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"In Silico Development and Evaluation of Doxorubicin Hybrids as Potent Anticancer Agents"

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Abstract
Doxorubicin remains a cornerstone in cancer chemotherapy; however, its clinical utility is hindered by dose-limiting toxicities and drug resistance. In this study, novel hybrid derivatives of doxorubicin were computationally designed and evaluated for their anticancer potential using in silico methods. Ligands were constructed using ChemSketch and assessed for physicochemical properties (Molinspiration), pharmacokinetic profiles (SwissADME), and biological activities (PASS). Toxicity was predicted via GUSAR, while molecular docking was performed using AutoDock Vina with key cancer-related protein targets. Docking results indicated favorable binding affinities, particularly with 5zad and 4zqf, suggesting enhanced therapeutic interactions. Visualization of ligand-protein interactions was conducted using Biovia Discovery Studio. This study demonstrates the potential of doxorubicin hybrids as promising leads for further development in anticancer therapy. Further in vitro and in vivo validation is warranted.

Keywords: *Doxorubicin hybrids, In silico drug design, Molecular docking, ADMET prediction, Anticancer agents, PASS analysis*

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I. INTRODUCTION

Doxorubicin, a widely used anthracycline antibiotic, has long been a cornerstone in cancer treatment due to its effectiveness against a range of malignancies, including breast cancer, lung cancer, leukemias, lymphomas, and brain tumors. Its anticancer activity stems from several mechanisms, such as histone displacement, ceramide generation, DNA-adduct formation, the production of reactive oxygen species, and modulation of calcium and iron balance.

However, despite its robust antitumor effects, the clinical use of doxorubicin is significantly restricted by various challenges:

1. Drug Resistance: Cancer cells can acquire mechanisms to evade the cytotoxic effects of doxorubicin, reducing its efficacy over time.
2. Toxicity: The drug is associated with serious side effects, including cardiotoxicity, which can progress to heart failure, along with nephrotoxicity and suppression of bone marrow function.
3. Pharmacokinetic Constraints: Doxorubicin's high hydrophilicity, short half-life, and extensive distribution in the body limit its bioavailability, often necessitating high doses that exacerbate toxic side effects.

To address these limitations, researchers have explored hybridizing doxorubicin with other chemotherapeutic agents. This approach seeks to enhance the drug's anticancer activity while reducing toxicity. Emerging doxorubicin-based hybrid compounds have shown promise, demonstrating increased efficacy and the potential to bypass resistance mechanisms.

In conclusion, while doxorubicin remains a critical component in cancer therapy, its associated challenges underscore the need for innovative strategies. The development of hybrid compounds based on doxorubicin offers a compelling solution to enhance therapeutic outcomes while minimizing adverse effects in cancer patients.

Dimerized doxorubicin refers to a form of doxorubicin, an anthracycline chemotherapy drug, that has been chemically modified to form dimers—pairs of molecules linked together. Doxorubicin is widely used in the treatment of various cancers, including leukemia, lymphoma, breast cancer, and ovarian cancer. The idea behind dimerization is to enhance the drug’s effectiveness by improving its ability to target and kill cancer cells while potentially reducing some side effects.

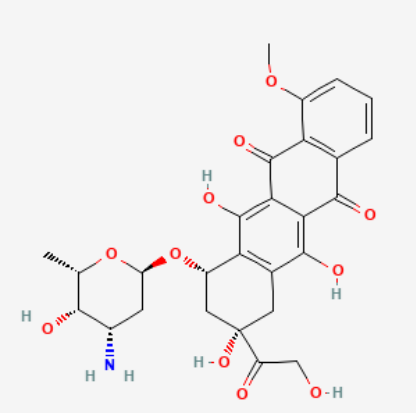

Fig 1: Structure of Doxorubicin

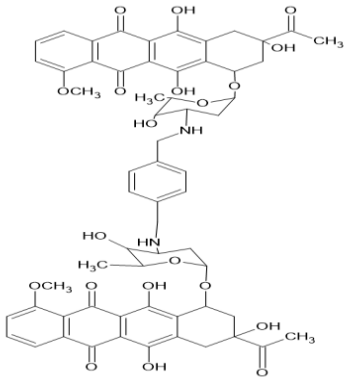

Fig 2: Structure of Di doxorubicin

II. MATERIALS AND METHODS:

Computational approaches were found to be the most reliable methods to start a research methodology. Hence, several computational tools were identified which rely on current research work for efficient strategies to develop novel compounds. The designed derivatives were studied using web tools to understand their physiochemical properties, biological activities and toxicological effects

Table I:

