Int J Pharm Bio Sci 2015 April; 6(2): (B) 1140 - 1154

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

MOLECULAR DOCKING STUDIES OF HERBAL LIGANDS SHOWING ANTI-TUMOR PROPERTIES

GIREESH KAMATH H[1*], DEECARAMAN[1] AND RAMESH KV[2]

[1]Dr. M. G. R. Educational and Research institute, Maduravoyal, Chennai-600095
[2] Center for Postgraduate Studies, Jain University, 18 / 3, 3rd Block, 9th Main, Jayanagar, Bangalore - 560011

ABSTRACT

In silico docking exercise of different herbal based ligands with anti-tumor properties, revealed that vinorelbine got docked onto breast cancer kinase protein with the lowest calculated interaction energy. Since the molecule was present in its unrefined form, it was further refined using geometrical optimization technique as implemented in GAUSSIAN software package. There was no substantial difference in the calculated interaction energy between unrefined and refined vinorelbine structure when docked onto the kinase protein; however, the protein complexed with the drug molecule, tamoxifen shared few residues that were interacting with refined vinorelbine structure, which was not seen in unrefined one.

KEY WORDS: Breast cancer protein, docking, herbal ligands, HEX, Vinorelbine.

*Corresponding author

GIREESH KAMATH H
Dr. M. G. R. Educational and Research institute, Maduravoyal, Chennai-600095

INTRODUCTION

Herbal medicine is in vogue for centuries for treating various ailments[1]. People have used these medicines to help themselves feel better. Further, these medicines are reported to assist in relaxation and also they can deal with anxiety, depression and other abnormalities such as hay fever, irritable bowel syndrome, menstrual problems etc. Some vegetables, fruit, and medicinal herbs, are known to possess a variety of anti-oxidant effects and other biological activities. Phenolic compounds in these plant materials are closely associated with their anti-oxidant activity, which is mainly due to their redox properties and their capacity to block the production of reactive oxygen species. Many of the spice-derived compounds which are potent anti-oxidants are of great interest to biologists and clinicians since they might help in protecting the human body against oxidative stress and inflammatory processes[2]. In general, low toxicity and wide acceptance of herbal medicines make them as a preferred substitute that may decrease the incidence of several diseases[3].It is a well known fact that natural phenolic compounds isolated from medicinal herbs and dietary plants play an important role in cancer prevention and treatment. These compounds include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. In addition to low toxicity of the herbal medicines, Kaefer and Milner (2011)[4] have also reported that various bio-activities of phenolic compounds are responsible for their chemo-preventive properties such as anti-oxidant, anti-carcinogenic, or anti-mutagenic and anti-inflammatory effects. In addition to this, phenolics also contribute in inducing apoptosis by (i) arresting cell cycle, (ii) regulating carcinogen metabolism and ontogenesis expression, (iii) inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation and (iv) blocking signaling pathways[5]. In the recent past, several studies have been conducted which demonstrates anti-tumor properties of several herbal plant extracts. Withaferine from *Withania somnifera*, four different anti-proliferative phytocompounds from *Wedelia chinensis*, nimbolide from *Azadirachta indica* and Diosgenin from *Dioscorea bulbifera* have been identified as potential ligands exhibiting anti-tumor properties[6,7,8,9]. Withaferin inhibits Notch-1 signaling and down regulates pro-survival pathways, such as Akt / NF-kappa B / Bcl-2 in different cancer cell lines[10]; this is also active against breast cancer[6]. Lin *et al,* (2007)[7], have reported phytocompounds such as indole-3-carboxylaldehyde, wedelolactone, luteolin and apigenin from *Wedelia chinensis* specifically inhibited the growth of androgen receptor (AR) dependent prostate cancer (PCa) cells and as a combination they also synergistically suppressed growth in AR-dependent PCa cells. Elumalai *et al* (2012)[11] investigated the cytotoxic effects of nimbolide on human breast cancer cells. Treatment with nimbolide resulted in inhibition of growth of MCF-7 and MDA-MB-231 cell lines. Further, intrinsic and extrinsic apoptotic signaling molecules expression were associated with increased levels of pro-apoptotic proteins with reduced levels of the anti-apoptotic proteins. Zhu-Jun Mao *et al* (2012)[12] in an *in vitro* study demonstrated the effect of diosgenin, against gastric cancer. Diosgenin is a potent candidate for decreasing the ability of invasion and survival in cobalt chloride treated BGC-823 cells. In addition, when combined with HIF-1α specific short hairpin RNA (shRNA), diosgenin can inhibit BGC-823 cells more effectively. The anti-invasion role of diosgenin may be related to E-cadherin, integrinα5 and integrinβ6. These results suggest that diosgenin may be a useful compound in controlling gastric cancer cells in hypoxia condition, especially when combined with down-regulated HIF-1α. There are several studies in the past which have used *in* silico approach for elucidating anti-cancer properties of many of the herbal ligands[13,14]. Abhinav *et al* (2010)[13] were successful in carrying out bio-computational studies with the aim of exploring the proteasome inhibition capability of herbal ligand, withaferin (WA). Docking of WA onto the structures of bovine and human proteasomes substantiate that this ligand has the ability to inhibit activity of mammalian 20S proteasomes by blocking the nucleophilic function of N-terminal Thr1. Thuo *et al* (2010)[14] using GOLD software, have reported that azadirachtin interacts with the Tumor Necrosis

