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INSIGHTING INTERACTION PATTERNS IN 7-ACETYLHORMINONE VIA DOCKING STUDIES AGAINST CDK2 AS ANTICANCER THERAPEUTIC TARGET

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ABSTRACT : 7-Acetylhorminone, abietane diterpene identified as anticancer CDK2 inhibitor. Molecular docking analysis has confirmed arrest of cell cycle in ATP binding subunit of CDK2 protein by 7-Acetylhorminone. Interaction pattern study reveals presence of quinone moiety in the hydrogen- bond (H-bond) formation of CDK2 - 7-Acetylhorminone docked complex aids in proving the antiproliferative and antineoplastic activity. The lead compound is showing comparable results with the standard drug "Dinaciclib" exhibiting more number of H-bonds and hydrophobic interactions. Tools like CDRUG and PASS server has proven the anticancer potency of the compound. Thus, in this scenario, 7-Acetylhorminone has been proposed as a better anti-cancer CDK2 inhibitor and may be preceded for further the biovalidation studies.

Key words : CDK2 inhibitor, molecular docking, 7-Acetylhorminone, interaction patterns, quinone moiety, anticancer lead compound.

INTRODUCTION

Cancer, an atrocious disease is crucially important to target so as to deflate the mortality rate increasing day by day worldwide (Kumar *et al*, 2017). Cancer cells are basically the resultant of deviant growth of cells/tissues inside the body. Cyclin dependent kinases 2 (CDK2) is a key controller in several phases (Resting phase (G0), Interphase (G1, S, G2) and the Mitotic phase (M)) of cell cycle. These phases are governed by the successive regulation of different types of CDKs. CDKs are the assemblage of serine/ threonine kinases that activates on associating with the regulatory proteins; cyclins. The standard cell cycle model suggests that CDK4or CDK6 linked with cyclin D controls events at early G1 phase, CDK2 combines with cyclin E to elicit S-phase while CDK2/CDK1 in complex with cyclin A regulates S-phase and CDK1- Cyclin B controls M-phase by phosphorylation of several proteins (Hochegger *et al*, 2008). Some other CDKs like CDK7/8/9 and CDK11/ 12/13 are known to regulate the transcription process (Mariaule and Belmont, 2014).

The conserved structure of CDKs has a specific ATP-binding cleft at its N-terminal, which binds to the substrates or ligands and alpha-helices rich cyclins to be complex for further cell cycle processes (Peyressatre *et al*, 2015). CDK2 is considered to be a therapeutic cancer target as it is intensively involved in various signaling pathways *viz.* centrosome duplication, synthesis of DNA, G1-S alteration, deviation in G2 phase processes. CDK2/ cyclin E complex activates p27 for proteasomal ubiquitination and degradation. Moreover CDK2/ cyclinA complex is involved in phosphorylation of Rb proteins which further initiates E2F release for transcription process. In tumorogenic environment, dysregulation of cyclins/CDK complexes results in interruption of the regular cell cycle and promoting overexpression of CDK2 or cyclins activity thereby resulting into activation of pre-apoptotic proteins in the absence of cyclin A-Cdk2 complex (Chohan *et al*, 2015; Peyressatre *et al*, 2015). From literature survey, various CDK2 inhibitors are at the clinical trial phases including synthetic (nilotinib, ADA, latuda, vilazodone, oxaliplatin, paliperidone, estradiol benzoate and azelastine hydrochloride) (Shi *et al*, 2015) as well as phytochemicals viz. Alvocidib (NSC-649890), BAY-1000394, R547, roscovitine, CYC-202, BMS-387032, TG02, Dinaciclib (Blachly and Byrd,

2013) are reported to possess several toxicities along with lesser anticancer activities. These compounds thus results in showing inhibitory actions towards multi CDKs sometimes along with other kinases too rather than the specific one (Law *et al*, 2015). Due to all these facts, there is a need to focus on the natural products or the phytochemicals to reduce the toxicity or side effects like baldness, myelosuppression, neurotoxicity, cardiotoxicity, and immunosuppression, mucositis (Singh *et al*, 2016). Phytochemicals recuperate their significance in drug discovery process (Sam *et al*, 2017). Their different classes: flavonoids, phytoalexins, carotenoids, terpenoids (monoterpenes, diterpenes, triterpenes, sesquiterpenes etc.) have been reported to obstruct several signaling pathways of cancer like alvocidib inhibits CDK2 activity via downregulating XIAP and MCL-1 (Lin *et al*, 2009). Terpenoids are documented as reserves of anticancer lead compounds as taxol/paclitaxel etc. (Wang and Lee, 1997). Various diterpenes are known to persist immense anticancer properties For example: Cavernenes A, B, C, D and kalihinenes E and F derived from Acanthella cavernosa showed cytotoxic effects in Human cancer cell lines (A549, HeLa and MDA-MB-231) (Xu *et al*, 2012). Trichodelphinines A, B, C, D, E isolated from Delphinium trichophorum in A549 cancer cell lines (Lin *et al*, 2014; Islam, 2017).

Abietane diterpenes from lamiaceae family have a charachteristic feature of an –ortho/-para-naphtholquinone and benzoquinone moiety and it is evident that quinone moiety has antiproliferative or antitumor activity against human cancer cell lines. These abietane dipterpenes are also known as *Tanshinones* revealing significant antioxidant, cytotoxic activities against cancer.