S.no	MATERIALS	METHODOLOGY
1.	Chemsketch	Generation of ligand structures
2.	Molinspiration	Molecular properties prediction
3.	Swiss ADME	Pharmacokinetic properties prediction
4.	PASS studies	Biological activity prediction
5.	GUSAR	Toxicological studies
6.	Autodock vina 1.5.7	Molecular docking
7.	Biovia Discovery studio 2021	Visualization of Interactions

III.METHODOLOGY

a. **Prediction of ADMET properties:**

Novel Drug discovery is found to be a complex and costly procedure, that comprises ailment collection, target identification, lead discovery and optimization, preclinical and clinical trials.Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) play crucial roles in novel drug development. A superior drug candidate must not only have satisfactory efficacy, but must also possess appropriate ADMET properties. In-silico methods are henceforth developed for estimation of ADMET properties. Though, it is still not laid-back to assess thedrug likeness of novel compounds in terms of severalADMET properties.

b. **Molinspiration:**

Molinspiration is a web tool that supports molecular management and processing, as well as SMILES and SD file translation, standardization of molecules, creation of tautomer, molecular fragmentation, calculation of numerous molecular properties desirable in QSAR studies, molecular modelling and novel drug design, higher quality molecule depiction, molecular database tools, auxiliary substructure and resemblance searches. The physicochemical properties predicted using this web tool are listed in the Table.

c. **SWISSADME:**

SwissADME allows us to calculate physicochemical properties and to forecast ADMET properties, pharmacokinetic parameters, drug likeliness and medicinal chemistry approachability of one or several small molecules to support novel drug discovery-72. Thus, obtained pharmacokinetic properties identification utilizing the swissADME tool are given in the Table.

d. **PASSSTUDIES:**

Prediction of activity spectra for novel substances (PASS) free web tool has thecapability to forecast 3678 pharmacological or biological effects; mechanisms and distinct toxicities of the novel molecule together with

teratogenicity, mutagenicity. carcinogenicity, and embryotoxicity. For each of the designed derivative PASS analysis was performed and obtained results are listed in Table.

e. **Molecular docking studies:**.

Computational methods are identical obligatory and beneficial resources in the course of drug development. With the initiation of computational tools, scheming, searching. assessment, modelling, binding energy calculation, pharmacokinetic properties and pharmacokinetic predictions, and the procedure of lead optimization turn out to be significantly easier Computational Molecular docking is a significant web tool in structural biology and computer aided drug design. The main goal of ligand target protein docking is to forecast the principal binding model (s) of a ligand molecule with a target protein of known three-dimensional molecular structure. Efficacious molecular docking methods are used to search high-dimensional spaces excellently besides utilizing a scoring function that will appropriately rank candidate dockings

i.Preparation of protein:

Based on the known molecular crystal structure of the protein molecules 6gqz, 5zad, 4zqf, 7kjs, 8a62, 4zvm, 6ftp inhibitors can be better validated by performing molecular docking Studies and molecular dynamics which exhibits significant features in ligand-receptor interactions. This crystalline structure was obtained from PDB database and all the water molecules, bounded ligands were removed by utilizing Auto dock vina software. Then the crystalline structures wase converted to. pdbqt format.

ii.Preparation of ligand:

The 2-dimensional structures (.mol) of the Hybrid Doxorubicin were drawn and analyzed using ChemDraw software. They were then converted to 3-dimensional form (.pdb) using Chem3D, desirable for studying ligand target interactions. For performing molecular docking studies, the .pdb format of ligand was converted to -pdbqt format.

iii.Performing docking:

Molecular docking was performed with AutoDock v4.5 (Morris et al. 2009) Preparation of ligand and the target protein was done by using Auto Dock vina tools. Molecular dockings studies were performed to find the active binding site and their interaction with ligand molecules. Auto Dock vina program was performed with6gqz (Petrobactin-binding engineered lipocalin), 5zad (Human topoisomerase II beta in complex with DNAs), 4zqf(Crystal structure of DOX-P Reductoisomerase fosmidomycin and magnesium), 7kjs (CDK2/cyclin E in complex), 8a26 (Lysophospholipase PlaA) and4zvm (Oxidized quinone reductase 2), 6ftp (Alpha1-antichymotrypsin, both ligand and macromolecule were selected and the rigid grid box was attained using Auto-grid. i.e. blind docking studies were performed as the active binding site for the newly synthesized compound was not known. The grid box dimensions were then documented in the config file as text document and saved in separate file for each target and each ligand. To predict the binding scores of these ligand target complex, command prompt was utilized. The desired syntax for the prediction and path for the results were given. Thus, obtained scores were obtained as the output file in the given file. Similarly, all the compounds scoring for different targets were obtained and tabulated in the table. The docked output files were used to study interactions. The pose with best binding affinity was visualized using biovia discovery studios

iv.**Visualization:**

Ligand target Molecular complex visualization is a significant aspect of the investigation and communication of modeling studies. It permits a mechanistic understanding of a molecular structure to be visualized. BIOVIA Discovery Studio Visualizer is a free web tool, feature-rich modeling application for observing, allocation and analyzing protein and other small molecular data. The output files obtained for every ligand against each target were utilized separately in Biovia discovery studio where the best scoring output amongst 7 conformers were visualized for their interactions of ligand molecule with amino acids of the target, visualizing the active site and 2D interactions to know which atom is bonding with which amino acid of target molecule. Thus, visualized complexes were saved as image files and represented in Table.

