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Research article

Molecular docking study of natural alkaloids as multi-targeted hedgehog pathway inhibitors in cancer stem cell therapy

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ABSTRACT

Cancer is responsible for millions of deaths throughout the world every year. Increased understanding as well as advancements in the therapeutic aspect seems suboptimal to restrict the huge deaths associated with cancer. The major cause responsible for this is high resistance as well as relapse rate associated with cancers. Several evidences indicated that cancer stem cells (CSC) are mainly responsible for the resistance and relapses associated with cancer. Furthermore, agents targeting a single protein seem to have higher chances of resistance than multitargeting drugs. According to the concept of network model, partial inhibition of multiple targets is more productive than single hit agents. Thus, by fusing both the premises that CSC and single hit anticancer drugs, both are responsible for cancer related resistances and screened alkaloids for the search of leads having CSC targeting ability as well as the capability to modulating multiple target proteins. The *in silico* experimental data indicated that emetine and cortistatin have the ability to modulate hedgehog (Hh) pathway by binding to sonic hedgehog (Hh), smoothened (Smo) and Gli protein, involved in maintenance CSCs. Furthermore, solamargine, solasonine and tylophorine are also seems to be good lead molecules targeting towards CSCs by modulating Hh pathway. Except solamargine and solasonine, other best lead molecules also showed acceptable *in silico* ADME profile. The predicted lead molecules can be suitably modified to get multitargeting CSC targeting agent to get rid of associate resistances.

1. Introduction

Cancer is a major health problem which is responsible for millions of global deaths each year (Raz et al., 2012). It has been estimated that 14.1 million new cases and 8.2 million cancer related death occurred during 2012 (Dosanjh et al., 2014). Moreover, more than five ten thousand cancer related deaths and approximately sixteen ten thousand new cases are predicted to occur only in United State during year 2014 (Siegel et al., 2014). Population throughout the world is suffering from the huge burden of this deadly disease. Due to technical advancements and increased understanding of the disease, cancer statistics have been slightly improved during the time period of the last two decades (Siegel et al., 2014). But still it is associated with a massive death rate, which indicates the lacunas related to its treatment. Among the various aspects resistance towards chemotherapy is the major factor responsible for massive cancer related death rate (Singh and Settleman, 2010). Almost all types of cancer cells have shown varying degree of drug resistance when exposed to chemotherapeutic agents (Luqmani, 2008). Several reports indicated that CSCs are the major factor behind drug resistance and relapse of disease (Bashyal Insan and Jaitak, 2014). CSC is a term used for a subpopulation of cancer cells, which show self renewal capacity, whole tumor regenerating capability, inbuilt resistance to chemotherapeutic agents and can produces a similar kind of tumor when transplanted into immune-compromised animal models (Bashyal Insan and Jaitak, 2014). CSC have been isolated from multiple cancer types such as leukemias, breast, liver, glioblastoma, pancreatic, prostate, head and neck cancers by using specific types of surface marker or cellular activity shown by these cells (Bashyal Insan and Jaitak, 2014). CSCs have stem like characteristics and is maintained significantly by several signaling pathways and among them intensity of hedgehog (Hh) signaling (Fig. 1) was found to impart significant impact on these cells (Bashyal Insan and Jaitak 2014; Zhao et al., 2009).

Hh pathway is a developmental pathway which play an active role in various developmental processes, such as cell fate, proliferation, survival and differentiation (Bashyal Insan and

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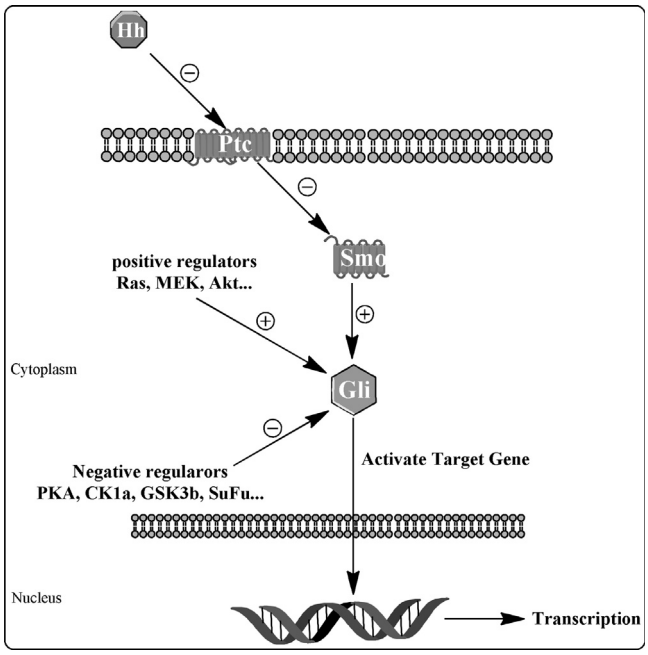

