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Exploring the Molecular and Structural Mechanism for Drug Induced Nephrotoxicity: *A Virtual Based Approach*

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ABSTRACT

Drug-induced nephrotoxicity is increasingly recognized as a significant contributor to acute kidney injury and chronic kidney disease. Acute kidney injury is a very common diagnosis, present in up to 60% of critical patients, and its third main cause is drug toxicity. Systematic and quantitative studies of nephrotoxicity have become increasingly important due to rising concerns of drug induced nephrotoxicity. Drugs frequently interact with more than one target, with hundreds of these targets linked to the side effects of clinically used therapeutics. This is based on the hypothesize that drugs with same side effects are likely to have similar targets (Zhang *et al*., 2017). Developing a computational model to predict drug induced nephrotoxicity will provide a screening tool for nephrotoxicity thereby minimizing the number of nephrotoxic drugs released to the market. The study was aimed at exploring the various molecular and structural mechanisms for drug induced nephrotoxicity using computer simulation techniques; pharmacophore studies, PASSONLINE target identification and molecular docking simulation techniques. Hydrogen bond donor and hydrogen bond acceptor were the features common to nephrotoxic drugs, kidney injury molecule 1, neutrophil gelatinase associated lipocalin and type IV collagen were the common nephrotoxic targets. The nephrotoxic drugs demonstrated excellent binding affinities against the common targets and superimpose with each other and the co-crystalized ligand in the active pocket of each of the targets. These findings imply that nephrotoxic drugs potentiate the effects of these targets and might be molecular mechanism responsible for the nephrotoxicity associated with drugs.

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INTRODUCTION

Drug-induced nephrotoxicity is increasingly recognized as a significant contributor to acute kidney injury and chronic kidney disease.[1] The kidney is the main organ required by the human body to achieve and perform different important functions including detoxification, regulation of extracellular fluids, homeostasis, and excretion of toxic metabolites.[2]

Nephrotoxicity can be defined as any renal injury caused directly or indirectly by medications, with acute renal failure, tubulopathies, and glomerulopathies as the common clinical presentations.[3] Some examples of drugs commonly associated with the acute reduction of glomerular filtration rate are anti-inflammatories, antibiotics, such as vancomycin and aminoglycosides, and chemotherapeutic agents, such as cisplatin and methotrexate. Cases of tubulopathy are very common with amphotericin B, polymyxins, and tenofovir, and cases of glomerulopathies are common with bisphosphonates, and immunotherapy.[4]

Acute kidney injury (AKI) is a global health challenge of vast proportions, as approximately 13.3 million people worldwide are affected annually.[5] Acute kidney injury is a very common diagnosis, present in up to 60% of critical patients, and its third main cause of drug toxicity. It has a high mortality rate of 1.7 million deaths per year.[6] Prospective cohort studies of AKI have documented the frequency of drug-induced nephrotoxicity to be approximately 14-26% in adult populations.[1] Nephrotoxicity is a significant concern in pediatrics with 16% of hospitalized AKI events being attributable primarily to a drug.[7]

Systematic and quantitative studies of adverse side effects have become increasingly important due to rising concerns of drug-induced toxicity and post-marketing withdrawal.[8] Concerns over drug induced nephrotoxicity have risen significantly from 10% to 20%.[9] It is time for drug developers to design new and accurate models to assess the nephrotoxic effect before costly human clinical trials.

Drugs frequently interact with more than one target, with hundreds of these targets linked to the side effects of clinically used therapeutics. This is based on the hypothesize that drugs with same side effects are likely to have similar targets. Developing a computational model to predict drug induced nephrotoxicity will provide a screening tool for nephrotoxicity thereby minimizing the number of nephrotoxic drugs released to the market. This will help researcher to screen out compounds more likely to be nephrotoxic in the early stage of drug discovery. It will also provide information on the likely structural substitution or modification to decrease the affinity of the compound for the target, hence increasing its selectively. This study was therefore aimed at exploring the various molecular and structural mechanism for drug induced nephrotoxicity using computer simulation techniques

METHODOLOGY

Ligand-Based Pharmacophore Screening

Ligand-based pharmacophore was generated using the selected hepatotoxic drugs as template.

