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Modeling and Docking Analysis of GPR87 with Anti-Cancer Drugs

Mukta Rani[1], Anuradha Nischal[1], Ganesh C Sahoo[2], Sanjay Khattri[1*]

[1]*Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow, U.P, India- 226003*
[2]*Biomedical Informatics Centre, Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna, India- 800007*

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Abstract

Orphan G-protein coupled receptor 87 (GPR87) in human is a very recently discovered orphan GPCR means that the search for their endogenous ligands has been a challenge. GPR87 was shown to be over expressed in squamous cell carcinoma (SCCs) or adenocarcinoma in the lung and bladder carcinomas. We have predicted the comparative account on 3Dstructures of GPR87 on the basis of PDB ID: 3ODU|A. The model was further validated by comparison with structural features of the template proteins by using Verify-3D, ProSA and ERRAT servers were used for determining the stereo-chemical parameters of 3Dstructure of GPR87 predicted by Ramachandran plot and good 3Dstructure compatibility as assessed by DOPE score. Molecular dynamics (MD) simulation of models is studied of protein by conjugate gradient method. The DRY-motif (Asp- Arg-Tyr sequence) at the end of helix 3 is highlighted, where the G-protein binds and thus the activation signals are transduced. Protein-ligand interactions shows highest dock score with doxorubicin is 96.654, and involved binding site residues of GPR87 are Phe67, Lys247, Lys249, Asn330 and Asp357. In search for a better inhibitor for GPR87, *in-silico* modification of some anti-cancer ligands shows doxorubicin has shown the highest binding affinity with GPR87. So, our study provides an early insight into the structure of major drug target GPR87, thus facilitating the inhibitor design.

Keywords: *GPCR, GPR87, Modeling, Docking, Anti-cancer.*

Introduction

GPCR represents the most efficient signalling system used by cells to establish relationships with the external environment. GPCRs are among the most heavily investigated drug targets in the pharmaceutical industry [1]. GPCRs modulate the regulation of several physiological processes involved in several diseases. Considering their popularity and functional importance, it's represent that ~60% of drugs target GPCRs in the market [2]. However the early stages of the drug discovery process suffer from lack of crystal structures of GPCR. There are more than 140 GPCRs have unknown endogenous ligands are called orphan GPCRs [3]. The identification of these endogenous ligands will show innovative designing of new drug targeted receptors with their physiological roles. Orphan GPCRs offers incredible promises, as they may provide novel therapeutic targets that may be more selective than currently known receptors [2]. Consequently, they may provide access to signal transduction pathways currently unknown, allowing for new strategies in drug design. Regardless, orphan GPCRs are critical area of an analysis, due to their characteristic structure and specific ligand binding ability; many orphan GPCRs have been extensively used as a target for therapeutic drug development and designing. GPCRs is one of the best target for inflammatory mediators, therefore it provides a link between chronic inflammation and cancer disease.

Orphan G-protein coupled receptor 87 (GPR87) in human is a very recently discovered orphan GPCR, it means that the search for their endogenous ligands has been a major challenge. GPR87 was shown to be over expressed in squamous cell carcinoma cell lines (SCCs) or adenocarcinoma in the lung and bladder carcinomas [4]. Orphan GPCR87 in human plays significant role in carcinoma, can also be searched which may be the best target to cure a cancer disease. Human G-protein

*Corresponding Author
Dr. Sanjay Khattri
Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow, UP, India.
Phone: +91 900515700
Email: drskhattri@gmail.com

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coupled receptor 87 (*hGPR87*) belongs to the orphans family of GPCR that determines its function and attempts to find a drug to modulate it by reverse pharmacology. The orphan is used as "hook" to "catch" the natural ligand from animal or human cells. The ligand is then used to learn about physiology and pathology related to the receptor [5]. GPR87 was classified in the $P2Y_{12}$ subgroup that contains $P2Y_{12}$, $P2Y_{13}$, $P2Y_{14}$, CysLT1, and CysLT2 receptors [6] and it is located in the human chromosome 3q25 region [7] in which the given P2Y receptor genes cluster. In the absence of a crystal structure, our study provides an early insight into the detailed 3Dstructure of a major drug target hGPR87 or developing new inhibitors for GPR87 will clearly be challenging work for the near future. GPR87 may be carcinogenicity associated to squamous cell carcinoma and should be further validated as a target of potential diagnostic, therapeutic or prognostic significance.