IV. RESULTS AND DISCUSSION

4.1. Computational studies:

Computer based drug discovery studies were effectively applied in the present study.
which recommends that these computerized methods will further highlight the role of computer-based drug discovery in the drug Research & Development plan.

4.2. Physicochemical Properties Results using Molinspiration:

Physicochemical properties were predicted using Molinspiration web tool which showed the following results listed in Table 4.01. molecular formula and molecular weight was obtained and listed. All the compounds showed 2 violations for Lipinski rule i.e., all the compounds have molecular weight more than 500 and number of hydrogen bond acceptors are also greater than 5. But most of the natural compounds deviate from Lipinski rule in molecular weight hence these compounds may be in the acceptance range. Log P Values are in the range of 5-8.

Table II: ADMET Properties of both compounds.

S.NO	FORMULA	MOL.WT	NHD	NHA	NRB	LOG P	VIOLATIONS
1	C9H7NO2S	193.22	0	3	2	2.12	1
2	C6H64N2O20	1157.17	10	22	14	3.39	12

Table III: Pharmacokinetics parameters using SwissADME.

FORMULA	logKp cm/s	Gi abs	BBB permeability	INHIBITORY interactions					
				P-gp substrate	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
C9H7NO2S	-5.9	High	Yes	No	Yes	No	No	No	No
C6H64N2O20	-9.46	Low	No	Yes	No	No	No	No	No

4.3. PASS Results:

The perception of the pharmacological activity range was acquainted to designate theproperties of biologically active moieties. The PASS (prediction of activity spectra forsubstances) free web tool, which helps in prediction of more than 300 pharmacologicalactivities and biochemical mechanisms on the roots of the structural/molecular formulaof the designed derivatives, which, was resourcefully used to find novel drug targets(mechanisms) for the newly designed derivatives. The pharmacological activitiespredicted by PASSsoftware for both compounds is listed inthe following tables respectively.

Table IV: PASS Biological data of Doxorubicin

Pa	pi	Activity
0,985	0,001	Cytostatic
0,979	0,001	DNA repair enzyme inhibitor
0,980	0,002	Caspase 3 stimulant
0,960	0,004	Antineoplastic
0,950	0,001	Alkylator
0,949	0,001	Gamma-glutamyl transferase inhibitor
0,950	0,003	Anti-infective
0,942	0,002	Anticarcinogenic
0,939	0,001	Leukopoiesis inhibitor
0,920	0,004	CYP2C9 substrate
0,907	0,000	RNA synthesis inhibitor
0,872	0,002	Aldose reductase substrate
0,873	0,008	CYP2H substrate
0,864	0,004	UDP-glucuronosyltransferase substrate
0,859	0,003	DNA synthesis inhibitor
0,842	0,003	Xenobiotic-transporting ATPase inhibitor
0,824	0,001	Antineoplastic antibiotic
0,818	0,003	Topoisomerase II inhibitor
0,807	0,009	CYP2C substrate

Table V: PASS Biological data of Di doxorubicin

Pa	Pi	Activity
0,985	0,001	Cytostatic
0,979	0,001	DNA repair enzyme inhibitor
0,980	0,002	Caspase 3 stimulant
0,960	0,004	Antineoplastic
0,950	0,001	Alkylator
0,949	0,001	Gamma-glutamyl transferase inhibitor
0,950	0,003	Anti-infective
0,942	0,002	Anticarcinogenic
0,939	0,001	Leukopoiesis inhibitor
0,920	0,004	CYP2C9 substrate
0,907	0,000	RNA synthesis inhibitor
0,872	0,002	Aldose reductase substrate
0,873	0,008	CYP2H substrate
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0,842	0,003	Xenobiotic-transporting ATPase inhibitor
0,824	0,001	Antineoplastic antibiotic
0,818	0,003	Topoisomerase II inhibitor
0,807	0,009	CYP2C substrate