Fig. 1. Hedgehog signaling pathway: Hedgehog protein (Hh) binds to patched (Ptc) and inactivate it. The Ptc is twelve transmembrane glycoprotein components that suppress the activity of Smothened (Smo) protein. When Ptc is inactivated by Hh protein, activation of Smo takes place, which results in increased concentration of Gli. The Gli is a transcription factor that mediate transcription related to cancer and CSCs. Some negative and positive regulators also modify Hh signaling by increasing or decreasing gli mediated activity.

Jaitak, 2014). Up-regulated Hh pathway was found to be significantly associated with multiple forms of cancers along with its involvement in CSC maintenance (Merchant and Matsui, 2010). Thus, various targeting approaches have been implemented to down regulate Hh pathways as a targeting strategy for cancer and CSCs. Hh pathway inhibitors have been developed which can be majorly categorized into inhibitors of Hh protein, Smo inhibitors and inhibitor of Gli proteins mediated activity (Peukert and Miller-Moslin, 2010). Among all categories of inhibitors only Smo inhibitors have gained significant considerations and several compounds such as cyclopamine derivative IPI 926 have entered in clinical trials (Bashyal Insan and Jaitak, 2014). But there is not any inhibitor of Hh and Gli protein is available, which is under clinical consideration (Bashyal Insan and Jaitak, 2014). Because of the availability of only Smo based Hh pathway inhibitors we don't have any alternate drugs to overcome resistance acquired by alteration in Smo protein. Furthermore, Hh pathway involves complex signaling mechanisms where several protein messengers are involved and there exists a complex crosstalk among multiple pathways, which further increase the possibility of drug resistance (Sengupta et al., 2007). Thus, multitargeted CSC therapy seems to be a more reasonable way to overcome possible drug resistance related problems associated with Hh pathway inhibitors (Bashyal Insan and Jaitak, 2014). According to network model partial inhibition of several targets are more efficient than complete inhibition of a single protein (Csermely et al., 2005). Among various drug like molecules, natural products being originated from natural phenomenon, having defined role in the body and thus seems to have strength to modulate several target proteins. Therefore, natural products may have potential for being developed as multitargeting drugs. Thus, taking in consideration the above mentioned facts, the aim of the current study is to investigate the natural drug like molecules that can be developed as multi-targeting inhibitors of Hh pathways. Among various natural products, anticancer potential of alkaloids is well established and thus included in the study. The alkaloids which were chosen are previously reported to have significant anticancer potential and further exploring them for their mechanistic prospective may provide us a good anticancer lead molecule.

2. Material and methods

2.1. Obtaining ligands and protein molecules

The alkaloids selected for the study are shown in Table 1. Alkaloids included in this study have shown promising anticancer potential and can be explored further for searching alternative anticancer lead. Required chemical structures were drawn manually using ChemBioDraw Ultra-12 by carefully considering the chiral centres using wedged/hashed bonds. Crystal structures of Smo, Hh and Gli protein used in this study were downloaded from protein data bank (PDB), have PDB ID 4JKV (Wang et al., 2013), 4C4 M (Whalen et al., 2013) and 2GLI (Pavletich and Pabo, 1993) respectively. Standard drugs used as a control in this study include GDC-0449, Robotnikinin and GANT61 which are the standard inhibitors of Smo, Hh and Gli mediated activity respectively (LoRusso et al., 2011; Pan et al., 2012; Stanton et al., 2009).