Factor (TNF) binding domain of its receptors and inhibits TNF-induced biological responses. Thymoquinone (TQ), an active ingredient of *Nigella sativa*, exhibits anti-oxidant, anti-inflammatory and anti-tumor activities through mechanism(s) that is not fully understood. Molecular docking analysis revealed that TQ formed interactions with 7 polar residues and 6 non-polar residues within the ligand-binding pocket of peroxisome proliferator-activated receptor gamma (PPAR-γ or PPARG) that are reported to be critical for its activity[15]. Among several plant-derived compounds which have been an important source of many clinically useful anti-cancer agents, Vinca plants are known to exhibit potential anti-tumor properties[16]. There are at least 86 alkaloids extracted from Vinca genus[17]. Among these, chemotherapy agent vincristine, vindesine and vinflunine extracted from *Vinca rosea* are used to treat leukemia, lymphomas and childhood cancers, as well as several other types of cancer and some non-cancerous conditions. Gonzalez-Angulo *et al,* (2007)[18] in their review have emphasized that breast cancer could be the most common cancer and the second leading cause of cancer death among women population. There are currently three main groups of medications used for adjuvant breast cancer treatment: (i) hormone-blocking agents[19], (ii) chemotherapy[20] and (iii) monoclonal antibodies[21]. Some breast cancers require estrogen to continue growing. They can be identified by the presence of estrogen receptors (ER+) and progesterone receptors (PR+) on their surface (sometimes referred to together as hormone receptors). These ER+ cancers can be treated with drugs that either block the receptors, e.g. tamoxifen, or alternatively block the production of estrogen with an aromatase inhibitor, e.g. anastrozole or letrozole. The use of tamoxifen is recommended for 10 years[22]. X-ray diffraction studies of the ligand binding domain of human estrogen receptor alpha (PDB ID: 3ERT) revealed that residues such as try[383], leu [384, 387, 391, 428, 525], met [388, 421], arg [394], phe[404], glu[419], ile [424], gly[420, 521] and his[524] of the protein were interacting with the ligand, 4 hydroxy tamoxifen[23]. According to the report issued from Health and Human Services' Agency for Healthcare Research and Quality, tamoxifen and other closely related drugs used for treating breast cancer though significantly reduces breast cancer in women, it also increases the risk of side effects. For instance, lower-limb lymphedema have been associated with the use of tamoxifen due to the blood clots and deep vein thrombosis caused by its inappropriate use[24]. Tamoxifen-treated breast cancer patients show evidence of reduced cognition, considered as a major side effect[25]. To offer more effective and less toxic treatment, selecting appropriate therapies requires careful consideration of patient's response to therapy, followed by clinical and molecular characteristics of the tumor under investigation[26]. Systemic treatment of breast cancer includes cytotoxic, hormonal, and immunotherapeutic agents. These medications are used in the adjuvant[27], neoadjuvant, and metastatic settings. In general, systemic agents are active at the beginning of therapy in 90 % of primary breast cancers and 50 % during metastases stage. According to recent review by Gonzalez-Angulo *et al* (2007)[18], if unnoticed, after some lapse of time, there is every possibility for the tumor to progress and thereby causing irreparable damage. At that point, resistance to therapy is not only common but expected. In addition, in their review, Gonzalez-Angulo *et al* (2007)[18] have further explained extensively regarding the general mechanisms of drug resistance, including multidrug resistance by P-glycoprotein and the multidrug resistance protein family in association with specific agents and their metabolism, emergence of refractory tumors associated with multiple resistance mechanisms, and resistance factors unique to host-tumor-drug interactions. Vinorelbine is a vinca alkaloid obtained by hemisynthesis, which makes the molecule lipophilic compared to others[28]. Based on the studies carried out by Nirmala et al (2011)[29], it is clear that the Vinca alkaloids are active against leukemia's, lymphomas, advanced testicular cancer, breast cancer and lung cancer. Vinorelbine, one of the extract from *Vinca rosea* is active against breast cancer protein transferase. The main objective of the present investigation is virtual screening of different herbal based ligands exhibiting anti-tumor properties, specifically against breast cancer through docking exercises.