Methodology

A. Homology modeling:

The translated amino acid sequences of G-protein coupled receptor 87 from *Homo sapiens* (Accession No: Q9BY21) have 358 amino acid lengths was taken for further study. The homologue sequence of GPR87 protein were retrieved from NCBI and BLASTP [8] search against Protein Data Bank (PDB) among these sequences, crystal structure of CXCR4 chemokine receptor in complex with a small molecule antagonist IT1t in I222 spacegroup (PDB ID: 3ODU|A) [9] was chosen as a best template by using SWISS-MODEL[10] servers. The analysis of the conformational correctness and reliability was carried out using Ramachandran plot in PROCHECK [11] is used to validate modeled structure. Molecular dynamic (MD) simulations were carried out using the CHARMm [12]. Based on intrinsic dynamic, structural stability and improved relaxation of the modeled GPR87 protein structure, the energy minimization by conjugate gradient methods of standard dynamic cascade protocol of DSv2.5 [13], which is an important step for the convergence of free MD simulation, were calculated. The different locations of the extracellular N-terminal domain and the transmembrane domains in amino acid sequences of GPR87 were predicted by thirteen different servers were published in Rani, M. et al, 2013 [14]. The prediction of different binding pockets i.e. active sites in GPR87 protein were predicted by MetaPocket [15] and CASTp [16] servers and compare their results with binding sites of template. The binding sites and the functional residues were identified and stored for further investigation.

B. Protein ligand interaction (Docking) analysis:

The LigandFit [17] docking protocol of Discovery Studio (DSv2.5) was used to dock anti-cancer ligands with GPR87 protein. The LigandFit docking algorithm combines a shape comparison filter with a Monte Carlo conformational search to generate docked poses consistent with the binding site of GPR87. These initial poses are refined by rigid body minimization of the ligand with respect to the grid-based calculated interaction energy using the Dreiding forcefield [18]. The receptor protein was kept fixed during docking. The docked poses were further minimized using all-atom CHARMm (version c32b1) force field and smart minimization method (steepest descent followed by conjugate gradient) until the RMS gradient for potential energy was less than 0.05 kcal mol−1 Å−1. The atoms of ligand and the side chains of the residues of the receptor within 5 Å from the center of the binding site were kept flexible during minimization. The final step in docking is the scoring of the refined docked poses. After that, the anti-cancer ligand which shows the highest dock score has used to predict the ADMET tests analysis by using DSv2.5 software. The initiation of predictive tools for screening of Absorption, Distribution, Metabolism, Excretion and Toxicity properties (ADMET) of drugs has revolutionized the drug discovery process. This elimination in early drug discovery process helps to decrease the number of drug failures in the clinical trials. Traditionally these predictive tools were applied at the end of the drug discovery process, but are now utilized during the initial phase of drug development. The ADMET descriptor and TOPKAT protocol available in DSv2.5 were used to predict these properties. The Lipinski's rule of 5 was also used to determine the biological activity or drug-likeness of the designed inhibitor [19-20].

Results and Discussion

The GPR87 homologue sequences were retrieved from NCBI-BLASTP [8] search against Protein Data Bank (PDB) shows that crystal structure of CXCR4 chemokine receptor in complex with a small molecule antagonist IT1t in I222 spacegroup (PDB ID: 3ODU|A) [9] was chosen as a best template, based on sequence identity (34%) and sequence similarity (53%) to construct 3Dstructure of GPR87 protein. The GPR87 model was further validated by DOPE scores by DSv2.5 (Profile3-D module) which shows a high DOPE score, i.e. -46755.238281. The modeled structure of GPR87 was compared with structural features of the CXCR4 chemokine receptor protein (template) using others servers PROCHECK, Verify-3D and ERRAT plots to determine the correct stereochemical parameters of energy minimized model of GPR87.