Importing ligands

Ligand-based pharmacophore modeling requires a set of two or more input ligands to generate characteristic pharmacophores. The input ligands could be training-set or test-set. The training- set molecules were used for the actual pharmacophore creation while the test-set ligands were used to verify the resulting pharmacophores.[10] The input ligands were imported by selecting the *Add Molecules* submenu in the *Ligand-Set* menu or via file menu. Another alternative was to add molecules by means of the Copy-board Widget to the ligand-Based Modeling Perspective. The ligand-based pharmacophores were generated from a set of ligands with no consideration to the structure of the macromolecule.[10]

Generating conformations for ligands

Ligands conformations is generated by selecting the "Generate Conformations for Ligand- Set" icon on the ligand set menu. A dialog box appeared and the ligand of interest was selected.[10]

Ligand-based pharmacophore creation in ligand scout

When the ligand-set is ready to use for the pharmacophore generation, the "Run Ligand-Based Pharmacophore Creation" icon was selected. A settings dialog appears where you can adjust the properties for the process. First, conformations of the Training-Set molecules were generated. After ranking the molecules according to their number of conformations (flexibility), pharmacophore features (lipophilic points, hydrogen bond donors and acceptors, positive and negative ionizable groups) were projected on these molecules and all their conformations. All conformations of the two-top ranked (i.e. the least flexible) molecules were then aligned using Ligand's molecular alignment algorithm.[10]

Generating shared feature pharmacophore

Two or more ligands were chosen from the "Alignment List" and the icon "generate merged feature pharmacophore" on the ligand menu was selected. Ligand Scout then calculate the several alignments. Ligand Scout chooses the best alignment and use it for merging the pharmacophores of the selected ligands. Finally, features which overlap too much was combined into a single feature. The aligned and merged pharmacophore appeared in the "Alignment List" and the "2D/3D Viewer".[11]

Results of ligand-based pharmacophore models

After the ligand-based pharmacophore generation is finished, the results were listed in the "Results Table". To see how well the resulting pharmacophore fits to the ligands in the 3D View, the "hierarchy View" make all ligands visible by selecting the Toggle Visibility icon for each ligand.[11]

Using PASS Online tool to predict targets for the Five selected Nephrotoxic drugs

PASS (Prediction of Activity Spectra for Substances) online software was used to select protein targets. Briefly, 3D structure of the selected drugs were uploaded into the query space of the software and a 'run' tab was clicked. An automated algorithm was generated by the software which was used to predict several biological targets. The targets common for the five drugs were then selected for molecular docking simulation.[12]

Development of Local Database of Protein Targets from Protein Data Bank

Protein Data Bank (PDB) is an archive of 3D structures of about 35,000-50,000 biological molecules (in PDB text format) protein targets were selected and downloaded from the PDB website (www.rcsb.org) and saved in PDB text format. A local database was created for the targets in my personal computer.[13] Briefly, The PDB ID of the macromolecule was typed in the search space of Protein Data Bank. The 3D structure of the macromolecule appeared in the left upper hand corner of the data bank. Then the "Download files" tool was selected displaying different downloading options and formats, and Pdb-gz format was selected since PyRx recognizes this format. This displayed the "opening pdb-gz box" with the option "to save". The macromolecules were saved in a computer local Database.

Development of Local Database for the Five Selected Nephrotoxic drugs from DrugBank

A Drug Bank is a drug Database that contains more than 4,000 compounds linked to about 14,000 molecular targets.

The drugs were downloaded from Drug Bank website (www.drugbank.ca) and saved in structural data format (SDF).[14]

Virtual Screening of Five (5) Nephrotoxic drugs against Selected Targets Using Pyrx Virtual Screening Tool

Importation of Macromolecules from the Local Database

To import macromolecule from local Database, File > Import molecule was selected, this displayed "import molecule wizard" carrying different options. Workspace Tarball> local File was then selected and "Next" button clicked followed by Finish button. Shortly an "Import Completed Successfully" dialog appeared; then OK button was clicked. The 3D structure of the macromolecule was displayed in the workspace and the protein ID appeared in the "molecule tab" of the navigator panel. Atoms of the macromolecule were viewed in the workspace by deselecting and selecting them in the "molecule tab" of the navigator panel. The macromolecule was inspected in the workspace by right clicking and holding the mouse. The binding site of the co-crystallized area examined, in shape, size, polarity and accessibility. The molecule can be toggle across different axis using the "view axis" in the "view panel" of the 3D scene and the full screen displayed using "display full screen" in the "view panel" of the 3D scene.[15]

