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Determination of potential phytochemical inhibitor for NDM-1 using different algorithms

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Abstract - New Delhi Metallo-beta-lactamase-1 (NDM-1)is an enzyme that makes bacteria resistant to a broad range ofbeta-lactam antibiotics. NDM-1 was first detected in a *Klebsiellapneumoniae* isolate from a Swedish patient of Indian origin in 2008. The resistance includes the antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacterial infections.The gene of NDM-1is one member of a large gene family that encodes beta-lactamase enzyme called carbapenemases. Carbapenemases are particularly dangerous resistance mechanisms, as they can inactivate a wide range of different antibiotics. The resistance conferred by the gene bla(NDM-1), aids the expansion of bacteria that carry it through a human host, since they will face less oppositionfrom populations of antibiotic-sensitive bacteria, which is diminished by the original antibacterial treatment.So to hinder the action of NDM-1,potential phytochemical compounds were identified using different docking algorithms. 300 antibacterial phytochemical compounds taken from Dr. Duke's Phytochemical and Ethanobotanical databasewere docked with the receptor of NDM-1(PDB_ID:4EXS) using iGEMDOCK and Accelrysligandfit.Thusthe top scoring compounds with the energy value greater than theknown inhibitor Captopril were shortlisted as probable inhibitors of NDM-1.The results obtained from both docking software's with similar top hits were checked for their molecular property and toxicity using molinspiration and lazar (LAZY STRUCTURE-ACTIVITY RELATIONSHIPS). This suggests that the top hits can block the action of NDM-1 and prevent it from flourishing in any organism if found. From the acquired results it can be inferred that these compounds can cease the lethal action of NDM-1 enzyme and may be impede the formation of a superbug.

Keywords: NDM-1, Carbapenemases, Phytochemical compounds, docking, molecular properties.

INTRODUCTION:

Antibiotics are types of antimicrobials used in treatment and prevention of bacterial infections. They may either kill or inhibit the growth of bacteria. Antibiotics revolutionized medicine in 20th century and have together with vaccination led to eradication of several diseases from the face of the earth. The effectiveness and easy access led to overuse, especially in livestock rising, prompting bacteria to develop resistance. This has led to widespread problems so much as to prompt the WHO to classify antibacterial resistance as serious threat.[1]
Antimicrobial resistance is the result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections. Several molecular mechanisms of antibacterial resistance exist. [2] Intrinsic antibacterial resistance may be part of the genetic makeup of bacterial strains. For example, an antibiotic target may be absent from the bacterial genome. Acquired resistance results from a mutation in the bacterial chromosome or the acquisition of extra-chromosomal DNA. [3]Antibacterial-resistant strains and species, sometimes referred to as "superbugs", now contribute to the emergence of diseases that were for a while well-controlled. For example, emergent bacterial strains causing tuberculosis (TB) those are resistant to previously effective antibacterial treatments pose many therapeutic challenges. Every year, nearly half a million new cases of multidrug-resistant tuberculosis (MDR-TB) are estimated to occur worldwide[4]. NDM-1 is a newly identified enzyme conveying bacterial resistance to a broad range of beta-lactam antibacterial.

The recent paper by RamananLaxminarayanet .al shows the importance on the *emergence of a new antibiotic resistance mechanism, New Delhi metallo-beta-lactmase1 (NDM1) in India, Pakistan andthe UK*may have sounded yet another wake-up call to counter the global menace of antibiotic resistance [5]. Multi drug-resistant pathogens exist in India as they do *in differentforms* globally including the western world with a death toll of over 2500 in the USA alone (more than the deaths due to AIDS) and some 2500 deaths in Europe every year.