7-Acetylhorminone (7á-acetoxyroyleanone, 7á-acetoxy-12-hydroxy-11,14-dioxoabieta-8,12-diene) compound are been isolated from *Hyptis martiusii Benth* roots (da Cruz Araújo *et al*, 2006). The study focuses on molecular docking studies of phytochemicals retrieved from preplated natural product library of 400 compounds targeting CDK2 protein. The process and protocol followed in the *in silico* experimental design as potential anti cancer strategy has been very well validated by other different studies (Arif *et al*, 2013 and Akhtar *et al*, 2011). 7-Acetylhorminone has shown better docking results against CDK2 thus suggested as a anticancer lead compound (Fronza *et al*, 2011).

MATERIALS AND METHODS

Materials

The objective of the study is to examine the ligand interaction sites for CDK2 and propose a best possible anticancer lead compound isolated from a natural compound library. The study has been done by PreADMET (Kwang, 2005; Lee *et al*, 2007), ChemDraw Ultra 10 (Mills, 2006), AutoDock 4.2 (Morris *et al*, 2014), BIOVIA Visualizer (BIOVIA, 2017), CDRUG (Li and Huang, 2012), PASS (Lagunin *et al*, 2000) server for validating the compound's anticancer potential against CDK2.

Methodology

Selection of a Library for screening

A library of natural product has been downloaded to carry out the filtration process for best lead compound. The selected dataset was a natural product library with approximately 400 compounds.

Screening through Drug-Like features

Lipinski Rule of five (RO5)

The basic features of the compounds have been calculated *via* Lipinski rule of five. Considering the facts and standards of the "Lipinski rule of five" proposed in 1997 by Christopher A Lipinski (Lipinski, 2004). He stated following set of rules which defines the physiochemical property of the compound to act as a "lead".

1. The molecular weight should be less than 500 kDa
2. The lipophilicity (log P- octanol-water partition coefficient) of compound should be less than 5
3. There must be less than 5 H-bond donors (i.e. the sum of OHs and NHs)
4. There must be less than 10 H-bond acceptors (i.e. the sum of Ns and Os)

ADME (absorption, distribution, metabolism and excretion)

Further screening has been done on the basis of ADME descriptor assessment tool – PreADMET (Kwang, 2005; Lee *et al*, 2007). Prediction of ADME standard has been an important factor to surpass the clinical trial levels of a lead compound for drug discovery. The seven parameters: BBB (Blood Brain Barrier), HIA (Human Intestinal Absorption), PPB (Plasma Protein Binding), Caco-2, CYP2D6, MDCK, P-gp_Inhibition specifying the features of ADME are listed in Table 1. The filtration of compound has been done on the basis of these characteristic features.

Toxicity prediction

Toxicity prediction of the compounds were checked through on online server PreADMET *ver* 2.0 (Kwang,

Table 1 : ADME Standards in PreADMET.

BBB (Blood Brain Barrier)		HIA (Human Intestinal Absorption)		PPB (Plasma Protein Binding)		Caco-2 Permeability		CYP2D6		MDCK (Madin-Darby Canine Kidney)		P-gp_ Inhibition	
Range	Features	Range	Features	Range	Features	Range	Feature	Feature	Acceptance	Range	Feature	Feature	Acceptance
More than 1	CNS active compound	0 ~ 20%	Poor absorption	More than 90%	Strongly Bounded	Less than 4	Lower	Non-inhibitor	Yes	Less than 25	Lower	Non-inhibitor	No
Less than 1	CNS inactive compound	20 ~ 70%	Moderate absorption	Less than 90%	Weakly Bounded	4 ~ 70	Moderate	Inhibitor	No	25 ~ 500	Moderate	Inhibitor	Yes
		70 ~ 100%	Higher absorption			More than 70	Higher			More than 500	Higher		

2005; Lee *et al*, 2007), which calculates the toxicity on the basis of Ames test, two year assay carcinogenicity test of rat and mouse.

Docking analysis

Molecular docking studies of receptor protein CDK2 with the standard and the sorted 30 compounds was performed using AutoDock 4.2 (Morris *et al*, 2014). Following are the steps involved in docking procedure.

Protein structure preparation

The crystal structure of the target protein was saved through protein databank (PDB), 4KD1 (CDK2-Dinaciclib) has been selected with resolution value of 1.7 Å without any missing loops or mutations.

The attached ligands and the water molecules were removed and protein structure was refined and minimized by applying CHARMm forcefield through UCSF Chimera (https://www.cgl.ucsf.edu/chimera/download.html), a visualization tool (Pettersen *et al*, 2004) for the analysis of macromolecules.

Ligand preparation

The standard compound was refined and minimized through UCSF chimera, while the SDF files of the screened ligands were converted into 3D PDB files using an accessible tool OpenBable converter – Open Babel v2.3.1 (O'Boyle *et al*, 2011). The unavailable structures were also drawn on ChemDraw Ultra 10 (Mills, 2006).