Importation of Ligands from the Local Database

To import ligands from the local Database, 'open babel' button was selected in the control panel of the PyRx tool. The 'insert new item' tabon the upper left-hand corner of the open babel panel was selected and a "choose open babel supported file" box appeared. The ligand of interest was then selected and imported into the PyRx. The selected ligands appeared in the 'open babel results table' displaying the drugs ID, formula, weight and Log P. Minimized atomic coordinates of the ligand was created using "the minimize all" widget. The minimized coordinate of the ligands right clicked and different options displayed. The option "Covert all to autodock ligand PDBQT" was selected. The PDPQT format of the ligand appeared in the ligand compartment of the autodock navigator area. **[15]**

Preparation of Ligands and Macromolecules

The molecule ID in the molecule tab of the navigator area was selected and right clicked, then "autodock" > "make macromolecule" selected. Shortly the macromolecule appeared in the macromolecule compartment of the autodock navigator panel. Here the molecule appeared in the PBDQT format, a format recognized by the PyRx virtual screening tool. Again, the molecule ID in the molecule tab of the navigator area was selected and right clicked, then "autodock" > "make ligand" selected. Shortly the ligand appeared in the ligand compartment of the autodock navigator panel.[15]

Running the Molecular Docking Simulation

Running an autodock/vina wizard includes "Start here", "Select molecule", Run vina/autodock, and "Analyze results". The "Start here" button was clicked to activate the autodock vina mode. The ligands of interest were selected from the autodock widget and "select ligand" button pressed, followed by the forward button. The ligands were automatically imputed into the ligand list in the control panel of PyRx software. Again, the macromolecules were selected from the autodock widget and "select macromolecule" button pressed followed by the 'forward' button and this automatically input macromolecules into the macromolecule list in the control panel.

To run vina, the "run vina" was clicked, and then forward button pressed. Finally, "analyze result" was selected then 'forward' button. This displayed the binding affinities of the various poses against the ligands. The lower the binding affinities the better the protein-ligand interaction, since molecules interact to conserve energy.[15]

Analysis of Results

The Analyze results page is where the final docking results were presented. The table was sorted according to the values of the binding energies. The table row was selected one by one to see the corresponding docking pose for each ligand-protein complex in the 3D scene. The numerical results were exported as a Comma-Separated Values (CSV) file compatible with excel.[15]

RESULTS

Pharmacophores for the Five (5) selected Nephrotoxic drugs

The generated pharmacophores for amphotericin B, vancomycin, gentamicin and captopril had multiple hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) (Figures 1-5).

