A study of the effect of Paclitaxel, Docetaxel and Tamoxifen citrate compounds found in *Taxus wallichiana* on the Beta-tubulin and Estrogen Receptor (ESR) proteins in humans using a bioinformatics approach

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Abstract. *Taxus wallichiana*, an indigenous plant of North East India contains bioactive compounds such as Tamoxifen citrate and taxol (paclitaxel and docetaxel) which inhibit the growth of cancerous cells. Tamoxifen citrate in its active form competitively inhibits the binding of estradiol or estrogen with estrogen receptor and results in tumor suppression whereas the taxol compounds stabilize the tubulin protein in microtubules and inhibit cell division in cancerous cells. In this approach, important domains and motifs of the protein receptors were studied and analyzed followed by homology modeling and its validation. Molecular docking studies were carried out via tubulin and estrogen receptor with paclitaxel, docetaxel, and endoxifen (an active form of tamoxifen citrate). Hence it was verified that tubulin and estrogen receptor contain binding sites for the bioactive compounds found in *T. wallichiana* and these bioactive compounds after binding with these receptors result in the prevention of the proliferation of the tumorous cells by stabilizing the microtubules and preventing the intake of steroidal hormones by cells respectively.

Keywords: Bioactive compounds, domains, homology modelling, molecular docking, taxol

INTRODUCTION

Taxol™, an anti-cancerous compound is present abundantly in conifer genera of *Taxus* and was first isolated from the bark of *Taxus brevifolia* (Pacific Yew) (Wani *et al.*, 1971), in 1969 with a yield of 0.01%. The molecule was further investigated for its isolation from the wood and the needles of the plant and consequently, the first structure was reported in 1971 (Kingston, 2007; Kingston, 1990).

Before its importance as an anti-cancer compound, various studies have been reported on the bioactive compounds especially known as

taxoids (Taxoid 2- α -hydroxylase, Taxoid 14- β -hydroxylase) from the genus *Taxus* (Wani *et al.*, 1971). One of the species of this genus, which is found specifically in the Himalayan region of India especially in the North-Eastern states, is *T. wallichiana* commonly known as East Himalayan Yew or Himalayan yew. This plant is declared as a threatened plant species by the International Union for Conservation of Nature (IUCN) (Thomas *et al.*, 2011). This is an evergreen tree is found at altitudes between 1800 and 3300 m above mean sea level (MSL) in the temperate

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Himalayas and the hills of Meghalaya and Manipur at an altitude of 1500 m. This plant also contains another anti-cancerous compound called Tamoxifen citrate (Juyal *et al.*, 2014; Sharma *et al.*, 2015).

T. wallichiana is an ethnobotanically important species and has been used by the native for treating various ailments. Its uses have been described in traditional medicines such as Ayurveda and Unani. Along with anti-cancerous compounds it also contains compounds which are anti-convulsant, analgesic, antipyretic, anti-inflammatory, anti-allergic, anti-bacterial, anti-fungal and many other properties (Juyal *et al.*, 2014; Nissar *et al.*, 2008; Khan *et al.*, 2011; Qayum *et al.*, 2012; Nissar *et al.*, 2008).

The first evidence of taxol activity with responses in melanoma was reported in 1987, in ovarian cancer in 1989 and in breast cancer in 1991. Overview of the Taxol biosynthetic pathway is given in Figure 1. The boxed MEP (Methylerythritol Phosphate) Pathway is the plastidial route from pyruvate and glyceraldehyde 3-phosphate (via methylerythritol phosphate) for C5 (IPP and DMAPP) precursor supply. The abbreviations are IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; IPPI, isopentenyl diphosphate isomerase; GGPPS, geranylgeranyl diphosphate synthase; TS, taxadiene synthase; and PAM, phenylalanine aminomutase. OPP denotes the diphosphate moiety; Ac and Bz denote acetyl and benzoyl groups, respectively. Because the relative order of C1 hydroxylation and oxetane formation is uncertain, the hypothetical intermediate illustrated could be at the level of an acylated hexanol rather than a heptanol bearing the C1 hydroxyl (Kovacs *et al.*, 2007; Amos *et al.*, 1999; Schiff *et al.*, 1980).