2.2. Ligand and protein preparation

For the purpose of ligand preparation all the forty five ligands and three standard drug molecules had been imported to the project table of Schrödinger Maestro 9.6 suite and using the LigPrep wizard application, ligands were made ready for docking (Singla et al., 2015). In ligand preparation step, the raw structures were incorporated with several structural modifications such as integrating hydrogen atom properly according to valency, appropriate fixation of charge as well as orientation of functional groups, bond length, bond angle correction, appropriate stereochemistry as well as tautomeric state generation. In ionization section of ligand preparation step, possible protonated state of ligands at pH range of 7 ± 2 was generatedusing Epik (Shelley et al., 2007). Furthermore generate tautomer command have been implemented while preparing ligands. While executing LigPrep module chiral centres of all the molecules were considered critically by implimenting retain specific chirality option available with the wizard. Maestro is provided with MMFFs and OPLS_2005 forcefield. OPLS_2005 is suitable and thus generally used for biological systems and organic molecules (Singla et al., 2015; Singla et al., 2015). Thus, minimization and then optimization of ligands using OPLS_2005 force field were performed to get one final 3D representative model of all ligands ready to dock. (Naik et al., 2011). For the preparation of protein molecules the crystal structures of protein were imported to the protein preparation wizard application of maestro 9.6 (Naik et al., 2011). The various step involved in protein preparation steps include preprocess, review, modiefy and refinement. During preprocess step basic modifications such as water molecules deletion, addition of hydrogen atoms, bond order assigning, creation of disulfide bonds and zero order bonds to metal were incorporated into the raw PDB structure. Thereafter, generate state option available in review and modiefy tab is executed considering pH range of 7 ± 3. Finally proteins are optimized and minimized with options availale in refine panel of protein preparation wizard. Optimization was done with default settings and then minimization step was executed. During minimization step conserve heavy atom to RMSD cutoff was taken as 30 Å with the implementation of OPLS_2005 force field. We have deleted associated DNA molecule in case of Gli (2GLI) and ligand in case of Hh (4C4 M) during protein preparation.

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Table 1
Alkaloids screened in this study.