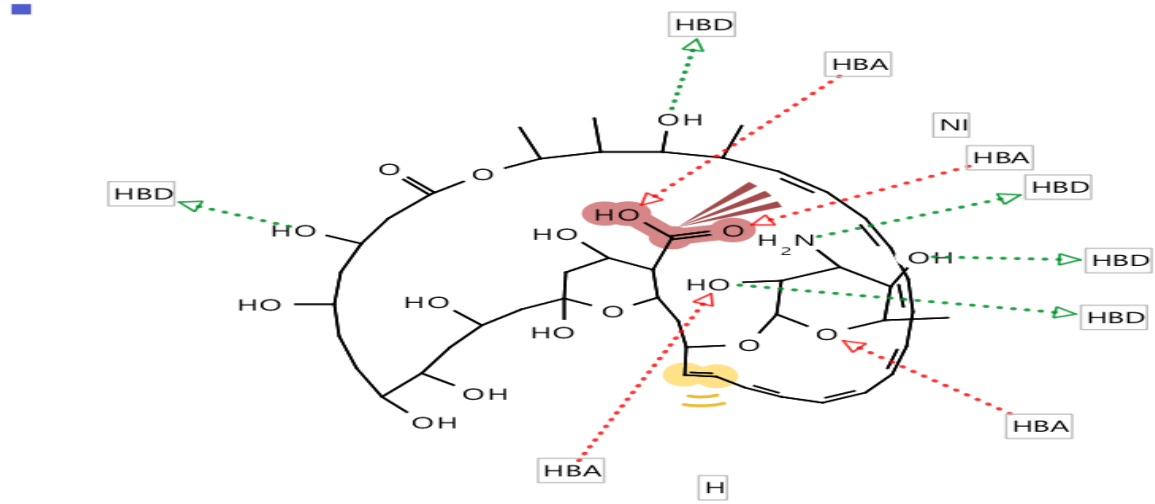

Figure 1: Pharmacophore of Amphotericin B

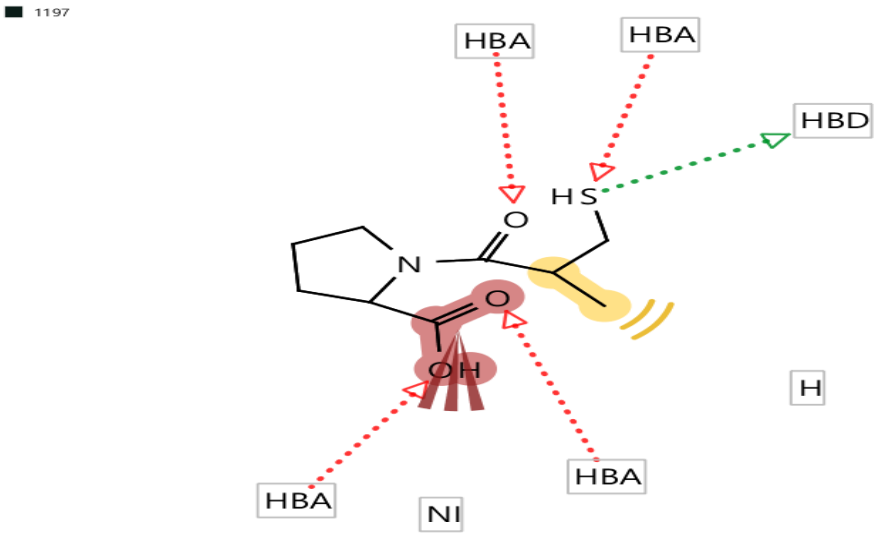

Figure 2: Pharmacophore of Captopril

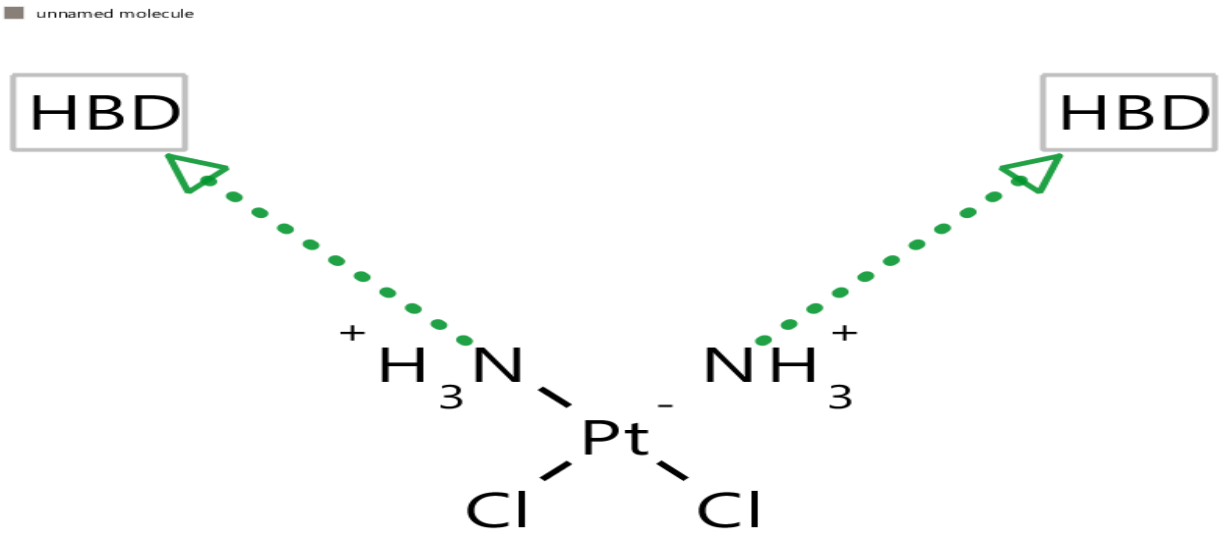

Figure 3: Pharmacophore of Cisplatin

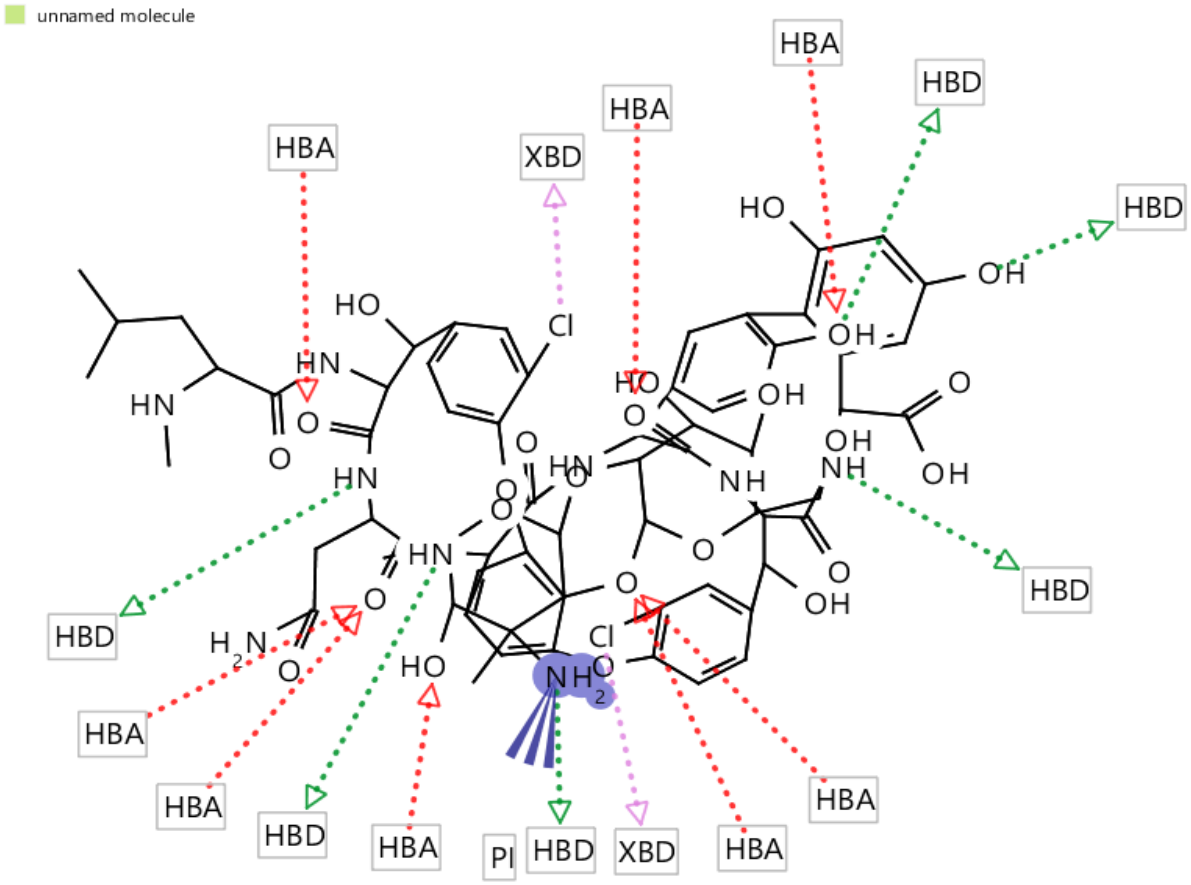

Figure 4: pharmacophore of Vancomycin

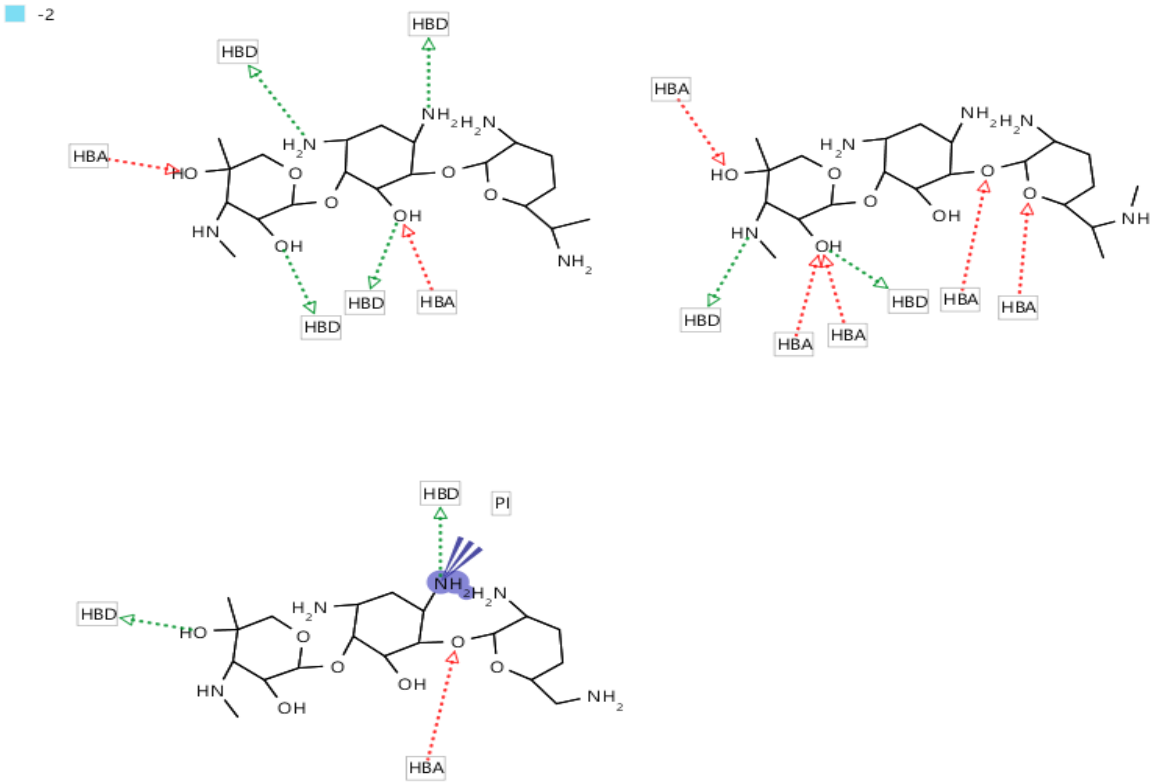

Figure 5: Pharmacophore of Gentamicin

Protein Targets common to the Five (5) selected Nephrotoxic drugs

The targets common to the drugs were; kidney injury molecule 1 (KIM-1), neutrophil gelatinase associated lipocalin (NGAL) and type IV collagen. Table 1

