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Research Article

MOLECULAR MODELLING STUDIES OF BINDING OF DACA DERIVATIVES INTO G-QUADRUPLEX DNA: COMPARISON OFFORCE FIELD AND QUANTUM POLARIZED LIGAND DOCKING METHODS

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ABSTRACT

The DNA G-qadruplexes are one of the targets being actively explored for anti-cancer therapy by inhibiting them through small molecules. This computational study was conducted to predict the binding strengths and orientations of a set of novel dimethyl-amino-ethyl-acridine (DACA) analogues that are designed and synthesized in our laboratory, but did not diffract in Synchrotron light.Thecrystal structure of DNA G-Quadruplex$(TGGGGT)_4$(PDB: 1O0K) was used as target for their binding properties in our studies.We used both the force field (FF) and QM/MM derived atomic charge schemes simultaneously for comparing the predictions of drug binding modes and their energetics. This study evaluates the comparative performance of fixed point charge based Glide XP docking and the quantum polarized ligand docking schemes. These results will provide insights on the effects of including or ignoring the drug-receptor interfacial polarization events in molecular docking simulations, which in turn, will aid the rational selection of computational methods at different levels of theory in future drug design programs. Plenty of molecular modelling tools and methods currently exist for modelling drug-receptor or protein-protein, or DNA-protein interactionssat different levels of complexities.Yet, the capasity of such tools to describevarious physico-chemical propertiesmore accuratelyis the next step ahead in currentresearch.Especially, the usage of most accurate methods in quantum mechanics(QM) is severely restricted by theirtedious nature. Though the usage of massively parallel super computing environments resulted in a tremendous improvement in molecular mechanics (MM) calculations like molecular dynamics,they are still capable of dealing with only a couple of tens to hundreds of atoms for QM methods. One such efficient strategy that utilizes thepowers of both MM and QM are the QM/MM hybrid methods. Lately, attempts have been directed towards the goal of deploying several different QM methods for betterment of force field based simulations, but with practical restrictions in place. One of such methods utilizes the inclusion of charge polarization events at the drug-receptor interface, that is not explicitly present in the MM FF.

Keywords: G-Quadruplex DNA, Molecular Docking, Force Field, Quantum Mechanics, Quantum Mechanics/Molecular Mechanics Hybrid Simulations.

INTRODUCTION

The Guanine tetrads (G-tetrad or G-Quadruplex) are guanine rich telomeric DNA structures which adapt a locked tetrad conformation in non-dividing phase of the cell. But, these locked conformations are resolved by appropriate resolvases to form single strands of DNA during cell division, such that they could be further processed by the cell replication machinery. Cancers are characterized by uncontrolled cell replication which can be benign or malignant. Hence, the small molecules stabilizing G-Quadruplex DNAs in cancer cells are being developed as potential anti-cancer agents. Acridine-4-carboxamides (DACA) analogues are planar topoisomerase poisons that are well known to intercalate into the DNA structure. The structure of the first parallel DNA Quadruplex – Drug complex (PDB ID: 1O0K) was resolved by single molecule crystallography[3], which revealed the binding mode for three daunomycins. The drug daunomycin possess a planar chromophore similar to that of DACA analogues, and hence a highly similar DNA binding orientation is expected for the latter. The daunomycin layers were packed tightly at one terminus of the G-Quadruplex DNA, while the daunosamine sugar moieties were placed within the negatively charged groove regions. The sugar moieties were found to have hydrophilic interactions via several H-bonds with the backbone oxygen atoms in order to stabilze the complex, while the planar chromophore surfaces had high amount of hydrophobic contacts with terminal the G-quadruplex bases through π-π stacking stabilization. One significant difference between the daunomycin and the studied acridineanalogues is that the latter had more flexible and aliphatic side chains/linkers than the former. As the crystals of the acridine derivative-DNA complexes did not diffract in powerful synchrotron light source, we decided to study their binding computationally, in order to understand their molecular mechanisms and possibly improve their binding strengths in future.