Docking procedure and analysis

The .pdbqt files *i.e* the modified version .pdb of receptor (R.pdbqt: having polar Hydrogen bonds and the charges) and the ligands (L.pdbqt: having rotatable bonds) were prepared through AutoDock 4.2 (Morris *et al*, 2014). The active sites of the receptor have been identified with the help of CASTp – Computed Atlas of Surface Topography of proteins (http://sts.bioe.uic.edu/castp/) (Binkowski *et al*, 2003). The ATP-binding pocket active residues were entered as GLU81, VAL64, PHE80, PHE82, LYS33, ALA31, VAL18, ALA144, LEU83, LEU134, ILE10, HIS84, GLN85, ASP86, GLU12, LYS89 and GLN131. Further a grid-box on the basis of selected interaction sites around CDK2 of dimension 60x60x60 with grid centre of 54.429, 78.901, 27.781 have been plotted. The grid parameter and dock parameter files (.gpf and .dpf) were prepared along with the default genetic and Lamarckian algorithmic values. The search parameter was set to 25 runs *i.e.* the ligand would bounded to receptor active sites through 25 conformational poses. The experiments were run the interaction map files through automated programs (.exe) files of *autogrid* and *autodock* resulting into .glg and .dlg files. The docking results (.dlg) can be analyzed by binding energy (kcal/mol), inhibition constant (Ki value-μM/nM), RMSD (root mean square deviation), respectively. The structure confirms its stability by the presence of hydrogen bonds (interaction between ligand - Aminoacid) in the complex and was visualized on Accelrys Discovery Studio Visualizer 2017 R2 (BIOVIA) v17.2.0.16349 (BIOVIA, 2017).

Prediction of anticancer properties

CDRUG (Li and Huang 2012), PASS server (Lagunin *et al*, 2000) are the *in silico* tools used to ensure of anticancer properties of the lead compounds.

CDRUG

CDRUG (Li and Huang, 2012) uses a novel molecular depiction method (corresponding frequency-weighted similarities) to implement the compound 'associates'. Then, a hybrid/cdrug_score was calculated to compute the similarity between the query and the active compounds.

Table 2 : List of compounds following RO5.

Compounds	LIPINSKI_RO5			
	milogP	**Mol.wt.**	**H_Acceptors**	**H_Donors**
Dinaciclib (Standard)	**1.29**	**396.495**	**6**	**2**
androstane-3,6,17-triol	2.22	308.456	3	3
17-methylandrostane-3,6,17-triol	2.39	322.482	3	3
pregnane-3,6,17-triol	3.12	336.509	3	3
alpha-Santonin	1.64	246.302	3	0
10(14)-Aromadendren-1-ol	2.27	222.323	2	1
4,10-Aromadendranediol	1.98	236.35	2	1
3-Hydroxyandrost-5-en-17-one	3.81	345.476	4	1
Carene	0.303	239.354	3	1
4-(10,13-dimethyl-3,12-dioxo-2,3,4,5,6,7,9,10,11,12,13,15,16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl) pentanoic acid	4.03	386.524	4	0
Ledol	3.42	222.366	1	1
Mecambrine	1.97	310.367	3	0
Acetyl-alpha-desmotroposantonin	3.09	288.338	4	0
7-Acetylhorminone	***3.06***	***374.471***	***5***	***1***
Jativatriol	3.94	446.576	6	0
Conchitriol	0.793	322.482	3	3
Sideritol	2.8	402.567	4	0
3,21-Dihydroxypregnan-20-one	3.2	334.493	3	2
methyl 3-(acetyloxy)-15-oxoandrostane-17-carboxylate	3.71	390.513	5	0
3,16-Dihydroxypregn-5-en-20-one	4.39	374.514	4	1
1-(acetyloxy)-3,11a-dimethyl-5,5a,5b,6,8,9,10,11,11a,11b,12, 13-dodecahydro-4aH-	3.58	430.534	6	0
21-[(chloroacetyl)amino]-20-oxopregna-5,14-dien-3-yl acetate	4.78	447.995	4	1
21-diazo-20-oxopregn-5-en-3-yl acetate	-1.64	384.512	3	0
Cholic acid	3.16	420.582	5	2
Ilicic acid (Vachanic acid)	1.45	252.349	3	2
Corypalline	1.58	193.242	3	1
Marrubiin (Marrubium bitter)	2.96	332.434	4	1

Finally, a confidence level (P-value) is calculated to predict whether the query compound have, or don't have the anticancer potency.

PASS (Prediction of Activity Spectra for Substances) Server

PASS server (Lagunin *et al*, 2000) works on immediate computation of several types of biological activities on the basis of the structure of the phytochemical or chemical compounds. It can be accessed online PASS takes SD file (.sdf) or MOL file (.mol) formats of compounds as input data and returns output in the form of Pa and Pi whose values ranges from 0.000 to 1.000. This server aids in proposing novel lead compounds for existing targets as well as innovative targets for available ligands. The activity spectrum can be analyzed with help of following set of rules :

- If Pa value is greater than 0.7 then the compound will manifest precise possibility of the biological activity in investigation with a very high risk of duplicity to already available compound or drugs.
- If Pa value is greater than 0.5 and less than 0.7 then the compound will manifest biological activity in investigation with less possibility and also with less risk of duplicity to already available compound.

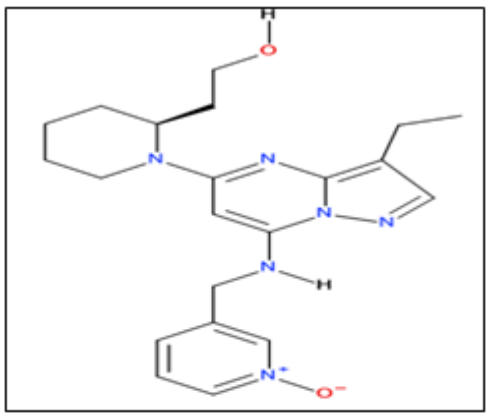

Fig. 1(a) : 2D structure of Dinaciclib.

