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DRUG RESISTANCE IN CANCER – AN ATTEMPT TO OVERCOME BY HOMOLOGY MODELING, VALIDATION AND DOCKING OF ERYTHROPOIETIN PROTEIN.

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Abstract *-:* The regulation of cell growth and tissue development significantly influence apoptosis, also known as programmed cell death. Carcinogenesis is accelerated and chemotherapeutic drugs become resistant due to defects in apoptotic processes. So, increased erythropoietin (EPO) enzyme level is the cause of drug resistance in cancer. Thereby an inhibitor targeting the EPO will reduce the drug resistance in cancer.

The focused approach is based on the understanding of the disease-causing protein. Erythropoietin (EPO) overexpression causes angiogenesis in VEGF therapy resistance. The protein sequence (Uniprot ID P01588) is used in the current work for template identification, alignment, the building of 3D model using the SWISS model, and validation. A protein's 3D structure has 93.9% of amino acids in the favorable region, 6.1% in the generously allowed zone, and 0% in the disallowed region. Cast P located the active site residues LYS167, ARG158, THR71, LYS179, ASP192, GLY185, and ARG193.These amino acids have a part in both the signaling process and the way the EPO protein attaches to its natural substrate (EPOR). If this amino acids are blocked, the drug resistance can be inhibited.

Researchers investigated the phytochemicals Dentatin (DEN), Nordentatin (NORD), and Quercetin (QUE) binding affinities for the EPO protein since these substances have shown anti-proliferative effects in a variety of cancer cell lines. These calculations showed that Nordentatin had a higher affinity for binding EPO than Quercetin and Dentatin (ΔG values of 7.4 and 6.8 kcal/mol, respectively), but also suggest that all three compounds can block erythropoietin and overcome resistance.

IndexTerms - Cancer, Drug resistance, Phytochemical and Insilico.

I. INTRODUCTION

Anti-VEGF-based medicines are used to treat variety of cancer types and more than a decade of clinical experience, the therapeutic effectiveness of anti-VEGF treatments is fairly poor (Cao, 2011). According to a recent research, anti-VEGF medication promotes the production of non-VEGF angiogenic proteins that promote tumour angiogenesis through VEGF-independent pathways (Casanovas, 2005). In addition to anti-VEGF drug-induced tumour hypoxia, hypoxia catalyses the compensatory mechanism for anti-VEGF resistance(Jain, 2014).

EPO administration in randomized studies to cancer patients is linked to a worse survival rate (Eckardt, 1992). EPO causes carcinogenesis in genetically altered animal models of breast cancer by increasing JAK/STAT signaling in breast tumor-initiating cells (TICs) and encouraging TIC self-renewal (Figure 1). Additionally, EPO has a pro-tumorigenic role in breast cancer tumors. Higher levels of endogenous EPO gene expression are also related to lower relapse-free survival(Bing, 2014).

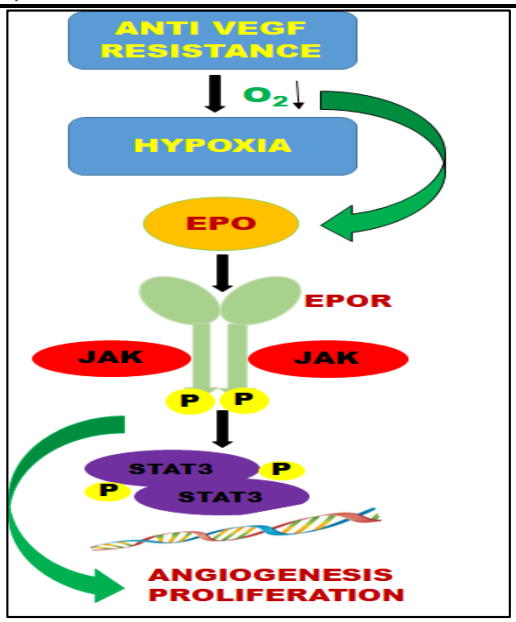

Figure 1. Schematic diagram of systemic anti-VEGF treatment-induced drug resistance. Anti-VEGF–induced vessel regression leads to tissue hypoxia, which induces transcription level target the Epo promoter for high production of EPO. EPO enters the circulation to stimulate angiogenesis and eventually contributes to antiangiogenic drug resistance. These findings provide mechanistic insights of off-tumor targets of antiangiogenic drugs in the cancer patients for development of drug resistance.

EPO activates the transmembrane EPO receptor (EPO-R) found in erythroid progenitor cells (Jelkmann, 2007). These findings support a possible function for the EPO pathway in the treatment of breast cancer by indicating an active involvement of endogenous EPO in tumor growth. The anti-VEGF medicine induces kidney hypoxia in mouse tumour models and cancer patients, with significant amplification of erythropoietin in the blood (Masaki Nakamura, 2017). EPO causes angiogenesis by directly influencing endothelial cells to encourage migration and proliferation, while it can also boost the expression of other angiogenic factors (Ribatti, 1999). It is critical to create a medication that targets the EPO protein since EPO inhibition provides a rationalization for diseases such as cancer, diabetic retinopathy, nephropathy, etc.

Recently, the occurrence of EPOR signaling and EPO-induced cellular proliferation was established in renal cancer cells (Wu , 2012), head and neck squamous cell carcinomas (Abhold , 2011), and cervical cancer cell lines (Lopez , 2011), as well as glioma cells (Pérès , 2011).Erythropoietin-induced proliferation of cancer cells was associated with the activation of JAK2, JAK3, STAT3, and STAT5 (Lopez , 2011), AKT phosphorylation (Abhold , 2011), ERK phosphorylation (Pérès , 2011), and phosphorylation by PI3K/AKT (Akiyama, 2011). Furthermore, the EPO-EPOR pathway stimulated the expression of cyclin D1, and led to a more rapid progression of renal cancer (Miyake, 2013).