Sr No	Compound	Reference	Mol Wt g/mol	H-bond Donor	H-bond Acceptor	Q P log P oct	Q P log Pw	Q P log Po/w	Q P log S Mol/dm^3
1	Aaptamine	Aoki et al. (2006)	228.25	1	3	11.416	6.565	2.63	-2.939
2	Ascididemin	Dassonneville et al. (2000)	283.289	0	5	13.551	9.261	2.287	-3.101
3	Belotecan	Li et al. (2008)	433.506	2	9.25	23.84	14.542	2.458	-4.134
4	Berbamine	Xu et al. (2006)	608.733	1	8	25.11	10.617	5.634	-4.121
5	Chelidonine	Panzer et al. (2001)	353.374	1	1	15.623	10.173	1.91	-1.986
6	Cortistatin	Aoki et al. (2007)	472.626	2	7.65	24.422	13.78	4.114	-5.587
7	Ecteinascidin-743	Pommier et al. (1996)	761.842	4	16.2	37.786	23.995	1.926	-2.42
8	Evodiamine	Liao et al. (2005)	303.363	1	4	15.694	9.119	3.556	-4.853
9	Glaucine	Konda et al. (1990)	355.433	0	5	14.253	5.938	3.776	-3.537
10	Lamellarian	Ballot et al. (2010)	499.476	3	7	24.43	14.693	3.859	-5.902
11	Matrine	Ma et al. (2008)	248.367	O	5	11.562	8.695	0.948	-0.32
12	Ningalin	Boger et al. (1999)	367.271	5	8	23.537	18.929	-0.724	-2.799
13	Piperine	Li et al. (2011)	285.342	0	4.5	12.077	5.888	3.261	-3.532
14	Tetrandrine	Fu et al. (2004)	622.76	0	8	23.765	8.405	6.136	-3.34
15	Tylophornidine	Zhen et al. (2001)	365.428	2	5.95	18.364	10.779	2.707	-3.469
16	Variolin b	Perry et al. (1994)	293.287	5	6.5	22.205	17.65	0.24	-2.922
17	Tylophorine	Wu et al. (2009)	393.482	8	5	15.922	6.223	4.392	-4.915
18	Boldine	Gerhardt et al. (2009)	327.379	2	5	16.128	9.452	2.427	-2.911
19	Lycorine	Lamoral-Theys et al. (2009)	287.315	2	6.9	15.344	11.541	0.693	-1.432
20	Ancistrotectorines	Boyd et al. (1995)	421.535	`1	5	18.961	8.012	5.185	-5.535
21	Nitensidine	Tajima et al. (2014)	193.291	3	2	12.949	7.468	2.148	-3.122
22	Tsitsikamm-amine A	Legentil et al. (2006)	303.32	3	5.25	18.433	13.173	1.625	-3.711
23	Penitrem A	Sallam et al. (2013)	634.211	4	7.65	30.949	15.682	6.196	-8.151
24	Coptichic aldehyde	Qian and Yang (2014)	367.314	0	8.75	15.869	14.337	-0.002	0.124
25	Coptichine	Qian and Yang (2014)	539.54	1	7.5	22.892	11.142	5.267	-6.126
26	13-carboxaldehyde-8-oxocoptisine	Qian and Yang (2014)	377.353	0	8	15.576	10.291	1.509	-2.02
27	Piperlonguminine	Bezerra et al. (2008)	317.341	0	5.25	13.558	6.632	3.153	-3.818
28	Emetine	Möller et al. (2007)	480.646	1	6.5	21.805	8.875	5.123	-5.119
29	Spongiacidin C	Yamaguchi et al. (2013)	246.225	4	6	17.318	14.688	-0.458	-2.149
30	Harmine	Ma and Wink (2010)	212.251	1	1.75	10.062	5.56	3.106	-3.572
31	Solasonine	Munari et al. (2014)	884.069	10	27.25	57.437	42.874	-0.754	-3.702
32	Solamargine	Munari et al. (2014)	868.069	9	25.55	53.889	39.314	0.128	-3.22
33	Antofine	Fu et al. (2007)	363.455	0	4.25	14.843	5.925	4.392	-4.489
34	Tryptanthrin	Yu et al. (2009)	248.24	0	6	12.624	9.886	1.016	-1.836
35	Granulatimide	Berlinck et al. (1998)	276.254	3	4.5	17.347	12.51	0.898	-2.624
36	Isogranulat-imide	Sturgeon and Roberge (2007)	276.254	2	4.5	15.16	11.094	1.304	-2.776
37	Galantamine	Tsvetkova et al. (2014)	287.358	1	5.2	13.726	8.254	2.046	-2.114
38	Noscapine	Chougule et al. (2011)	413.426	0	8.75	17.501	10.29	1.905	-1.622
39	Pityriacitrin	Zhang et al. (2011)	311.342	0	1	13.651	5.509	5.087	-6.164
40	3,5-Dihydroxy-2,4-dimethoxyaristolactam	Chanakul et al. (2011)	311.293	3	5.5	16.796	12.159	1.525	-2.72
41	Pityriacitrin B	Irlinger et al. (2005)	355.352	1	3	17.335	9.135	4.344	-5.887
42	Deoxypodophy-llotoxin	Kim et al. (2002)	398.412	0	6.75	15.89	8.112	2.95	-2.84
43	Cernumidine	Lopes et al. (2011)	304.348	5	6	21.816	15.012	1.434	-3.406
44	Distichamine	Nair et al. (2012)	329.352	0	7	13.594	8.254	1.343	-1.272
45	Narciprimine	Ingrassia et al. (2009)	271.229	2	4.5	13.472	10.092	1.129	-2.332

2.3. Grid parameter selection and validation

Fully prepared proteins were carried further for grid generation step. The grid was generated at place of preexisting ligand for Smo (4JKV) protein using default settings and re-dock work was performed to validate the parameters. Acceptable RMSD of 2 Å was obtained from re-dock study and thus same parameters were used for docking the test ligands. In case of Hh and Gli proteins, because of the absence of appropriate inbuilt ligand SiteMap application of maestro 9.6 was the best way to calculate the druggable pockets within the proteins. Thus, SiteMap application was used with default settings which have anticipated just one possible druggable pocked within both the proteins. Thus for both the proteins only one druggable pocket is present which was included in grid box using rest of parameters same as used in case of Smo. Initially possible druggable pockets was also predicted for Gli without deleting the associated DNA molecules and got several binding sites. In this case, predicted binding sites was found to be made up of by residues of both Gli proteins as well as DNA molecules. But investigating the interaction pattern of ligand with Gli protein only, excluding DNA is important. This is because, it is more reasonable to target transcription factor Gli before it binds to DNA and thus activate transcription process. Thus we have selected the grid with deleted DNA molecule. Notably, selected binding caviety was situated distinctly apart from DNA molecule, thus presence or absence of DNA seems to have little impact on docking studies.

2.4. Docking and binding energy estimation

Molecular docking studies were conducted using glide docking module of Schrö6;dinger Maestro 9.6 suite. Glide docking was performed using flexible extra precession mode provided by Maestro glide (Naik et al., 2011). Docking results were obtained in the form of Glide score which were finally re-scored in the form of binding energy using prime MM-GBSA module provided with Maestro 9.6 (Lyne et al., 2006).