4.4. Molecular Docking Results

7 Target proteins were selected from literature review i.e. 6gqz (Petrobactin-binding engineered lipocaline), 5zad (Human topoisomerase II beta in complex with DNAs), 4zqf(Crystal structure of DOX-P Reductoisomerase fosmidomycin and magnesium), 7kjs (CDK2/cyclin E in complex), 8a26 (Lysophospholipase PlaA) and 4zvm (Oxidized quinone reductase 2), 6ftp (Alpha1-antichymotrypsin). Against these targets the unsubstituted/parent molecule was docked using Auto-Dock 4.5 and the results of docking scores are listed in the table the parent molecule showed highest score with 5g0s (enoyl-acyl carrier protein reductase) protein target i.e., doxorubicin & Di doxorubicin and Scei (cyclin dependent kinase) also gave good score. Hence these targets were selected for further docking with all the derivatives.

Table VI:

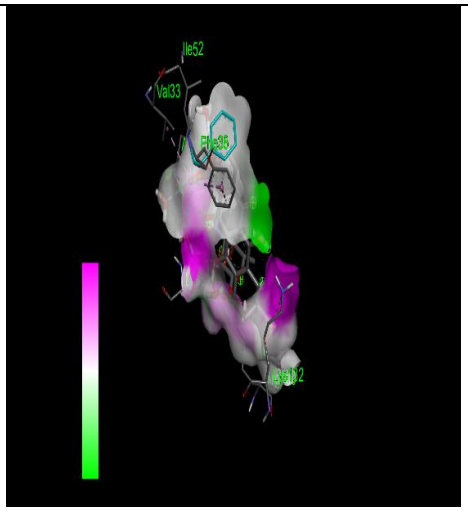
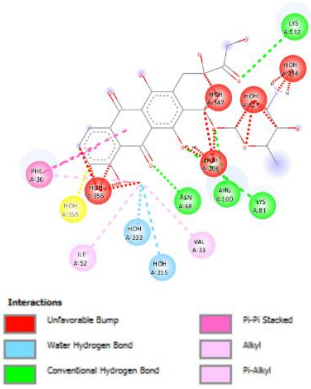
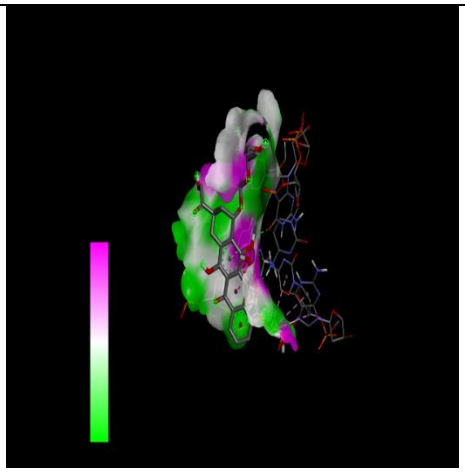
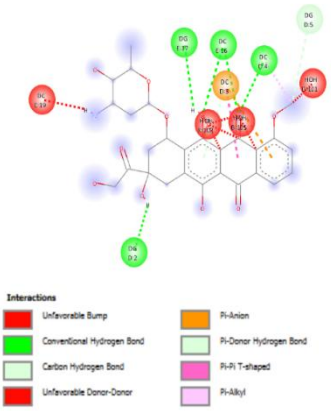
			Affinity of target proteins				
DOXORUBICIN	6gqz	5zad	4zqf	7kjs	8a62	4zvm	6ftp
1	-8.6	-10.3	-9.2	-8.2	-7.5	-7.6	-8.3
2	-8.3	-10.2	-7.8	-8.2	-7.5	-7.4	-7.9
3	-8.2	-9.9	-7.1	-7.8	-7.5	-7.4	-7.9
4	-7.9	-9.7	-6.9	-7.4	-7.4	-7.3	-7.9
5	-7.9	-9.7	-6.8	-7.2	-7	-7	-7.3
6	-7.9	-9.6	-6.7	-7.1	-6.9	-6.9	-7.2
7	-7.7	-9.6	-6.7	-7	-6.9	-6.9	-7
8	-7.3	-9.4	-6.7	-7	-6.8	-6.8	-6.9
9	-7.1	-9.4	-6.6	-7	-6.7	-6.8	-6.9