The molecular mechanics force field based modeling techniques prove to be invaluable tools in biophysical studies and current drug discovery pipelines. But, their inherent inaccuracies due to high level of approximations lay limitations on their applications. For example, studying the receptor-drug binding interactions via techniques like docking, virtual screening, etc., is a highly applied area where the approximations originating from multiple sources limit the qualityof predicted results. Such limitations include suitability of a force field to the molecule(s) in question, inefficient sampling or presence of extremely vast and impractical conformational space, description of theatomic charge fields via point charges for different atom types, inaccuracies of scoring functions, inefficient treatment of receptor flexibility, inability to account for water mediated interactions, etc. The fixed FFparameters omit induced charge polarization or incorrectly describe it in a mean-field manner at the drug-receptor interface, which changes in reality,depending on the chemical nature of drug molecule and receptor atoms.These changes can alter the potential energy of receptor-ligand complexes dramaticallyas a consequence to the change in atomic chargesat interface by ignoring theinduced polarization effects. To address this bottleneck, several novel attempts have been aimed at using different quantum mechanics techniquesfor calculating the polarized ligand charges in receptor environment. Accounting for charge polarization of ligands in the receptor environment possibly provides a platform for improvement in finding the right drug candidates from a set of putative ligands more accurately through better description of force field energy parameters. But, the usage of these charges in combination with standard force field parameters for the given geometries are expected to result in enhanced quality of drug binding pose predictions and their energies.Applications of such QM/MM combination methods have been reported in the recent literature[1-2]that aim to capture the polarization effects only on theligand charges while the receptorcharges were kept unaltered. Such methods typically takes the resultsfrommolecular mechanics software as inputs for intoquantum mechanics packages andthe results beingre-used again in the molecular mechanics programs in a iterative manner. Such quantum polarization techniques might need intensive computational power, but it can pay off via theenhanced accuracies in predictions.

We addressed two main questions in this study, which are: i) What would be the possible optimal binding modes of the novel DACA analogues and a phenelene derivative *(Fig. 1)*, which were successfully co-crystallized with G-tetrads but failed to diffract in synchrotron light, ii) Does the inclusion of induced charge polarization scheme in receptor environment lead to betterment of the docking results obtained by using fixed force field charges? In order to accomplish this, we used a uniform grid box for blind docking of drug molecules that enclosed the whole DNA. The docking study was conducted at three levels: a) Glide XP[4] Force Field docking, b) Quantum Polarized Ligand Docking(QPLD) using Jaguar[5] with B3LYP[6-9] density functional method and LACVPbasis sets, c) QPLD docking with B3LYP density function method and LACVP* basis sets. Ultimately, the results from each of these methods were compared and contrasted for obtaining useful insights.