Table 1: Three (3) Selected Protein Targets common to Nephrotoxic drugs

S/N	PDB ID	TARGETS
1	5F7H	Kidney injury molecule 1
2	INGL	Neutrophil gelatinase associated lipocalin
3	IM3D	Type IV Collagen

Binding affinity of the Nephrotoxic drugs and the Co-crystalized ligand against the three selected targets

Amphotericin B and gentamicin had the best binding energy (-9.7 each) against KIM-1, amphotericin B and captopril, the best binding energy (-5.8 each) for neutrophil gelatinase associated lipocalin while amphotericin B and vancomycin the best affinity (-5.8 each) for type IV collagen. Table 2

Table 2: Binding affinity of the five (5) nephrotoxic drugs and the Co-crystalized ligand against the three selected targets

		TARGETS		
S/N	Drugs	5F7H	INGL	IM3D
1	Co-crystalized ligand	-10.8	-5.9	-7.0
2	Amphotericin B	-9.7	-5.8	-5.8
3	Captopril	-9.5	-5.8	-4.9
4	Cisplatin	-9.2	-5.7	-4.0
5	Gentamicin	-9.7	-5.2	-6.0
6	Vancomycin	-8.0	-5.0	-5.8

The Five (5) Nephrotoxic drugs superimposing to the active site of the protein targets

The five selected nephrotoxic drugs superimpose with each other and the co-crystalized ligand in the active pocket of each of the nephrotoxic target. Figures 6-8

