Open Science Journal of Bioscience and Bioengineering
2018; 5(2): 6-16
http://www.openscienceonline.com/journal/bio
ISSN: 2381-3806 (Print); ISSN: 2381-3814 (Online)

In silico Virtual Screening of 10-Hydroxycamptothecine Against Different Cancer Proteins

Govindappa Melappa*, Kalavathi Murugan Kumar, Ramakrishna Devappa

Natural Product Laboratory, Department of Biotechnology, Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bengaluru, India

Email address

dravidateja07@gmail.com (G. Melappa), drgovindappa-bt@dayanandasagar.edu (G. Melappa)

*Corresponding author

To cite this article

Govindappa Melappa, Kalavathi Murugan Kumar, Ramakrishna Devappa. *In silico* Virtual Screening of 10-Hydroxycamptothecine Against Different Cancer Proteins. *Open Science Journal of Bioscience and Bioengineering.* Vol. 5, No. 2, 2018, pp. 6-16.

Received: April 12, 2018; **Accepted**: May 18, 2018; **Published**: July 2, 2018

Abstract

To know the role of 10-hydroxycamptothecine in anticancer activity, we have selected 45 cancer proteins for in silico study. 10% of the world population death is from different types of cancer and it indicates need of better therapeutic against which are less toxic. 10-hydroxycamptothecine was identified from different plants and endophytic fungal species and exhibiting antioxidant, antimicrobial, anti-inflammatory and anticancer activities. The present research was aimed to know interaction studies of 10-hydroxycamptothecine with various cancers virulent proteins using computer aided virtual screening using iGEMDOCK. The ADMET test clarifies its drug like properties. In present study, selected 45 different cancers proteins were selected based on virulent functions for interaction studies and was identified based on binding energy. The 10-hydroxycamptothecine have shown highest binding energy to cancer virulent proteins viz., BCl-Xl BAK (-100.19 kcal/mol), small cell lung cancer (-115.4 kcal/mol), BAX (-108 kcal/mol), reps1 EH domain (-107.94 kcal/mol), Hodgkin lymphoma (-107.35 kcal/mol), MCF (-94.8 kcal/mol), two ribosome-inactivating proteins (-107.51 kcal/mol), epidermal growth factor (EGF) (-99.9 kcal/mol), Malignant pleural mesothelioma (-94 kcal/mol), MCF-9-EGFR tyrosine kinase (-102.9 kcal/mol), CDK4 in complex with a d-type cyclin (-102.2 kcal/mol), Gastric cancer (-98.01 kcal/mol), Ovarian Cancer (-109 kcal/mol), Oral cancer (-93.5 kcal/mol), HER-2 (-100.28 kcal/mol), BCl-2 (-101.2 kcal/mol), BCl-2 protein (-111.8 kcal/mol), BH3 domain (-102 kcal/mol), BID (-94 kcal/mol), Bcl-2 alpha beta-1 LINEAR complex (-94.8 kcal/mol), MCl-1 (-97.8 kcal/mol), topoisomerase (-101.29 kcal/mol), Topoisomerase 2A (-104.79 kcal/mol), caspase -3 (-118.9 kcal/mol), topo 4 (-103.35 kcal/mol), caspase 9 (-94.1 kcal/mol). The ligand has more binding energy growth factors and receptors, gastric, oral, ovarian cancer and MCl-1 proteins. BCl-2 and its proteins have more attracted to ligand. The ligand has ability to bind topoisomerase, 2A and caspase 3 and 9. The study results concluded that 10-hydroxycamptothecine has higher interacting properties to different cancer proteins and enzymes and therefore it can be used as novel inhibitory agents against different cancers. Molecular docking studies strongly support the experimental results and results of the present data concluded that 10-hydroxycamptothecine clearly shows how it interacts with different cancer proteins and cancer enzymes can lead as potential nontoxic anticancer agent.

Keywords

10-Hydroxycamptothecine, Anticancer Agent, Virtual Screening, Cancer Proteins, Cancer Enzymes

1. Introduction

Camptothecine and 10-hydroxycamptothecine are two antitumour alkaloids isolated from *Camptotheca acuminata* Decne (Nyssaceae), have a unique quinolone skeleton with a highly conjugated five ring system. 10-Hydroxycamptothecine (HCPT) is used clinically in China for the treatment of stomach

and liver cancers. The ^{13}C NMR analysis was done by Lin and Cordell [1]. CPT is an enzyme responsible for the unwinding of DNA during replication and transcription [2]. Because of this property, CPT is one of the most cytotoxic compounds [2-3] and several derivatives of CPT are being used in the treatment of a wide variety of cancers, including ovarian, small lung and refractory ovarian cancers. Few important medicinal plants contain various cytotoxic alkaloids, such as camptothecine (CPT) and 10-hydroxycamptothecine (HCPT) (9-hydroxycamptothecine) [4]. Camptothecine have reported from *Nothapodytes foetida* (*Mappia foetida*) (Iccacinaceae), *Morrilliodendron megacarpum* (Icacnaceae). *Ophiorrohiza mungus*, *Ophiorrohiza pumila* (Rubiaceae), *Ervatamia heyneana* (Apocynanceae) and *Mostuea brunonis* (Loniaceae). Few endophytic fungal species are also abe to produce camptothecine from *Colletotrichum gleosporiodes*, *Xylaria* sp., *Fusarium solani*, and unidentified fungi from *Camptotheca acuminata*, *Enterphosphora infrequens*, *Neurospora* sp. from *Nothapodytes foetida* and *Fusarium solani* from *Apodytes dimidiata* (Table 1).

Table 1. *List of plants and endophytes able to produce 10-hydroxycamptothecine.*

Plants name	Family
Nothapodytes foetida (Mappia foetida)	*Iccacinaceae*
Morrilliodendron megacarpum	
Ophiorrohiza mungus	*Rubiaceae*
Ophiorrohiza pumila	
Ervatamia heyneana	*Apocynaceae*
Mostuea brunonis	*Loganiaceae*
Endophytes	Host
Colletotrichum gloeosporioides (strain XSXY05)	
Xylaria sp	*Camptotheca acuminata*
Fusarium solani INFU/Ca/KF/3	
an unidentified endophytic fungal strain XK001	
Entrophospora infrequens RJMEF001	*Nothapodytes foetida*
Entrophospora infrequens 5124	
Neurospora sp. ZPSSE	
Fusarium solani MTCC9667	*Apodytes dimidiata*
Fusarium solani MTCC 9668	

The study was aimed to evaluate the short-time effects and toxicity of combination of HCPT plus L-OHP regimen in treatment of advanced colorectal cancer [5]. Topical application of 0.1 mg/ml HCPT could effectively prevent fibroblast proliferation and reduce epidural adhesion after laminectomy in rats [6]. Morphological variations of apoptosis were observed at 48 hours treated with HCPT by AO staining and apoptosis body was observed under electron microscope. The results indicated that HCPT can suppress the growth of human leukemia cell line K562 and induce apoptosis of the cells [7]. The upregulated expression of Raf kinase inhibitor protein in colorectal cancer cells inhibited cell invasion and metastasis, while down-regulation of RKIP reduced chemosensitivity by inhibiting apoptosis induced by hydroxycamptothecine [8] The camptothecine inhibits topoisomerase I catalytic activity, they stabilize the DNA/ protein complex by forming topoisomerase I-DNA adduct so that the normal, rapid process of strand division, disentangling and rejoing is arrested at mid stage [9-10]. The camptothecine have showed typical apoptotic morphology, such as condensed chromatin, irregular nuclei, and apoptotic body formation. The mRNA and protein levels of caspase-3 and caspase-9 were upregulated, while caspase-8 was unchanged [11]. Cancer is causing more deaths in the world due to its abnormal growth of cells. In 2012, the worldwide burden of cancer rose to an estimated 14 million new cases per year, expected to rise to 22 million annually within the next two decades. Over the same period, cancer deaths are predicted to rise from an estimated 8.2 million annually to 13 million per year. Globally, in 2012 the most common cancers diagnosed were those of the lung (1.8 million cases, 13.0% of the total), breast (1.7 million, 11.9%), and large bowel (1.4 million, 9.7%). The most common causes of cancer death were cancers of the lung (1.6 million, 19.4% of the total), liver (0.8 million, 9.1%), and stomach (0.7 million, 8.8%). According to recent report estimated the number of people living with cancer disease is around 2.5 million and every year, new cancer patients registered are over 7 lakh and cancer related deaths are 5, 56, 400 may reach to between 30-69 years. Men are more prone to cancer compared to women. The 60-80% cancer cases in India are diagnosed late and 60% patients do not have access to quality treatment (Cancer Statistics in India, 2014). This makes a more prominent requirement for management of cancer.