MATERIALS AND METHODS

Information related to herbal ligands was obtained from extensive literature search; some of them showing a wide range of anti-tumor properties. Using this data as the basis, followed by survey of PDB databank (www.rcsb.org), cancer related target proteins were segregated into transferases, isomerases or oxidoreductases. Herbal ligands specific against transferases were considered since these proteins were present in larger proportion, whose mol2 files were subsequently downloaded from ZINC database (www.zinc.docking.org) (Table 1) for docking exercise. Plant extracts exhibiting anti-tumor properties against several cancer types is presented in Table 2. Docking studies: The 3D structures of all the chosen herbal ligands were docked onto the crystal structures of two randomly selected kinase (kinases belong to larger family of phosphotransferases) complexes 1KDT[30] (cytidine monophosphate kinase) and 1J1B[31] (human tau-protein kinase) deposited in protein data bank. Docking was carried out using HEX software[32]. Docked conformations and interaction energies were recorded at the end of the docking exercise. During the dock operation, the total energies were calculated based on shape as well as electrostatics using a default grid spacing of 0.6 Å. Deep View package[33] was used for later visualizing the best docked output. The interacting residues within 5 Å for each ligand were identified. Geometry optimization as well as vibrational frequency of the herbal ligand having the lowest calculated interaction energy was carried out using Gaussian package[34] installed on SGI Altix UV10. For optimizing the structure, Hatreefock theory with basis set "3-21g" was considered. Standard orientation of the optimized structure generated was visualized using ARGUS lab package and the structure was saved in PDB format. Geometrically optimized structure was once again docked onto the same kinase receptor protein(s) using HEX software as explained earlier. The docked output was later validated by comparing the calculated interaction energy with the crystal structure of protein complexed with anti-tumor drug / ligand molecule deposited in the protein data bank (PDB Id. 3ERT).

RESULTS

An *in silico* investigation was carried out to screen different herbal based ligands exhibiting anti-tumor properties, specifically against breast cancer through docking exercises. Among the 18 anti-tumor herbal ligands that were selected for docking, vinorelbine – unrefined (ur) got docked with the lowest calculated interaction energy onto the kinase proteins (1J1B and 1KDT). While the molecule got docked with calculated interaction energy of -362.07 kcal mol^{-1} onto 1J1B, it got docked onto 1KDT with a calculated interaction energy -297.60 kcal mol^{-1} (Table 3, Fig 1a and 2a). Analysis of the binding site of vinorelbine – ur and tamoxifen with 1J1B revealed both the ligands got docked at different locations. While ile^{62}, phe^{67}, $val^{70,135}$, arg^{141}, $lys^{85,183}$, gln^{185}, $asn^{64,186}$, leu^{188}, cys^{199}, $gly^{63,65,\ 68,\ 202}$, $ser^{66,203}$ and $asp^{181,200,764}$ of 1J1B interacted with vinorelbine-ur, $gly^{259,565}$, glu^{268}, val^{267}, gln^{265}, pro^{268}, tyr^{221}, $arg^{220,720}$, $ser^{261,719}$, $asp^{260,264,681,700}$, $asn^{564,686}$, $lys^{271,585,683}$ and phe^{567} of 1J1B made contact with tamoxifen (Fig 1b). Visualization of 1KDT protein docked with vinorelbine – ur and tamoxifen through DeepView package disclosed both the ligands appears to occupy the same binding site of the protein and thereby shared few common residues which include: asp^{10}, lys^{11}, tyr^{12}, arg^{20} and gln^{49} (Fig 2b).Since vinorelbine used for docking purpose is not a refined structure, it was subjected to geometry optimization using GAUSSIAN package. The structure which got optimized at the end of 10 cycles recorded energy of -1585.89 AU (Fig 3). In addition to this, maximum force, RMS force, maximum displacement and RMS displacement computed by the package were less than the set threshold values, suggesting the molecule has converged to global minimum energy.There was no difference in the calculated interaction energy when the kinase protein (1J1B) was docked with geometrically optimized vinorelbine (-362.07 kcal mol^{-1}) (Fig 4a); however, the protein complexed with tamoxifen molecule shared few residues (val^{267}, lys^{271}, asn^{564}, gly^{565}, ser^{566}) that were interacting with refined vinorelbine structure (Fig 4b), which was not obtained with unrefined vinorelbine structure. Though geometrically optimized vinorelbine was successfully docked onto the crystal structure of

estrogen receptor (3ERT), calculated interaction energy was considerably less (-270.50 kcal mol^{-1}) (Fig 5a) compared to the value of tamoxifen docked onto the same protein (-288.29 kcal mol^{-1}) (Fig 6a). Analysis of the residues of the protein interacting with these two ligands strongly suggests that they occupy two different binding sites (Fig 5b and 6b).