Taxol and its semisynthetic analog docetaxel (Taxotere™) are now used (either as single agents or in combination with other drugs such as cisplatin) for the treatment of ovarian cancer, breast cancer, and non-small-cell lung cancer (Weirnik *et al.*, 1987; McGuire *et al.*, 1989; Holmes *et al.*, 1991; Guirritte *et al.*, 1986; Piccart *et al.*, 2003; Ozols, 2003). Paclitaxel consists of an eight-member taxane ring with a four-member oxetane ring and a bulky ester side chain at C-13 that is necessary for antitumor activity (Thomas *et al.*, 2011). The chemical formula of paclitaxel is

C₄₇H₅₁O₁₄ and its molecular weight is 853.9 g/mol. Docetaxel differs from paclitaxel in the 10-position on the baccatin ring and in the 3'-position of the lateral chain and has a chemical formula of C₄₃H₅₃NO₁₄ and a molecular weight of 807.9 g/mol. The compounds found in *T. wallichiana* are Paclitaxel and Docetaxel (Davies *et al.*, 2003), which bind at the taxane pocket of the beta chain of the tubulin protein, which in return stabilizes the microtubules during cell division. The disintegration of microtubules is required for the formation of two new daughter cells at the end of cell division, and thus stabilizing these microtubules prevents further cell division and hence prevents cancerous cells from dividing rapidly (Long *et al.*, 1994; Sakkaraiyyan *et al.*, 2005).

On the other hand, Tamoxifen citrate in its active form i.e endoxifen competitively binds with estrogen receptor α (ER- α) instead of estradiol and results in the lowering of cell division of the cells. Studies have shown that there is a direct connection between the increased responses of ER- α with most cases of cancer in mammary glands. Endoxifen thus blocks the working of ER- α and significantly lowers the rate of cancerous cell division (Fisher *et al.*, 1989; Fisher *et al.*, 1996; Yu *et al.*, 2001; Rowinsky *et al.*, 1995; Suffness *et al.*, 1993). Biosynthesis of the anticancer drug Taxol in *Taxus* (yew) species involves 19 steps from the universal diterpenoid progenitor geranylgeranyl diphosphate (Croteau *et al.*, 2006).

Beta-Tubulin is a subunit of tubulin. Tubulin is one of several members of a small family of globular proteins. It is the major constituent of microtubules. There are two of the most common members of the tubulin family: alpha-tubulin and beta-tubulin, and together their dimers form microtubules. The dimers of alpha- and beta-tubulin bind to GTP and assemble onto the (+) ends of microtubules while in the GTP-bound state. After the dimer is incorporated into the microtubule, the molecule of GTP bound to the beta-tubulin subunit eventually hydrolyzes into GDP through inter-dimer contacts along the microtubule protofilament. Beta-tubulin faces the plus end of the microtubule while alpha-tubulin faces the minus end (Heald *et al.*, 2002; Howard *et al.*, 2003). In the context of cancer, the tubulin family of proteins is recognized as the target of the tubulin-binding chemotherapeutics, which

suppresses the dynamics of the mitotic spindle to cause mitotic arrest and cell death. Also, changes in the microtubule stability and the expression of

different tubulin isotypes as well as altered post-translational modifications have been stated for a variety of cancers (Amelia *et al.*, 2014).

Figure 1. Overview of the Taxol biosynthetic pathway (Croteau *et al.*, 2006).

MATERIALS AND METHOD

Data set

The taxol compounds, Paclitaxel and Docetaxel have PubChem CID 36314 and 148124 respectively. And Tamoxifen citrate along with its active form Endoxifen has PubChem CID 2733525 and 10090750. These compounds were all retrieved from the PubChem compound (Wang *et al.*, 2009). The FASTA sequence for

Estrogen Receptor (ESR) and Beta-tubulin protein of *Homo sapiens* were retrieved from NCBI (National Center for Biotechnology Information) with accession number AAA52399.1 and AAB59507.1 respectively and Gene Information - 182193 and 338695 respectively.

Domain and motif analysis

After the sequences for the proteins Estrogen Receptor and Beta -tubulin were retrieved, their domain and motifs were analysed using cDART

(Conserved Domain Architecture Retrieval Tool) and Motif Scan (Geer *et al.*, 2002; Marchler *et al.*, 2007). The domains and their superfamilies were accessed using NCBI Conserved Domain Database (Pagni *et al.*, 2007).

Homology modelling

Using Swiss-Model server (Guex *et al.*, 2009), protein homology modelling was carried out for both Estrogen Receptor (ESR) and Beta-tubulin after their FASTA sequences were retrieved from NCBI (Biasini *et al.*, 2014; Bienert *et al.*, 2017; Bertoni *et al.*, 2007). And these structures were further validated using Ramachandran Plot via PROCHECK (Laskowski *et al.*, 1993; Laskowski *et al.*, 1996). The templates generated were 4ZNH and 5IJ0 as PDB IDs respectively which had the highest similarity percentage and were viewed using Chimera 1.11.2 (Pettersen *et al.*, 2004).