Malignancy is represented by qualitative and quantities of proteins with different functions. These proteins can go about as critical destructive variables that can restrain the pathway with the help of ligands. These ligands have capacities to disturb the disease pathway and accordingly keeping its unsafe impacts to the host cell. 10-hydroxycamptothecine has been one such ligand which has great pharmacological action against various sorts of malignancies [12].

In study review, we aimed to screen the inhibitory properties of 10-hydroxycamptothecine against different tumour proteins by computer supported virtual screening. We have broken down the association of 10-hydroxycamptothecine with receptors of various kind of growth which incorporates gastric tumour, prostate disease, dangerous pleural mesothelioma, glioma, little cell lung malignancy, endometrial tumor, bosom tumor and so on. We have performed mutlireceptor docking with 10-hydroxycamptothecine.

2. Materials and Methods

2.1. Selection of Receptors

The receptors were chosen in light of their capacity in the pathway of different sorts of tumours. Distinctive cancer pathways were examined from Kyoto Encyclopedia of Genes and Genomes [13] which incorporates cervical tumor, gastric malignancy, colorectal growth, endometrial malignancy, thyroid tumor, hepatocellular carcinoma, oral growth, esophageal tumour, bladder growth, choriocarcinoma, glioma, laryn-geal disease, ovarian disease, bosom tumour, cholangiocarcinoma, alveolar rhabdomyosarcoma, prostate tumour, harmful pleural mesothelioma, synovial sarcoma, Hodgkin lymphoma, little cell lung malignancy and vulvar malignancy and receptors which assume enter part in the

pathway were chosen. The receptors chose for study reviews have appeared in Table 2. The three dimensional structures of these receptors were accessible in their local shape in PDB [14] database. The three dimensional directions of the chose receptors were recovered from PDB database.

Table 2. *The ligand and receptor interaction data obtained from docking studies. The binding energy between 10-hydroxycamptothecine and the confirmation of various cancer drug targets is analysed in kcal/mol.*

PDB Number	Type of cancer	Binding energy (kcal/ mol)	Amino acid binding sites
1axc	Cervical cancer	-88.71	glu 109, glu 192, ser 109, pro 140, glu 143, glu 193, glu 193, ser 223
1bxl	BCl-Xl BAK	-100.19	arg6, glu 7, asp 29, asn 33, thr 35, gln 3, arg 6, glu 7, val 10, arg 34, thr 35
1d5r	small cell lung cancer	-115.4	cys124, lys125, gly127, arg130, thr167, gln171, asp92, his93, lys125, lys126, gly129, arg130, gln171
1f16	BAX	-108	gly11, thr 14, ser16, ser16, arg9, arg9, gly10, gly11, gly12, pro13, thr14, thr14, ser15, ser15
1fl6	*reps1 EH domain*	-107.94	gly11, thr14, ser16, ser16, arg9, arg9, gly10, gly11, gly12, pro13, thr14, thr14, ser15, ser15
1g5j	BAD	-97.1	asp33, glu11, val14, asp33, val34, glu35, glu35, glu36, thr39
1ikn	Hodgkin lymphoma	-107.35	lys28, glu49, ser51, asp53, lys28, ser51, thr52, thr52, asp53, asp53, glu225, pro275, pro275, ser276
1jdh	Colorectal cancer	-84.39	arg225, his265, his223, his223, his224, his265, ala42, lys45
1kz7	MCF	-94.8	ala13, val14, gly15, val14, gly15, cys18, phe28, val33, gln116, ala159
1mox	Gastric cancer	-85.46	asn32, asn32, asn33, fuc601, fuc601, lys5, lys5, asn32, asn33, asn33
1mrk	two ribosome-inactivating proteins	-107.51	asn110, tyr111, glu112, ser159, tyr70, asn110, asm110, tyr 111, tyr111, ile 155, glu160
1nql	epidermal growth factor (EGF)	-99.9	asn314, asn331, his334, asn314, glu32o, phe321, thr330, asn331, his334, his334, phe335
1p4o	Malignant pleural mesothelioma	-94	gln1084, lys1081, gln1084, glu1088, asp1116, phe1117, phe1117, thr1118, glu1241
1tgr	Malignant pleural mesothelioma	-90.4	cys6, cyc6, leu10, cys42, cys6, cys6, leu10, cys37, cys38, cys42
2a2r	Prostate cancer	-74.32	ala22, gln26, ser27, trp28, lys29, phe192, glu197
2ax6	Prostate cancer	-73.15	trp741, met745, arg752, gln711, gln711, leu712, leu712, trp741, trp741, trp741, leu744, met745
2axi	Penile cancer	-76.37	gln72, his73, leu24, gln72, his73, his73, val93, lys94, glu22, glu22, leu24
2j5r	MCF-9-EGFR tyrosine kinase	-102.9	gly729, leu730, pro741, leu1001, met1002, asp1003, asp1003, glu1004, met1007, met1007, val1010
2r7g	retinoblastoma protein	-91.57	arg418, asn478, met484, asn522, asn522, lys524, arg418, arg418, asp421, asn480
2w3l	Small cell cancer	-91.58	arg66, arg68, arg68, arg69, arg69, asp70, asp70, arg68, arg68, arg69, arg69
2w39	Ovarian cancer	-92.93	thr37, arg87, ser90, asn151, arg61, thr37, ala39, pro40, arg87, leu91, lys149, asn151
2w96	cdk4 in complex with a d-type cyclin	-102.2	lys35, asp97, asp99, arg101, asp158, lys35, phe93, val96, asp99, glu144, leu147, asp158
3b2t	Gastric cancer	-98.01	tyr566, asp644, asp644, leu487, val495, lys517, tyr566, ala567, ser568, gly570, leu633
3co6	FoxO1 DBD	-88.2	ala177, arg180, gla185, ser176, ser176, glu178, leu181, gln185, trp189, trp236
3hhm	Ovarian Cancer	-109	val851, asn853, ser854, trp780, tyr836, val851, arg852, asn853, ser854, met922, ile932
3mjk	Growth factor precursor	-90.2	ile88, glu89, lys97, arg86, thr100, glu126, lys125, lys127, glu176
3poz	Oral cancer	-93.5	arg841, pro877, ser720, gly721, phe723, arg841, lys875
3ppo	pancreatic cancer	-73.61	asn72, thr94, tyr217, gln39, gly71, tyr91, tyr137, asp169, ala171, tyr217
4hrl	HER-2	-100.28	his9, asp13, phe45, gln134, ile162, ser12, ser12, phe45
4qnq	BCl-XL	-87.9	phe97, asn136, arg139, glu96, arg100, tyr101, asn138, gly138, arg139, arg139
1gjh	BCl-2	-101.2	thr7, his186, thr7, gly8, tyr9, his186, gln190, trp195
1g5m	BCl-2 protein	-111.8	tyr9, gln190, trp195, thr7, tyr9, his186, ile189, gln190, gln190, gly193, gly194, trp196, trp195
2roc	MCl-1 with PUMA	-90.5	ser164, arg165, arg168, lys178, glu161, arg165, arg165, arg168, lys178, lys178
2vof	BH3 domain	-102	ile144, asp147, ser43, asn51, gln151, glu47, asn51, ile144, glu47, lys50, asn51, gln151
4yk3	BID	-94	cys232, ala199, glu200, gly210, arg211, thr218, thr218, asn219, asn219, ala220
5agx	Bcl-2 alpha beta-1 LINEAR complex	-94.8	arg20, arg21, arg8, asp12, phe14, tyr108, ala19, arg20, arg8, arg8, ile10, ile10, asp12
5jsb	MCl-1	-97.8	gln189, lys194, arg214, arg108, gly192, lys194, thr226, arg108

2.2. Selection of Ligand

Structure of 10-hydroxycamptothecine was obtained from NCBI Pubchem database [15]. To do the near review, one ligand was additionally chosen, taxol. The pharmacokinetics properties were screened utilizing Pre-ADMET device [16]. Sedate likeliness, ADME profile and lethality examination were anticipated for all the three ligands.