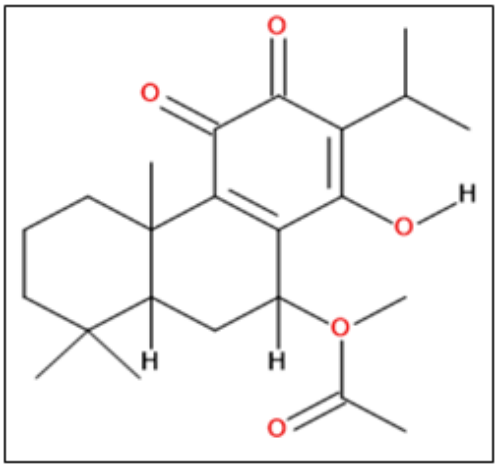

Fig. 1(b) : 2D structure of 7-Acetylhorminone.

- If Pa value is less than 0.5 then the compound will manifest very low biological activity in investigation thus due to this feature the compound can be confirmed as a new entry in the chemical compound's library.

RESULTS

Drug- like feature test: Lipinski filters (RO5) and ADME/T

RO5 (Table 2) is the test, which basically tells whether the compound exhibit the oral absorption property or not. It determines the pharmacological activity of the lead compounds. According to the standards mentioned above, the library was screened and preceded for the ADME screening. Besides the drug likeliness filters, a noteworthy obstacle left behind in drug development pipeline. In this scenario, quantitative assessment of the lead compound becomes an important step to overcome the ADME barriers (Hou *et al*, 2004). These barriers decipher the pharmacokinetics marking the behavior of drug inside the body. ADME can be elaborated by given standards features *viz.* BBB (Blood Brain Barrier), HIA (Human Intestinal Absorption), PPB (Plasma Protein Binding), Caco-2, CYP_2D6 inhibition, MDCK (Madin-Darby Canine Kidney), P-gp_inhibition.

Out of 400 compounds, 26 isolated leads along with the standard compound; Dinaciclib following the drug likeliness and ADME properties are listed in Table 3.

Toxicity profile

Toxicity was checked by an online server PreADMET (Kwang, 2005) to predict the mutagenecity, carcinogenicity by Ames test and hERG_inhibition values. 7-Acetylhorminone was the only one out of 26 compounds (Table 3) illustrated better results. The standard compound Dinaciclib was mutagenic but non carcinogenic thereby is at medium risk level. Out of 26 compounds, 7-Acetylhorminone is non-mutagenic, non-carcinogenic and it is also at lower risk. Therefore, this compound may be proposed as anticancer lead compound targeting CDK2 protein.

Docking results

Docking studies of all the sorted 26 compounds were performed and compared to the standard compound i.e Dinaciclib. The binding energy of Dinaciclib and CDK2 was -7.18 kcal/mol with the inhibition constant of 5.47 µM. These compounds were further subjected to the toxicity profiling. 7-Acetylhorminone because of its comparable druglikeliness, ADME profiling and better docking results have been proposed as anticancer lead compound targeting CDK2 as shown in table 4.

Molecular Structure of standard drug "Dinaciclib" and 7-Acetylhorminone/7alpha-Acetoxyroyleanone

The 2D structure of the sorted compound 7-Acetylhorminone (Pubchem ID: 494501) surpassing Lipinski, ADME/T analysis.

Tools for Anticancer activity

CDRUG (cancer drug)

This tool (Li and Huang, 201) basically estimates the anticancer activity in a chemical compound. It requires the SMILES ID of the compounds in order to calculate the hybrid score (H-score/ cdrug_score) on the basis of similarities in query and the chemical compound thereby resulting into a P-value determining the anticancer possibility. Possibility of the compound demonstrated by three colours: grey(less possible), black (possible), green (highly possible).

Herein, 7-Acetylhorminone showed higher anticancer compound possibility than the standard drug (Table 5).

PASS Server

Pass server (Lagunin *et al*, 2000) estimated biological activity chart of 7-Acetylhorminone as mentioned in Table

Table 3 : List of compounds: ADME/T Screening.

Compounds	PreADMET_ADME							PreADMET_Toxicity			
	BBB	**HIA**	**PPB**	**Caco-2 Permeability**	**CYP2D6**	**MDCK**	**Pgp inhibition**	**Ames_test**	**Carcino_mouse**	**Carcino_rat**	**hERG_inhibition**
Dinaciclib (Standard)	**0.0187328**	**94.731497**	78.414262	33.9329	**Non**	**15.9534**	**Non**	**mutagen**	**negative**	**negative**	**medium_ risk**
androstane-3,6,17-triol	2.9143	86.564775	94.48447	17.8604	Non	130.776	Inhibitor	mutagen	negative	positive	low_risk
17-methylandrostane-3,6,17-triol	0.76484	87.224283	92.466658	18.4436	Non	159.827	inhibitor	non-mutagen	negative	positive	low_risk
pregnane-3,6,17-triol	0.162253	87.190036	91.881046	20.5476	Non	66.1278	Non	non-mutagen	negative	positive	low_risk
alpha-Santonin	1.11155	98.20521	87.283439	23.5051	Non	76.9187	Inhibitor	mutagen	negative	positive	medium_ risk
10(14)-Aromadendren-1-ol	1.43927	94.869364	100	24.2042	Non	376.635	Non	mutagen	negative	positive	medium_ risk
4,10-Aromadendranediol	3.33178	90.162429	74.038917	27.1438	Non	115.669	Non	non-mutagen	negative	positive	low_risk
3-Hydroxyandrost-5-en-17-one	3.81617	95.571781	100	20.8186	Non	204.652	Inhibitor	non-mutagen	negative	positive	medium_ risk
Carene	5.5333	100	100	23.6313	Non	304.815	Inhibitor	mutagen	negative	positive	medium_ risk
4-(10,13-dimethyl-3,12-dioxo-2,3,4,5, 6,7,9,10,11,12,13,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl) pentanoic acid	0.0246363	98.512585	96.94441	21.2946	Non	0.230983	Inhibitor	mutagen	negative	positive	low_risk
Ledol	7.56612	100	100	54.5722	Non	218.828	Inhibitor	non-mutagen	negative	positive	low_risk
Mecambrine	1.9205	97.997814	32.777195	42.33	Inhibitor	117.085	Inhibitor	mutagen	positive	negative	medium_ risk
Acetyl-alpha-desmotroposantonin	2.10449	98.218936	89.32026	23.3124	Non	84.854	Inhibitor	mutagen	negative	positive	low_risk
7-Acetylhorminone	**1.04951**	**96.259043**	94.679981	21.9159	**Non**	**213.043**	**Inhibitor**	***non-mutagen***	***negative***	***negative***	***low_risk***
Jativatriol	2.4536	88.018084	86.982234	21.2243	Non	70.151	Inhibitor	mutagen	negative	positive	medium_ risk