New Delhi Metallo-beta-lactamase-1 (NDM-1) is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics. NDM-1 was first detected in a *Klebsiellapneumoniae* isolate from a Swedish patient of Indian origin in 2009 and hence the name New Delhi Metallo-beta-lactamase-1 [6]. The resistance includes the antibiotics of the carbapenem family, which are a mainstay for the treatment of antibiotic-resistant bacterial infections. The gene of NDM-1 is one member of a large gene family that encodes beta-lactamase enzyme called carbapenemases. Carbapenemases are particularly dangerous resistance mechanisms, as they can inactivate a wide range of different antibiotics. The resistance conferred by the gene bla(NDM-1), aids the expansion of bacteria that carry it through a human host, since they will face less oppositionfrom populations of antibiotic-sensitive bacteria, which is diminished by the original antibacterial treatment.

It is standard practice to test bacteria for sensitivity to antibiotics. Strains that produce NDM-1 will show resistance to penicillins, cephalosporins, and carbapenems. Because carbapenem resistance is still relatively rare, resistance to these agents should raise suspicion of NDM-1, although not all of these resistant strains will be NDM-1 strains.[7] If the patient has recently been to an area where NDM-1 is common, like India or Pakistan, this increases the probability that the strain is producing NDM-1.

Much as our national sentiments are tickled by the NDM-1 report, the development is alarming. The tragedy is replicated frequently. It is high time that we put in place practices and institutions that regulate antibiotic therapy. Essentially, the practices should aim at diminishing environmental antibiotic levels and inhibiting the spread of resistance factors.[8] Various studies to suppress the reaction of NDM1 have been done. In these studies various inhibitors have been identified along with the now known inhibitor Captopril.Captopril is an angiotensin-converting enzyme (ACE) inhibitor used for the treatment of hypertension and some types of congestive heart failure. Biological and mass spectroscopy results revealed that D-captopril can effectively inhibit the enzymatic activity of NDM-1 by binding to its active site with high binding affinity. The unique features concerning the primary sequence and structural conformation of the active site distinguish NDM-1 from other reported metallo-β-lactamases (MBLs) and implicate its role in wide spectrum drug resistance.[9]Tightbinding of the ligand to the enzyme as well as the binding energy plays a crucial role and it affects the catalytic capability of the enzyme.[10] So, an inhibitor should be found that can bind more effectively to NDM-1 than any commonly available competitive inhibitor at lower binding energy, thus acting as a competitive inhibitor.

In this study, 300 curated phytochemical compounds with antimicrobial activity were considered for the molecular docking using iGEMDOCK and for ligand fit docking using Accelyrs. Thus the top scoring compounds with the energy value greater than the known inhibitor Captopril were shortlisted as probable inhibitors of NDM-1.The results obtained from both docking softwares with similar top hits were checked for their molecular property ad toxicity using molinspiration and Carcinogenic potency Databases (CPDB). This suggests that the top hits can block the action of NDM-1 and prevent it from flourishing in any organism if found. From the acquired results it was inferred that these compounds can cease the lethal action of NDM-1 enzyme and may be impede the formation of a superbug.

MATERIALS AND METHODS

SELECTION OF PHTYOCHEMICAL COMPOUNDS:

Phytochemical compounds areplant components with discrete bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to be potential inhibitors.[11] For this studycurated phytochemical compounds were taken from **Dr. Duke's Phytochemical and Ethanobotanical Database.**[12] Around 300 phytochemical compounds were selected for their antibacterial activity.These phytochemical compounds were then subjected to docking studies.

iGEMDOCK:

(http://gemdock.life.nctu.edu.tw/dock/igemdock.php)

Pharmacological interactions are useful for understanding ligand binding mechanisms of a therapeutic target. These interactions are often inferred from a set of active compounds that were acquired experimentally. [13] Moreover, most docking programs loosely coupled the stages (binding-site and ligand preparations, virtual screening, and post-screening analysis) of structure-based virtual screening (VS). An integrated VS environment, which provides the friendly interface to seamlessly combine these VS stages and to identify the pharmacological interactions directly from screening compounds, is valuable for drug discovery.