We further validate 3Dstructure of GPR87 by Ramachandran plot (the Φ/Ψ distribution of the backbone conformational angles for each residue of the 3-D structure) and structure was analyzed by PROCHECK program. It was revealed that phi-psi angles of 86.1% residues are in the most favored regions, 11.2% residues in additional allowed regions, 1.8% residues in generously allowed region and rest 0.9% in disallowed region. The disallowed residues were further validated by various structural refinements protocols by DSv2.5 using loop modeling, side chain refinement and energy minimization to increase the model reliability. The secondary structure of GPR87 proteins (α-helices and β-sheets) have 12 α-helices including with 7 transmembrane helices (7TMs), 4 small 3_{10} α-helices and 2 anti-parallel β-sheets shown in Figure 1. The total

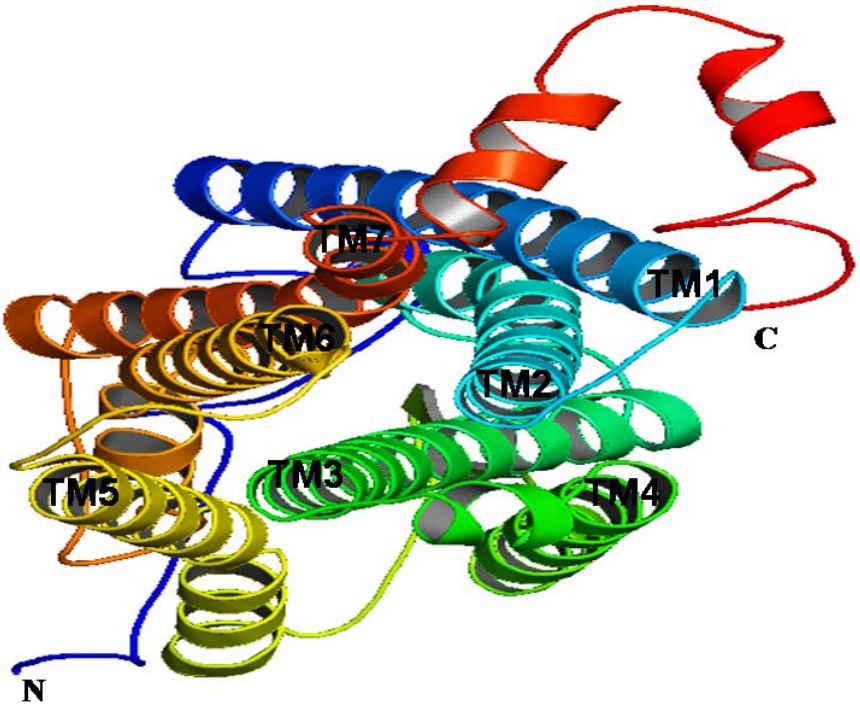


Figure 1: Three dimensional (3D) modeled structure of GPR87 protein which showing N-terminal in blue color and C-terminal in red color. The modeled structure contains two antiparallel β-sheets, twelve α-helices including 7 transmembrane helices (7TMs) and four 3_{10} helices. The α-helices are shown in the cylinder, β-sheets as arrows and the rest are the loops. This figure is generated using PyMol v0.99.