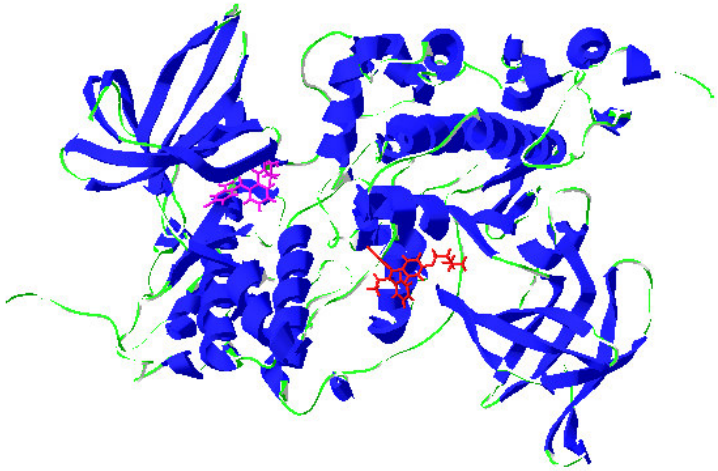


Figure 1a

Docking of vinorelbine (colored magenta) and tamoxifen (colored red) onto the crystal structure of kinase protein (PDB ID: 1J1B). While vinorelbine got docked with dock energy of -362.07 kcal mol^{-1}, tamoxifen got docked with dock energy of -257.67 kcal mol^{-1}. The images were generated using Deep View package

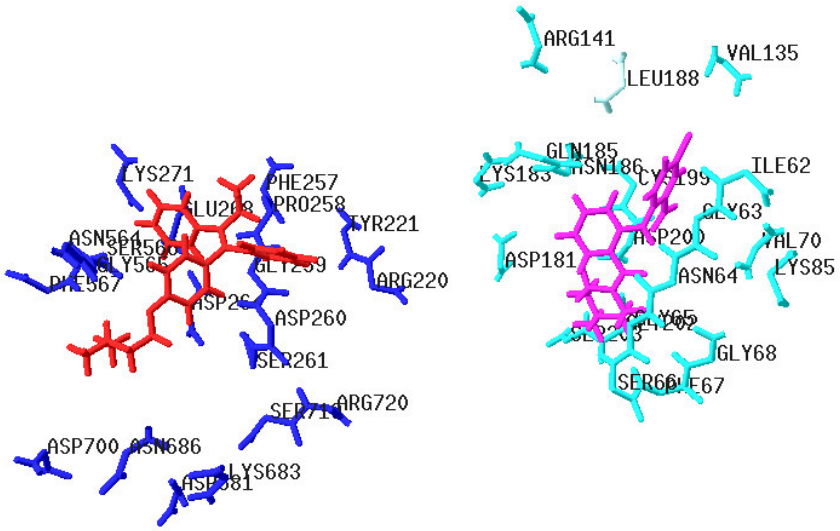


Figure 1b

Active site residues of the crystal structure of kinase protein (PDB ID: 1J1B) interacting with vinorelbine (colored magenta) and tamoxifen (colored red) within a distance of 5Å. The images were generated using Deep View package.

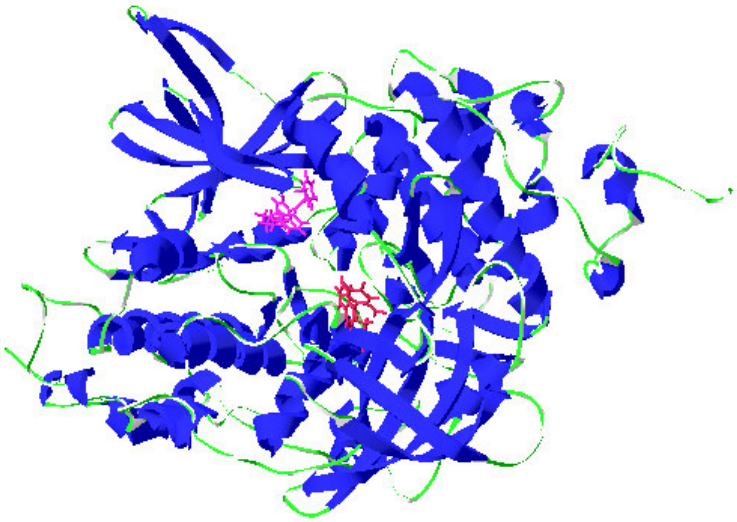


Figure 2a

Docking of vinorelbine (colored magenta) and tamoxifen (colored red) onto the crystal structure of kinase protein (PDB ID: 1KDT). While vinorelbine got docked with dock energy of 297.6 kcal mol^{-1}, tamoxifen got docked with dock energy of -220.7 kcal mol^{-1}. The images were generated using Deep View package