Erythropoietin was also involved in cell growth, invasion, survival, and sensitivity to the multikinase inhibitor sunitinib and cisplatin in renal cancer cells (Wu , 2012) and in head and neck squamous cell carcinoma (Abhold, 2011), respectively. *In vivo*, EPO increased lung metastases in MDA-MB-231 or MDA-MB-435 cells and treated with chemotherapy relative to mice treated with chemotherapy alone (Hedley, 2011).The study of Yasuda et al. (Yasuda, 2002) revealed that normal human cervix and endometrium, as well as ovary malignant tumors of female reproductive organs produce EPO and EPOR, and that the tumor cells themselves and capillary endothelial cells are sites responsive to the EPO signal.

Based on recent clinical studies, the adverse effect of EPO in cancer patients during anti-cancer, such treatments are long termed and rarely single dosed. It was suggested that the concurrent use of anti-cancer treatment as the main reason for EPO negative effects (side effects) on response to anti-cancer therapy, overall survival, and disease recurrence.

Bioinformatics tools deliver a new area of personalized medicine aimed at more reliable and effective diagnosis and treatment of human diseases. This will operate in a short period of time, which will greatly increase the quality of treatment, reduce healthcare costs, precise molecular knowledge on the pathology of protein levels in diseases, and a promising potential method for healthcare system. Hence the proposed work deals with the identification of new small inhibitor molecules of pathogenic angiogenesis by considering EPO protein as a novel target.

II. RESEARCH METHODOLOGY

2.1 Homology Modeling of EPO Protein

Homology modeling is a computational method to determine the 3D structure of proteins, in the absence of PDB either by X-ray crystallography, NMR, or Electron spectroscopy (Brigitte, 2003). The present study involves Homology Modeling, Evaluation and refinement of predicted 3D structure, Active site identification and virtual Screening and ADME prediction(Navaneetha, 2012).

The fasta sequence of EPO protein (267 amino acids) is retrieved from Uniprot of Expert Protein Analysis System (ExPASy) Server with Uniprot ID Q9P0G3. A protein sequence (Template) similar to EPO protein sequence is identified using NCBI-BLASTp (Gasteiger, 2003), JPred (Drozdetskiy, 2014), Phyre (Kelley, 2015) servers based on the parameters of sequence similarity, secondary structure, fold recognition and E-value obtained (Bhargavi, 2010). Pairwise alignment of the EPO protein sequence with the template is carried out using Clustal W (Ahola, 2006). The 3D structure of EPO protein is built using the Modeller 9v9 program (Sali, 1995). Among the generated 200 models, the model with the lowest modeller objective function is selected for further refinement.

For additional analysis and visualization, SPDBV 4.10 was used (Johansson, 2012). The model was then verified using servers from Verify3D (Eisenberg, 1997), ProSA (Wiederstein, 2007), and PROCHECK (Laskowski, 1993). SWISS-MODEL uses the QMEAN scoring algorithm to evaluate the modelling mistakes and provide estimates of the expected model correctness (Guex, 1997). To determine the quality of each residue globally and individually, QMEAN uses statistical potentials of mean force. The Ramachandran plot, Verify3D profile evaluation, What check, and PROCHECK plots provide graphical representations of these data (Abraham, 2015; Eisenberg, 1997; Melo, 1998).

The EPO protein's most stable conformation is examined for its secondary structural characteristics and reliable active site areas. An important stage in drug design is the identification of the active site region. The active site regions of the EPO protein is ascertained using the tools the Computed Atlas of Surface Topography of Proteins (CASTp) server (Dundas, 2006), and Active Site Prediction Server. The evaluation of the active site domain of these server measures the surface area and volume of each cavity. Utilizing the Patch Dock server programme (Schneidman-Duhovny, 2005), the protein-protein docking is carried -out between the 3D model of EPO protein and its receptor (EPOR), which is its natural substrate (PDB ID: 4Y5X). The docked EPO-EPOR complex is evaluated to verify the potential binding interactions.

2.2 Docking of ligands with protein

The PubChem Compound Database (National Center for Biotechnology Information; https://pubchem.ncbi.nlm.nih.gov/) was used to download the structures of Dentatin, Nordentatin, and Quercetin in the Spatial Data File (.SDF) file format. The ligands' physicochemical characteristics satisfied Lipinski's rule of five, also known as Lipinski's rule of drug-likeness (Lipinski, 2016). Particularly, compounds with >5 hydrogen-bond donors, >10 hydrogen-bond acceptors, >500 molecular masses, and computed log P (CLog P) values of >5 are more likely to be permeable and active as ligands (Zhang, 2007). We investigated the phytochemicals Dentatin, Nordentatin, and Quercetin in silico binding affinities for EPO because they have shown antiproliferative effects in numerous cancer cell lines. PyMol, Discovery Studio Biovia 2017, Auto Dock Vina, and Auto Dock Tools version 1.5.4 were used to conduct molecular docking investigations (Trott, 2010).

Chemical structures in the SDF format were converted to the PDB format using Discovery Studio Biovia 2017. ADT was then used to investigate ligand structures in terms of combinations with nonpolar hydrogens, additions of Gasteiger changes, and rotatable bonds. Preparation of macromolecule structures of the protein (the 3D model of EPO protein) where the water molecules were removed, and ADT software was used to prepare the required files for AutoDock Vina by assigning hydrogen polarities, calculating Gasteiger charges to protein structures, and converting protein PDB structures to PDBQT format (Jaghoori, 2016).