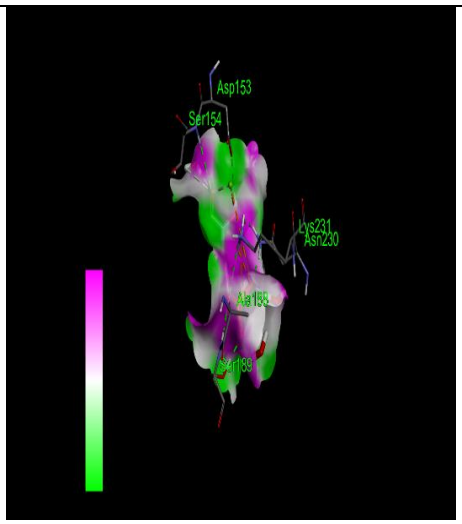
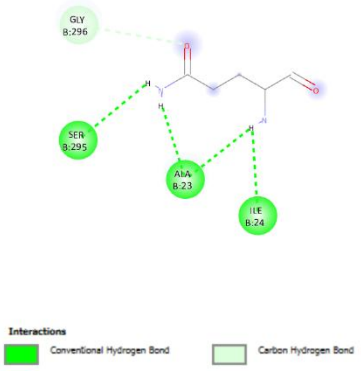
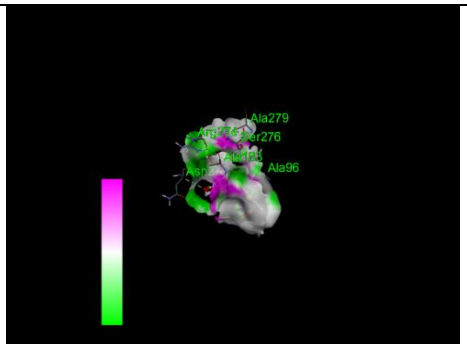
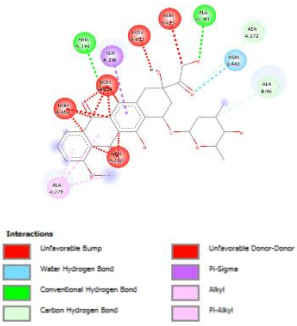
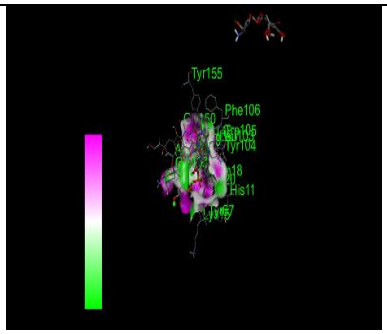
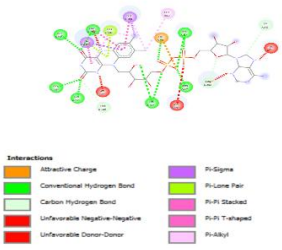
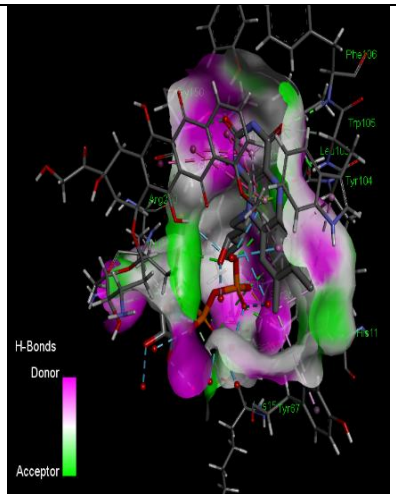
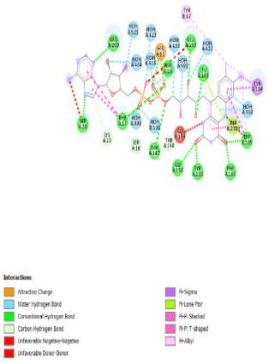
Table VII:

			Affinity of target proteins				
DIDOXORUBICIN	6gqz	5zad	4zqf	7kjs	8a62	4zvm	6ftp
1	-11.2	-9.5	-10.6	-8.2	-7.4	-8.8	-7.4

2	-11	-8.7	-10.5	-8.2	-7.2	8.7	-7.2
3	-10.5	-8.4	-10	-7.8	-7.2	-8.1	-7.2
4	-10.4	-8.3	-9.9	-7.4	-7.1	-8	-7.1
5	-10.3	-7.5	-9.8	-7.2	-7	-8	-7
6	-10.1	-7.2	-9.6	-7.1	-6.7	-8	-6.7
7	-10	-6.9	-9.5	-7	-6.6	-7.8	-6.6
8	-9.9	-5.6	-9.5	-7	-6.6	-7.7	-6.6
9	-9.5	-9.4	-9.5	-7	-6.5	-7.4	-6.5

Table VIII: VISUALIZATION with Doxorubicin

TARGET PROTIEN-PDBID	**Target ligand complex**	**2D interactions**	
6gqz			ASN A:68-Conventional Hydrogen Bond ARG A:100-Conventional Hydrogen Bond LYS A:81-Conventional Hydrogen Bond HOH A:215- Water Hydrogen Bond VAL A:33-Pi -Alkyl
5zad			DG D:2-Conventional Hydrogen Bond DG E:17-Conventional Hydrogen Bond DC E:16-Conventional Hydrogen Bond Dc D:3-PI-Anion