2.5. ADME profile

Pharmacokinetics of drug like molecules is an important aspect to be considered during the drug development process. Multiple good drug candidates are unable to pass clinical trials because of their poorer ADME profile. Thus, pharmacokinetic properties of all

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Table 2
Binding data of lead compounds based on MMGB-SA binding energy study

Receptor	Compound	δG_Bind (Kcal/mol)	δG_Bind Hbond (Kcal/mol)	δ_GBind Coulomb (Kcal/mol)	δ_GBind Lipo (Kcal/mol)	δ_GBind vdW (Kcal/mol)	Key Protein ligands interaction
4C4M	Emetine	−82.59	-0.68	−24.49	−48.98	−51.39	Ser 80, Arg 62, Glu 64, Arg 73, Glu 76, Thr 78, Thr 100, Gln 101, Arg 102, Arg 145, Lys 187, Glu 189, Asn 190, Ser 191
	Robotnikinin*	−61.72	−0.64	−12.49	−32.22	−39.86	Arg 62, Glu 64, Glu 74, Glu 189
	Solamargine	−61.02	−2.86	−10.52	−37.91	−40.95	Glu 64, Lys 75, Gln 101
	Cortistatin	−56.21	−0.72	45.65	−38.43	−35.32	Asn 190, Ser 191
4JKV	Cortistatin	−91.44	−0.5	−22.4	−81.77	−52.53	Tyr 394, Trp 480,
	GDC−0449*	−84.12	−0.36	−0.33	−45.54	−58.2	Asn 219, Phe 484, Glh 518
	Emetine	−66.85	−0.17	−21.17	−86.36	−31.56	Asp 384, Lys 395, Asp Arg 400, 473, Glu 481
2GLI	Solamargine	−84.78	−2.16	−33.5	−39.3	−44.55	Glu 119, Lys 168, Ser 180
	Solasonine	−78.65	−4.57	−8.98	−38.78	−46.24	Glu 119, Glu 167, Pro 169, Lys 171
	Emetine	−57.8	−1.94	2.9	−28.35	−38.42	Glu 119, Lys 168, Pro 169. His 170, Ser 180
	Tylophorine	−53.34	−1.91	14.94	−28.19	−33.85	Arg 162, Glu 167
	Cortistatin	−50.63	−0.69	−19.24	−30.13	−28.18	Hie 123, Lys 168
	GANT61*	−34.67	−0.56	52.92	−22.75	−30.34	Glu 167

the compounds have been studied using QikProp application of Maestro 9.6. QikProp is an easy to use application of Schrödinger which can predicts several useful pharmacokinetic parameters such as permeability towards the blood brain barrier and thus overall CNS activity, skin permeability, percentage oral absorption and binding to human serum albumin and many more parameters (Vanjari et al., 2012).

3. Results and discussion

3.1. Binding ability of lead molecules

The compound with the best binding ability can be estimated by the binding energy yielded during Prime MM-GBSA study. Furthermore, ligand receptor complex formed during such study provided valuable information concerning role of key ligand receptors interactions such as hydrogen bonding, electrostatic interactions, lipophilic interactions, vanderwall interaction in context to binding ability of ligands which is represented in Table 2. The data represented here describe the ligands with best binding ability in term of MM-GBSA total binding energy (δG_Bind). Contribution of individual interaction pattern in total binding energy such as hydrogen bonding interactions (δG_Bind Hbond), electrostatic interactions (δG_Bind coulomb), lipophilic interactions (δG_Bind Lipo) and vanderwall interactions (δG_Bind vdw) have also been represented here.

Among various docked compound emetine **(1)** (Fig. 2) seems to be a good lead molecule which represents binding energy of −82.59 Kcal/mol in case of Hh receptor that is significantly good compared to standard drug robotnikinin which is −61.72 kcal/mol. Same molecule compound **(1)** also have good binding ability towards Gli, having binding energy of −57.80 Kcal/mol which is comparatively better than binding energy of standard drug GANT61 showing binding energy of −34.67 kcal/mol. Furthermore, in case of Smo receptor the binding energy of compound **(1)** was found to be −66.85 Kcal/mol which is although less but comparable better then other ligands. Thus, compound **(1)** appears to be a good lead molecule which modulates Hh as well as Gli proteins. Moreover, binding energy towards Smo is also significant and may be further improved by slight structure modification.