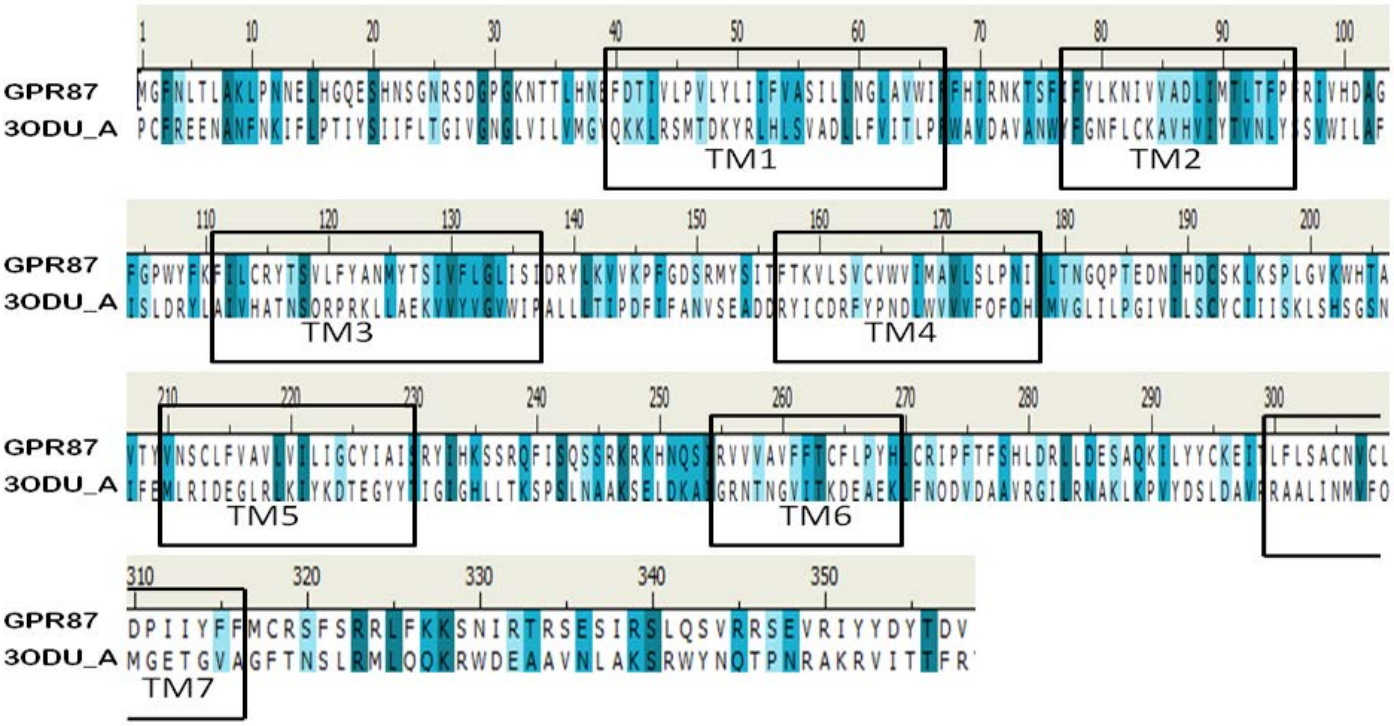


Figure 2: The sequence alignment analysis of GPR87 with template i.e. CXCR4 chemokine receptor PDBID: 3ODU|A are shown and locations of seven of transmembrane helices. A region marked by the dark cyan color indicates that sequences are conserved, light cyan and light sky blue are shown as strong and weak similarities respectively. The sequences in white color are not aligned with each other. The figure was prepared by using software by using DSv2.5.

percentage of alpha helices and 3_{10} helix comes out 61.5% and 2.8%, similar with template 63% and 4% respectively. In addition of one significant motif i.e. DRY-motif (an Asp-Arg-Tyr sequence) is located at the end of transmembrane helix 3 (138-140aa) is shown which is an important site for binding of G-protein and activation of signals which were transduced shown in Figure 2. The sequence alignment analysis of GPR87 with template PDBID: 3ODU|A are giving 36 conserved residues and locations of seven of transmembrane helices are represented in Figure 2. The comparative analysis of transmembrane helices prediction programs showed that the lowest range and higher range of transmembrane helices in first TM is 42-71 residues, 74-99 in second TM, 111-149 in third TM, 155-180 in fourth TM, 206-232 in fifth TM, 252-278 in sixth TM and 292-322 in seventh TM were published [14].