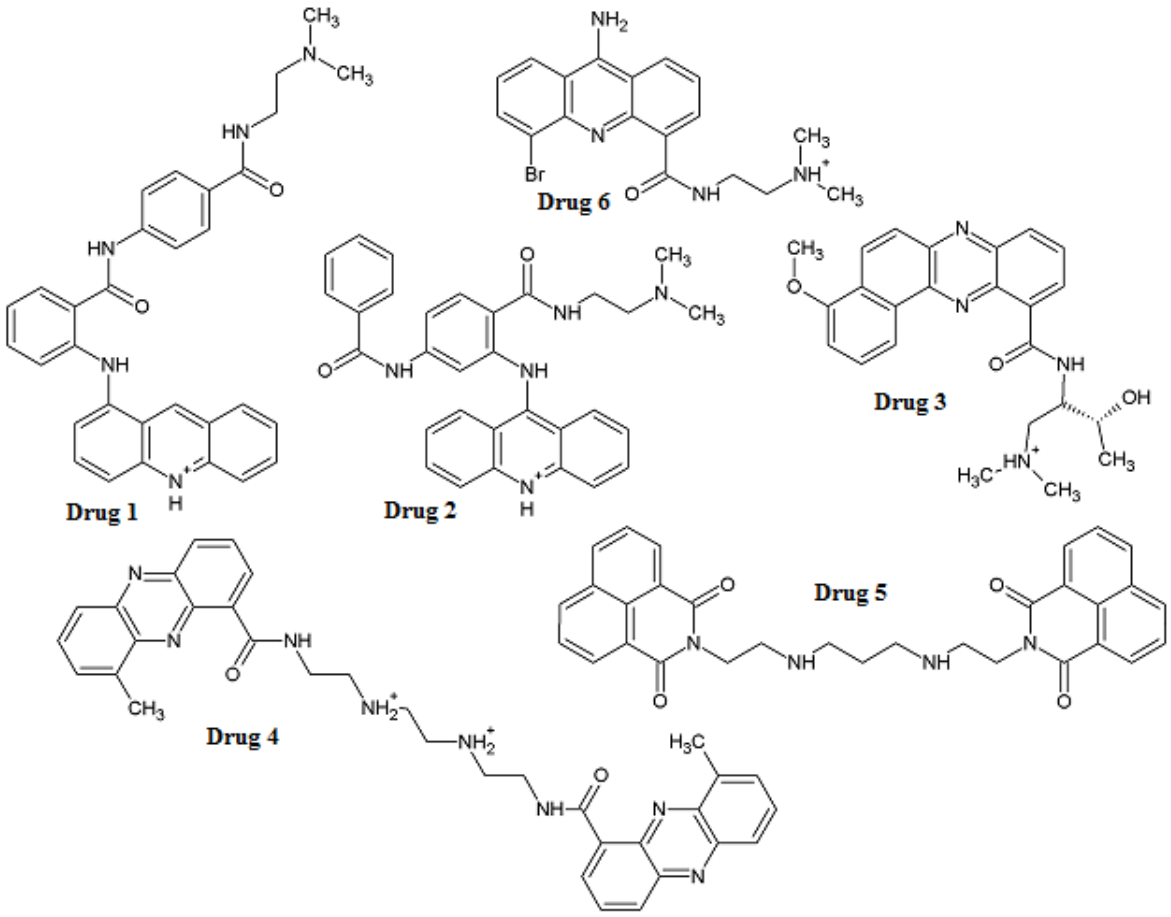

Fig. 1: The drug molecules used in this study. All of these molecules are DACA derivatives, except drug 5, which is a phenalene derivative that was used as a test compound for the purpose of comparison

METHODS

Preparation of Receptor and Ligand Molecules for Docking

The G-quadruplex DNA was extracted from PDB structure 1O0K and the ions and waters were removed manually. The ligand molecules were sketched in Accelrys DS viewer academic version and their structures were cleaned to remove any van der Waals clashes before being used in the docking program Glide.Several different conformations were generated for each ligandby varying the torsional degrees of freedom via Ligprep[11]algorithm in Schrodinger suite and upto 1000 energetically low conformations were stored, which were then converted into individual ligand conformational databases. Initially, each of these conformations in the databases was docked via Glide XP algorithm using fixed point charges and the results were pipelined to single point quantum mechanics optimizations in the Jaguar module. The quantum polarized ligand ligandchargeswere then obtained by fitting to the QM/MM calculation results via proprietary scripts embedded in the QPLD workflow of Schrodinger suite. Theresulting polarized ligand charges were stored and used for re-docking the same ligand molecules into the receptor by combining with the standard OPLS 2005[11-12] force field parameters for geometries of ligands and for the geometries and charges of the DNA receptor. The predictions were finally ranked based on least docking scores of drug bound DNA complexes and the results were compared.

The Docking Methodologies

The Glide module was used for force field docking via the XP algorithm. The Glide XP scoring function, which is a modified version of Chemscore wasused to assess the binding ofsmall molecules into DNA. The XP scoring functioncontains four main components that accounts for: i) Coulomb energy of the interacting atoms, ii) the van der Waal's energy of atoms, iii) A collection of terms that favor binding interactions, and iv) A collection of terms that hinder binding interactions. The supremacy of XP over SP methodology is attributed to the inclusion of more number of terms than the SP scoring function, which leads to more accurate description of ligand binding into the receptor.

$$\text{XP GlideScore} = E_{coul} + E_{vdW} + E_{bind} + E_{penalty}$$

Where; $\boldsymbol{E_{bind}} = E_{hyd\_enclosure} + E_{hb\_nn\_motif} + E_{hb\_cc\_motif} + E_{PI} + E_{hb\_pair} + E_{phobic\_pair}$.

$$\boldsymbol{E_{penalty}} = E_{desolv} + E_{ligand\_strain} \quad .$$

Meanwhile, the quantum polarized ligand docking was performed through the QPLD workflow deployed in the Schrodinger suite, using B3LYP DFT functional with two different basis sets.