The ADME incorporates rate of retention, appropriation, digestion system and discharge. Pre-ADMET predicts poisonous quality in view of the AMES parameters and rat cancer-causing nature tests of rodent and mouse [16].

2.3. Multireceptor Docking

Molecular docking is performed to concentrate the receptor-ligand association which is viewed as the reason for structure based medication revelation. Docking studies were performed via iGEMDOCKv4.2 [17]. The reactant and restricting site of the objective has been identified via AutoGrid. The structure and synthetic properties of the dynamic destinations permit the acknowledgment and authoritative of the ligand. Around 2,500,000 bioactive adaptations were created by 10 emphases and the best compliances were screened regarding most minimal restricting vitality produced in the grouping histogram. The communications of 10-hydroxycamptothecine with selected receptors were further contrasted and the association of those receptors with their normal ligands. Advance, the approvals of the docking results were done utilizing set of known atoms for every malignancy receptors from accessible writing. The ascertained docking vitality was contrasted and measured trial restricting vitality connected with known atoms for every receptor.

2.4. ADME Test

The ADME/toxicity parameters compliance was evaluated by screening through admetSAR, a commercial tool. The admetSAR is system pharmacology or system chemical biology and toxicology platform designed for the assessment of would be therapeutic indications, off-target effects and potential toxic end points of natural products. In the studied work, this database/tool was used to predict and evaluate the human metabolism compliance, toxicity risk assessment and mode of action by using standard experimental data.

3. Results and Discussion

3.1. Selection of Receptors

Based on their functions in different types of cancers, the receptors were selected. Thirty seven receptors and eight different caspase receptors were selected based on the functional role in the pathways. Cyclin Dependent Kinase Inhibitor 1A (CDKI1A) playing important role in negative control of cell cycle progression [18] and are crucially involved in cell cycle arrest at G1 phase. Machin et al. [19] have explained that beta 1 (CTNNB1) along with cadherin associated protein interacts with other proteins it leads to variety of processes of canrconogenesis such as control of ageing and survival, regulation of circadian rhythm and lysosomal sorting of G protein-coupled receptors.

Epidermal development components (EGF) introduce in the extracellular space of film bound proteins and are included in arrangement of disulphide bonds [20]. Changes can bring about over expression and prompts to disease. Epidermal development figure receptor (EGFR) [21], changing development consider, alpha (TGFA) [22], insulin-like development component 1 receptor (IGF1R) [23] and erythroblastic leukemia viral oncogene homolog 2 (ERBB2) [24] comprises of areas and make up the bilobal ligand restricting site.

Akt murine thymoma viral oncogene homolog 2 (AKT2) [25] and fibrobroblast development factor receptor 2 (FGFR2) [26] have a place with serine/threonine protein kinases. They frame reactant area and are included in protein phosphorylation. Forkhead box O1 (FOXO1) [27] has HNF-3/fork head DNA-acknowledgment theme takes after histone H5. Capacity of glutathione S-transferase pi1 (GST-PI) [28] is conjugation of lessened glutathione to an assortment of targets. Insulin-like development element 1 (IGF1) [29] are emitted administrative hormones. They are disulfide rich alpha overlay. Androgen receptor (AR) [30] shapes DNA restricting space of an atomic hormone receptor. Mdm2, p53 E3 ubiquitin protein ligase homolog (MDM2) is an inhibitor of the p53 tumor silencer quality [31] restricting the transactivation space and down directs the capacity of p53 to enact interpretation. Atomic component of kappa (NFK-BIA) [32] is a light polypeptide quality enhancer in B-cells inhibitor, alpha contains rehash space of layer restricting intervenes most restricting exercises of protein. Platelet-inferred development figure alpha (PDGFA) polypeptide and platelet-determined development consider beta (PDGFB) [33] polypeptide are included in flag transduction and is an endogenous inhibitor of protein phosphatase-1. PIK3CA - phosphatidylinositol-4, 5-bisphosphate 3-kinase, reactant subunit alpha have Ras-restricting spaces in their N-ends.

PTEN - phosphatase and tensin homolog assume a key part in film official. Retinoblastoma 1 (RB1) [34] is required for high affinity official to E2F-DP edifices what's more, for maximal constraint of E2F-responsive promoters, in this manner going about as a development silencer by obstructing the G1-S move of the cell cycle. Cyclin D1 (CCNDI) controls cyclin subordinate kinases (CDKs) [35]. B-cell CLL/lymphoma 2 (BCL2) [36] smothers apoptosis in an assortment of cell frameworks including component subordinate lymphohematopoietic and neural cells and directs cell passing by controlling the mitochondrial film penetrability.

3.2. Selection of Ligands

Ligands were anticipated for pharmacokinetic properties utilizing Pre-ADMET apparatus. Tranquilize resemblance,

ADME and poisonous quality forecasts were performed. We have seen that 10-hydroxycamptothecine is very much qualified regarding pharmacokinetic elements, for example, human intestinal assimilation, $CaCo_2$ (heterogeneous human epithelial colorectal adenocarcinoma) cell porousness, MDCK (Madin-Darby canine kidney) cell penetrability, skin porousness and blood mind obstruction entrance. The poisonous quality reviews uncovered that 10-hydroxycamptothecine observed to be non-cancer causing agent and non-mutagen anticipated by ADME test and mouse carcinogenicity show separately (Table 3).

Table 3. *The binding energy between 10-hydroxycamptothecine and the confirmation of various caspases targets is analysed in kcal/mol.*

PDB Number	Type of cancer	Binding energy (kcal/ mol)	Amino acid binding sites
On different caspase enzymes			
1bgw	Topoisomerase	-101.29	arg364
3qx3	Top2β-DNA-etoposide complex	-83.04	arg820, tyr821, arg820, arg820, tyr821, gly776, glu777, gln778, gln778
1zxm	Top2A	-104.79	lys83, arg241, asp246, trp62, tyr82, arg241, arg242, ile311, ala318, ser320
2ar9	Caspase 3	-61.66	thr181, arg355, thr181, trp354, arg355, arg355, asp356, asp356, pro357
2x70	Caspase -3	-118.9	arg5, asp29, asp29, arg180, thr181, gly0, his2, his3, arg28, asp28, tyr208, pro209, glu263
1d3y	Topo 4	-103.35	glu143, asp144, ala226, ser199, lys222, glu143, asp144, lys222, ser199, lys222, lys222
1i4o	Caspase 7	-84.82	arg102, lys254, gln303, leu67, arg102, ser103, leu104, gly105, lys254, phe301
5juy	Caspase 9	-94.1	cys158, lys160, ser161, val162, tyr361, gly153, lys160, ser161, ser161, asp244, trp246, tyr359, asp360, tyr361

Henceforth, the present study demonstrated that 10-hydroxycamptothecine, qualifying the greater part of the tenets can be a perfect medication applicant. Pharmacological forecasts of this compound were similarly low contrasted with 10-hydroxycamptothecine. There was an infringement in lead of 5; be that as it may, forces high blood cerebrum boundary entrance. The medication likeliness properties and pharmacokinetic elements are appeared in Table 4.