The AutoDock Vina programme was used to carry out molecular docking. Each ligand was individually docked to the receptor using grid coordinates (grid center) and grid boxes of a specific size. When the ligand interacted with macromolecules in rigid circumstances, it was in a flexible state. It was necessary to use ADT to set the size and the Centre of the grid box as well as to prepare the input PDBQT file for EPO. The EPO structure contained polar hydrogen atoms and Kollman charges. The grid size was set at 22 × 12 × 20 (x, y, and z) points, and the grid center was designated at x, y, and z dimensions of -20.0407, 4.8705, and -22.1005, respectively, with a grid spacing of 1000 Å. Ligand-binding affinities were predicted as negative Gibbs free energy (ΔG) scores (kcal/mol), which were calculated on the basis of the AutoDock Vina scoring function. Post-docking analyses were visualized using Discovery Studio Biovia 2017.

IV. RESULTS AND DISCUSSION

Human erythropoietin, a hematopoietic cytokine, is an essential protein for the development of precursor cells into red blood cells. Cells are activated by an intracellular phosphorylation cascade spurred on by two binding and orientated erythropoietin receptors (EPOR) on the cell surface. EPO is a novel target in the current study to identify new treatment approaches for preventing pathological angiogenesis. The three-dimensional (3D) structure of the protein is evaluated using bioinformatics techniques. Docking in the conserved domain area to identify the active site residues and novel lead structures.

The target protein's fasta sequence is downloaded from the ExPASy server and submitted through a template search on several servers (NCBI – Blast, Phyre & JPred). When using the EPO protein as a template, the Position Specific Iterative Basic Local Alignment Search Tool (PSI-BLAST) is used to find amino acid sequences that are similar to it (Johnson, 2008). A low e-value indicates a criteria of high biological importance concerning the EPO amino acid sequence. The EPO family templates, specifically 1EER - A, are compatible with the secondary structure model produced by the Jpred4 server tool. Based on the low E-value, homologous secondary structure prediction and fold recognition, the estimated template structure is 1 EER – A (Cuff, 1999).

Table 1 shows the e-Scores (Expectation Values) of template from different search servers and 1EER - A (retrieved from the RSC protein data library) was verified as a trustworthy template for modelling EPO protein based on the outcomes of NCBI-BLASTp, JPred4 analysis, and Phyre.

Table 1. Template selection for EPO protein.

S.No.	Server	Parameter(s)	E-Value	PDB CODE
1.	BLAST	Sequence Specificity	3e-116	1EER -A
2.	JPred 4	Secondary Structure	7e-89	1EER –A
3.	Phyre	Fold recognition	100% (Confidence)	1EER -A

Using CLUSTALW, the EPO protein's sequence was aligned paired-wise with the template protein sequences (Figure 2).

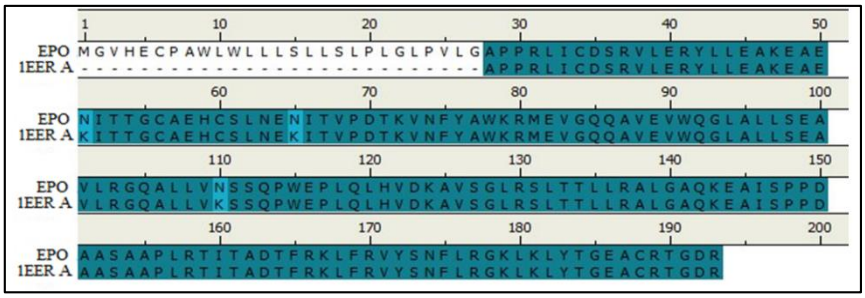

Figure 2. Alignment of EPO protein with template. Dull blue represents the identical residues, Cyan strongly similar residues

As a result, the EER protein sequence is thought to be sufficiently matched with the phylogenetically related EPO protein sequences to produce a precise and trustworthy 3D structure. With the help of loop modelling and energy minimization, the 3D model is further improved using 4.1.0 Swiss PDB Viewer (Kaplan, 2001). Figure 3 depicts the 3D homology inferred model.

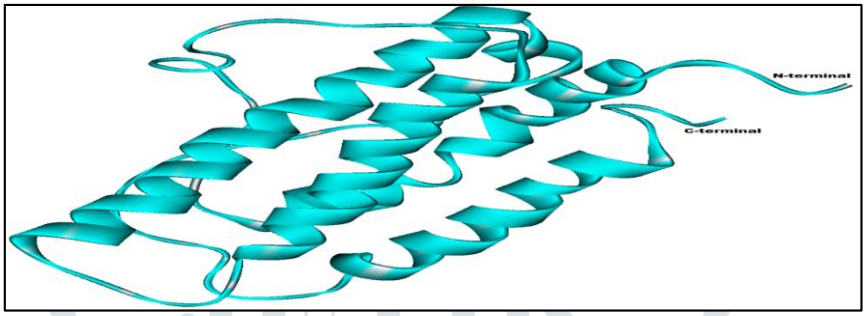

Figure.3 Three of EPO protein Dimensional (3D) Structure**.**

The Ramachandran plot (Figure 4) from the PROCHECK server, verifies the stereochemical quality of the EPO protein structure. The 3D model of EPO protein is validated by Ramachandran plot statistics showing 93.9% and 6.1%of residues are in most energetically favored region, additionally allowed region respectively and none of the residues found in disallowed region which shows that the predicted 3D model of EPO protein is a good quality model.