The first step of our study was to subject the 300 curated phytochemical compounds taken from Dr. Duke's phytochemical and ethnobotanical database to iGEMDOCK for the docking studies. The natural inhibitor Captopril was also docked along with these compounds to the protein. This step would act as our control.The result of this docking study

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was a set of 40 of the 300 phytochemical compounds showing higher binding energy than the natural inhibitor captopril.

ACCELRYS DISCOVERY STUDIO (LIGAND FIT):

(http://accelrys.com/products/collaborative-science/biovia-discovery-studio/visualization-download.php)

Discovery studio is a suite of software for simulating small molecules and macromolecule systems.It is developed and distributed by Accelrys.[14]The product suite has a strong academic collaboration programme,supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community.

The second step of our study was to again subject the 300 curated phytochemical compounds to Ligand fit docking done by accelrys.The result obtained were again a set of compounds with a good docking score.

MOLINSPIRATION:

(http://www.molinspiration.com)

Molinspiration offers broad range of cheminformatics software tools supporting molecule manipulation and processing, including SMILES and SDfile conversion, normalization of molecules, generation of tautomers, molecule fragmentation, calculation of various molecular properties needed in QSAR, molecular modelling and drug design, high quality molecule depiction, molecular database tools supporting substructure and similarity searches. Our products support also fragment-based virtual screening, bioactivity prediction and data visualization. Molinspiration tools are written in Java, therefore can be used practically on any computer platform.

To find the molecular property of the compounds found after the docking studies, the online tool MOLINSPIRATION was used .The compounds with best fits common in both the docking studies were taken and the molecular properties for these compounds were hence studied.

LAZAR-(LAZY STRUCTURE- ACTIVITY RELATIONSHIP):

(http://lazar.in-silico.ch/predict)

Lazar (lazy structure–activity relationships) is a modular framework for predictive toxicology. Similar to th*read across* procedure in toxicological risk assessment, lazar creates local QSAR (quantitative structure–activity relationship) models for each compound to be predicted. Model developers can choose between a large variety of algorithms for descriptor calculation and selection, chemical similarity indices, and model building. This paper presents a high level description of the lazar framework and discusses the performance of example classification and regression models[15].

The final step of our study was to check for the toxicity of the compounds found. Properties like carcinogenicity and mutagenicity were considered as vital. Compounds showing a range from 0-1 were inferred to show non carcinogenic and non-mutagenic property. Hence these compounds were finally considered to be potential phytochemical inhibitor for NDM-1.

RESULTS AND DISCUSSION

RETRIEVAL OF THE PROETIN AND 300 PHYTOCHEMICAL COMPOUND:

The first step of our study was the retrieval of the protein and the compounds from various online sources available. The protein used for the study was the NDM-1 strain with the PDBID:4EXS, Beta-lactamase NDM-1 is a hydrolase taken from the organism source *Klebsiella pneumoniae*. The compound was retrieved from PDB.

The 300 curatedphytochemical compounds were shortlisted on the basis of their antibacterial activity on Dr. Duke's Phytochemical and Ethanochemical database. These 300 compounds were then individually downloaded from the pubchem database for their submission in the further docking studies.

DOCKING USING iGEMDOCK:

The second step of our study was to subject the 300 curated phytochmical compounds extracted from Dr. Duke's phytochemical and Ethanobotanical Database for a docking simulation done by the software iGEMDOCK. The natural inhibitor of NDM1, Captopril was also docked along with the 300 antibacterial compounds and this would act as the control to our study.

As the docking was done few compounds showed the docking energy score greater than that of Captopril which indicates that these compounds have a better binding to the protein than its natural inhibitor. Hence these compounds were shortlisted.