Multi-Ligand Biomolecular Association (EMBRACE) Calculations

The EMBRACE minimization tool deployed in the Macromodel module is used to study the association of ligands with the receptor, using simple minimizations. The energy difference mode was used to calculate the binding strengths, whereby the differences in total energies between the drug bound DNA complexes and their uncomplexed DNA were studied. The calculation is performed first on the receptor, then on the ligand, and finally on the complex, as described below:

$$\Delta E = E_{\text{complex}} - E_{\text{ligand}}, - E_{\text{protein}}.$$

These calculations include the full effects of relaxation and solvation through an implicit solvation model.

RESULTS

The DNA G-tetrad surface is characterized by presence of negative charges in the central pore region formed between the guanine tetrads and in the groove regions due to backbone oxygens. Typically the acridines stack with the terminal nucleotides via π-π interactions, while the side chains penetrate into the shallow groove regions. Hence there clearly exist high possibilities for polarization of ligand molecules, especially in the functional groups lining chromophore and side chain regions. Although the common trends exhibited by the crystallographic ligand binding poses were present in the binding modes predicted by both techniques, we found that the top ranking poses of XP docking results did not remain the same in most cases when re-docked with induced polarized charges. This is because the polarized charges obtained using quantum mechanics optimization is aimed at capturing thestrengths of dipole – dipole, dipole – monopole and monopole – monopole interactions, which should remain standard in ff point charge scheme. Comparing the force field docking and QPLD dockingrevealed subtle differences in the electrostatic potential maps*(Fig. 3)*of ligands bound to DNA G-tetrad and several parameters used in Glide XP scoring function highlighted these differences.

FF Point Charges Vs. QPLD: Comparison of Predicted Binding Orientations

The molecular docking results revealed that agreement between fixed charges and polarized charges varied to different extents depending on the chemistry of drug molecules. Although there was some overlap in drug 3chromophores, theydid not align completely.Meanwhile, their side chains got placed in different grooves, thus revealinghuge differences in their binding predictions whencompared to the chromophore placement.Whereas the chromophoreof drug 3aligned nicely between the QPLD1 and QPLD2, highlighting a good consistency between these two methods. On the other hand, the binding orientations of drugs 2, 4 and 5 deviated heavily, which were further analyzed for comparing the efficiencies of different methods used in the study.The stacking interactions of DACA chromophorein ff docking seemed to be quite irrational for drugs 2and 4, wherein the flat and hydrophobic acridinechromophore was placed away from the tetrad surface, just above the groove regions in drug 2, whichled to reduction in the quantity of hydrophobic contacts between acridine and the terminal bases tremendously.Meanwhile, this binding orientation also led to a complete lack ofπ-π stacking interactions. The drug 4ff docking predictions revealed quite contradicting binding orientation as the less favorable aliphatic side chains/linkers were shown to be placed above the G-tetrad surface while the big and rigid chromophores were docked into the shallow grooves. The phenaline derivative drug 5binding orientation was also very poorly predicted as it mimicked the ffdocking characteristics of drug 4 by placing the rigid hydrophobic chromophores out of the G-tetrad surface, exhibitingonly partial stacking interactions.

