionization states, ring conformations and stereoisomer to produce broad chemical and structural diversity from a single input structure.

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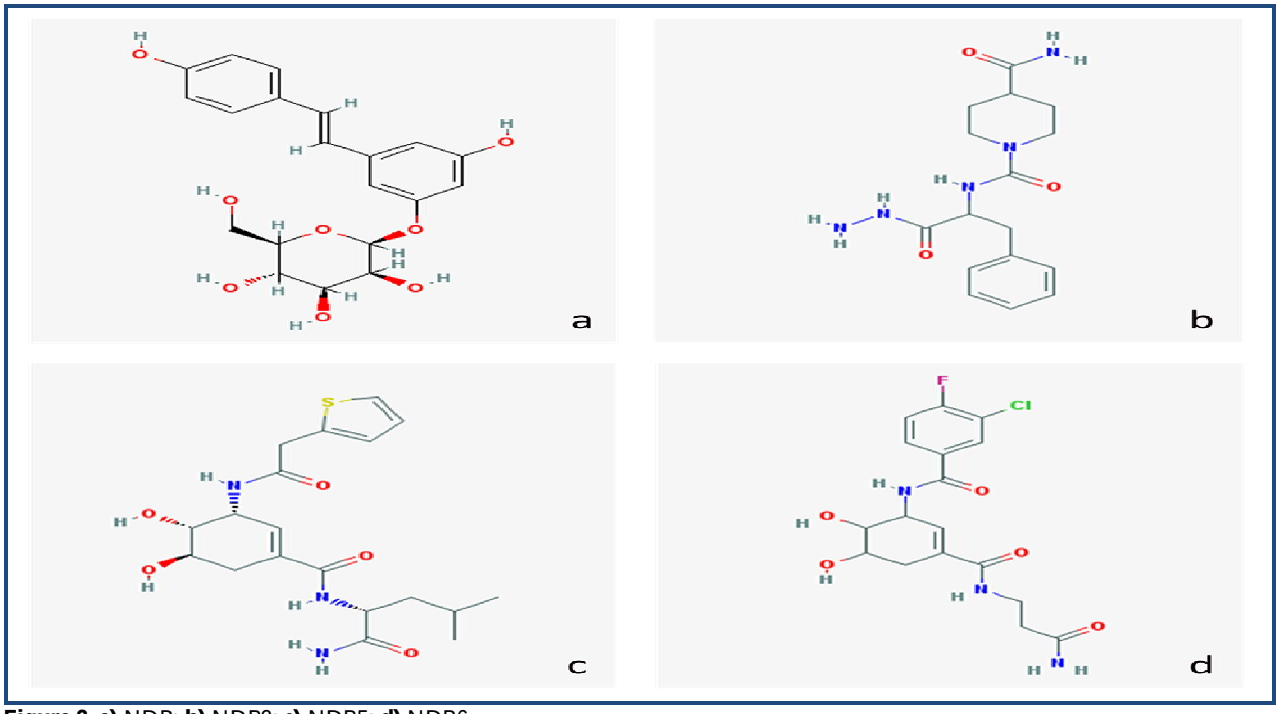