	Compound	Energy	E-S HIS 122	E-S ZN 302	H-W HIS 120	H-S HIS 120	H-S HIS 122	H-W GLN 123	H-W ASP 124	H-S ASP 124	H-W LYS 125	H-W HIS 189	H-S HIS 189	H-W CYS 208	H-W LEU 209	H-S LYS 211	H-W SER 217	H-W LEU 218	H-W GLY 219
1	cav4EXS_X0Z-313-0.pdb	-103.3	0	0	0	0	0	0	0	0	0	0	-0.9	0	0	0	0	0	0
2	cav4EXS_X0Z-0004-0.pdb	-133	0	0	0	0	-11	-9.2	-9.3	-2.5	0	0	-6.9	0	0	-3.5	0	0	0
3	cav4EXS_X0Z-337-1.pdb	-92.9	0	0	0	0	-3.5	0	0	0	0	0	0	0	0	0	0	0	0
4	cav4EXS_X0Z-455-1.pdb	-131.7	0	0	0	0	0	-5.2	-3.4	-2.5	0	0	-4.9	0	0	-7	0	-2.5	0
5	cav4EXS_X0Z-0005-0.pdb	-120	0	0	-3.6	0	-1.7	-3.2	-3.5	-4.9	-2.5	0	0	0	0	0	0	0	0
6	cav4EXS_X0Z-948-1.pdb	-119.8	0	0	0	0	-3.5	-3.5	0	-0.8	0	0	-10.4	0	0	-4.8	0	-5	-3.3
7	cav4EXS_X0Z-534-1.pdb	-115.3	0	0	0	0	-3.5	-3.2	-3.2	-5	0	0	-1.8	0	0	-5.8	0	-2.5	0
8	cav4EXS_X0Z-774-0.pdb	-112.5	0	0	0	0	0	-3.5	0	0	0	0	0	0	0	-3.5	0	0	0
9	cav4EXS_X0Z-0461-0.pdb	-108.3	0	0	0	0	-3.5	-3	-3.3	-2.5	0	0	-8	0	0	-2.7	0	0	0
10	cav4EXS_X0Z-0647-0.pdb	-107.7	0	0	0	0	-1.1	-6.9	-3	-7.5	0	0	0	0	0	-3.5	0	0	0
11	cav4EXS_X0Z-036-1.pdb	-107.4	0	0	0	0	-9.7	-3.5	-3.5	-7.5	0	0	-0.8	0	0	0	0	0	0

Figure 1:A representation of iGEMDOCK result

The above **figures 1** are the outcome of the docking of 300 curated phytochemical compounds and the natural compound Captopril. In the above result it is evident that around 40 compounds showed the interaction with the protein better than the already existent ligand Captopril.

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DOCKING USING ACCELYRS:

The second step of our study was to subject the same 300 curated phytochemical compounds extracted from Dr. Duke's phytochemical and Ethanobotanical Database for a ligand fit docking study using the software Accelyrs discovery studio along with the natural inhibitor of NDM1, Captopril as this would act as our control. Ligand fit docking employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions [16]. A shape comparison filter is combined with a Monte Carlo conformational search for generating ligand poses consistent with the active site shape. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein-ligand interaction energies.

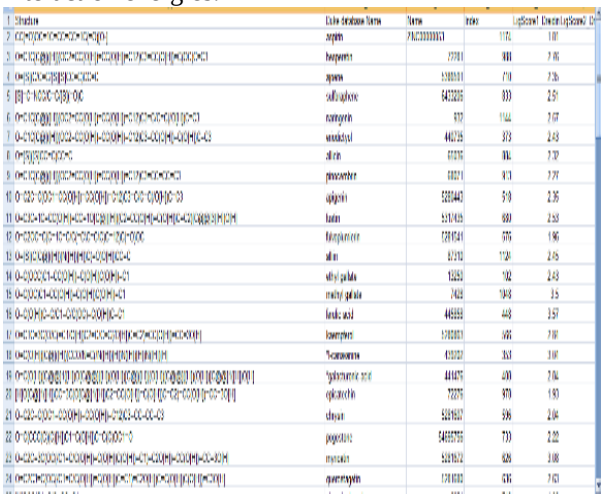

Figure 2. A representation of Accelrysdocking result.