4zqf			ALA A:183- Conventional Hydrogen Bond SER B:295- Conventional Hydrogen Bond ALA A:23 Conventional Hydrogen Bond GLY B:296- Carbon Hydrogen Bond.
7jks			ASN A 272- Carbon Hydrogen Bond. ALA B 96-Carbon Hydrogen Bond ALA A:183 Conventional Hydrogen Bond ALA A:229- Pi-Alkyl HOH B:440- Water Hydrogen Bond
8a62			PHE A:90 Conventional Hydrogen Bond TYR A:155 Conventional Hydrogen Bond ASN A:203 Carbon Hydrogen Bond TYR A:86 - Pi-Sigma
4zvm			PHE A:106- Conventional Hydrogen Bond TRP A: 105- Conventional Hydrogen Bond HOH A:416- Water Hydrogen Bond HOH A:404- Water Hydrogen Bond TYR B:67- Pi-Alkyl

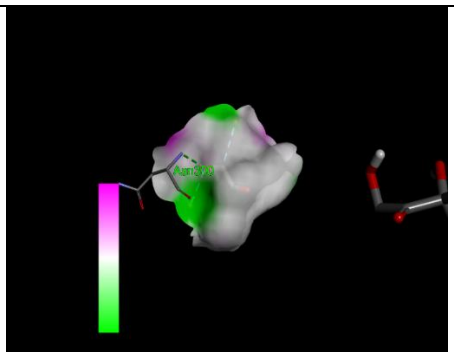
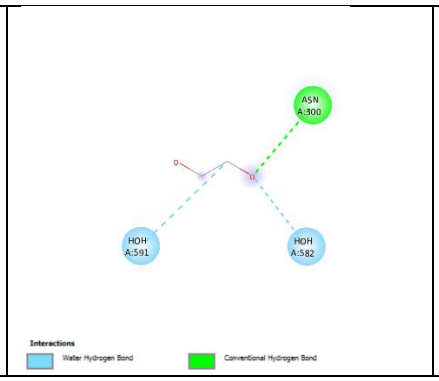