Figure 3: a) NDB; **b)** NDB2; **c)** NDB5; **d)** NDB6

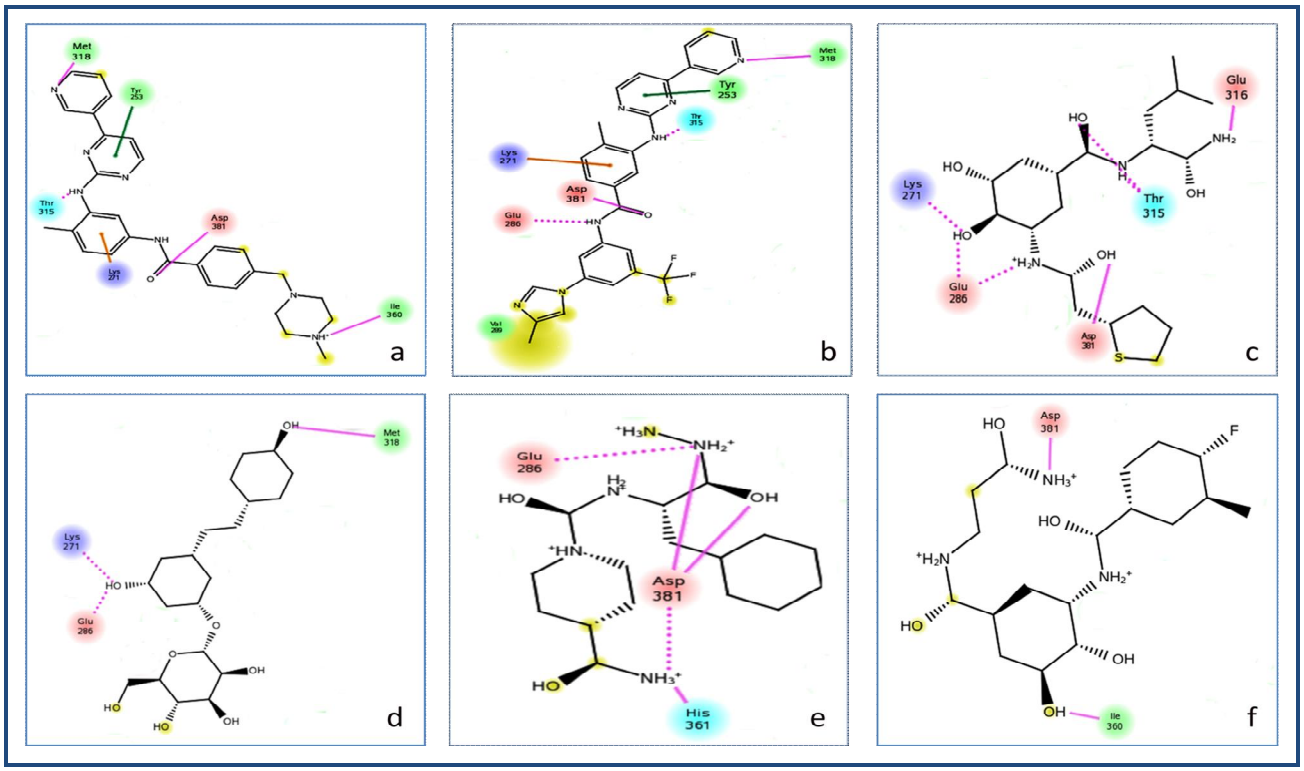

Figure 4: a) Active site of c ABL-kinase; **b)** Nilotinib with c ABL kinase ... H bond side chain, ____ H-bond backbone, ___ π – cation, ___ π- π stacking; **c)** N-(1-carbamoyl-3-methyl-butyl)-4,5-dihydroxy-3-[2-(2-thienyl)acetyl amino-cyclohexene-1-carboxamide (NDB5); **d)** cis Resveratrol 3-O-D-glucopyranoside (NDB); **e)** N-[(1S)-1-benzyl-2-hydrazino-2-keto-ethyl]piperidine-1,4-dicarboxamide (NDB2); **f)** N-[3-(2-carbamoylethylcarbamoyl)-5,6-dihydroxy-1-cyclohex-2-enyl] 3-chloro-4-fluoro-benzamide (NDB6)

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Designing of compounds

Compounds were screened using Nilotinib as model compound. From the resulting list of 1437, eleven most similar molecules were retrieved. Eleven compounds were subsequently docked with c-ABL to find its binding affinity. The compounds listed in **(Figure 3 a, b, c &d)** showed binding affinities with BCR-ABL.