Table 3 continued..

Table 3 continued..

Conchitriol	2.45362	88.017938	88.829153	20.7874	Non	70.151	Inhibitor	non-mutagen	negative	positive	low_risk
Sideritol	2.67354	88.022203	80.334214	21.3247	Non	289.066	Non	non-mutagen	positive	positive	low_risk
3,21-Dihydroxypregnan-20-one	0.928132	92.142962	100	20.5023	Non	182.951	Inhibitor	non-mutagen	negative	negative	low_risk
methyl 3-(acetyloxy)-15-oxoandrostane -17-carboxylate	0.0162632	98.317769	96.194954	21.585	Non	0.0641658	Inhibitor	mutagen	negative	positive	low_risk
3,16-Dihydroxypregn-5-en-20-one	0.535465	92.478487	98.981286	19.7837	Non	77.0478	Inhibitor	non-mutagen	positive	negative	low_risk
1-(acetyloxy)-3,11a-dimethyl-5,5a,5b,6,8,9, 10,11,11a,11b,12,13-dodecahydro-4aH-	0.0549381	98.741288	87.715079	34.5517	Non	0.292989	Inhibitor	non-mutagen	positive	positive	low_risk
21-[(chloroacetyl)amino]-20-oxopregna-5, 14-dien-3-yl acetate	0.203181	96.220434	99.230738	21.3044	Non	0.0482024	Inhibitor	non-mutagen	negative	positive	medium_ risk
21-diazo-20-oxopregn-5-en-3-yl acetate	0.0282621	98.167055	100	20.9075	Non	0.819049	Inhibitor	non-mutagen	negative	positive	medium_ risk
Cholic acid	0.401474	86.409987	92.711644	21.1097	Non	0.345149	Inhibitor	mutagen	negative	positive	low_risk
Ilicic acid (Vachanic acid)	0.967007	93.778057	93.103297	2.66556	Non	188.982	Inhibitor	mutagen	negative	positive	low_risk
Corypalline	0.805417	95.394445	51.319709	43.7348	Inhibitor	364.659	Non	mutagen	positive	negative	low_risk
Marrubiin (Marrubium bitter)	0.436966	95.786026	100	28.0415	Non	36.9549	Inhibitor	mutagen	negative	positive	low_risk

Table 4: Docking analysis.

Compounds	Binding Energy (Kcal/mol)	Inhibition Constant (ìM/nM)
Dinaciclib (Standard)	**-7.18**	**5.47**
androstane-3,6,17-triol	-7.78	1.97
17-methylandrostane-3,6,17-triol	-8.05	1.25
pregnane-3,6,17-triol	-8.37	727.4
alpha-Santonin	-7.62	2.5
10(14)-Aromadendren-1-ol	-6.39	20.79
4,10-Aromadendranediol	-5.95	43.53
3-Hydroxyandrost-5-en-17-one	-7.09	6.33
Carene	-6.17	29.95
4-(10,13-dimethyl-3,12-dioxo-2,3,4,5,6,7,9,10,11,12,13,15,16,17-tetradecahydro -1H-cyclopenta[a]phenanthren-17-yl)pentanoic acid	-8.94	279.89
Ledol	-6.27	25.5
Mecambrine	-7.44	3.53
Acetyl-alpha-desmotroposantonin	-8.01	1.34
7-Acetylhorminone	***-8.96***	***270.58***
Jativatriol	-7.52	3.08
Conchitriol	-7.06	6.63
Sideritol	-6.38	21.24
3,21-Dihydroxypregnan-20-one	-6.98	7.7
methyl 3-(acetyloxy)-15-oxoandrostane-17-carboxylate	-8.39	709.6
3,16-Dihydroxypregn-5-en-20-one	-7.56	2.87
1-(acetyloxy)-3,11a-dimethyl-5,5a,5b,6,8,9,10,11,11a,11b,12,13-dodecahydro-4aH-	-7.82	1.86
21-[(chloroacetyl)amino]-20-oxopregna-5,14-dien-3-yl acetate	-5.62	76.24
21-diazo-20-oxopregn-5-en-3-yl acetate	-6.06	36.28
Cholic acid	-8.22	940.03
Ilicic acid (Vachanic acid)	-7.01	7.22
Corypalline	-5.27	136.30
Marrubiin (Marrubium bitter)	-7.26	4.79

Table 5 : CRDUG Prediction.