Table 4. *ADMET Prediction profile for 10-hydroxycamptothecine.*

Model	Result	Probability
Absorption		
Blood-Brain Barrier	BBB-	0.9148
Human intestinal absorption	HIA+	0.8689
Caco-2 permeability	Caco2-	0.7358
P-glycoprotein substrate	Substrate	0.7084
p-glycoprotein inhibitor	Non-inhibitor	0.8601
	Non-inhibitor	0.9867
Renal organic cation transporter	Non-inhibitor	0.8750
Distribution		
Metabolism		
CYP450 2C9 substrate	Nonsubstrate	0.8700
CYP450 2D9 substrate	Nonsubstrate	0.8243
CYP450 3A4 substrate	Substrate	0.5140
CYP450 1A2 inhibitor	Inhibitor	0.8107
CYP450 2C9 inhibitor	Noninhibitor	0.8592
CYP450 2D6 inhibitor	Noninhibitor	0.8811
CYP450 2C19 inhibitor	Noninhibitor	0.8916
CYP450 3A4 inhibitor	Inhibitor	0.6208
CYP inhibitory promiscuity	Low CYP inhibitor Promiscuity	0.6193
Excretion toxicity		
Human ether-a-go-go related gene inhibition	Weak inhibitor	0.9948
	Non-inhibitor	0.8250
AMES toxicity	Non AMES toxic	0.5413
Carcinogens	Non-carcinogens	0.8341
Fish toxicity	High FHMT	0.9248
Tetrahymena Pyriformis toxicity	High TPT	0.9396
Honey Bee toxicity	Low HBT	0.7814
Biodegradation	Not ready biodegradable	1.0000
Acute oral toxicity	II	0.5498
Carcinogenecity (three class)	Non-required	0.5098
Absorption		
Model	Value	Unit
Aqueous solubility	-2.9300	LogS
Caco-2 permeability	0.8648	LogPapp, cm/s
Distribution, Metabolism, excretion, toxicity		
Rat acute toxicity	2.9852	LD50, mol/kg
Fat toxicity	1.4090	pLC50, µg/L

3.3. Multireceptor Docking of 10-Hydroxycamptothecine

To analyse inhibitory action of 10-hydroxycamptothecine with different cancer receptors performed the multireceptor docking studies and it was considered as probable drug target. Selected the best docked conformations based on the highest negative binding energy of docked complex, number of interacting residues and number of hydrogen bonds. The present study reveals that, 10-hydroxycamptothecine having best biding properties against all tested receptors. The three receptors (EGF, GST-PI and PDGFA) were highly inhibited by 10-hydroxycamptothecine. The EGF is considering for identifying drug target in gastric cancer [33], GST-PT helps in progression of prostate cancer [28] and PDGF showing virulent function in malignant pleural mesothelioma and glioma [33]. The binding energy of these receptors with ligand was -99.9, -74.32, and -90.2 kcal/ mol respectively. Interaction with 10-hydroxycamptothecine with EGF and PDGFA was more due to their hydrogen bonds. The main residues interacting with 10-hydroxycamptothecine in EGF are ASN314, ASN331, HIS334, ASN314, GLU32O, PHE321, THR330, ASN331, HIS334, HIS334, PHE335 (Figure 1) and in PDGFA are ILE88, GLU89, LYS97, ARG86, THR100, GLU126, LYS125, LYS127, GLU176 (Figure 2). The interacting residues of GST-PI with 10-hydroxycamptothecine are ALA22, GLN26, SER27, TRP28, LYS29, PHE192, GLU197 (Figure 3). All the docked conformation of different cancer drug targets with 10-hydroxycamptothecine is listed in Table 2.

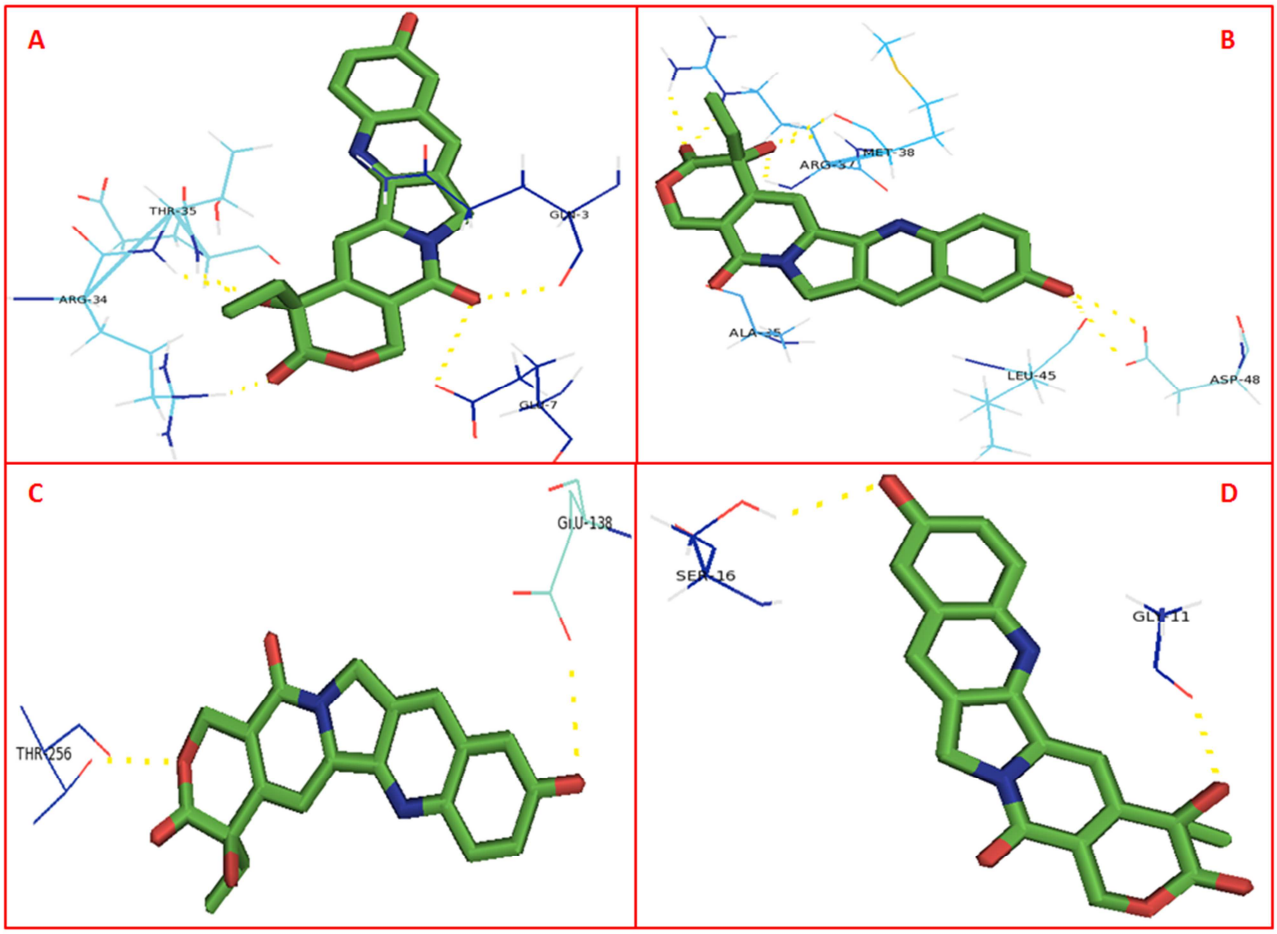

Figure 1. *Molecular docking studies of 10-hydroxycamptothecine and cancer drug targets. (a). BCl-xl BAK (1bxl+Camptothecine), (b). Resps1 EH domain (1f16 Bax+ Camptothecine), (c). Hodgkin lymphoma (1mrk- two ribosome-inactivating proteins), (d). BAX proteins (1IKN+ Hodgkin lymphoma). The binding energy found to be -100.19 kcal/mol, -108 kcal/mol, -107.35 kcal/mol and -107.51 kcal/mol respectively.*