A similar trend has been obtained in case of cortistatin **(2)** which is the best docked compound with respect to binding energy in case of Smo. The binding energy obtained for Smo was found to be −91.44 Kcal/mol which is better than −84.12 Kcal/mol obtained for standard drug GDC-0449. In case of Gli, compound **(2)** has shown binding energy of −50.63 Kcal/mol which is although not best but better as compared to standard drug. The binding energy obtained for compound **(2)** in case of Hh was −56.21 Kcal/mol

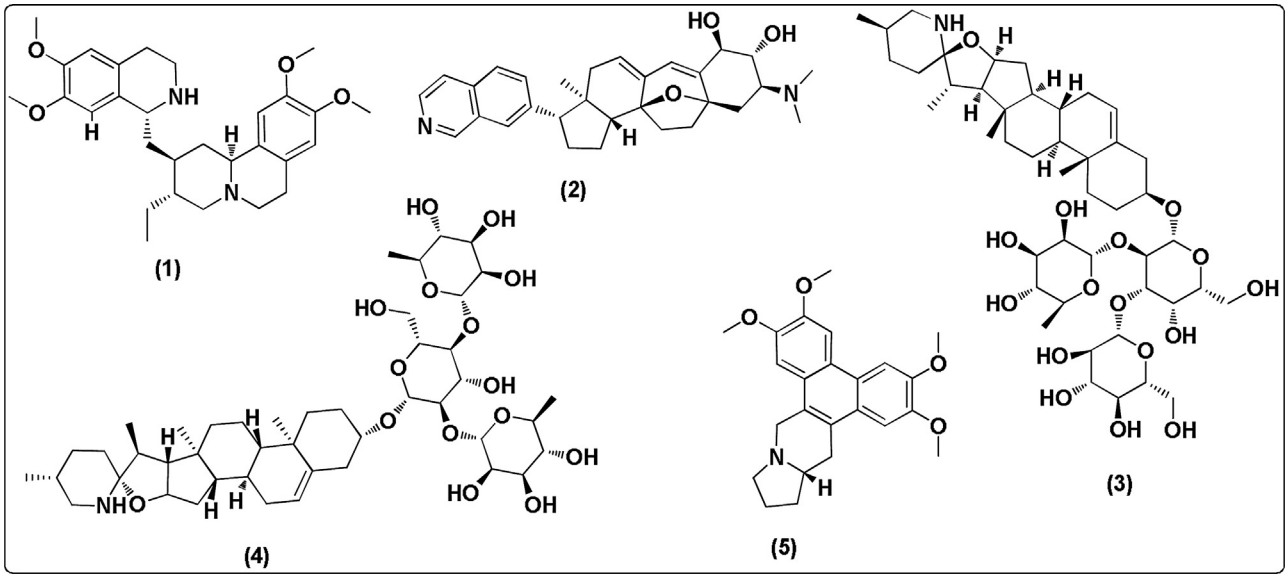

Fig. 2. Chemical structure of lead compounds.