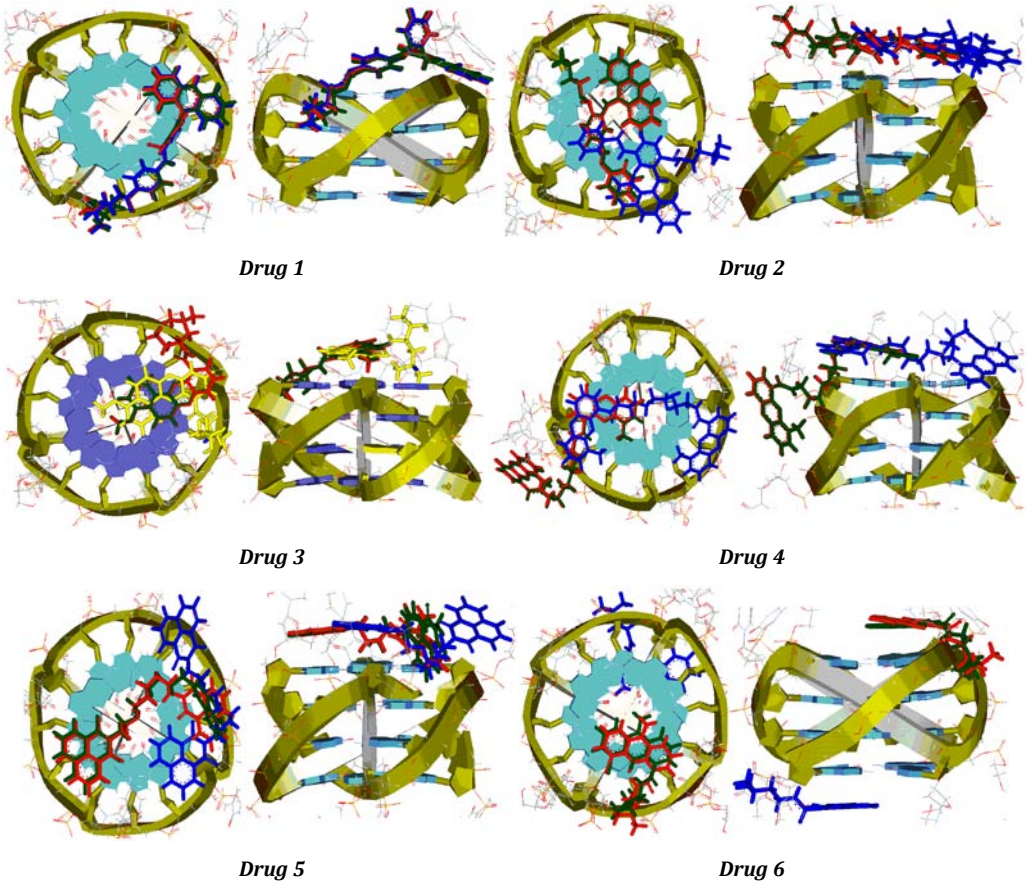

Fig. 2: The molecular docking poses of studied drug molecules. Color Code: Blue = XPDock,Red = QM/MM 3-21G/LACVP, Green = QM/MM 6-31G*/LACVP*

FF Point Charges Vs. QPLD: Comparison of Ligand Charges via Electrostatic Potential (ESP) Surfaces

The ligand charge distributions of ff and QPLD are depicted in fig. x. The drugs drug 1 and drug 6 revealed few differences in charge polarization (Fig. 3), but only within their chromophores. As the charge differences were low and there were no side chains attached to the chromophore, their binding orientations also remained highly similar between ff and QPLD predicted poses. Also, there were hardly any charge polarization events seen in the ESP surfaces ofdrug 2 and drug 5. Meanwhile, drug 3 and drug 4 highlighted some polarization in the chromophore, terminal functional groups and linker regions. The drug 4 possesses quite positively charged connecting linker due to the presence of several amino groups. Though the polarized charge surface was not strikingly different than the ff charges, the aliphatic linker was placed across the G-tetrad, which left one of the chromophores to have less interactions at the G-Quad terminus in XP docking. But, this binding mode changed tremendously in QPLD, where the linker was docked into one of the negatively charged shallow groove region. This mode of binding has been reported several times with other similar flexible drug molecules, which is in good agreement with the predicted results of QPLD. Overall, the positive charge distributions were seen to be affected by QPLD compared to the negative charges. This could be because of the prevalent negative charge surfaces present in the central pore and groove regions of the G-tetrad.