Compounds	P-value	Cdrug_score/H-score	Anticancer property possibility
Dinaciclib (Standard)	0.647	0.091	Grey (less possible)
7-Acetylhorminone	**0.0976**	**0.295**	**Black (possible)**

6.

DISCUSSION

The study reveals 7-Acetylhorminone / 7alpha-Acetoxyroyleanone, an abietane diterpenes (secondary metabolite) as a potent anticancer lead compound targeting CDK2 and showing better results than the standard drug Dinaciclib. Dinaciclib is currently in phase trial II and III showing inhibitory effects alone or in combinations with the other drugs such as cisplatin (Chen *et al*, 2015). Although, there are many other CDK2 inhibitors studied in literature and tested in *in silico* studies (Khan *et al*, 2017) including alvocidib (flavopiridol- phase II), roscovitine (seliciclib – terminated at phase trial II), R547, SNS-032, TG02 (completed phase I of clinical trials) with many side-effects as several patients get deterioted after their few doses and flavopiridol has been proved inhibitory effect on Ser/Thr kinases (Blachly and Byrd, 2013), while Dinaciclib is known for its specific inhibition on CDK2/CDK1, CDK5/CDK9 protein and is the most recent potent

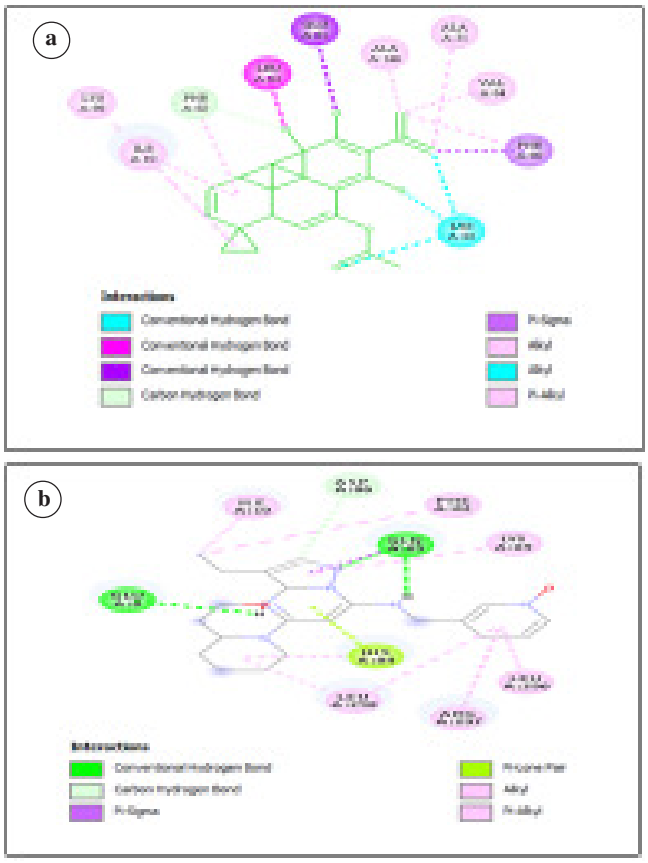

Fig 2 (a) : Ligand interaction pattern diagram of CDK2-7-Acetylhorminone docked complex **(b)** Ligand interaction pattern diagram of CDK2- Dinaciclib docked complex.

Table 6 : Biological Activity chart of 7-Acetylhorminone / 7alpha-Acetoxyroyleanone.

Pa	Pi	*7-Acetylhorminone / 7alpha-Acetoxyroyleanone*
0,879	0,005	Antineoplastic
0,701	0,015	Apoptosis agonist
0,674	0,016	UDP-glucuronosyltransferase substrate
0,639	0,027	HIF1A expression inhibitor
0,573	0,010	Antineoplastic (lung cancer)
0,577	0,019	Caspase 3 stimulant
0,510	0,013	Myc inhibitor
0,486	0,011	Prostate cancer treatment
0,349	0,009	Transcription factor NF kappa B inhibitor
0,323	0,192	Antineoplastic (non-Hodgkin's lymphoma)

drug with some toxicity issues (Kalra *et al*, 2017). The library of 400 natural compounds was subjected to Lipinski (Lipinski 2004) and ADME rule (Hou *et al*, 2004; Kwang, 2005) to screen the lead compounds. Out of which 26 compounds passed these filters.

7-Acetylhorminone, an abietane diterpenes, reveals hopeful outcome targeting CDK-2 as a potential G1/S