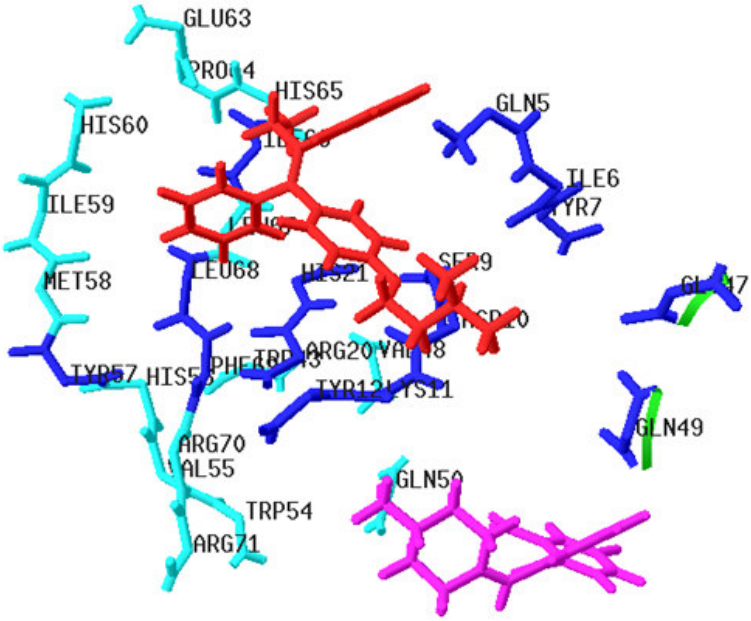
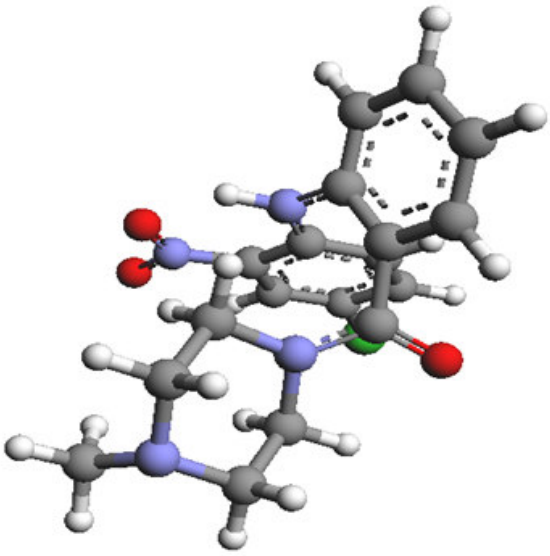


Figure 2b

Active site residues of the crystal structure of kinase protein (PDB ID: 1KDT) interacting with vinorelbine (colored magenta) and tamoxifen (colored red) within a distance of 5Å. The images were generated using Deep View package.



Item	Value	Threshold	Converged?
Maximum Force	0.000021	0.000450	YES
RMS Force	0.000004	0.000300	YES
Maximum Displacement	0.000984	0.001800	YES
RMS Displacement	0.000237	0.001200	YES

Figure 3

3D structure of vinorelbine geometrically optimized using GAUSSIAN software package [E (RHF) = -9.9x10^{5} kcal mol^{-1} after 10 cycles]. Hatreefock theory with 3-21g" as the basis set was used. The data presented in the table summarizes maximum force, RMS force, maximum displacement and RMS displacement is less than the set threshold value.

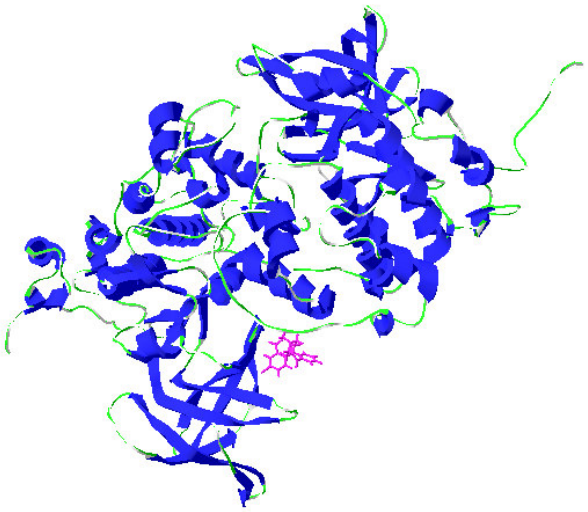


Figure 4a

Docking of optimised vinorelbine onto the crystal structure of kinase protein (PDB ID: 1J1B). Vinorelbine got docked with dock energy of -362.07 kcal mol^{-1}. The images were generated using Deep View package.

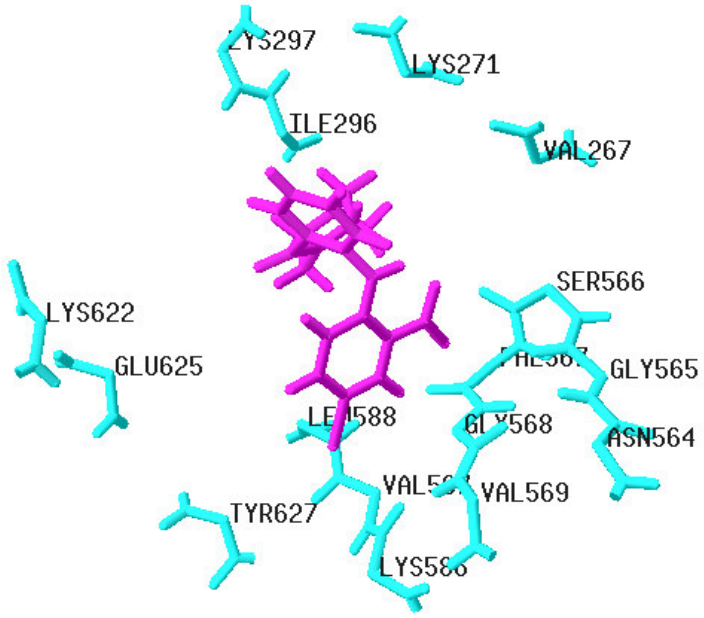


Figure 4b

Active site residues of the crystal structure of kinase protein (PDB ID: 1J1B) interacting with optimized vinorelbine within a distance of 5Å. The images were generated using Deep View package.