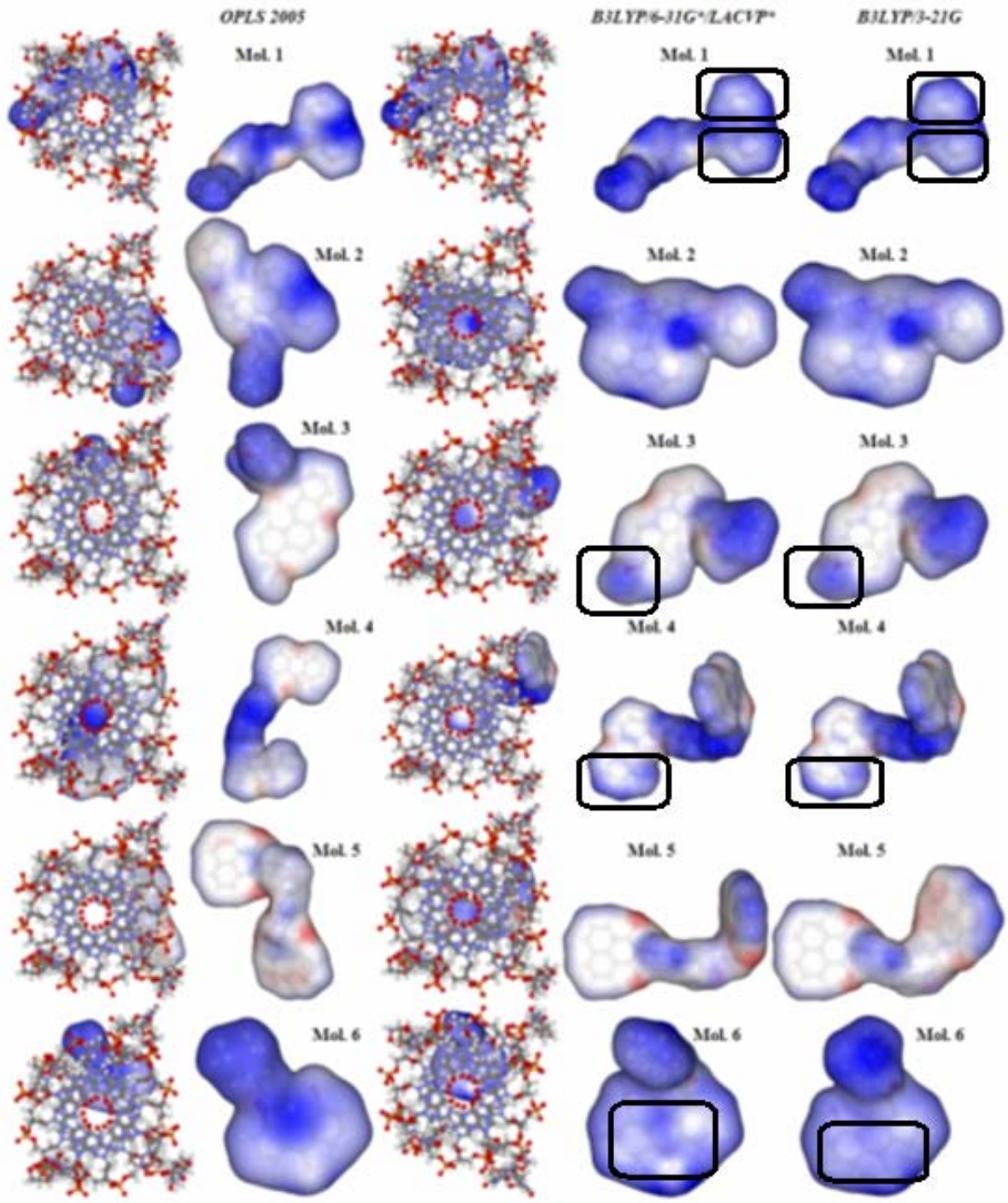

Fig. 3: The charge surface maps of studied drug molecules. Left = Surfaces mapped on the standard FF charges, Right = Surfaces mapped on the QPLD derived charges. The differences in charge maps shows the polarization effects at the drug – receptor interfaces