Docking

Protein preparation is relaxation of the receptor structure so that it at least accommodates the ligand or inhibitors. We employed the standard Schrodinger protein preparation utility for this purpose. Glide calculation performs Grid based ligand docking with energetics and searches for favorable interactions between one or more typically small ligand and a larger receptor molecule usually a protein. After ensuring that the protein and ligands are in correct form for docking the receptor grid files were generated using Grid Receptor generation programme. The ligand docking calculations were done in the standard precision mode of GLIDE. During the docking process, the receptor was treated as fixed while ligand was flexible. In the minimization of ligands, we have used a distance-dependent dielectric constant with a value of 2.0 and a conjugate gradient algorithm with small 100 steps. All of the inhibitors were passed through a scaling factor of 0.80 and partial charge cutoff of 0.15 **[21, 22].**

Discussion:

A grid was generated at the centroid of the active sites consisting of residues Asp-381, Ile-360, Thr-315, Glu-286, Lys-271 and Met-318 **(Figure 4a)** and the ligands were docked into the active site using Glide.

Docking of Tyrosine kinase inhibitors

The ligands were docked with the active site using Standard Precision (SP) Glide algorithm. The docking results of these ligands are given in **Table 1 (see supplementary material)**. The ranking of ligands was based on the glide score. The goal of SP Glide methodology is to semi quantitatively rank the ability of candidate ligands to bind to a specified conformation of the protein receptor. The purpose of scoring procedure is the identification of the correct binding pose by its lowest energy value and the ranking of protein ligand complexes according to their binding affinities. In the protein receptor complex (1IEP), whether the ligand fits appropriately into the receptor is judged by the ability to make key hydrogen bonding and hydrophobic contacts. Glide SP scoring function can be enumerated by the displacement of waters by the ligand from hydrophobic regions of the protein active site, protein –ligand hydrogen bonding interactions as well as other strong electrostatic interactions such as salt bridges, desolvation effects, entropic effects due to the restriction on binding of the motion of flexible protein or ligand groups and also interaction of the ligand with metal ions **[23].** Our docking results showed that Nilotinib ranked among top among the compounds with the best GLIDE score -18.35 **(Figure 4b)**. The glide energy term is very small, which indicates that there is a very low energy penalty when the ligand is buried in the active site.

When we analyzed the receptor-ligand interaction nilotinb sits deeply within the binding site and interacts with protein via hydrogen bond with Asp 381, Met318 and Glu286 and via pi-pi stacking interaction with Thr315, Tyr253 and pi-cation with Lys-271. Next to Nilotinib an analogue of Bosutinib had a glide score of -13.05 binds with almost same amino acids except Met318; instead it showed interaction with Val269. Glide provided the best docking results, with the most accurately predicted binding around the active site. So we selected Nilotinib as model to develop pharmocophore models. The pharmacophore features selected for creating sites were hydrogen bond acceptor (A), hydrogen bond donor (D), molecular weight, and hydrophobic region. Using nilotinib as a model, the best pharmacophore models were obtained from Molecular database of Supercomputing facility, IIT, Delhi. Eleven compounds were selected out of 1457 substances which have mutual pharmacopohre features with nilotinib. These eleven compounds were chosen to dock with BCR-ABL to determine its binding affinities. The top four compounds which showed best binding affinities were selected for further analysis.

Docking of Nilotinib like- molecules

Out of ten compounds studied only four compounds binds with BCR-ABL and produced docking score. The glide score of compound NDB5 is -12.197 **(Figure 4c)** and it binds with amino acids Glu286, Asp381 and His361 with the docking energy of -61.443. NDB binds with Met318, Lys271 and Glu286 with the glide score of -8.555 and its docking energy is -46.754 **(Figure 4d).** NDB2 and NDB6 bind with docking score of -8.436 and -8.335 **Figure 4e, f** & **Table 2 (see supplementary material)**.