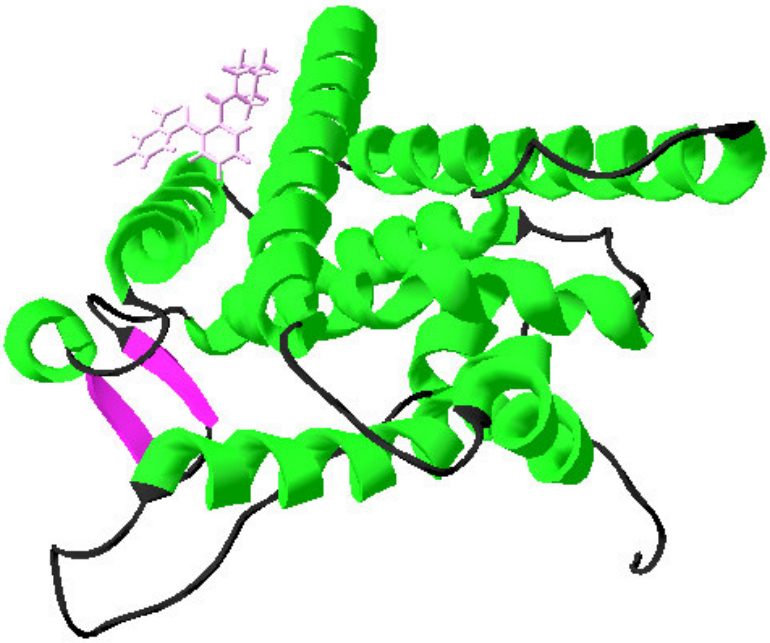


Figure 5a

Docking of vinorelbine onto the crystal structure of estrogen receptor protein (PDB ID: 3ERT). Vinorelbine got docked with dock energy of -270.50 kcal mol^{-1}. The images were generated using Deep View package.

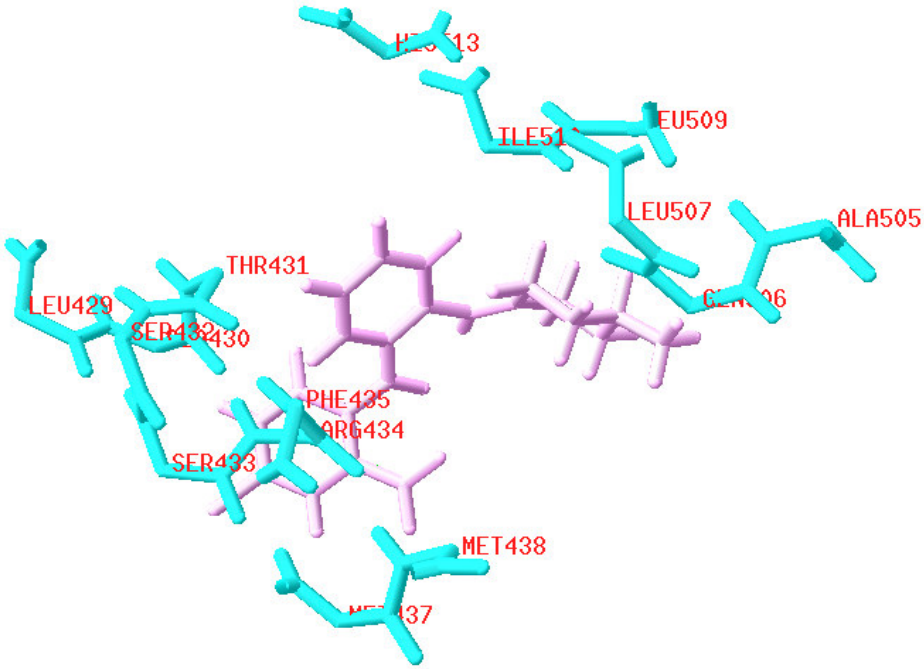


Figure 5b

Active site residues of the crystal structure of estrogen receptor protein (PDB ID: 3ERT) interacting with vinorelbine within a distance of 5Å. The images were generated using Deep View package.