The above **figure 2** are the compilation of the results of ligandfit docking studies done using Accelrys Discovery studio. Out of the 300 curated compound subjected for docking a total of 84 compounds were selected as they showed better binding energy than Captopril.These compounds were therefore taken for the comparison studies with the compounds found in iGEMDOCK to find the best hits.

The docking results hence obtained consisted of number of hits with variant docking energies. The compounds with the docking energy better than that of Captopril were shortlisted. A set of 84compounds with a better docking than Captopril were identified.

COMPARISON OF THE DOCKING STUDIES:

The next approach for this study was done by comparing the two docking results obtained when the 300 curated compounds were docked along with Captopril using different docking algorithm. The comparison was done on the basis of the top hits obtained in both docking programs. The 40 compounds obtained using iGEMDOCK and the 84 compounds obtained using Accelrys were compared for the top hits and the result was again narrowed down to 6 compounds showing best hits.

According to the results obtained a total number of 6 compounds showed top hits in both docking algorithms (iGEMDOCK, ACCELYRS).

Table 1: A tabular representation for results from iGEMDOCKandAccelyrs.

		Accelrys Discovery Studio		***iGEM***	
NUMBER	**COMPOUND**	**INTERACTIONS (Bond)**	**DOCKSCORE (in kcal/mol)**	**INTERACTIONS**	**DOCK ENERGY**
	Kievitone	LYS211 (1)	19.97	HIS122, GLN123, ASP124, HIS189, LYS211, ASN220, ZN302	-108.25
	Gossypetin	LYS211 (1)	20.836	GLN123, ASP124, LYS211, ASN220, ZN303	-107.71
	Taxifolin	LYS211 (1)	23.018	GLN123, ASP124, LYS211, ASN220, HIS250	-101.59
	Galacturonic	LYS211 (2)	30.161	GLN123, ASP124, LYS211, ASN220, HIS250, ZN303	-101.33
	Kaempferol	-	29.818	GLN123, ASP124, LYS211, ASN220, ZN303	-100.14
	Eriodictyol	LYS211 (1)	35.852	GLN123, ASP124, HIS189, LYS211, ASN220, HIS250	-96.18
	Captopril	-	3.967	HIS122, ASP124,	-90.9

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				HIS189, ZN303	

The above **Table1** shows the result of the compounds which showed top hits in both the docking studie.These compounds had dock energy greater than the natural ligand Captopril. The H bond interactions of these 6 compounds were also similar to the H bond interactions of Captopril. The tables a and b also give us an insight of the interaction sites of the 6 shortlisted compounds.

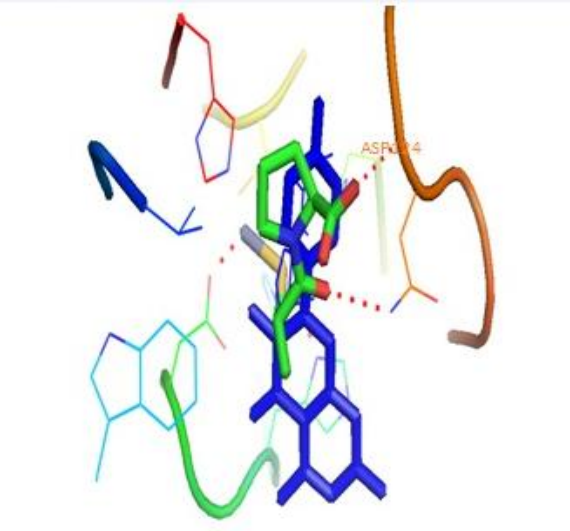

Figure 3: The docked poses of the ligands Captopril and Galacturonic acid with the protein 4EXS viewed in pymol

The above **figure 3** shows the docked poses of the known ligand Captopril and the phytochemical ligand Galacturonic acid to the protein 4EXS(Beta-lactamase-NDM-1). Captopril as well as Galacturonic showed common binding to the site ASP 124. Galacturonic acid also showed the interaction with the proteins in sites such as GLN123, LYS211, ASN220, HIS250.

Hence, these compounds were put forward for the molecular properties and toxicity prediction analysis to find the best phytochemical inhibitor for NDM-1.