The compounds obtained after docking were subjected to determine their pharmacokinetics properties using QikProp module of Schrodinger and compared with nilotinib. We analyzed 44 physically significant analogues of these four compounds among which are molecular weight, H-bond donors, H-Bond acceptors, logPo/w (octonal/water), skin permeability Kp , aqueous solubility (logS) , Predicted IC50 value for blockage of HERG K+ channels (logHERG) , apparent Caco-2 cell permeability in nm/sec (PPCaco), brain/blood partition coefficient (PlogBB) ,apparent MDCK cell permeability in nm/sec(PPMDCK) and percentage of human oral absorption. In this study, out of 4 compounds, one compound (NDB) showed allowed values for the properties analyzed and exhibited drug-like characteristics **[24]**. For NDB, the partition coefficient (QPlogPo/w) and water solubility (QPlogS), critical for estimation of absorption and distribution of drugs within the body, ranged between -2 to -6.5 and -6.5 to 0.5 respectively and cell permeability (QPPCaco), a key factor governing drug metabolism and its access to biological membrane is 49.449. Overall, the percentage human oral absorption for the compounds ranged from ~ 25 to ~ 80% **[25].** All these pharmacokinetic parameters are within the acceptable range defined for human use. When compared with nilotinib NDB showed better ADME properties and it could be a potential inhibitor of BCR-ABL **Table 3 (see supplementary material).** Combining the results of pharmacophore, drug-likeness, ADMET, molecular docking studies, and the novelty search, we have found NDB (cis Resveratrol 3-O-D-glucopyranoside) as possible virtual lead to design novel human BCR-ABL inhibitor.

Conclusion:

The development of novel and potent kinase inhibitors is a challenging task. As an attempt to develop inhibitors we have

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employed pharmacophore modeling and docking studies to identify potential inhibitors against BCR-ABL. Pharmacophore models were generated with nilotinib as a model according to Lipinski's rule (i.e ME <500,H-Bond donor<=5, H-bond acceptor<=10,log P <=5). Further the compounds were docked with BCR-ABL using Glide. Best hit was identified on the basis of target affinity, molecular docking, and scoring and binding affinity predictions. Further QikProp was used to evaluate the drug likeness of the lead molecules by assessing their physiochemical properties. All pharmacokinetic properties were within the acceptable range for cis Resveratrol 3-O-D-glucopyranoside.When compared with nilotinib it showed better ADME properties and it can be a potential inhibitor of BCR-ABL and further analysis can be performed through various experimental studies.

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Supplementary material:

Table 1: Tyrosine kinase inhibitors Docking Score

S.No	Ligand	Name	G-score	Energy	Hbond	Good VDW	Bad VDW	UglyVDW
1	16757572	Nilotinib	-18.35	-77.5	2	468	16	0
2	5328940	Bosutinib	-13.05	-45.5	1	441	16	2
3	3062316	Dasatinib	-12.14	-57.2	1	318	21	0
4	10302451	AZD0530	-8.74	-52.8	1	414	12	2
5	5494449	MK-0457	-7.37	-47.0	1	313	13	2

Table 2: Nilotinib like molecules docking score

Title	Name	G- score	G-energy
NDB5	N-(1-carbamoyl-3-methyl-butyl)-4,5-dihydroxy-3-[2-(2-thienyl)acetyl]amino cyclohexene-1-carboxamide	-12.19722	-61.443
NDB	cis Resveratrol 3-O-D-glucopyranoside	-8.555196	-46.754
NDB2	N-[(1S)-1-benzyl-2-hydrazino-2-keto-ethyl] piperidine-1,4-dicarboxamide	-8.436406	-59.420
NDB6	N-[3-(2-carbamoylethylcarbamoyl)-5,6-dihydroxy-1-cyclohex-2-enyl] 3-chloro-4-fluoro-benzamide	-8.335115	-54.425

Table 3: ADME properties of compounds

Title	QPlogPo/w (-2 to -6.5)	QPlogS (-6.5 to 0.5)	QP logHERG concern below -5	QPPCaco <25 poor >500 high	QPlogBB (-3.0 to 1.2)	QPPMDCK <25poor >500great	QPlogKp (-8.0 to-1.0)	Human oral Absorption	Percent Human Oral Absorption >80% high <25%low
Nilotinib	5.847	-9.24	-8.232	591.087	-1.062	1219.978	-1.684	3	84.87
NDB5	-1.606	0.972	-7.043	1.102	-1.912	0.651	-8.276	1	5.339
NDB	-0.474	-2.267	-3.995	49.449	-2.411	19.182	-4.841	2	41.535
NDB2	-2.813	2	-8.129	0.58	-1.977	0.212	-9.107	1	0
NDB6	-2.061	0.793	-7.185	0.558	-2.181	0.315	-9.043	1	0

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