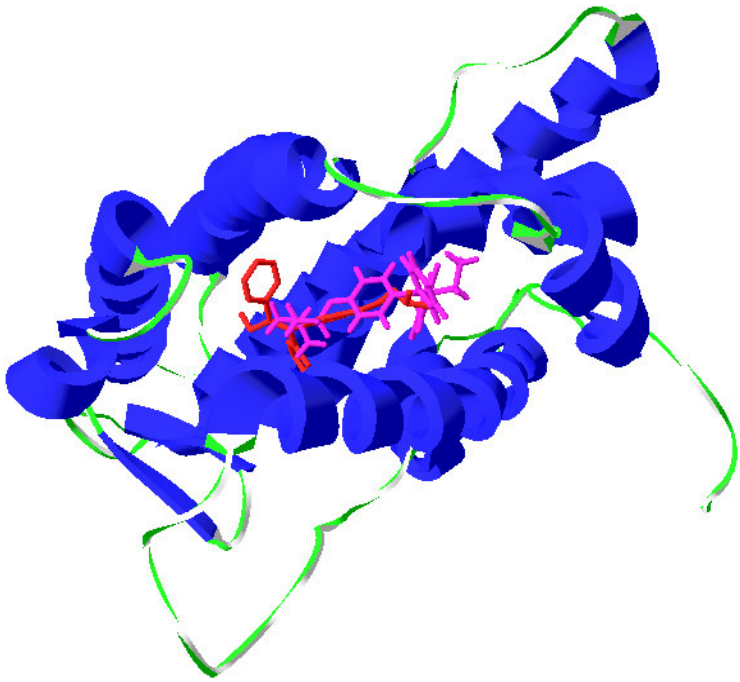


Figure 6a

Docking of tamoxifen onto the crystal structure of estrogen receptor protein (PDB ID: 3ERT). Tamoxifen got docked with dock energy of -288.29 kcal mol^{-1}. The images were generated using Deep View package.

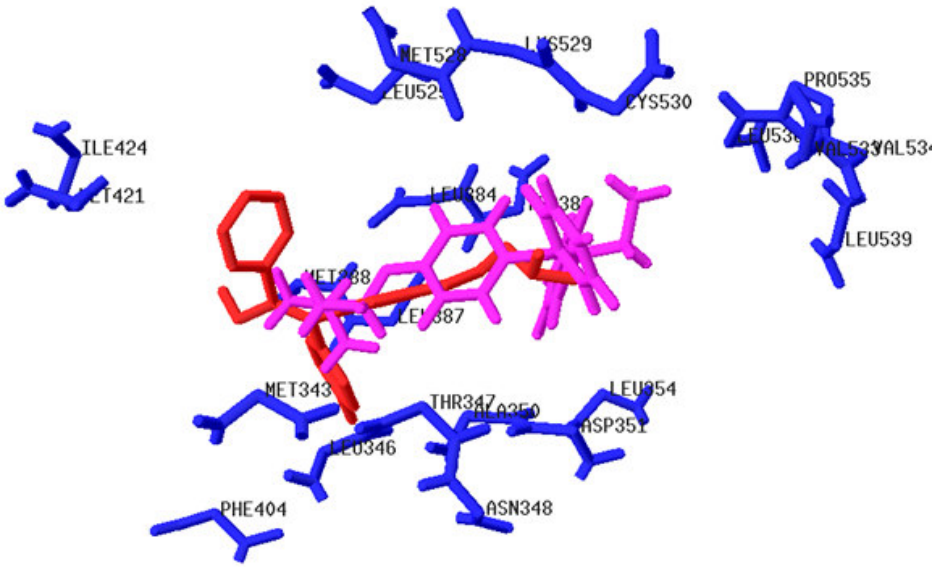


Figure 6b

Active site residues of the crystal structure of estrogen receptor protein (PDB ID: 3ERT) interacting with tamoxifen within a distance of 5Å. The images were generated using Deep View package.

Table 1

mol2 files of anti-tumor herbal ligands specific against transferase downloaded from ZINC database.

Vinorelbine	Daizdzin 2
Daizdzin	Diosgenin
Chalnone	Brassinin
Betulinic acid	Xanthohumol
Wedalactone	Withaferine
Theophyline-pheoforbide	Sinapic acid
Silymarin	Quercetiin_1
Pheopharbide	Osthole
Nimbolide	Mellissic acid
Geniposide	Gallic acid

Table 2

Summary of plant extracts showing anti-tumor properties.

Name of the ligand	Plant source	Active against cancer type	Reference
Melissic acid	*Wedelia chinensis* (Pitabhrngaraja)	Prostate cancer (putative)	Kaefer and Milner 2011[4]
Withaferine	*Withania somnifera Linn* (Ashwagandha)	Breast Cancer,	Singh *et al* 2010[6]
Nimbolide	*Azadirachta indica* (Neem)	Choriocarcinoma (putative), colon cancer	Elumalai *et al* 2012[11]
Vinorelbine	*Vinca rosea Linn. (Periwinkle)*	Breast Cancer, Lung Cancer, Ovarian Cancer, non-small cell lung cancer	Yu *et al* 2004[35]
Diosgenin	*Dioscorea bulbifera* (Dukkar kand)	Colon cancer, breast cancer (putative)	Li *et al* 2006[36]
Gallic Acid	*Humulus yunnanensis* (Hop plant)	Lung Cancer (putative),	Yi-Chen Chia *et al* 2010[37]
Chalcone	*Glycyrrhiza glabra* (Yashti-madhuka)	Breast, Prostrate and colon carcinoma	Koneni *et al*[38]
Geniposide	*Gardenia jasminoides* (Gandharaj,)	Glioma	Koo *et al* 2004[39]
Osthole	*Cnidium monnieri* (She chuang zi)	Lung and Various cancers	Chou *et al* 2007[40]
wedalolactone	*Eclipta alba (L.)* (False daisy)	Various cancers	Mithun and Shashidhara[41]
pheoforbide	*Carpinus betulus* (Common hornbeam)	Various cancers	Cieckiewicz *et al* 2011[42]
Xanthohumol	*Humulus yunnanensis* (Hop plant)	hepatocellular carcinoma (putative), leukemia (putative)	Gerhauser *et al* 2007[43]
Sinapic acid	*Various plants*	Breast cancer	Janakiraman *et al* 2014[44]
daidzin	Leguminous plants	Various cancer	Kaufman *et al* 1997[45]
Betulinic acid	*Ziziphus mauritiana* (Madhuraphala)	Several cancers	Mullauer *et al* 2011[46]