Molecular docking

Molecular docking studies were carried out using the PATCHDOCK server (Duhovny *et al.*, 2002; Schneidman-Duhovny *et al.*, 2005) and the energy minimization was carried out with QMEAN server (Benkert *et al.*, 2009) and the molecular docking results were further refined with the help of FIREDOCK (Andruiser *et al.*, 2007; Mashiach *et al.*, 2008). The refined docked structures were viewed using Chimera 1.11.2 (Pettersen *et al.*, 2004) and further analysis of the amino acids present in the active site was analyzed using Pymol (DeLano, 2002). Further analysis means the positions of the amino acids in the docking area in their respective chains. Energy minimization methods can precisely locate minimum confirmation by mathematically "homing in" on the energy function minima. The goal of energy minimization is to find a route from an initial confirmation to the nearest minimum-energy confirmation using the smallest number of calculations possible. Energy minimization can repair the distorted geometric by moving atoms to release internal constraints. The refinement of docked structures aims at side chain optimization. This method targets the problem of flexibility and scoring of solutions (docked structures).

RESULTS

Domain and motif analysis

After the analysis of the domains of the protein receptors, it is found that Estrogen receptor has four domains of four different superfamilies whereas tubulin protein has only one domain. The four superfamilies of the estrogen receptor are Oestrogen receptor superfamily, DNA binding domain of nuclear receptors, ligand binding domain of nuclear receptors and oestrogen type nuclear receptor final C-terminal. The total architecture was found to be 265. These domains are found in the taxonomic spans of tetrapoda, gnathostomata, metazoan, bilateria, dipnoterapodomorpha and other taxonomic categories (Figure 2a). Tubulin protein has only one domain of tubulin superfamily and is found in euteleostomi, poecilia, eutheria, thaumarchaeota, gossypium and in other cellular organisms. The total architecture is found to be 45 (Figure 2b). According to the motif analysis, the motifs of each protein are located along with the analysis of these proteins. Result of the motif analysis of Estrogen receptor, Ligand-binding domain of nuclear hormone receptor -pos.: 351-543, raw-score =177.9, N-score =60.047, E-value =1.9e-53 (Figure 3a). (Oest_recep) Oestrogen receptor - pos.: 1-181, raw-score = 464.0, N-score =144.636, E-value = 4.9e-138 (Figure 3b). (SapB_2) Saposin-like type B, region 2- pos.: 527-547, raw-score = 9.5, N-score = 10.122, E-value = 0.0016 (Figure 3c). (zf-C4) Zinc finger, C4 type (two domains)- pos.: 183-258, raw-score = 178.2, N-score = 60.984, E-value = 2.2e-54 (Figure 3d). Results of the motif analysis of tubulin protein, Sigma-54 interaction domain (Sigma54_activat) - pos.: 367-381, raw-score = 4.9, N-score = 9.735, E-value = 0.0039 (Figure 4a). Tubulin/FtsZ family, GTPase domain - pos.: 3-243, raw-score = 338.0, N-score =111.096, E-value = 1.7e-104 (Fig 4b). Tubulin/FtsZ family, C-terminal domain-pos.: 246-383, raw-score = 279.9, N-score = 89.212, E-value = 1.3e-82 (Figure 4c). Coagulation Factor V LSPD Repeat (LSPR) - pos.: 284-292, raw-score = 1.4, N-score = 6.746, E-value = 3.8 (Figure 4d, Table 1).

Figure 2. (a) Domain analysis of Estrogen receptor shows 265 total architectures encompassing Oest_recep, NR|_DBD_like, NR_LBD, ESR1_C superfamily, (b) Domain analysis of Tubulin shows 45 total architecture encompassing Tubulin superfamily.

Figure 3. Motif analysis of tubulin protein (a) sigma – 54 interaction domain (b) GTPase domain (c) C-terminal domain and (d) Coagulation factor VLSPD Repeat (V LSPR), from investigating databases which includes HAMAP profiles [hamap], Pfam HMMs (local models) [pfam_fs], Pfam HMMs (global models) [pfam_ls].

Figure 4. Motif analysis of Estrogen receptor. (a) ligand binding domain of nuclear hormones (b) Oestrogen receptor (c) Saposin like type B (d) Zinc finger, from investigating databases which includes HAMAP profiles [hamap], Pfam HMMs (local models) [pfam_fs], Pfam HMMs (global models) [pfam_ls].

Table 1. Showing the motifs/domains present in the Estrogen Receptor and also in the Tubulin protein. It includes their position(s), raw score, N-score, and E-value.