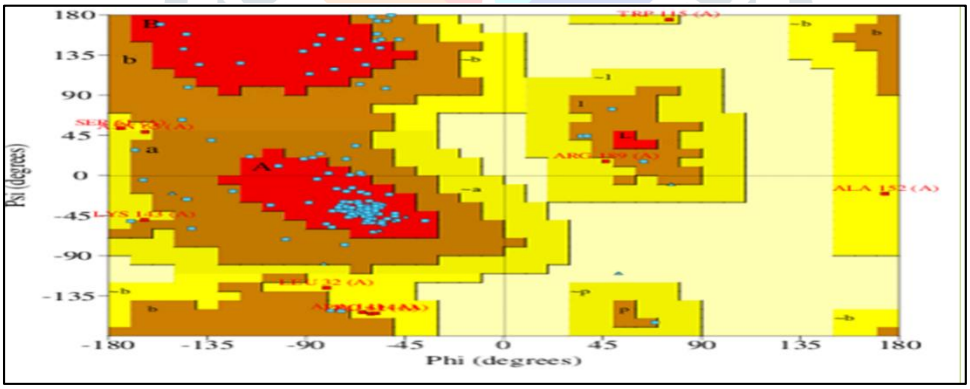

Figure.4 Stereo chemical quality of the EPO protein.

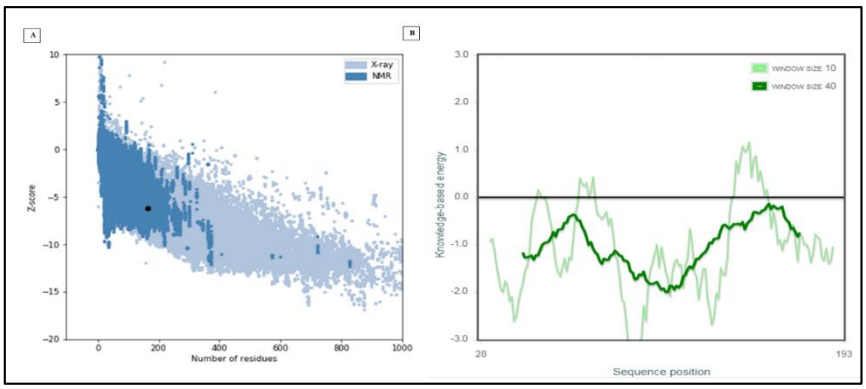

Figure 5A) Overall and local model quality assessment of the EPO. The ProSA plot represented for all the proteins in PDB determined by X-ray crystallography (light blue) and by NMR Spectroscopy (dark blue). 5B) The knowledge-based energy profile of the individual amino acids on a window size 10 amino acids (light green) and window size 40 amino acids (dark green).

The ProSA server assess the EPO's overall model quality (Fig. 5A & B). By comparing the target protein's overall and local model quality to other proteins with similar-length amino acid sequences deposited in the PDB, the ProSA web service confirms the quality of those models. The Z-score (Black dot) in ProSA plot for the EPO protein in **Figure 5A**, which represent the total energy difference between the EPO structure and the crystal structures of other proteins with the same number of amino acids, is -6.15. All the amino acids are in the negative region of **Figure 5B**'s knowledge-based energy profile of the individual amino acids, demonstrating the stability and dependability of the 3D model.

Figure 6 illustrates the conformational compatibility of an atomic model of the EPO protein (3D - 1D profile) with its amino acid sequence (1D) score of 81% (A score of 0.2 for >80% of the amino acid residues)as acquired from the Verify 3D server (Eisenberg, 1997) is considered as reliable.

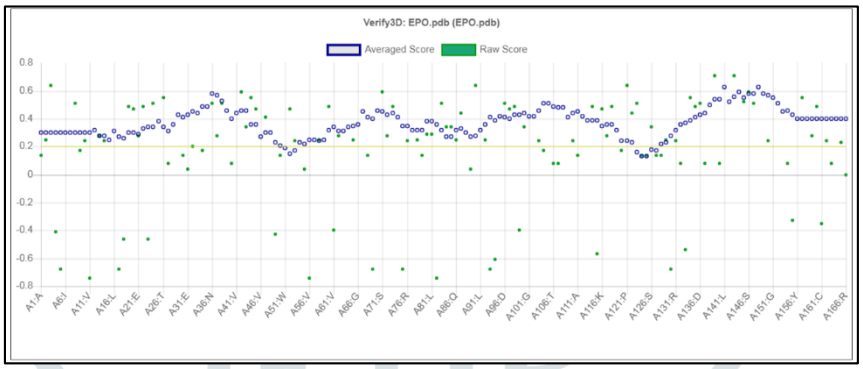

Figure 6. 3D – 1D profile of EPO protein from Verify 3D**.**

The 3D model of EPO protein is submitted to PDBsum server for secondary structure analysis as shown in **Figure 7** has 5 helices, 7 helix – helix interactions, 13 beta sheets, 1 gamma turn and 2 disulphides. Figure 7 show the secondary structure, the amino acid range of helix H1 is from Ser36 to Thr53, helix H2 is from Phe75 to Trp78, helix H3 is from Glu82 to Asn110, helix H4 is from Gln119 to Ala138, and helix H5 is from Thr164 TO Cys188. A hydrophobic Pi Sigma interaction A:TYR42 - A:LEU39:CA (3.62305Å), and several Pi Anion interactions such as A:LEU182:CD1 - A:TRP78 (3.26498Å), A:ARG31 - A:CYS34 (4.26478 Å), A:LEU32 - A:LEU139 (5.37706 Å), A:ILE33 - A:LEU135(4.75592 Å), A:VAL68 - A:LYS167 (4.62884 Å), A:VAL68 - A:VAL171 (4.60492 Å), A:PRO69 - A:LEU96 (4.83894 Å), A:PRO69 - A:LEU97(4.35685 Å), A:LEU39 - A:LEU180 (5.49437 Å), A:PRO69 - A:VAL171 (5.19369 Å), A:ALA87 - A:LYS143(5.43371Å), A:ALA49:C,O;GLU50:N - A:PHE169 (5.00348 Å) A:VAL90 - A:LYS179(4.31806 Å), A:LEU94 - A:LEU132 (5.33403 Å) etc., Salt bridges A:LYS124:NZ - A:GLU45:OE2 (2.65839 Å), A:ARG170:NH1 - A:GLU50:OE1 (3.22652 Å) and A:ARG189:NH1 - A:GLU186:OE1 (3.26755 Å). And two electrostatic interactions A: ARG41:NH2 - A: GLU45:OE2 (5.4641 Å) and A: LYS179:NZ - A:GLU89:OE2 (4.16365 Å) and two Disulphides bridges between CYS 34 – CYS 188 & CYS 56 – CYS 60 which add extra stability to the structure.