Table 3

Summary of dock energies computed by HEX software while docking the anti-tumor herbal ligands onto the crystal structures of 2 randomly selected kinase complexes, 1KDT and 1J1B

Ligand from herbal source	Dock energy(kcal mol^{-1})	
	1J1B	1KDT
Vinorelbine	-362.07	-297.60
Withaferine	-292.07	-249.54
Nimbolide	-282.38	-230.54
Melissic acid	-278.63	-283.64
Diosgenin	-264.61	NA
Betulinic acid	NA	-233.80
Chalcone	-206.32	-184.61
Wedalolactone	-228.05	-212.91
Osthole	-239.75	NA
Quercetiin_1	-241.65	-201.63
Daidzin	-259.89	-229.41
Sinapinic acid	-203.26	-179.46
Xanthohumol	NA	-229.71
Gallic Acid	-167.92	-134.37
Daidzin-2	-63.36	-249.42
Geniposide	NA	-216.64
Theophylline- pheoforbide	-157.03	NA
Brasinin	NA	-188.59
Tamoxifen	-257.67	-220.71

DISCUSSION

Among the various herbal ligands that were screened for anti-tumor properties, specifically against beast cancer, vinorelbine structure present in *Vinca rosea* got docked onto the crystal structure of kinase protein (1J1B) with the lowest calculated interaction energy. Since the molecule was present in its unrefined form, it was further refined using geometrical optimization technique as implemented in the GAUSSIAN software package. There was no substantial difference in the calculated interaction energy between unrefined and refined vinorelbine structure when docked onto the kinase protein; however, the protein complexed with tamoxifen molecule shared few residues that were interacting with refined vinorelbine structure, which was not seen in unrefined one. In a study conducted by Suvannang *et al* 2011[48], aromatase inhibitors were geometrically optimized using appropriate model chemistry implemented in GAUSSIAN software, before attempting molecular docking. This explains the fact that docking program needs to have the ligands with the right molecular mechanics parameters and atom types or the results may not be accurate enough. Nirmala *et al* (2011)[29] has reported that vinorelbine extracted from *Vinca rosea* is active against breast cancer protein transferase. Though the refined vinorelbine structure got successfully docked onto the crystal structure of estrogen receptor, tamoxifen drug molecule showed more affinity towards the protein. However, dock output shows that these two ligands occupy two different binding sites. The structural analogues of ATP, Adenosine diphosphate (ADP) and phosphoaminophosphonic acid-adenylate ester (AMP-PNP) are complexed with 1J1B[30]. The active site residues of this ligand are almost same as that of Tamoxifen when docked on IJ1B. Since, Vinorelbine has docked onto a different binding site, different binding modes of vinorelbine and tamoxifen onto the kinase. Therefore, vinorelbine being an herbal based molecule, can serve as a better alternative to tamoxifen, which is a synthetic drug with more side effects. Paganini-Hill and Clark (2000)[25] have reported that tamoxifen-treated breast cancer patient show reduced cognition, considered as a major side effect. The structural analogue of ATP, Adenosine diphosphate (ADP) is complexed with 1J1B. The active site residues of this ligand are almost same as that of Tamoxifen when docked on IJ1B. Since, Vinorelbine has docked onto a different binding

site, different binding modes of vinorelbine and tamoxifen onto the kinase.

CONCLUSION

Herbal extracts are considered as one of the cheapest source of medicine for treating innumerable number of diseases. In this context, vinorelbine extracted from *Vinca* plant may be a good alternate for the presently marketed drug "tamoxifen" for the breast cancer. Vinorelbine upon geometrical optimization had the lowest calculated interaction energy which also shared the binding site occupied by tamoxifen. To conclude, vinorelbine may be better and cheaper alternative and hence, may be used as a alternate for the existing drug after confirmation through testing in the *in vitro and in vivo* studies.

ACKOWLEDGEMENT

Sincere thanks to Syngene International Limited for providing the opportunity to complete this paper.

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