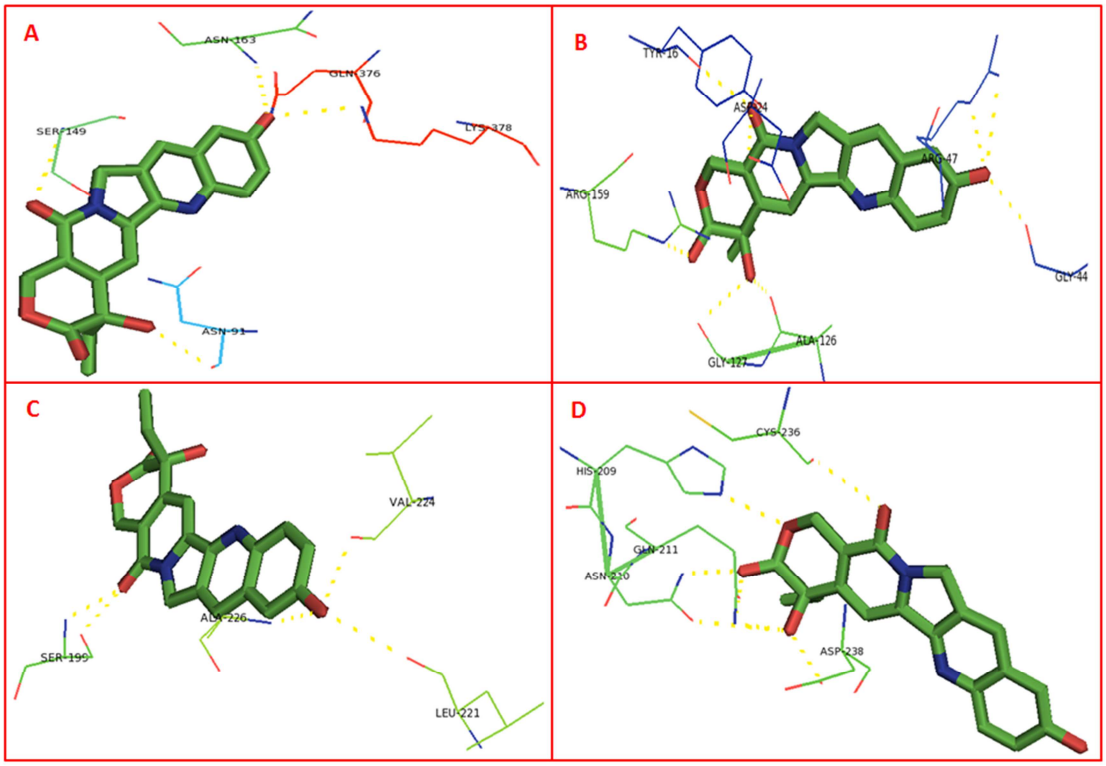

Figure 2. *Molecular docking studies of 10-hydroxycamptothecine and cancer drug targets. (a). Topoisomerase 2A (1zxm-Topo 2A), (b). PTEN- Phosphatase and tensin homolog (1d5r-PTEN- Phosphatase and tensin homolog), (c). Topoisomerase 4 (1d3y-Topoisomerase 4), (d). EGF-Epidermal Growth Factor (1nql-EGF (Epidermal Growth Factor)). The binding energy found to be -104.70 kcal/mol, -115.4 kcal/mol, -103.35 kcal/mol and -99.9 kcal/mol respectively.*

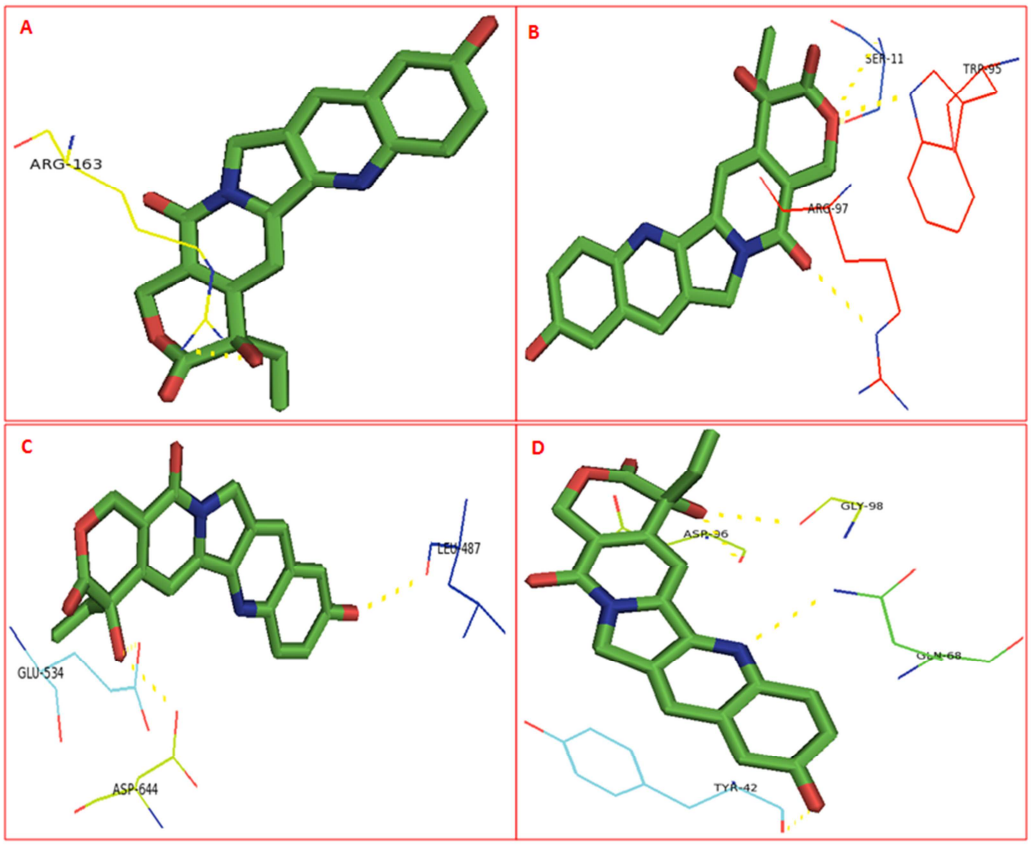

Figure 3. *Molecular docking studies of 10-hydroxycamptothecine and cancer drug targets. (a). CDK4 in complex with a D-type cyclin (2w96-CDK4 in complex with a D-type cyclin), (b). Caspase-3 (2x70-Caspase-3), (c). Gastric cancer-FGFR2 (3b2t-Gastric cancer-FGFR2), (d). HER-2 (4hrl-HER-2). The binding energy found to be -102.2 kcal/mol, -118.9 kcal/mol, -98.1 kcal/mol and -100.28 kcal/mol respectively.*

The more energy binding number was found when interacted with 10-hydroxycamptothecine by PTEN (phosphatase and tensin homolog) at CYS124, LYS125, GLY127, ARG130, THR167, GLN171, ASP92, HIS93, LYS125, LYS126, GLY129, ARG130, GLN171 (-115.4 kcal/ mol), Reps1 EH Domain at GLY11, THR14, SER16, SER16, ARG9, ARG9, GLY10, GLY11, GLY12, PRO13, THR14, THR14, SER15, SER15 (-107.94 kcal/ mol), nuclear factor of kappa light polypeptide gene enhancer at LYS28, GLU49, SER51, ASP53, LYS28, SER51, THR52, THR52, ASP53, ASP53, GLU225, PRO275, PRO275, SER276 (-107.35 kcal/ mol), MCF-9 EGFR tyrosine kinase at GLY729, LEU730, PRO741, LEU1001, MET1002, ASP1003, ASP1003, GLU1004, MET1007, MET1007, VAL1010 (-102.9 kcal/ mol), CDK4 complex with D-type cyclin at LYS35, ASP97, ASP99, ARG101, ASP158, LYS35, PHE93, VAL96, ASP99, GLU144, LEU147, ASP158 (-102.2 kcal/ mol), Phosphatidylinositol-1, 5-biphosphate 3-kinase (PIK3CA) at VAL851, ASN853, SER854, TRP780, TYR836, VAL851, ARG852, ASN853, SER854, MET922, ILE932 (-109 kcal/ mol) (Figure 4).