FF Point Charges Vs. QPLD: Comparison of Parameters in the Scoring Function

In order to analyze the impact of changes in the overall docking score through charge polarization by QPLD,we monitored five different parameters "Lipo" (lipophilic contact score), "RotB" (penalty for freezing rotatable bonds), "Coul" (Coulombic energy), "CvdW" (Coulombic + van der Waal's energy), and "Internal" (Internal energy). These parameters areincluded in the final scoring function of XP algorithm and hence are useful in discriminating the performance of the studied methods. Histograms comparing these values between the FF and QPLD based techniques are depicted in Fig. 4. Two of these terms,"Lipo" and "RotB" revealed relatively high variance for some drug molecules*(Table. 1)*, while the differences in other terms were either marginal or non-discriminatory in nature.However, there was a distinction observed by the QPLD charges, wherein the QPLD methods using LACVP and LACVP* basis sets were quite deviating from the FF docking terms, but theygenerally revealed a high level of agreement between the two QPLD methods. The drug 1 and drug 6 revealed a high "Lipo" value, which were predicted to be in positive range for the phenalinederivative drug 5. This is suggestive of loss of lipophilic interaction mediated stabilization. The drugs drug 2, drug 3, and drug 4 also revealed a similar trend, wherein the "lipo" scores were very poor and hence a poor binding profile is expected for these drug molecules. The 1st ranking binding mode orientations from the QPLD/LACVP* docking simulations were extracted and their ligand binding strengths were evaluated using the EMBRACE minimization method of Macromodel module (Table. 2). Thus, the binding profiles of the studied ligands were evaluated based on their difference in potential energies between the drug unbound DNA and drug-DNA complexes.The top 3 best binding acridine derivatives, viz., drug 1, drug 6 and drug 4were suggested for future development such that a high affinity drug-DNA complex could be formed, which possibly are amenable for X-ray diffraction in the future development programs.

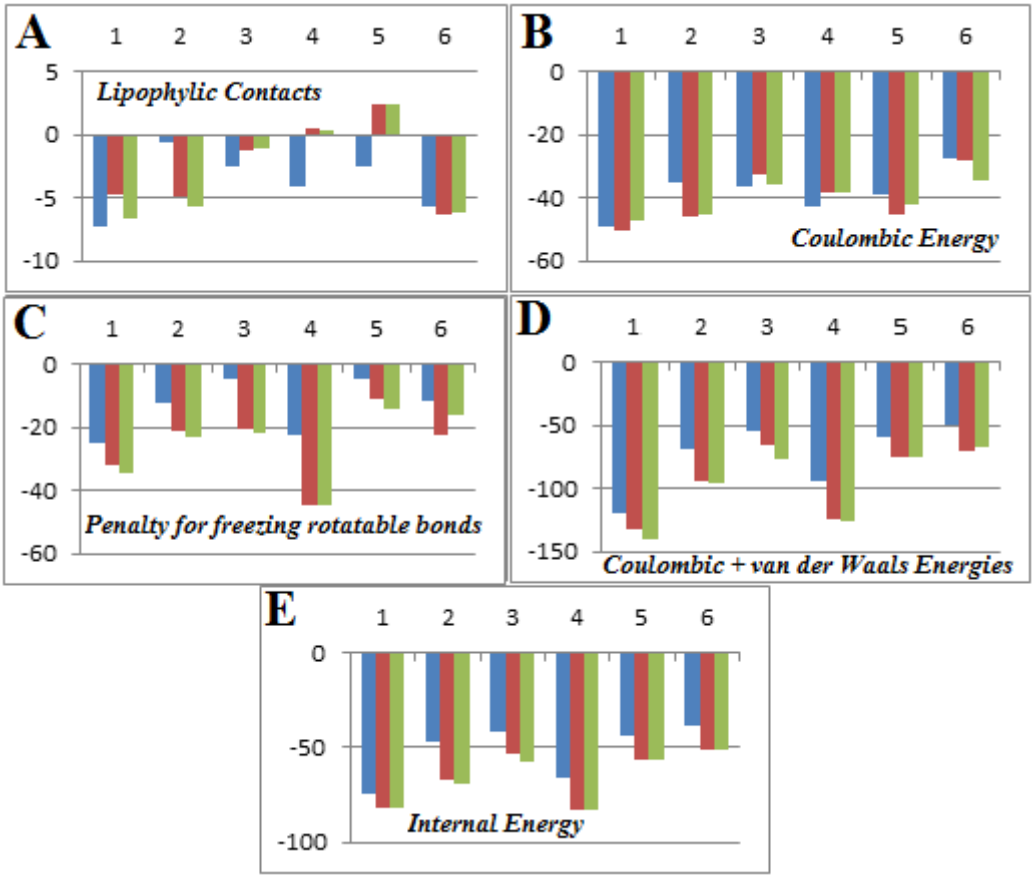

Fig. 4: The histogram plots of various parameters used in the scoring function of the Glide docking algorithm. Color Codes: Blue = XP Docking, Red = QPLD with LACVP basis sets, Green = QPLD with LACVP* basis sets