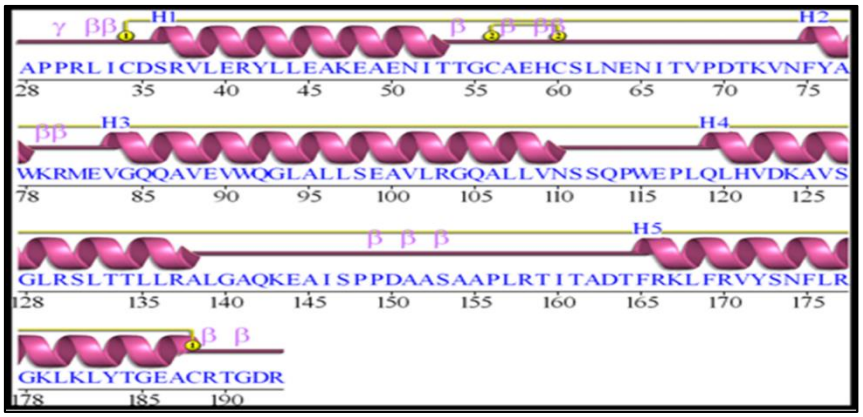

Figure 7. The secondary structure obtained by PDBSUM server.

The NCBI – BLASTp server results show that the target EPO protein sequence has EPO_TPO super family domain from amino acid 31 to 193 in the N-terminal region, suggesting a EPO loop domain as shown in **Figure. 8**. By examining the two interfaces in the protein-protein complexes of EPO and EPOR, it is possible to understand the binding site area in EPO that is crucial for the activation of ERs implicated in angiogenesis. The EPO binding pockets involved in the

interaction with its native receptor for the signaling cascade are identified by the CASTp server and active site prediction server.

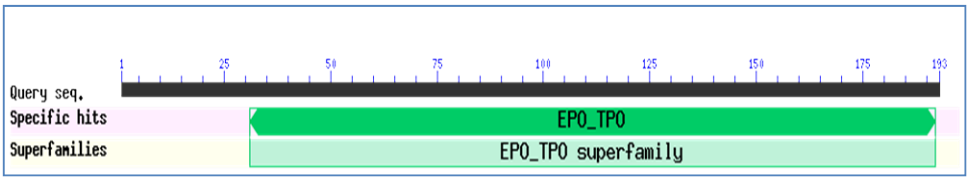

Figure. 8 Conserved domain of the EPO protein.

Table 2. Active site residues.

Server	Cavity Volume	Active Site Residues[$]
Castp	174.029 147.664	PRO69, ASP70, VAL73, ASN 74, ALA77, TRP78, MET81, GLU89, GLN92, GLY93, LEU96, ALA152, PRO156, LEU157, ARG158, THR159, LYS179.
Active site prediction	-	ALA, PHE, TYR, LEU, ARG, GLU, VAL, LSY, SER, GLY, THR, ILE, ASP, PRO

[$]*Amino Acid residues represented as three letter codes.*

Two binding pockets found in the conserved region, as indicated in Table 2.To identify the specific amino acid residues involved in binding, the Protein-Protein Interaction (PPI) studies were conducted between EPO and EPOR using the Patch Dock server. The results of PPI are shown in table 3. The interactions between EPO and its receptor (PPI) involve THR71, ARG158, LYS167, LYS179, GLY185, ASP192, and ARG193 residues are identified as active site residues as shown in Figure 9.

Table 3. Interactions of Protein-Protein docking of EPO with its receptor.

Type of Bond	Interactions (Donor – Acceptor)*	Distance (Å)
Salt Bridge	A:LYS167:NZ - B:ASP61:OD1	3.13519
Salt Bridge	A:ARG170:NH1 - B:GLU97:OE1	2.63382
Salt Bridge	A:LYS179:NZ - B:GLU117:OE2	1.30536
Hydrogen Bond	A:THR71:OG1 - B:GLU60:OE1	1.71888
Carbon Hydrogen Bond	B:ARG155:CD - A:ASP192:O	3.78749
	B:PRO203:CD - A:GLY185:O	3.06597
Carbon Hydrogen Bond	A:GLY185:CA - B:SER204:OG	3.39711
	A: ARG158:CA - B: ILE174:O	3.54721
Carbon Hydrogen Bond		
Carbon Hydrogen Bond		