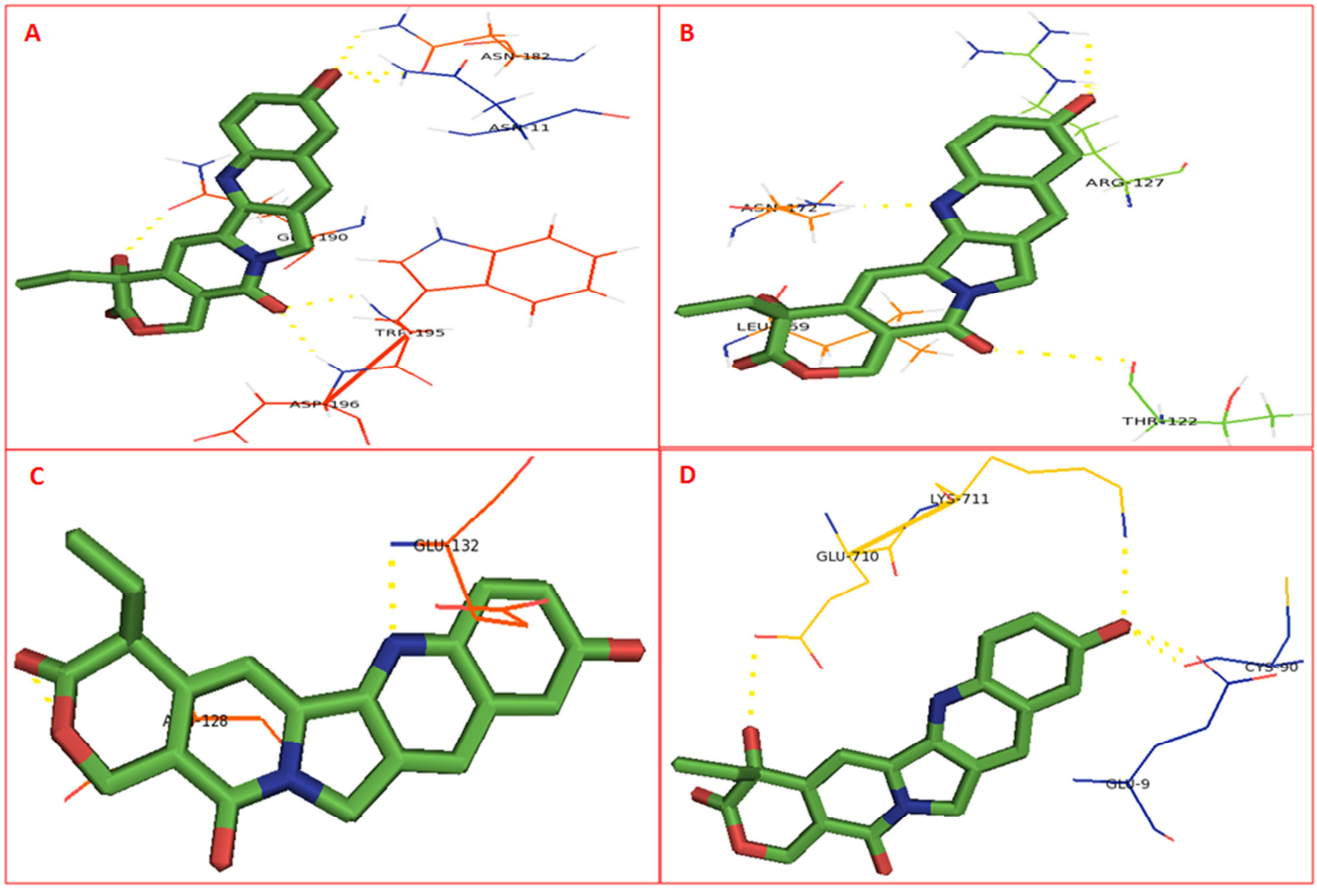

Figure 4. *Molecular docking studies of 10-hydroxycamptothecine and cancer drug targets. (a). BCl-2 inform 1 (Ig5m-BCl-2 inform 1), (b). BCl-2 inform 2 (1gjh-BCl-2 isoform 2), (c). BH3 domain (2vof-BH3 domain), (d). PIK3CA-Phosphatidylinositol-4,5-bisphosphate 3-kinase (3hhm-PIK2CA-Phosphatidylinositol-4,5-bisphosphate 3-kinase). The binding energy found to be -101.2 kcal/mol, -111.8 kcal/mol, -102 kcal/mol and -97.84 kcal/mol respectively.*

The more interaction studies were observed with caspase enzymes by 10-hydroxycamptothecine, topoisomerase at ARG364, (-101.29 kcal/ mol), Top2A at LYS83, ARG241, ASP246, TRP62, TYR82, ARG241, ARG242, ILE311, ALA318, SER320 (-104.79), caspase -3 at ARG5, ASP29, ASP29, ARG180, THR181, GLY0, HIS2, HIS3, ARG28, ASP28, TYR208, PRO209, GLU263 (-118.9 kcal/ mol), topo 4 at GLU143, ASP144, ALA226, SER199, LYS222, GLU143, ASP144, LYS222, SER199, LYS222, LYS222 (-103.35), caspase 9 at CYS158, LYS160, SER161, VAL162, TYR361, GLY153, LYS160, SER161, SER161, ASP244, TRP246, TYR359, ASP360, TYR361 (-94.1 kcal/ mol) (Figure 5).