The MD simulations snapshots of the dynamics trajectory at 0, 200, 500, 1000, 1500, 2000, 2500, and 3000 and so on of the production run are shown in Figure 3. The result indicates that the variation in total energy is -19607.42067 KJ/Mol to -20705.31756 KJ/Mol at 1500 ps and then in steady state were calculated by conjugate gradient method at 3000 ps dynamics trajectory was retrieved. Each step calculates Van der Waals energy, electrostatic energy and RMS gradient energy of GPR87 protein. Van der Waals energy did not show much variation, by conjugate gradient method (-2251.28272 KJ/Mol to -2306.85742 KJ/Mol). Conjugate gradient method showed that the electrostatic energy and RMS gradient of modeled GPR87 protein become stable at -21453.97372 KJ/Mol from -20452.43179 KJ/Mol and 1.11005 KJ/Mol to 0.09504 KJ/Mol respectively. The potential ligand binding sites (LBSs) of orphan GPCR 87 protein of human have found by CASTp server and frequently involved amino acid residues involved in forming the active sites are Lys36, Asn37, Leu45, Cys49, Tyr58, Ala64, Ile55, Leu62, His693, Lys81, Phe94, Gly105, Cys114, Phe121, Gly148, Thr158, Ser173, Thr183, Gly200, Val201, Lys204, Val210, Arg245, Phe261, Leu281, Tyr292, Lys295, Phe301, Phe325, Arg329, Lys337, Arg345, Tyr352 and Val358 are responsible for protein- ligand interaction studies of drug designing approaches [14].

Protein ligand interaction (Docking) analysis:

After perceptive the binding sites of GPR87 protein, we examine the anti-cancer ligands were docked with 3D model of GPR87 to estimate the site of interaction on the ligand molecule and the binding energy and their interaction analysis by using Ligandfit protocol of DSv2.5. There are four different anti-cancer ligands which shows the highest dock score are predicted as up to doxorubicin has 96.654, methotrexate has 85.238, paclitaxel has 78.098 and lower as 5-fluorouracil has score 68.654 score as a binding affinity are shown in Figure 4. The active site residues of GPR87 which involved in different ligands interaction like in doxorubicin has Phe67, Lys247, Lys249, Asn330 and Asp357, methotrexate has Phe67, Thr74, Ile77, Phe78 and Lys81, 5-fluorouracil Lys32, Asn330, Asp354, Thr356, Asp357 and Val358 and paclitaxel has Arg246, Lys247, Lys249, His250, Ser253, Ser320, Phe316 and Phe321. The ADMET predictions indicate that the doxorubicin is likely to have good oral bioavailability, absorption and permeation as identified from Lipinski's rule of five. We have predicted the ADMET analysis with doxorubicin because it shows the highest