**Amino Acid residues represented as three letter codes.*

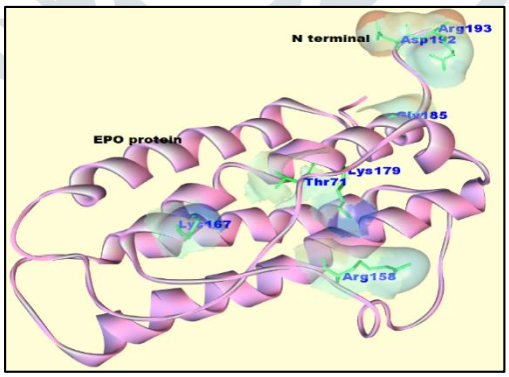

Figure 9. Active site residues in EPO protein. EPO protein

Phytochemical compounds such as coumarins and flavonoids have a wide range of pharmacological effects, including anticancer activities (Pratheeshsh, 2012). Among coumarins, dentatin (C20H22O4) and nordentatin (C19H20O4) from Clausena excavata have demonstrated strong cytotoxicity against several cancer cell lines (Sripisut, 2012; Arbab, 2012; Promsuwan, 2013). Moreover, the flavonoid quercetin (3,3 ,4 ,5,7-pentahydroxy-flavone) is commonly found in fruits and vegetables and affects various biological functions, in part culminating in antineoplastic properties (Hashemzaei, 2017).

Three anti – apoptotic compounds (Nordentatin, Dentatin, and Quercetin) were subjected to docking with the 3D model of EPO protein using the PyRx Virtual Screening software and AutoDock Vina. Figure 10 shows the docking interaction of the target protein with ligands.According to docking data, the compounds are docked with the predicted 3D

model of EPO in a range of -6.8 to -7.4 kcal/mole binding energy. The Nordentatin bind with the target protein with the highest binding affinity as shown in Table 4.

Table 4. Interactions of EPO amino acid residues with ligands at receptor sites.

S. No	Ligand (UNK)	Binding Energy, (kcal/mol)	Interactions (Donor – Acceptor)	Distances
1	DEN	-6.8	A:LYS179:HZ1 - :UNK:O15	2.3170
2	NOR	-7.6	A:ARG158:HE - :UNK:O12	2.4743
			A:ARG158:NH1 - :UNK	6.3144
			A:ARG158:NH1 - :UNK	5.5002
			A:ARG158:NH2 - :UNK	4.2256
			A:LYS179:NZ - :UNK	4.9616
			A:LYS179:NZ - :UNK	6.0318
3.	QUE	-7.4	:UNK:H19 - A:GLN92:O	2.5530
			:UNK:H20 - A:ASP70:OD2	2.4064
			A:GLY93:CA - :UNK:O18	3.3231
			A:LYS179:NZ - :UNK	4.3197

The molecular interaction investigations also revealed that the EPO model is bound with more than one active site residue (ARG158 and LYS 179) for Nordentatin, whereas Dentatin and Quercetin is bound with only one active site residue as shown in Figure 10. Nordentatin and Quercetin have docking sites that substantially overlap with the protein pockets, and could be considered as a possible therapeutic drugs. Molecular docking algorithms has become an increasingly common technique to drug research, due to the lower time and financial expenses of in silico drug screening compared to traditional laboratory experiments.

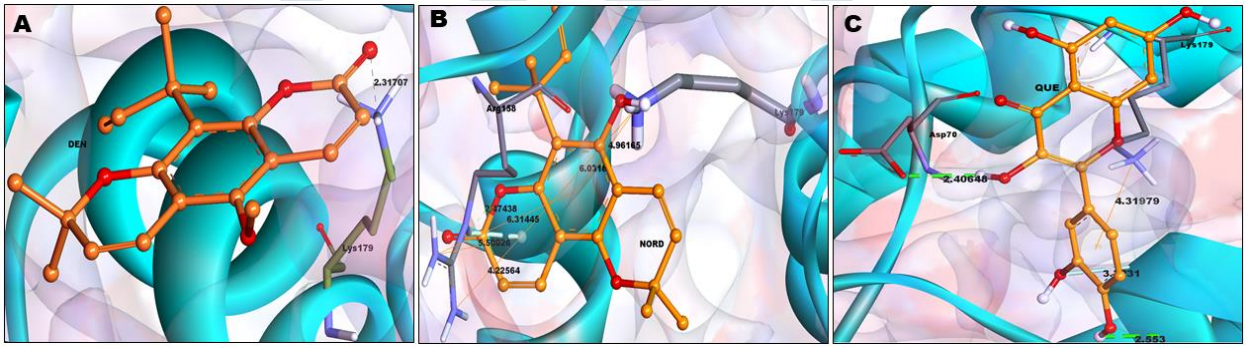

Figure 10. Docked interactions of EPO with ligands. EPO protein represented as Cyan ribbon, Ligands A) DEN, B) NORD, and C) QUE.

The Nordentatin-EPO interactions in the current study exhibited the biggest negative G values, but significant negative changes in G levels suggested that Quercetin and Nordentatin had high binding affinity. We conclude that all of the ligands that have been studied have the capacity to form solid, long-lasting complexes with the EPO protein.

The results specifically show that Nordentatin formed hydrophobic interactions with only three residues (A: ASP70, A: ARG158, A: LYS179), but only one (LYS179) of these residues as those involved in binding to Dentatin and two residues (A: ASP70, A: LYS179) for Quercetin. In particular, LYS179 was identified in all hydrophobic interactions of all ligands with EPO.