MOLECULAR PROPERTIES AND TOXICITY PREDICTION.

The final step of our study was to predict the toxicity of the 6 compounds shortlisted by the docking algorithms. For this study the online tools molinspirationand lazar were used to predict the molecular property and toxicity of the compounds. The compounds

Kievitone, GalacturonicacidandEriodictyol were found to be most suitable inhibitors that can be used to prevent the proliferation of NDM-1 in microorganisms.

Table 2: A tabular representation for the results from molinspiration and lazar

Compound	Log p Value	Polar Surface Area	DSSTox Carcinogenic Potency DBS MultiCellCall	DSSTox Carcinogenic Potency DBS SingleCellCall	DSSTox Carcinogenic Potency DBS Mutagenicity	Kaziu s-Bursi Salmonella mutagenicity
Kievitone	0.76	110.05	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Gossypetin	1.44	151.58	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Taxifolin	0.71	127.44	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Galacturonic	-2.77	127.44	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Kaempferol	2.17	111.12	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Eriodictyol	1.63	107.22	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic
Captopril	-1.09	57.61	Non Carcinogenic	Non Carcinogenic	Non Mutagenic	Non Mutagenic

Table 2. The above results shows the toxicity of the 6 compounds that have been shortlisted from the two different docking studies. The properties LogP and Polar Surface Area are taken from the results of molinspiration whereas the properties DSSTox Carcinogenic Potency DBS Multicellcall,SinglecellCall, Mutagenicity and Salmonella mutagenicity were properties taken from the lazar(lazy structure activity relationship).The 6 phytochemical compounds along with Captopril were subjected to toxicity study and it was be inferred that three of the compounds

Gossypetin,Toxfolin and Kaempferol showed the confidence value which was higher for carcinogenicity and mutagenicity . These three compounds were therefore discarded. The remaining three compounds **Kievitone, Galacturonic acid** and **Eriodictyol**showed a confidence value higher for non-carcinogenicity and non-mutagenicity. Hence these compounds were considered safe to be used as potential inhibitors of the protein NDM-1.

CONCLUSION

Antimicrobial resistance is the result of microbes changing in ways that reduce or eliminate the effectiveness of drugs, chemicals, or other agents to cure or prevent infections.New Delhi Metallo-beta-lactamase-1 (NDM-1) is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics is one of the most deadliest threat that the has hindered the growth of potential antibiotics. Since such an immense effect of NDM-1, the identification of potential inhibitors is the need of the hour. Captopril being one of the known inhibitor of protein is prone to side effects as it is also an hypertensive drug. Hence a potential phytochemical inhibitor for the enzyme is found using various principles in this study. Phytochemical compounds are the natural product of plants, a total of 300 curated of these antibacterials where taken from Dr. Duke's Phytochemical and Ethanochemical database. These compounds were hence subjected to two different docking studies,iGEMDOCK and ACCLEYRS Discovery Studio. Both of these docking algorithms have a different approach to find the top hits.The purpose of repeating docking with two different algorithm was to find the robustness to the hits shortlisted. As a resultfew of the compounds out of the 300 phytochemicals showed better docking results than the already know inhibitor Captopril. Out of these compounds the ones with the top hits were selected which narrowed down the results to just 6 compounds namely,Keivitone,Gossypetin,Taxifolin,Galaturinicacid,Kaempferol and Eriodictyol.These 6 compounds were then again tested for their molecular properties and toxicity levels using the online sources, molinspiration and lazar. Out of the six compounds three compounds showed higher confidence value for non-carcinogenicity,non- mutagenicity and better log p,TSPA values than the rest. These compounds were **Kievitone, Galacturonic acid** and **Eriodictyol**. Hence considering the properties binding energy,interaction sites, molecular properties and toxicity we can infer that the compounds **Kievitone, Galacturonic acid** and **Eriodictyol**can be considered as potential inhibitors of the superbug NDM-1.

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