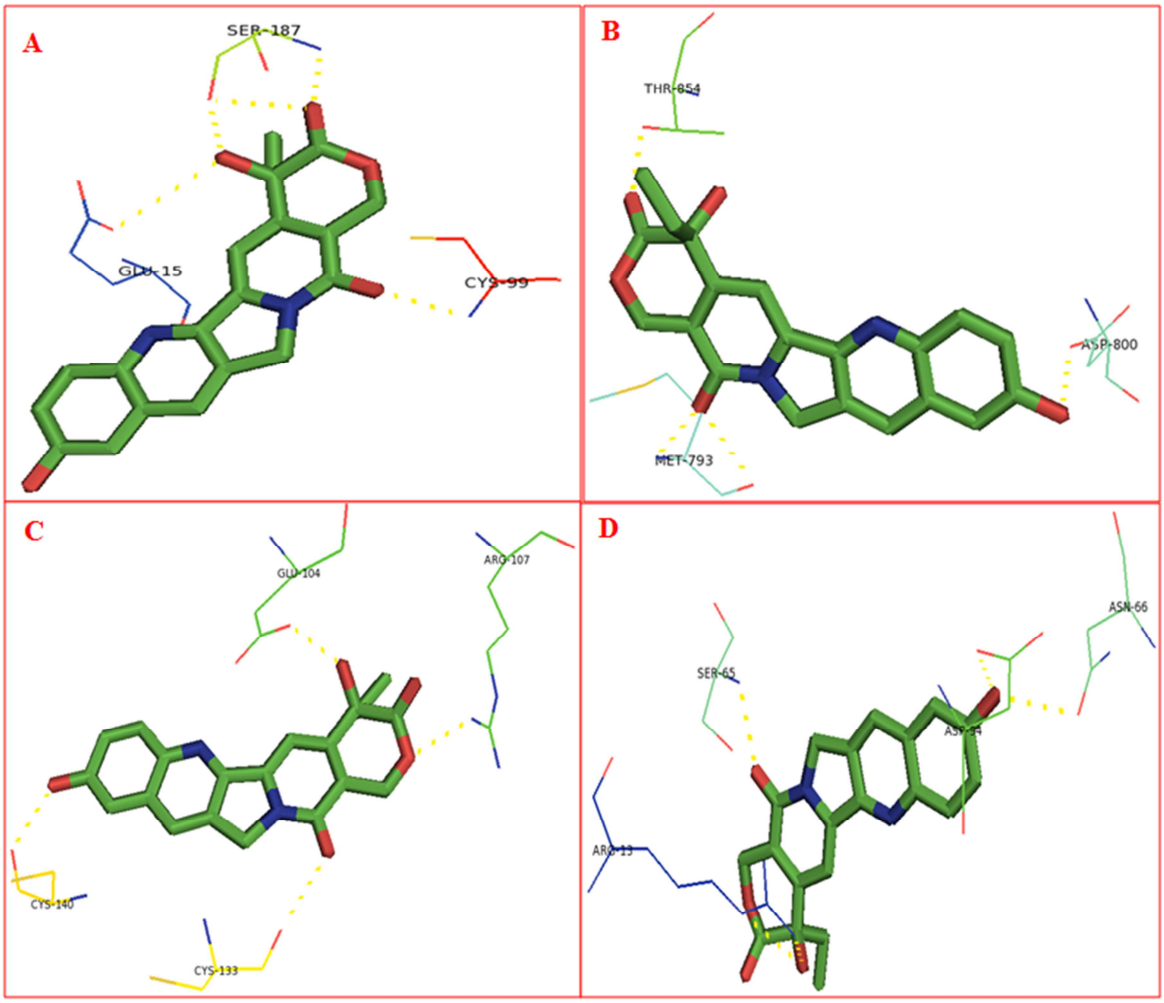

Figure 5. *Molecular docking studies of 10-hydroxycamptothecine and cancer drug targets. (a). PDGFB-Platelet Derived Growth Factor Beta Chain (3mjg-PDGFB-Platelet Derived Growth Factor Beta Chain), (b). EGFR-Epidermal Growth Factor Receptor Kinase (3poz-EGFR-Epidermal Growth Factor Receptor Kinase), (c). Platelet derived growth factor alpha chain (PDGFA) (3mjk-Platelet derived growth factor alpha chain (PDGFA), (d). Glutathione-S-Transferase Pi gene (2a2r-Glutathione-S-Transferase Pi gene). The binding energy found to be -103.58 kcal/mol, -90.13 kcal/mol, -102 kcal/mol and -106.93 kcal/mol respectively.*

DNA topoisomerases Type II α (topo II α) and II Beta catalyze the ATP-subordinate transport of one in place DNA twofold helix through another [37]. Topo II α assumes a key part in DNA replication with primary capacities are chromosome isolation, chromosome build up, capture in meiosis I and recombination suppression [38]. It is perceived that topo II α abnormal state expression relate with medication affectability and topo II-α low level correspond with medication resistance [39]. Increased topo II α expression is associated with an aggressive form of breast cancer predicts disease-related death, lymph node metastasis, and advanced tumor stage as prognostic markers [40].

DNA topoisomerase I (Top1) is over-expressed in tumour cells and is an important target in cancer chemotherapy. It relaxes DNA torsional strain generated during DNA processing by introducing transient single-strand breaks and allowing the broken strand to rotate around the intermediate Top1 – DNA covalent complex [41].

DNA topoisomerase I plays important role in DNA replication by managing the topological state of the DNA in the cell, allowing selected regions of DNA to become sufficiently untangled and relaxed to permit its replication, recombination, repair, and transcription [42-43]. There are two major types of DNA topoisomerases: DNA topoisomerase I initiates the cleavage of a strand of DNA molecule while DNA topoisomerase II cleaves both DNA strands [44].

Caspases are crucial mediators of programmed cell death (apoptosis). Among them caspase-3 is frequently associated protease, catalyzing the target specific cleavage of many key cellular proteins [45]. Caspase -7 is executioner caspases, activates the intrinsic apoptotic pathways through cleavage of BID to induce efficient cell death, apoptotic cell development. Caspase-9 initiates apoptosis by cleaving and thereby activating executioner caspases, mitochondrial morphological changes, ROS production by cleaving and activating BID into tBID [46]. The caspase-3 enzyme plays a major role in the apoptotic signalling cascade and was found to be activated in multiple signalling pathways of apoptosis and hence chosen as potential target in this study [47-48].

From above study result, the 10-hydroxycamptothecine has broad spectrum properties against different cancer drug targets. The pharmacokinetics, inhibitory and ADME properties of 10-hydroxycamptothecine can used for inhibition of various types of cancer proteins. The results are only based on computer aided virtual screening evidences for all previous work has been done on in vivo studies and they tried the same ligand on few cancer cell lines. The study results may be proved significant insights to work on various types of cancer to produce a single compound can be used for treating various types of cancer.

4. Conclusion

Every day the cancer incidence is increasing in world due to various factors and the synthetic chemicals have lot of adverse effects. The natural products may not show their activity immediately but they are potential and have no side effects. The 10-hydroxycamptothecine is producing various types of plants and fungal endophytes showing all most biological activities. Based on *in silico* virtual screening using computer software, the 10-hydroxycamptothecine has shown more potent in inhibition of various types of cancer proteins by showing highest binding energy. The study also evident that 10-hydroxycamptothecine was an ideal drug agent with better drug likeliness and pharmacokinetics properties. Further, the compound inhibited the more virulent genes or proteins of different cancer. The compound can be used as therapeutic molecule for different types of cancers when the treatment in cancer fails to manage. The present research is the exhibit information clear vital points of interest for further reviews to approve 10-hydroxycamptothecine as promising medication applicant against different malignancies.

Conflict of Interest Statement

All the authors do not have any possible conflicts of interest.

Acknowledgements

The authors thank Dr Kiran S, HOD of Department of Biotechnology of DSCE, Dr CPS Prakash, The principal of DSCE and The Management and The Secretary of Dayananda Sagar Institutions, Bengaluru, India for their encouragement.

References

[1] Lin, L. Z; Cordell, G. A. (1990). 13C-NMR assignments of camptothecine and 10-hydroxycampthocine, J Natural Prod, 53 (1): 186-189.

[2] Hsiang, C. Y; Ho, T. Y; Lin, C. H; Wu, K; Chang, T. J. (1996). Analysis of upregulated cellular genes in pseudorabies virus infection: use of mRNA differential display. J Virol Methods, 62: 11–19.

[3] Pommier, Y. (2006). Topoisomerase I inhibitors: camptothecines and beyond, Nature Rev Cancer, 6: 789–802.

[4] Li, P; Chen, Q; Yu, F; Xie, X; Wang, S. (2015). Simultaneous determination of camptothecin and 10-hydroxycamptothecine in the *Camptotheca acuminata*, its medicinal preparation and in rat plasma by liquid chromatography with fluorescence detection, Biomed Chromatography, 29 (10): 1522-1526.

[5] Sun, Y; Zhao, H; Lin, F; Cai, X; Tang, X; Yao, Y. (2007). The short-time effects of chemotherapy with combinations of hydroxycamptothecine and oxaliplatin in treatment of advanced colorectal cancer, The Chinese-German J Clin Onc, 6 (1): 40–43.

[6] Sun, Y; Wang, L; Sun, S; Liu, B; Wu, N; Cao, X. (2008). The effect of 10-hydroxycamptothecine in preventing fibroblast proliferation and epidural scar adhesion after laminectomy in rats, Eur J Pharm, 593 (1-3): 44-48.

[7] Shao, S. L; Zhang, W. W; Zhao, W; Wu, G. H. (2012). Apoptosis of human leukemia cell line K562 cells induced by hydroxycamptothecine, Adv Mater Res, 535-537: 2420-2424.

[8] Nie, F; Cao, J; Tong, J; Zhu, M; Gao, Y; Ran, Z. (2015). Role of Raf-kinase inhibitor protein in colorectal cancer and its regulation by hydroxycamptothecine, J Biomed Sci, 5: 22: 56.

[9] Covey, J. M; Jaxel, C; Kohn, K. W. (1989). Cancer Res, 48: 5016.

[10] Hsiang, Y. H; Lihou, M. G; Liu, L. F. (1989). Cancer Res, 49: 5077.

[11] Tang, W; Zhang, Y; Oian, C; Yuan, Z; Du, J. (2012). Induction and mechanism of apoptosis by hydroxycamptothecin in human Tenon's capsule fibroblasts, Invest Ophthal Visual Sci, 53: 4874-4880.