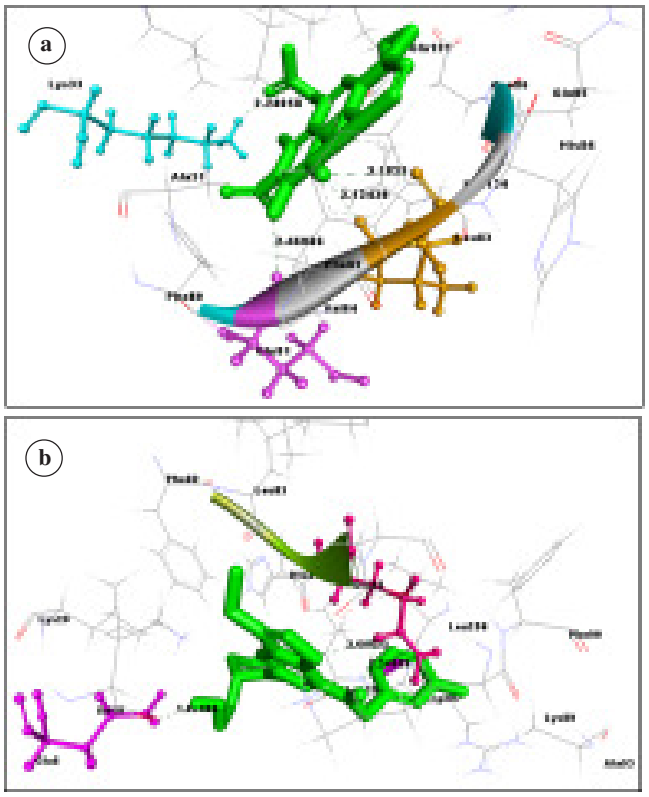

Fig. 3 (a): Hydrogen bond interaction in CDK2-7-Acetylhorminone docked complex, **(b)** Hydrogen bond interaction in CDK2-Dinaciclib docked complex.

phase inhibitor. The milogP value of 7-Acetylhorminone is about 3.06 and that of Dinaciclib is approximately 1.29. Hence, the likelihood of 7-Acetylhorminone to be absorbed is comparatively much better than to Dinaciclib. As per the standard rule of five, 7-Acetylhorminone followed all the parameters of Lipinski's rule, milogP value was 3.06, the molecular weight was 374.471 Dalton, No of hydrogen bond acceptor was 5 and the number of hydrogen bond donor was 1. Further on demonstrating ADME profiling and all its parameters (BBB, HIA, PPB_level, Caco-2, CYP2D6_inhibition, MDCK, Pgp_inhibition). 7-Acetylhorminone was found to be in harmony with the standard drug Dinaciclib. The ADME/T parameters like BBB, HIA, MDCK values are much better and are in the acceptable ranges of drug discovery pipeline as compared to 'Dinaciclib'. CYP2D6 computes the inhibitory action of cytochromeP450 enzyme *i.e.* it should show a non-inhibitory action. Here, 7-Acetylhorminone is showing a non- inhibitory effect while Dinaciclib is an inhibitor of cytochrome P450 enzyme. Literature suggests the chemical which enhances P-gp inhibition have been proved to exhibit anti drug resistant properties in several cancer cell lines. Table 3 clearly pictures the Pgp inhibitory activity by 7-Acetylhorminone mounting anti MDR activity while

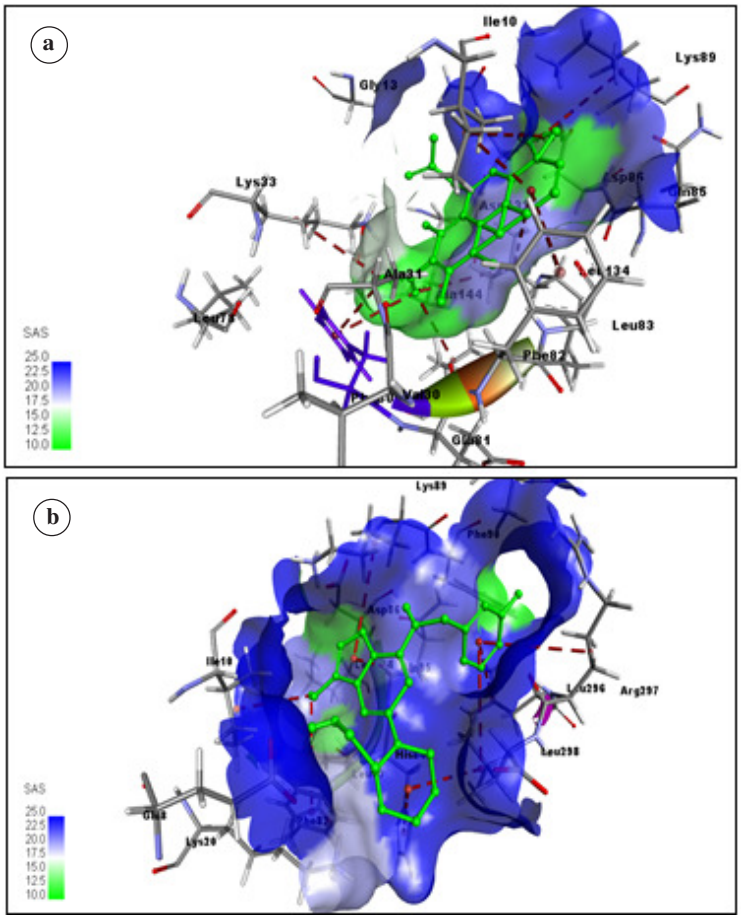

Fig. 4 (a): Hydrophobic interactions in CDK2-7-Acetylhorminone docked complex, **(b)** Hydrophobic interactions in CDK2- Dinaciclib docked complex.

Dinaciclib is a non-inhibitor of Pgp or ABC transporters (Nanayakkara *et al*, 2018).