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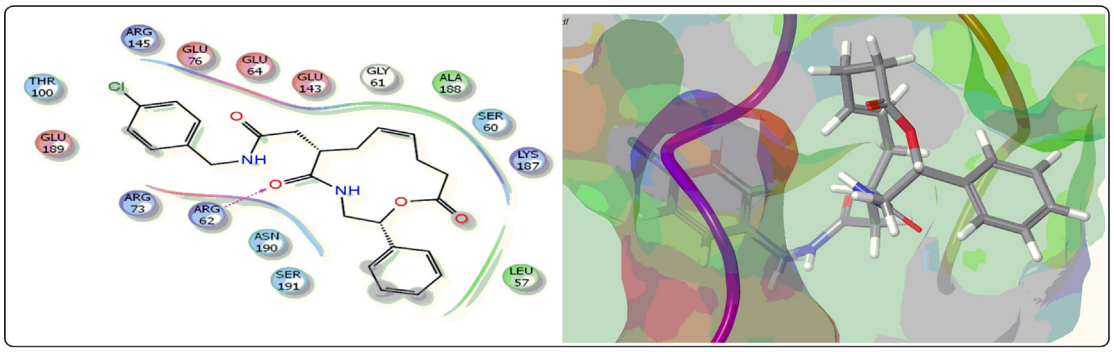
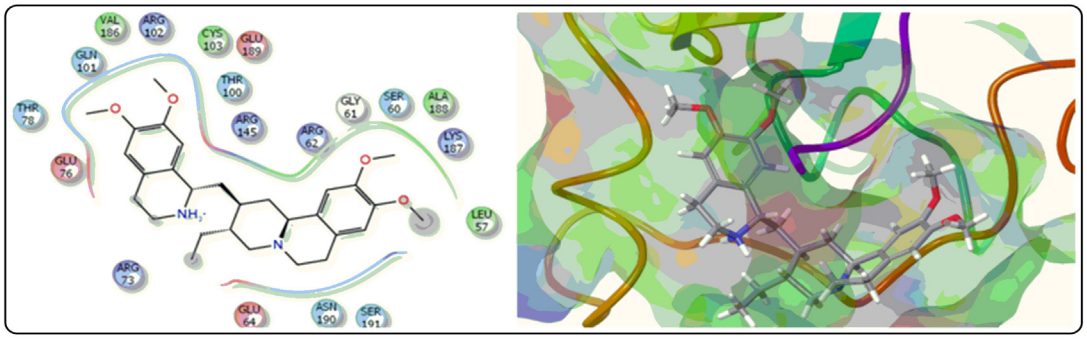
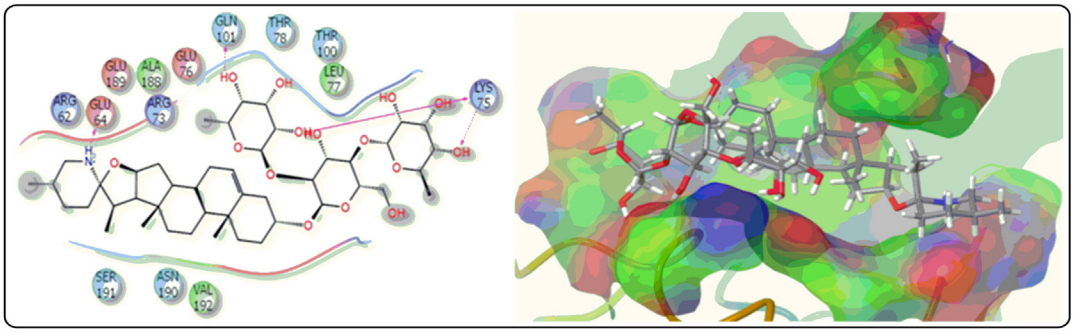


(A)



(B)



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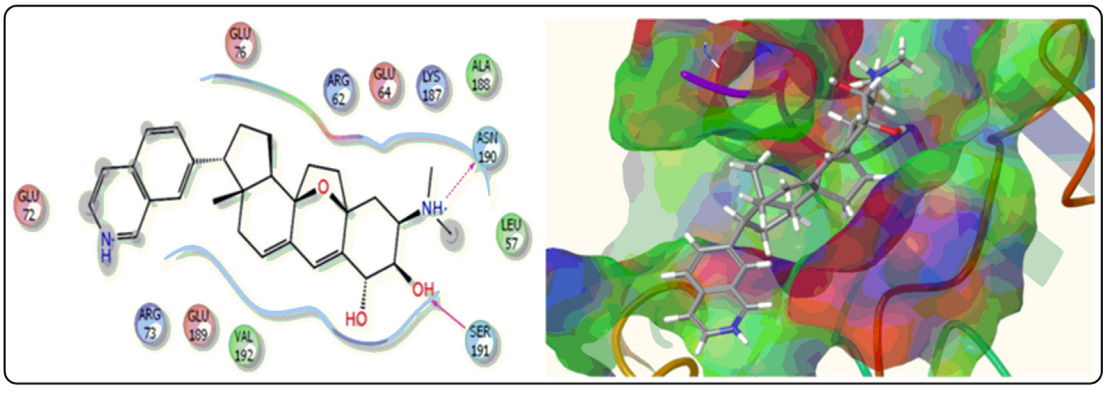

(D)

Fig. 3. Interaction pattern and pose of Standard drug robotnikinin **(A),** emetine **(B),** solamargine **(C)** and cortistatin **(D)** with sonic hedgehog protein.