	Motif(s)	Position	Raw score	N score	E-value
Motif analysis of the Estrogen Receptor	Ligand binding domain of nuclear hormone receptor	351-543	177.9	60.047	1.9e-53
	Oestrogen Receptor	1-181	464.0	144.636	4.9e-138
	Saposin -like type B, region 2	527-547	9.5	10.122	0.0016
	Zinc finger, C4 type domain	183-258	178.2	60.984	2.2e-54
Motif analysis of Tubulin protein	Sigma -54 interaction domain	367-381	4.9	9.735	0.0039
	Tubulin/FtsZ family, GTPase domain	3-243	338.0	111.096	1.7e-104
	Tubulin/FtsZ family, C-terminal	246-383	279.9	89.212	1.3e-82
	Coagulation Factor V, LSPD Repeat (LSPR)	284-292	1.4	6.746	3.8

Homology modeling and validation

In the present work, the protein molecules i.e. Estrogen Receptor and Beta Tubulin were initially explored for the best homology modeling which determines the overall 3D structure of the amino acids present in the enzyme molecule. The homology modeling using the Swiss-Dock server depicted the best model of the enzyme molecule Estrogen Receptor (Figure 5a) and Beta Tubulin (Figure 6a). These structures were further validated by the corresponding Ramachandran Plot for Estrogen Receptor (Figure 5b) and Beta tubulin (Figure 6b) respectively. For Estrogen Receptor, the Ramachandran Plot depicted 1 of amino acids in the disallowed region with the maximum number of amino acid molecules in the favorable region. Most favorable regions showing 95.4% having 414 residues, additional allowed region showing 4.1% having 18 residues and generously allowed region showing 0.2% having only 1 residue. There was 1 residue found in the disallowed region. The Ramachandran Plot analysis further showed 484 as the total number of residues, the number of Glycine (Gly) and Proline (Pro) are 20 and 14, respectively, and the number of end residues (excluding Gly and Pro) was 16. For Beta tubulin protein, the Ramachandran Plot depicted that none of the

amino acids were in the disallowed region with the maximum number of amino acid molecules in the favorable region. Most favorable regions showing 94.3% having 707 residues, additional allowed region showing 5.1% having 38 residues and generously allowed region showing 0.7% having only 5 residues. There was no residue found in the disallowed region. The Ramachandran Plot analysis further showed 856 as the total number of residues, the number of Glycine (Gly) and Proline (Pro) are 63 and 39, respectively, and the number of end residues (excluding Gly and Pro) was 4.

Computation of docking score between the ligands and receptor

Protein and other chemical molecule interaction outputs compute data which could be an advantage to understand the interaction between the ligands and the receptor. The ligands docetaxel, paclitaxel bind to β chain of tubulin protein and the ligand endoxifen (an active form of tamoxifen) binds to Estrogen Receptor α (ER α). We kept the clustering RMSD value restricted to 2 and the complex type was kept as the protein-small ligand. Figure 7, Table 2. The top 10 docking results are provided in a tabular format. Table 3.

Figure 5. (a) Three-dimensional structure of Estrogen Receptor modeled using the Swiss-Model server with its natural ligands. (b) Ramachandran Plot of 4ZNH.

Figure 6. (a) Three-dimensional structure of Beta Tubulin modeled using the Swiss-Model server with its natural ligands. (b) Ramachandran Plot of 5IJO.

Figure 7. Molecular docked structures of (a) Docetaxel and tubulin protein, (b) Paclitaxel and tubulin protein, (c) Endoxifen and estrogen receptor.

Table 2. Detailed docking analysis result showing interaction of receptors and ligands. The amino acid residue position of the receptors interacting with the ligands are also provided.

Ligand	Receptors		
	β chain of tubulin	Estrogen Receptor α	Estrogen Receptor α
Docetaxel		Paclitaxel	Endoxifen
Global Energy	-257.13	-275.19	- 42.55
ACE	-63.32	-56.47	-12.94
Score	312	306	3906
Area	39.50	41.80	518.10
Attractive VdW	-104.28	-123.78	-18.99
Repulsive VdW	0.00	0.00	4.08
Hydrogen Bonding	0.00	0.00	0.00
Positions (Residue)	Pro-32, Gly-34, Lys-58, Pro-80, Phe- 81	Pro-272, Leu- 289	Gly-323, Pro-324, Pro-325, Ile-326, Leu- 327, Glu-353, Ile-386, Leu – 387, Gly-390, Trp-393, Arg – 394, Gly – 442

Table 3. The top ten molecular docking results of Beta tubulin and Docetaxel, Beta Tubulin and Paclitaxel, and for Estrogen Receptor and Endoxifen.