Although we identified an amino acid that was involved in electrostatic interactions between NOR and QUE and EPO (A: ARG158, A: LYS179), no electrostatic interactions were present in DEN – EPO complexes. Increasing evidence indicates that EPO is a unique member of Trypsin like serine protease family and is highly expressed and induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in numerous human cancers (Ribatti, 1999). Hence, restoring apoptotic activities by targeting EPO is widely considered as a viable strategy for inhibiting cancer cell growth and overcoming the anti VEGF resistance. EPO overexpression in cancer cells was also inactivated or blocked in previous studies of small molecules such as siRNA, monoclonal antibodies, and synthetic or natural compounds. In keeping with these findings, our in silico analyses show that the natural compounds Dentatin, Nordentatin, and Quercetin have potential as ligands that inhibit the anti-apoptotic EPO protein

Conclusion - To achieve optimistic results in inhibition of drug resistance by blocking of EPO protein and a deep understanding of protein at structural level is essential. In the present study, EPO is selected as target and its 3D Structure, active site identification, docking and the physicochemical properties were carried out using in silico techniques. 3D structure (Tertiary) is generated by considering 1EER – A template. The 3D model generated has 93.9% and 6.1%of residues in most energetically favored region and additionally allowed region of Ramachandran Plot, with a z score of -6.15 in ProSA indicating a similarity to NMR resolved protein structure. I*n silico* tool Verify 3D shows that >80% amino acids are in the acceptable energy range indicating the reliability of the 3D Model of EPO.

The binding energy of the three compounds docked with the 3D model of EPO are in a range -6.8 to -7.4 kcal/mole. The Nordentatin bind with the target protein with the highest binding affinity. In silico analyses show that the natural compounds Dentatin, Nordentatin, and Quercetin are potential inhibitors for restoring apoptotic activities in cancer cells and act through interactions with anti-apoptotic EPO protein, and can be further modified. Further in vitro and in vivo experiments are required to validate these in silico results.

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REFERENCES

[1] A. Šali, L. Potterton, F. Yuan, H. van Vlijmen, M. Karplus, 1995. Proteins Struct. Funct. Genet.23, DOI 10.1002/prot.340230306.

[2] Abhold E, Rahimy E, Wang-Rodriguez J, Blair KJ, Yu MA, Brumund KT, et al. Recombinant human erythropoietin promotes the acquisition of a malignant phenotype in head and neck squamous cell carcinoma cell lines in vitro. *BMC Res Notes* (2011) 4:553. 10.1186/1756-0500-4-553

[3] Abraham MJ. Murtola T., Schulz R., Páll S., Smith J.C., Hess B., and Lindahl E. 2015. "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," SoftwareX **1–2:** 19–25.

[4] Akiyama M, Kawano T, Mikami-Terao Y, Agawa-Ohta M, Yamada O, Ida H, et al. Erythropoietin activates telomerase through transcriptional and posttranscriptional regulation in human erythroleukemic JAS-REN-A cells. *Leuk Res* (2011) 35(3):416–8. 10.1016/j.leukres.2010.11.002

[5] Arbab I A, et al. 2012 Dentatin induces apoptosis in prostate cancer cells via Bcl-2, Bcl-x survivin downregulation, caspase-9, -3/7 activation, and NF-κB inhibition Evid. Based. Complement. Alternat. Med. 2012 1–15

.

[6] Bing Zhou, Jeffrey S. Damrauer T. , Sean Bailey, Tanja Hadzic, YoungtaeJeong, et al. 2014. Erythropoietin promotes breast tumorigenesis through tumor-initiating cell self-renewal. J Clin Invest. 124(12): 553 - 563.doi:10.1172/JCI69804.

[7]Brigitte B. Amos B., Rolf A, Marie-Claude B, Anne E, Elisabeth G. 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003, Nucleic Acids Research. 31 (1): 365–370.

[8] Cao Y, et al. 2011. Forty-year journey of angiogenesis translational research. SciTransl Med. 3:114rv3.

[9] Casanovas O, Hicklin DJ, Bergers G, Hanahan D, 2005. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors.Cancer Cell. 8:299–309.

[10] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov and H.J. Wolfson. 2005. Patch Dock and Symm Dock: servers for rigid and symmetric docking. Nucleic Acids Res. 33 (2): W363 – W367.

[11] DrozdetskiyA, Cole C, Procter J & Barton GJ, 2015. Jpred4: a protein secondary structure prediction server. nucl. acids res. 1;43(w1):w389-94. doi: 10.1093/nar/gkv332.

[12] Du X, Li Y, Xia Y L, Ai S M, Liang J, Sang P, Ji X L and Liu S Q. 2016Insights into protein– ligand interactions: mechanisms, models, and methods. Int J Mol Sci. 17: 144.

[13] E. Gasteiger, 2003.EXPASy: The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res. 31: 3784 – 3788.

[14] Eckardt KU, Ratcliffe PJ, Tan CC, Bauer C, Kurtz A. 1992. Age-dependent expression of the erythropoietin gene in rat liver and kidneys. J Clin Invest. 89:753–760.

[15] Eisenberg D, Lu¨thy R, Bowie JU. VERIFY3D: assessment of protein models with three-dimensional profiles. In: Abelson J, Simon M, Verdine G, Pyle A, editors. Methods in enzymology, vol. 277. New York: Academic Press; 1997. p. 396–404.

[16] Guex, N., and Petish, M.C.1997. SWISS-MODEL and the Swiss PdbViewer: an environment for comparative protein modelling. Electrophoresis. 18: 14-23.

[17] Hashemzaei M, et al. 2017 Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncol. Rep. 38 819–28

[18] J. Dundas, Z. Ouyang, J. Tseng, A. Binkowski, Y. Turpaz and J. Liang.2006.CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. Nucleic Acids Res. 34: W116 – W118.

[19]J.A. Cuff and G.J. Barton, 1999. Evaluation and improvement of multiple sequence methods for protein secondary structure prediction. Proteins. 34: 508 – 519.