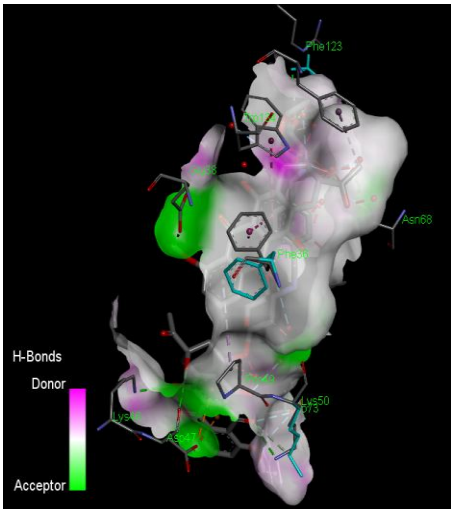
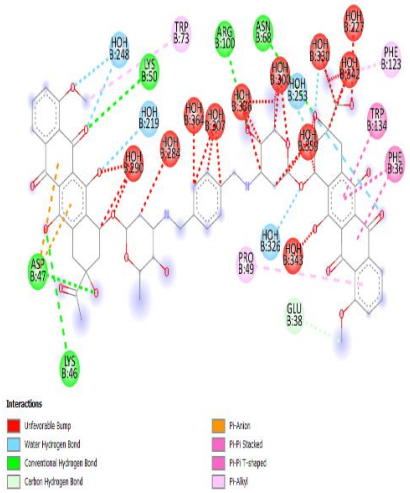
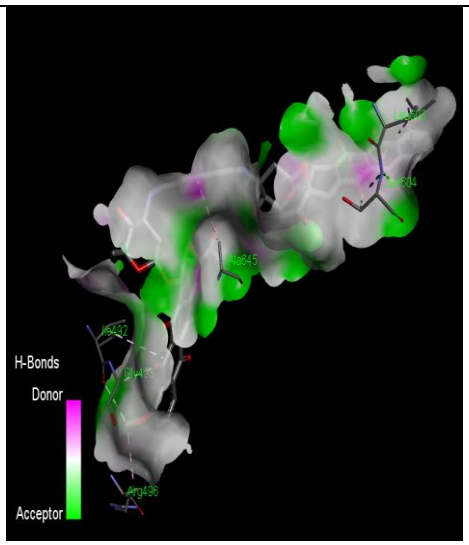
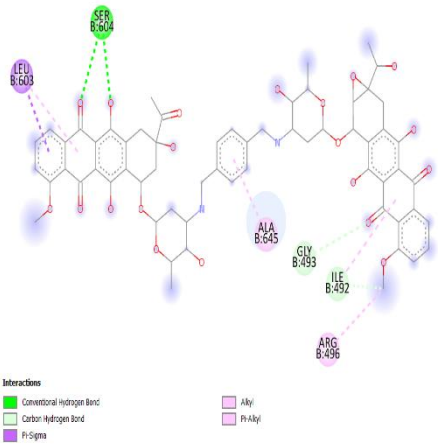
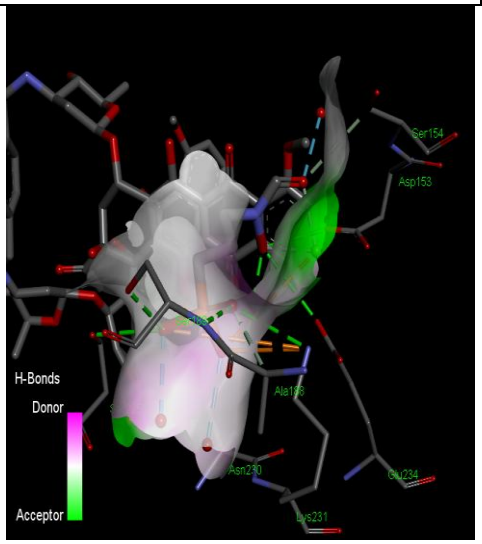
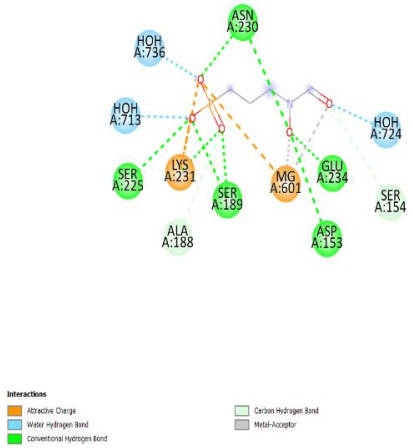
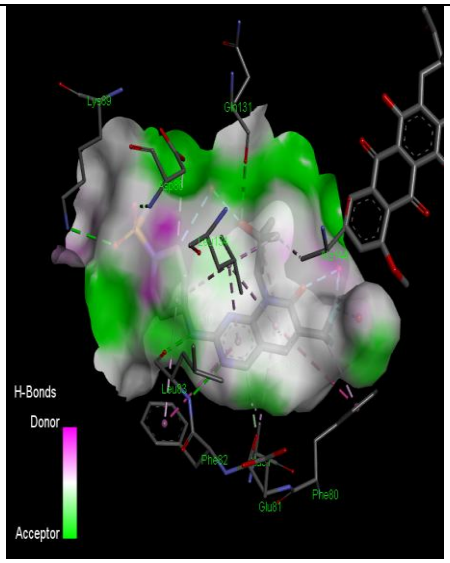
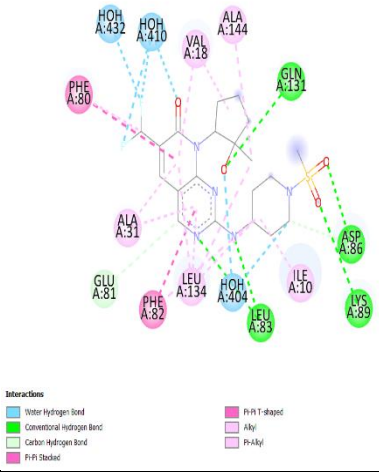
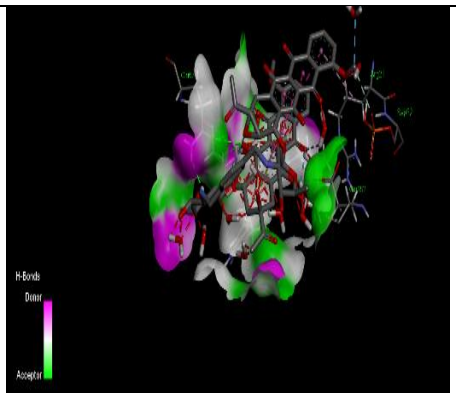
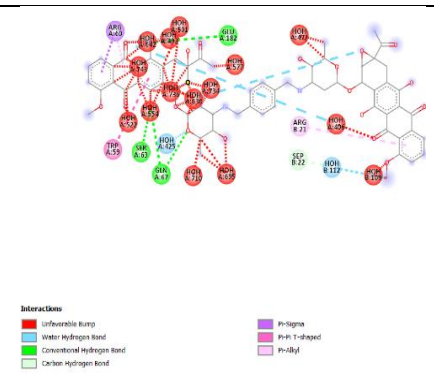
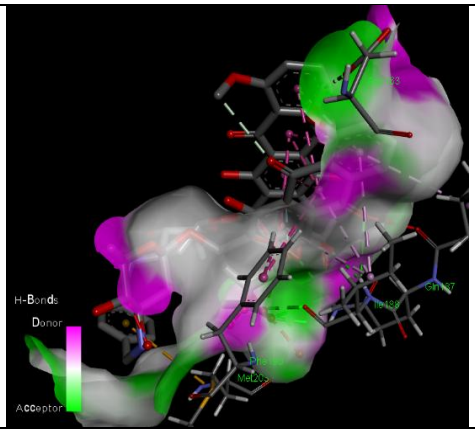
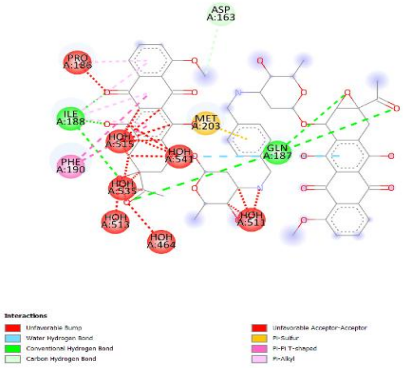
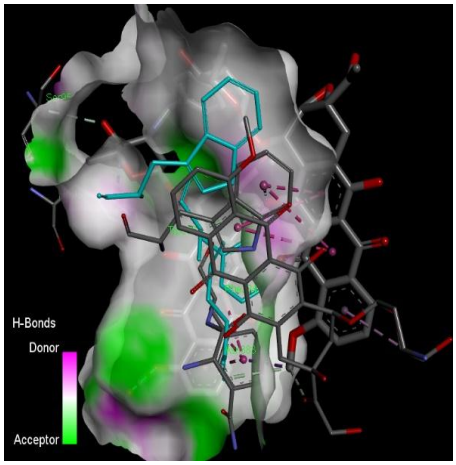
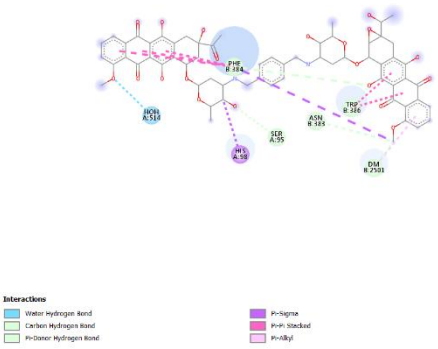
6ftp			HOH A:582- Water Hydrogen Bond HOH A:591- Water Hydrogen Bond ASP A:300- Conventional Hydrogen Bond