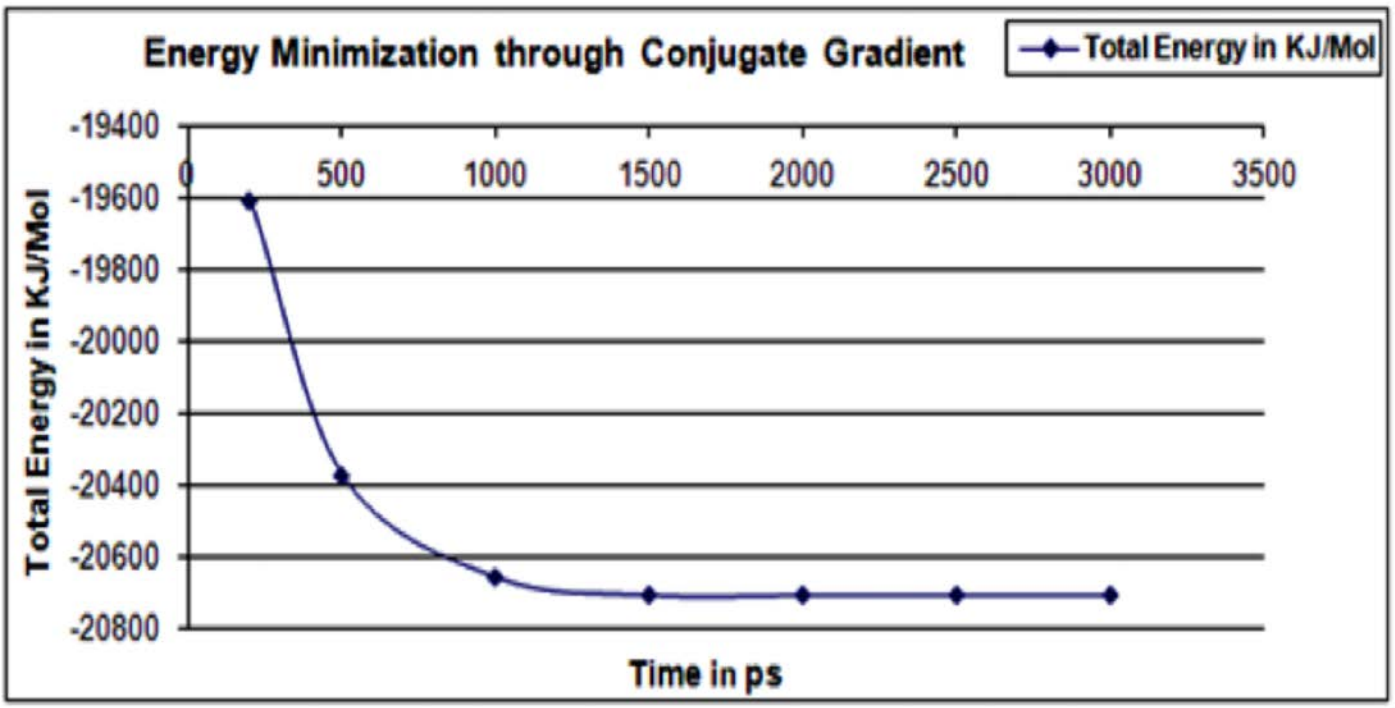
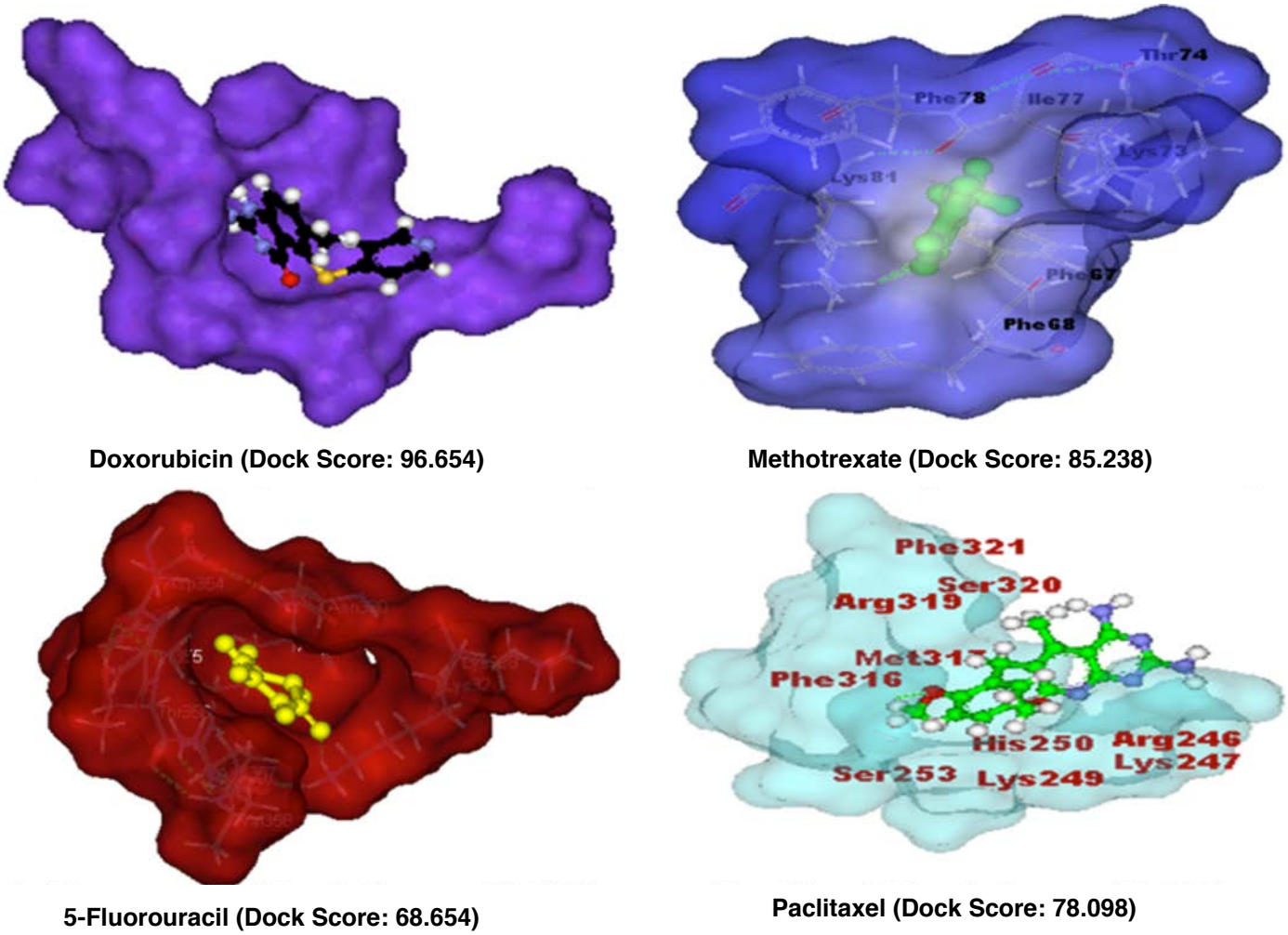