Table 2: Evaluation of the binding strengths of the studied ligands using the EMBRACE biomolecular ligand association tool

Rank	Ligand	Δ*E* Total. Kcal/mol	RMS Deviation	vdW*E*. Kcal/mol
1	Drug 1	-182.5	0.05	-149.24
2	Drug 6	-148.9	0.09	-137.07
3	Drug 5	-133.74	0.05	-244.57
4	Drug 4	-118.81	0.04	-189.23
5	Drug 2	-106.87	0.05	-161.6
6	Drug 3	-85.25	0.05	-162.11

Δ*E*represents the energy change upon association of the drug molecule into the DNA, which is a similar measure to that of the Gibbs free energy, but obtained with a rather simple minimization. The root mean squared (RMS) deviation describes the change in ligand structure before and after EMBRACE minimization, expressed in Å. The drug molecules are finally ranked based on their Δ*E* values, expressed in Kcal/mol

Table 1: The values for various parameters used in the scoring function of Glide XP docking algorithm. The different QPLD methods are described as: QPLD with LACVP basis sets = QM Med, QPLD with LACVP* basis sets = QM High

Ligand	Method	Lipo	Coul	RotB	CvdW	Intern
		Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol	Kcal/mol
Drug 1	XP	-7.27	-49.0	-25.0	-119.7	-74.0
	QM_Med	-4.81	-50.4	-31.9	-132.7	-82.3
	QM_High	-6.62	-47.3	-34.3	-139.9	-81.6
Drug 2	XP	-0.57	-35.1	-12.4	-69.7	-47.5
	QM_Med	-4.9	-45.8	-21.5	-94.1	-67.3
	QM_High	-5.63	-45.6	-23.2	-96.5	-68.9
Drug 3	XP	-2.5	-36.5	-5.0	-54.7	-41.4
	QM_Med	-1.28	-32.6	-20.4	-65.5	-53.0
	QM_High	-1.07	-35.9	-22.4	-77.0	-57.8
Drug 4	XP	-4.16	-43.1	-22.8	-94.2	-65.9
	QM_Med	0.53	-38.2	-44.8	-125.0	-83.0
	QM_High	0.37	-38.6	-44.4	-125.9	-83.0
Drug 5	XP	-2.54	-39	-4.8	-59.0	-43.9
	QM_Med	2.41	-45.3	-11.1	-75.2	-56.4
	QM_High	2.43	-42.0	-14.6	-74.6	-56.6
Drug 6	XP	-5.64	-27.4	-11.5	-49.9	-38.9
	QM_Med	-6.36	-28.4	-22.5	-69.9	-50.9
	QM_High	-6.13	-34.7	-16.5	-66.9	-51.3

CONCLUSIONS

Our results were able to shortlist three drug molecules for future optimization and also highlighted that the inclusion of receptor induced charge polarization showed a direct influence on the choice of top ranking binding poses predicted by the XP docking algorithms. As observed in drugs drug 1 and drug 6, the results were well consistent with the theory that there should be no differences in binding pose predictions between FF and QM/MM docking results when there were no polarization events taking place in receptor-drug interface, and vice versa. The QPLD workflow seems to have captured atleast the crucial polarization events in the drug charge surface through QM/MM SP optimization. As expected, there wassome polarization events observed in the drug molecules.Especially, drugs drug 1 and drug 6 were found to be the strongest binding drugs via their docking scores and their charge surfaces looked alike. A good agreement was seen between the predicted binding poses of QPLD1 and QPLD2 because their methodological changes were limited only to the choice of basis sets. Though there are no crystal structures available for comparing the QPLD1/QPLD2 predicted orientationsof studied drug molecules, theirpredicted binding modes agreed between each other. But, the correlation between the FF and QPLD results were present only for few drug molecules, while they differed in the others.

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