[12] Aggarwal, S; Takada, Y; Singh, S; Myers, J. N; Aggarwal, B. (2004). Inhibition of growth and survival of human head and neck squamous cell carcinoma cells by curcumin via modulation of nuclear factor-kB signalling, Int J Cancer, 111: 679-692.

[13] Kanehisa, M; Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes, Nucl Acid Res, 28: 27-30.

[14] Berman, H. M; Westbrook, J; Fengm Z; Gilliland, G; Bhat, T. N; Weissig, H; Shindyalov, I. N; Bourne, P. E. (2000). The Protein Data Bank, Nucl Acid Res, 28: 235-242.

[15] Wang, Y; Xiao, J; Suzek, T. O; Zhang, J; Wang, J; Zhou, Z. (2012). PubChem's BioAssay Database, Nucl Acid Res, 40: D400-412.

[16] Seal, A; Aykkal, R; Babu, R. O; Ghosh, M. (2011). Docking study of HIV-1 reverse transcriptase with phytochemicals, Bioinformation, 5: 430-439.

[17] Sharmila, R; Subburathinam, K. M; Aishwarya, S; Margret, A. A. (2013). *In silico* analysis of andrographolide against cancer, Int J Pharm Sci Drug Res, 5 (2): 56-61.

[18] Jalili, A; Wagner, C; Pashenkov, M; Pathria, G; Mertz, K. D; Widlund, H. R; Lupien, M; Brunet, J. P; Golub, T. R; Stingl, G; Fisher, D. E; Ramaswamy, S; Wagner, S. N. (2012). Dual suppression of the cyclin dependent kinase inhibitors CDKN2C and CDKN1A in human melanoma, J Natl Cancer Inst, 104: 1673-1679.

[19] Machin, P; Catasus, L; Pons, C; Munoz, J; Matias-Guium, X; Prat, J. (2002). CTNNB1 mutations and beta-catenin expression in endometrial carcinomas, Hum Pathol, 33: 206-212.

[20] Stoscheck, C. M; King, L. E. Jr. (1986). Role of epidermal growth factor in carcinogenesis, Cancer Res 46: 1030-1037.

[21] Mendelsohn, J; Baselga, J. (2006). Epidermal growth factor receptor targeting in cancer, Semin Oncol 33: 369-385.

[22] Greten, F. R; Wagner, M; Weber, C. K; Zechner, U; Adler, G; Schmid, R. M. (2001). TGF alpha transgenic mice. A model of pancreatic cancer development, Pancreatology, 1: 363-368.

[23] Hewish, M; Chau, I; Cunningham, D. (2009). Insulin-like growth factor 1 receptor targeted therapeutics: novel compounds and novel treatment strategies for cancer medicine, Recent Pat Anticancer Drug Discov, 4: 54-72.

[24] Sauter, G; Moch, H; Moore, D; Carroll, P; Ker-schmann, R; Chew, K; Mihatsch, M. J; Gudat, F; Waldman, F. (1993). Heterogeneity of erbB-2 gene amplification in bladder cancer, Cancer Res 53 (10): 2199-2203.

[25] Cicenas, J. (2008). The potential role of Akt phosphorylation in human cancers, Int J Biol Markers, 23: 1-9.

[26] Byron, S. A; Pollock, P. M. (2009). FGFR2 as a molecular target in endometrial cancer, Future Oncol, 5: 27-32.

[27] Maekawa, T; Maniwa, Y; Doi, T; Nishio, W; Yoshimura, M; Ohbayashi, C; Hayashi, Y; Okita, Y. (2009). Expression and localization of FOXO1 in non-small cell lung cancer, Oncol Rep, 22: 57-64.

[28] Re, A; Aiello, A; Nanni, S; Grasselli, A; Benvenuti, V; Pantisano, V; Strigari, L; Colussi, C; Ciccone, S; Mazzetti, A. P; Piercontim F; Pinto, F; Bassi, P; Gallucci, M; Sentinelli, S; Trimarchi, F; Bacchetti, S; Pontecorvi, A; Lo Bello, M; Farsetti, A. (2011). Silencing of GST-PI, a prostate cancer prognostic gene, by the estrogen receptor-β and endothelial nitric oxide synthase complex, Mol Endocrinol, 25: 2003-2016.

[29] Heidegger, I; Pircher, A; Klocker, H; Massoner, P. (2011). Targeting the insulin-like growth factor network in cancer therapy, Cancer Biol Ther, 11: 701-707.

[30] Heinlein, C. A; Chang, C. (2004). Androgen receptor in prostate cancer, Endocr Rev, 25: 276-308.

[31] Chene, P. (2003). Inhibiting the p53-MDM2 interaction: An important target for cancer therapy, Nat Rev Caner, 3: 102-109.

[32] Curran, J. E; Weinstein, S. R; Griffiths, L. R. (2002). Polymorphic variants of NFKB1 and its inhibitory protein NFKBIA, and their involvement in sporadic breast cancer, Cancer Lett 188: 103-107.

[33] Liu, Z; Liu, L; Li, M; Wang, Z; Feng, L; Zhang, Q; Cheng, S; Lu, S. (2011). Epidermal growth factor receptor mutation in gastric cancer, Pathology 43: 234-238.

[34] Chinnam, M; Goodrich, D. W. (2011). RB1, development, and cancer, Curr Top Dev Biol, 94: 129-169.

[35] Takano, Y; Takenaka, H; Kato, Y; Masuda, M; Mikami, T; Saegusam, M; Okayasum, I. (1999). Cyclin D1 overexpression in invasive breast cancers: Correlation with cyclin-dependent kinase 4 and oestrogen receptor over expression and lack of correlation with mitotic activity, J Cancer Res Clin Oncol, 125: 505-512.

[36] Hockenbery, D. M. (1994). BCl-2 in cancer, development and apoptosis, J Cell Sci Suppl 18: 51-55.

[37] Wang, J. C. (2002). Cellular roles of DNA topoisomerases: a molecular perspective, Nature Rev 3: 430-438.

[38] Watt, P. M; Hickson, I. D. (1994). Structure and function of type II DNA topoisomerases, Biochemical J 303: 681-695.

[39] Lynch, B. J; Donald, G; Guinee, Jr; Holden, J. A. (1997). Human DNA topoisomerase IIα: A new marker of cell proliferation in invasive breast cancer, Human Pathology 28 (10): 1180-1188.

[40] Depowski, P. L; Rosenthal, S. I; Brien, T. P; Stylos, S; Johnson, R. L; Ross, J. S. (2000). Topoisomerase II α expression in breast cancer. correlation with outcome variables, Modern Pathology, 13 (5): 542–547.

[41] Drwal, M. N; Agama, K; Wakelin, L. P. G; Pommier, Y; Griffith, R. (2001). Exploring DNA Topoisomerase I Ligand Space in Search of Novel Anticancer Agents. *PLoS One* 2011; 6 (9): e25150.

[42] Champoux JJ. DNA topoisomerases: structure, function, and mechanism, Annu Rev Biochem, 70: 369-413.

[43] Nobili, S; Lippi, D; Witort, E; Donnini, M; Bausi, L; Mini, E; Capaccioli, S. (2009). Natural compounds for cancer treatment and prevention, Pharm Res, 59: 365-378.

[44] Ewesuedo, R. B; Ratain, M. J. (1997). Topoisomerase I inhibitors, The Oncol, 6: 359-364.

[45] Porter, A. G; Janicke, R. V. (1999). Emerging roles of caspases in apoptosis, Cell Death and Differentiation, 6 (2): 99-104.

[46] Mcllwain, D. R; Berfest, G; Mak, T. W. (2013). Caspase functions in cell death and diseases, Cold Spring Harbor Perspective in Biology, 1-29.

[47] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, J Immunol Methods, 65: 55-63.

[48] Adinarayana, K. P. S; Babu, P. A; Palakeerthi, S. K. (2012). *In silico* lead identification by virtual screening and *in vitro* anti-cancer activities by MTT assay, Inter J Comp Bioinf In Silico Model, 1 (5): 55-57.