Figure 3: It shows the comparisons and calculates energy versus time plot by using Conjugate gradient methods (B) of energy minimization protocol of DSv2.5 software. The X-axis is Time (ps) and Y-axis Total energy in KJ/Mol.



Doxorubicin (Dock Score: 96.654)

Methotrexate (Dock Score: 85.238)

5-Fluorouracil (Dock Score: 68.654)

Paclitaxel (Dock Score: 78.098)

Figure 4: The 3Dstructure of the GPR87 was shown the best interactions with these four anti-cancer ligands are doxorubicin has 96.654, methotrexate has 85.238, paclitaxel has 78.098 and 5-fluorouracil has score 68.654. The GRASP model surface representation of GPR87 is shown and four anti-cancer ligands in ball and stick model. Side chains of the amino acids contributing to hydrogen bond formation are represented as a stick model and the residue names and numbers shown next to them. These pictures were generated from by using DSv2.5.

Table 1: It shows the ADMET test analysis of best suited anti-cancer ligand i.e. doxorubicin.

ADMET Properties	Doxorubicin
ADMET Solubility	-2.359
ADMET Solubility Level	2
ADMET Hepatotoxicity probability	0.486
ADMET CYP2D6 probability	0.358
ADMET PPB_level	4
ADMET AlogP98	8.265
ADMET PSA_2D	200.325