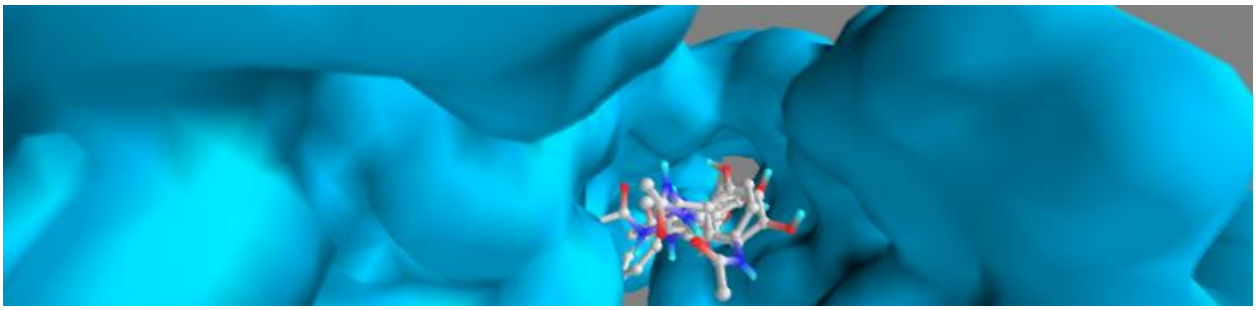

Figure 6: The Five Nephrotoxic drugs superimposing and binding to the active site of Kidney injury molecule

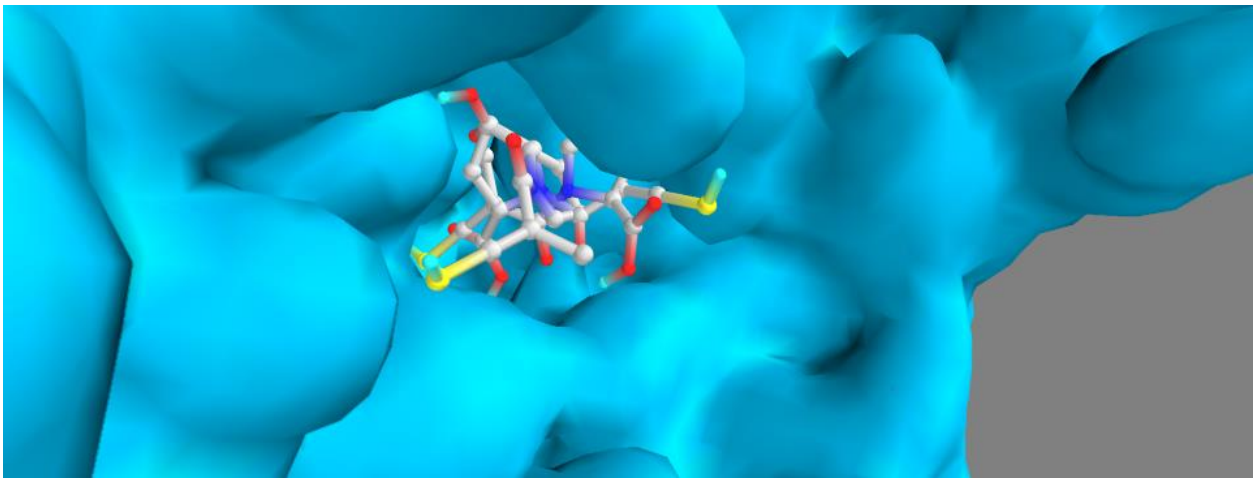

Figure 7: The Five Nephrotoxic drugs superimposing and binding to the active site of Neutrophil gelatinase associated lipocalin