Table VIII: VISUALIZATION with Doxorubicin

Table IX: VISUALIZATION for Di Doxorubicin

TARGET PROTIEN-PDBID	**Target ligand complex**	**2D interactions**	
6gqz			ARG B:100- Conventional Hydrogen Bond ASN B:68- Conventional Hydrogen Bond HOH B :219- Water Hydrogen Bond PRO B:49- Pi-Alkyl TRP B:73- Pi-Alkyl GLU B:38- Carbon Hydrogen Bond

5zad			SER B:604- Conventional Hydrogen Bond LEU B:603-Pi-sigma ALA B:645-Alkyl GLY B:493- Carbon Hydrogen Bond ILE B:492- Carbon Hydrogen Bond
4zqf			ASN A:230- Conventional Hydrogen Bond SER A:189- Conventional Hydrogen Bond MG A:601-Attractive Charge HOH A:724- Water Hydrogen Bond ALA A:188- Carbon Hydrogen Bond
7jks			GLN A:131- Conventional Hydrogen Bond ASP A:86- Conventional Hydrogen Bond HOH A:432- Water Hydrogen Bond ALA A:31- Pi-Alkyl LEU A:134- Pi-Alkyl PHE A:82-Pi-Pi T-shaped
8a62			GLU A:182- Conventional Hydrogen Bond

			SER A:63- Conventional Hydrogen Bond HOH B :112- Water Hydrogen Bond ARG B:21- Pi-Alkyl SEP B:22- Carbon Hydrogen Bond
4zvm			GLN A:187- Conventional Hydrogen Bond ILE A:188- Conventional Hydrogen Bond ASP A:163- Conventional Hydrogen Bond PHE A:190- Pi-Alkyl MET A:203- Pi-Sulfur
6ftp			HOH A:514-Water Hydrogen Bond PHE B:384- Pi-Donor Hydrogen Bond SER A:95 - Pi-Donor Hydrogen Bond ASN B:383-Carbon Hydrogen Bond TRP B:386- Carbon Hydrogen Bond

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