[20] Jaghoori M M, Bleijlevens B and Olabarriaga S D, 2016. 1001 Ways to run AutoDock Vina for virtual screening J Comput Aided Mol. 30: 237–249

[21] Jain RK. 2014. Antiangiogenesis strategies revisited: From starving tumors to allevi-ating hypoxia. Cancer Cell. 26:605–622.

[22] Jelkmann W, 2008. Developments in the therapeutic use of erythropoiesis stimulating agents. Br J Haematol.141(3):287–297.

[23] Johansson MU, Zoete V, Michielin O, Guex N. 2012. Defining and searching for structural motifs using DeepView/SwissPdbViewer. BMC Bioinform. 13:173.

[24] Johnson M., Zaretskaya I., Raytselis Y., Merezhuk Y., McGinnis S., & Madden TL, 2008. NCBI BLAST: a better web interface. Nucleic Acids Res. 36(Web Server issue): W5–W9.

[25] Hedley BD, Chu JE, Ormond DG, Beausoleil MS, Boasie A, Allan AL, et al. Recombinant human erythropoietin in combination with chemotherapy increases breast cancer metastasis in preclinical mouse models. *Clin Cancer Res* (2011) 17(19):6151–62. 10.1158/1078-0432.CCR-10-3298.

[26] K. Bhargavi, P.K. Chaitanya, D. Ramasree, M. Vasavi, D.K. Murthy and V. Uma. 2010. Homology modeling and Docking studies of Huma Bcl – 2L10 protein. J. Biomol. Struct. Dyn. 28: 379 - 391.

[27] Kaplan, W., &Litt Kaplan, W., & Littlejohn, T. G. 2001. Swiss-PDB Viewer (Deep View). Briefings in bioinformatics. 2(2): 195–197.

[28] Lopez TV, Lappin TR, Maxwell P, Shi Z, Lopez-Marure R, Aguilar C, et al. Autocrine/paracrine erythropoietin signalling promotes JAK/STAT-dependent proliferation of human cervical cancer cells. *Int J Cancer* (2011) 129(11):2566–76. 10.1002/ijc.25935.

[29]LA Kelley et al, 2015. The Phyre2 web portal for protein modelling, prediction and analysis, Nature Protocols, 10; 845 – 858.

[30] Laskowski RA, MacArthur MW, Moss DS, Thornton JM, 1993. PROCHECK: a program to check the stereochemical quality of protein structures. J ApplCrystallogr.26:283–91.

[31] Lipinski CA. 2016. Rule of five in 2015 and beyond: target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions Adv. Drug. Delivery. Rev.101: 34–41.

[32] Masaki Nakamura, Yin Zhang, Yunlong Yang, CeylanSonmez, Wenyi Zheng, Guichun Huang, Takahiro Seki, et al. 2017. Off-tumor targets compromise antiangiogenic drug sensitivity by inducing kidney erythropoietin production. PNAS. E9635–E9644.

[33] Melo,F. and Feytmans,E. 1998. Assessing protein structures with a non-local atomic interaction energy. J. Mol. Biol.277: 1141–1152.

[34] Miyake M, Goodison S, Lawton A, Zhang G, Gomes-Giacoia E, Rosser CJ. Erythropoietin is a JAK2 and ERK1/2 effector that can promote renal tumor cell proliferation under hypoxic conditions. *J Hematol Oncol* (2013) 6:65. 10.1186/1756-8722-6-65
.

[35] Navaneetha N., Kiran Kumar M, Vasavi M, Bhargavi K, SaritaRajender P et al. 2012. Angiogenesis: an insilico approach to angiogenic phenotype. J Phar Res. 5 (1): 583 - 588.

[36] Pratheeshkumar P, et al. 2012 Cancer prevention with promising natural products: mechanisms of action and molecular targets Anticancer agents Med. Chem. 12 1159–84
.

[37] Pérès EA, Valable S, Guillamo JS, Marteau L, Bernaudin JF, Roussel S, et al. Targeting the erythropoietin receptor on glioma cells reduces tumour growth. *Exp Cell Res* (2011) 317(16):2321–32. 10.1016/j.yexcr.2011.06.011

[38] Promsuwan P and Yenjai C 2013 Synthesis and cytotoxicity of coumarin derivates and nordentatin Asian J. Chem. 25 3629–32

[39] Ribatti D, et al. 1999. Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood. 93: 2627–2636.

[40] Sripisut T, Cheenpracha S and Ritthiwigrom T 2012 Chemical constituents from the roots of Clausena excavata and their cytotoxicity Rec. Nat. Prod. 6 386–89.
.

[41] Trott O and Olson A J. 2010. AutoDockVina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem. 31: 455– 461.

[42] V. Ahola, T. Aittokallio, M. Vihinen and E. Uusipaikka. 2006. A Statistical score for assessing the quality of multiple sequene alignments. BMC Bioinformatics. 7: 484.

[43] Wiederstein M, Sippl MJ. 2007. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 35(suppl_2):W407–10

[44] Wu P, Zhang N, Wang X, Zhang C, Li T, Ning X, et al. The erythropoietin/erythropoietin receptor signaling pathway promotes growth and invasion abilities in human renal carcinoma cells. *PLoS One* (2012) 7(9):e45122. 10.1371/journal.pone.0045122

[45]Yasuda Y, Fujita Y, Masuda S, Musha T, Ueda K, Tanaka H, et al. Erythropoietin is involved in growth and angiogenesis in malignant tumours of female reproductive organs. *Carcinogenesis* (2002) 23(11):1797–805. 10.1093/carcin/23.11.1797

[46] Zhang M Q and Wilkinson B. 2007. Drug discovery beyond the “rule-of-five”. CurrOpin Biotech. 18: 478–488.