7-Acetylhorminone has better docking results (B.E= -8.96 kcal/mol) and inhibition constant (Ki= 270.58 nM) compared to the standard drug Dinaciclib (B.E = -7.18 Kcal/mol; Ki= 5.47ìM). Along with this, on inspecting the interaction patterns (Table 7) of standard "*Dinaciclib*" and the ligand "7-Acetylhorminone", docked complex (CDK2-7-Acetylhorminone) has four H-bonds (Fig. 3a), out of which three bonds have distance of less than 3Å determining more stability of the compound and twelve hydrophobic interactions (Fig. 4a) including one pi-sigma (UNK1:C22 - A:PHE80), three pi- alkyl (UNK1:C22 - A:LYS33, A:PHE80 - :UNK1:C19, A:PHE82 - :UNK1) and eight alkyl interactions maintaining the stability of the compound (Fig. 2). On the other hand, in docked complex (Dinaciclib - CDK2), there are three H-bonds (Fig. 2b) with nine hydrophobic interactions (Fig. 4b) comprising of one pi-sigma (A:GLN85:HA - :UNK1), five alkyl (A:LEU296 - :UNK1, A:ARG297 - :UNK1, A:LEU298 - :UNK1, A:LEU298 - :UNK1, :UNK1:C16 - A:ILE10) and three pi-alkyl (A:PHE82 - :UNK1:C16, A:HIS84 - :UNK1, :UNK1 - A:LYS89).

Table 7 envisioned the interaction pattern chart of the lead compound '7-Acetylhorminone' and the standard 'Dinaciclib' docked complexes.

The quinone moiety of 7-Acetylhorminone is uniquely reported to be accountable for its anti-cancer and antineoplastic activity in cancer cell lines. Here also it is seen that the quinone moiety of 7-Acetylhorminone is sturdily involved in forming hydrogen bond interaction with Glutamic acid (GLU 81) of CDK2 (Fig. 2a and Table 7) suggesting the strong association of cytotoxic effects and antitumor activity with the compound targeting CDK2 (Bana *et al*, 2015).

Moving ahead to the toxicity check, 7-Acetylhorminone demonstrates the non-mutagenic, non-carcinogenic, non-toxic compound in comparison to the Dinaciclib, a mutagen, a non-carcinogen and may be a toxic compound.

Exceeding with the CDRUG (Li and Huang, 2012) and PASS (Lagunin *et al*, 2000) server results, 7-Acetylhorminone (lead) compound has proven ability of sustaining the anticancer activities. CDRUG data evaluation reveals 7-Acetylhorminone has more possibility of being an anticancer lead than *Dinaciclib* (Table 5). PASS server (Lagunin *et al*, 2000) also discloses many biological activities *i.e* 7-Acetylhorminone is antineoplastic (lung cancer, non-Hodgkin's lymphoma), Apoptosis agonist, Myc inhibitor, prostate cancer treatment TNF-êâ inhibitor (Table 6). Myc inhibitor and essentially an apoptotic agonist, Caspase 3 stimulant which would be an add-on feature for a CDK2 inhibitor. 7-Acetylhorminone also shown some antiangiogenic property as PASS predicted that it is an inhibitor of Hypoxia inducible factor-1 alpha (HIF1A) expression inhibitor (Masoud and Li, 2015). Thus, the study concludes that inhibiting CDK2 overexpression using 7-Acetylhorminone can be a promising approach in cancer chemoprevention.

CONCLUSION

In conclusion, the *in-silico* studies conducted on library of natural products, suggested Abietane diterpenes of Lamiaceae family can thus be proposed as anticancer compounds. The novel identified compound 7-Acetylhorminone showed enhanced results in comparative molecular docking, toxicity profiling as well as the anticancer potency (CDRUG analysis) of the compound. The investigations may be further put forward for *in vitro*

Table 7 : Comparative ligand interaction pattern studies in CDK2 - 7-Acetylhorminone and standard Dinaciclib-CDK2.

Compound	H bond interaction	Distance (Å)	Hydrophobic interaction	Distance (Å)
CDK2-7-Acetylhorminone	UNK1:O5 - A:LEU83:OUNK1:O4 - A:GLU81:OA:LYS33:HZ1 - :UNK1:O2A:LEU83:H - :UNK1:O5	3.1825 2.460857 2.24058 2.13439	A:PHE82 - :UNK1 (Pi-Alkyl)A:PHE80 - :UNK1:C19 (Pi-Alkyl)UNK1:C22 - A:LYS33 (Alkyl)UNK1:C19 - A:LEU134 (Alkyl)UNK1:C19 - A:VAL64 (Alkyl)UNK1 - A:LYS89 (Alkyl)UNK1 - A:ILE10 (Alkyl)A:ALA144 - :UNK1:C19 (Alkyl)A:LEU134 - :UNK1 (Alkyl)A:ALA31 - :UNK1:C22 (Alkyl)A:ILE10 - :UNK1 (Alkyl)UNK1:C22 - A:PHE80 (Pi-Sigma)	5.191570 4.077500 4.573793 4.895911 3.723224 5.432533 4.814165 3.120642 5.256713 3.610773 3.861637 3.624648
Dinaciclib-CDK2	UNK1:N5 - A:GLN85:OE1UNK1:H16 - A:GLN85:OE1UNK1:H15 - A:GLU8:OE1	2.606318 2.340275 1.874058	A:GLN85:HA - :UNK1 (Pi-Sigma)A:LEU296 - :UNK1 (Alkyl)A:ARG297 - :UNK1 (Alkyl)A:LEU298 - :UNK1 (Alkyl)A:LEU298 - :UNK1 (Alkyl):UNK1:C16 - A:ILE10 (Alkyl)A:PHE82 - :UNK1:C16 (Pi-Alkyl)A:HIS84 - :UNK1 (Pi-Alkyl):UNK1 - A:LYS89 (Pi-Alkyl)	2.817396 5.175343 5.058064 4.747324 5.296741 4.216671 5.216510 4.999382 5.042373

and *in vivo* studies as unique anticancer lead against CDK2 protein for cure of cancer.

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