Complex	Rank	Solution No.	Global Energy	Attractive Van der Waals	Repulsive Van der Waals	ACE	Amino acid position(s)
Beta tubulin and Docetaxel	1	51	-257.13	-104.28	0	-63.32	
	2	56	-234.82	-85.86	0	-66.39	
	3	15	-228.12	-102.25	0	-46.71	Cys-12, Asp-177, Tyr-222,
	4	70	-227.39	-120.25	0	-29.46	Thr-178, Ser-138, Asn-99,
	5	8	-224.42	-101.82	18.48	-55.53	Ala-97, Gly-142, Gly-144,
	6	76	-222.63	-99.35	1.30	-47.06	Thr-143, Gln-11, Gly-141,
	7	32	-220.00	-92.24	0	-51.32	Asn-204, Val-169, Ile-16,
	8	74	-216.80	-87.05	0	-54.33	Asn-226, Gln-15
	9	68	-216.79	-87.04	0	-54.33	
	10	13	-216.30	-91.40	0	-49.59	
Beta tubulin and Paclitaxel	1	76	-275.19	-123.78	0	-56.47	Val-418, His-524, Met-528,
	2	17	-264.64	-121.23	31.02	-67.47	Met-421, Met-343, Leu-525,
	3	79	-251.83	-109.57	0	-54.92	Met-421, Met-343, Leu-525,
	4	84	-249.08	-109.99	0	-53.12	Leu-384, Glu-419, Gly-420,
	5	70	-245.79	-129.57	0	-32.24	Gly-521, Ile-424, Phe-425,
	6	13	-237.79	-99.34	0	-55.58	Leu-428, Met-388, Arg-394,
	7	74	-233.89	-93.68	0	-58.78	Glu-353, Leu-387, Leu-349,
	8	68	-233.83	-93.66	0	-58.78	Leu-391, Phe-404, Ala-350,
	9	11	-231.74	-120.98	34.75	-49.31	Trp-383, Leu-540, Leu-536,
	10	63	-220.07	-116.48	0	-28.31	Thr-347, Leu-346
Estrogen Receptor and Endoxifen	1	62	-42.55	-18.99	4.08	-12.94	
	2	5	-42.45	-19.28	6.23	-13.71	
	3	36	-40.98	-21.72	6.88	-10.40	Tyr-222, Cys-12, Gln-15,
	4	8	-40.23	-17.22	6.92	-14.59	Asn-226, Ile-16, Asn-204,
	5	83	-39.53	-17.27	6.02	-14.96	Val-169, Gly-141, Gln-11,
	6	87	-38.26	-19.63	5.08	-10.42	Thr-143, Gly-144, Gly-142,
	7	38	-37.29	-20.84	10.63	-11.01	Ala-97, Asn-99, Ser-138,
	8	3	-36.93	-20.61	7.77	-9.33	Thr-178, Asp-177
	9	61	-36.21	-16.94	7.69	-13.84	
	10	55	-36.11	-21.42	9.93	-9.05	

DISCUSSION AND CONCLUSION

Taxus wallichiana is an ethnobotanical plant found in the Himalayan range of the Indian subcontinent and is traditionally parts of the plant that have been used to various ailments and it is found to have anti-convulsant, analgesic, antipyretic, anti-inflammatory, anti-allergic, anti-bacterial, anti-fungal and also anti-cancerous properties. The anti-cancerous properties are found to be due to the interaction of anti-cancerous compounds found specifically in the bark of the plant with proteins and as a result, controls the rapid growth of cancerous cells. Out of these compounds, Paclitaxel (TaxolTM) and Docetaxel (TaxotereTM) interact with tubulin protein and stabilizes the microtubules during cell division and prevents cell division. Whereas endoxifen, the active form tamoxifen binds with estrogen receptor and prevents uncontrolled cell division in steroidal cells. The ligands that are found in *Taxus wallichiana* are proved to have interacted with the receptors as a result of which it shows anti-cancerous properties. This plant is found in 1800 and 3300 m above mean sea level (MSL) in the temperate Himalayas and the hills of Meghalaya and Manipur at an altitude of 1500 m. According to the Forest & Environment Department, Government of Meghalaya, *Taxus wallichiana* is found in abundant in Mawphlang Sacred Groves of Meghalaya at latitude 25.449433707307875, longitude - 91.75763019314581 and elevation is 1845 m. *Taxus wallichiana* is an endangered plant according to IUCN Red List (Thomas et al., 2011) and further care should be taken during the extraction of these anti-cancerous compounds.

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