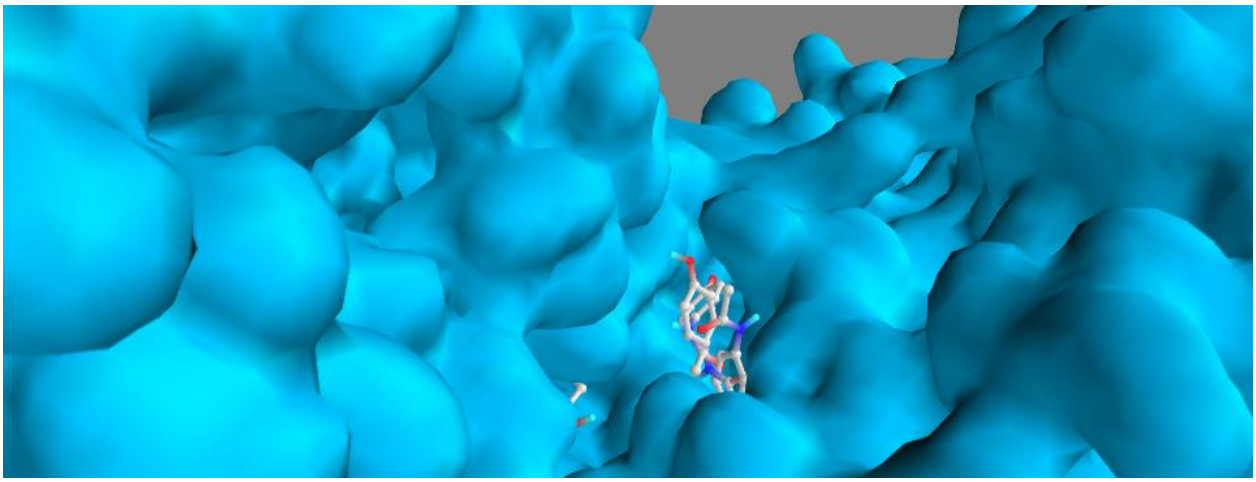

Figure 8: The Five Nephrotoxic drugs superimposing and binding to the active site of Type IV Collagen

DISCUSSION

Determination of potential drug toxicity and side effects in early stages of drug development is important in reducing the cost and time of drug discovery. Several drugs bind to "off-target" proteins, potentially leading to unwanted side or toxic effects.[16] More appropriate models must be developed to take advantage of complex molecular responses of drugs in cells, by exploiting fully the relationships between chemical compounds, protein targets, and side effects observed at the physiological level.[17] In this work, we explore a computational method for predicting potential nephrotoxicity of small molecules. The study was aimed at exploring the various molecular and structural mechanisms for drug induced nephrotoxicity using computer simulation techniques; pharmacophore studies, PASSONLINE target identification and molecular docking simulation techniques.

Ligand Scout was used to generate the ligand-based pharmacophore for each of the five selected nephrotoxic drugs. Hydrogen bond donor and hydrogen bond acceptor were the features common to the five drugs, implying that hydrophilicity is implicated in nephrotoxicity. The hydrophilicity might prolong the retention of the drug nephrotic cells thereby increasing the chances of nephrotoxicity. Again, PASSONLINE software was used to predict various targets with affinity for each of the five nephrotoxic drugs. The nephrotoxic targets common to the drugs were; kidney injury molecule 1 (KIM-1), neutrophil gelatinase associated lipocalin (NGAL) and type IV collagen. KIM-1 is a transmembrane glycoprotein that is elevated following ischemic or toxic injury. Elevated urine KIM-1 levels are highly specific for kidney injury, because it is only expressed in injured kidney.[18] Some studies suggested KIM-1 as an indicator of acute kidney Injury transition to chronic kidney disease, because high levels of KIM-1 are maintained during the disease progression.[19-22] NGAL is a 25 kDa protein that binds to gelatinase in particular neutrophil granulocytes. It is synthesized in the maturation process of granulocytes and often induced in epithelial cells by inflammation or tumorigenesis [23]. Its expression is increased in proximal tubule cells by drug-induced nephrotoxicity or ischemia. NGAL was regarded as a sensitive biomarker for the early diagnosis of acute kidney injury [24]. Type IV collagen, a main component of the basement membrane, is a sensitive indicator for glomerular changes in the structure of the extracellular matrix and thus an important biomarker of nephrotoxicity.[23]

Furthermore, Pyrx virtual screening tool was used to predict binding affinities of the drugs against the selected targets. The five nephrotoxic drugs demonstrated excellent binding affinities (when compared to the co-crystalized ligands) against the three selected nephrotoxic targets. Furthermore, the five drugs superimpose with each other and the co-crystalized ligand in the active pocket of each of the nephrotoxic target. These findings imply that the five selected nephrotoxic drugs potentiate the effects of these targets and might be molecular mechanisms responsible for the nephrotoxicity associated with drugs.

CONCLUSION

It can be concluded that, Kidney injury molecule (KIM-1), neutrophil gelatinase associated lipocalin and type IV collagen might be molecular mechanism responsible for drug induced nephrotoxicity.

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