dock score i.e. 96.654 with GPR87 protein. The results of ADMET test shows that doxorubicin has molecular weight of 543.51 Dalton, calculated AlogP value is 2.6, six hydrogen bond donors, twelve hydrogen bond acceptors and 5 rotatable bonds and satisfies all the criteria of Lipinski's "rule of 5"[19-20] summarized in table 1. The ADMET solubility which predicts the solubility of each compound in water at 25°C, is -2.359 and ADMET solubility level is 2, it means that the values is -2.0 < log(Sw) 0.0 shows the optimal in nature i.e. doxorubicin is most favorable ligand. The ADMET Hepatotoxicity probability score is 0.486 have shown non-toxic in nature, it defines the potential organ toxicity for a wide range of structurally diverse compounds. The ADMET-cytochrome P450 2D6 (CYP2D6) probability score of doxorubicin is 0.358 have shown non-inhibitor in nature. The ADMET Plasma Protein Binding (PPB_level) of doxorubicin is 4; it means that the binding is < 90% (No markers flagged and AlogP98 < 4.0). it defines that the compound (doxorubicin) is likely to be highly bound to carrier proteins in the blood. The ADMET AlogP98 value of doxorubicin is 8.265 and the ADMET 2D polar surface area (PSA_2D) is 200.325. The ADMET analysis of anti-cancer ligand (doxorubucin) indicates that it is likely to be a drug candidate. Therefore doxorubicin is designed as a suitable lead molecule for the development of novel GPR87 inhibitors as anti-cancer drugs.

Conclusions

This is the first wide-ranging study, which highlighted the structural features of potential drug target, i.e. orphan GPR87. Therefore we concluded that doxorubicin and their analogues might have potential for better inhibition efficiency as a synergistic compound for treatment of squamous cell carcinoma. Thus the doxorubicin is a suitable lead compound for the development of a novel class of selective drugs for anti-cancer therapy.

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Authors Column

Mukta Rani is a Ph.D Student in Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, India. She is an active member of Research Group in Department of Pharmacology. In research group, her interest of area is in Computer aided drug designing (CADD), protein modeling and dynamics studies. She has worked indomitably in Structure Based Drug Designing (SBDD) which is very much useful to optimize the drug parameters in their specialized areas. During research work, fifteen research papers were published in reputed peer reviewed international journals. I have presented posters on my research work in proceedings of National/International conferences.

Dr Anuradha Nischal is currently working as an Associate Professor in Pharmacology, King George's Medical University, Lucknow, Uttar Pradesh, India. She has been associated with institutions like the Lady Harding Medical College, New Delhi; Institute of Human Behavior and Allied Sciences, New Delhi; Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India. With the learning and experiences at these places, she has written a book Viva questions in pharmacology for the benefit of students (published 2011). She has eight national and international publications to her credit. She has supervised and co-supervised various MD and PhD thesis. Her chief area of interest is Neuro-Psychopharmacology.

Dr. Ganesh Chandra Sahoo was completed Ph.D. in science from Jadavpur University, India in 2008. He has expertise in bioinformatics, virology and nanotechnology. Presently he is involved in research carried out at BioMedical Informatics Center of Rajendra Memorial Research Institute of Medical Science (RMRIMS), Patna, India and Editor and members of reviewing committee of various international journals related to bioinformatics, drug discovery, HIV, nanotechnology, next generation sequencing technology, databases. He has visiting faculty to various national universities in India and attended various international workshops and conferences such as APBC and IUMS. He has organized national level workshops on bioinformatics involving next generation sequence analysis by using NextGene programand SOLiD BioScope software (ABI). He has published more than 50 research articles in various international journals and conferences.

Prof. Sanjay Khattri is a Professor in Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, India. He has expertise in Clinical Pharmacology, especially in monitoring of ADR, therapeutic drug monitoring and rationale prescribing. I am establishing a lab setup on "Clinical Pharmacolog Unit & Therapeutic Drug Monitoring Centre" in our department on support of state government. It is my endeavour to establish a endeavour to establish a Clinical Pharmacology Section in the department is fully equipped with modern equipments for the pharmacokinetics studies. I have more than 11 years of experience in teaching students of various medical courses like Bachelor of Medicine and Bachelor of Surgery (M.B.B.S.), Doctor of Medicine (M.D.) in Pharmacology, Bachelor of Dental Surgery (B.D.S.), Master of Dental Surgery (M.D.S.) and Diploma in Pharmacy. He has published more than 50 research articles in various international journals and conferences.