which is very close to robotnikinin with binding energy of −61.72 kcal/mol. Thus, above mentioned fact indicates that along with emetine, cortistatin may also have the ability to alter Hh signaling by modulating Hh, Smo as well as Gli protein. In case of Gli protein solamargine **(3)** have shown best binding energy of −84.78 kcal/mol, which is very high as compared to standard

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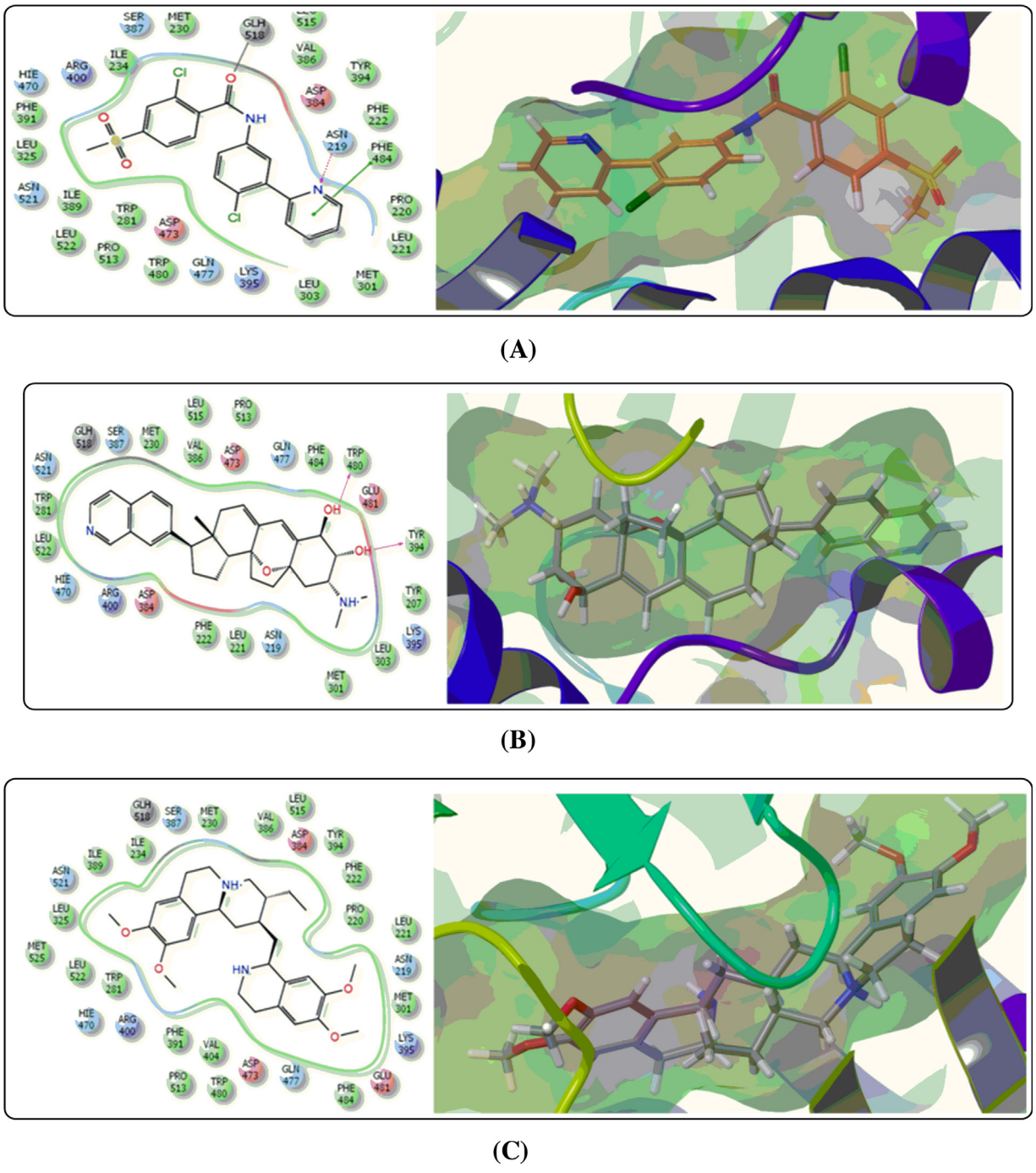

Fig. 4. Interaction pattern and pose of Standard drug GDC-04; 49 **(A),** cortistatin **(B)** and emetine **(C)** with smoothened protein.

inhibitor GANT61. Furthermore compound **(3)** have also shown good binding energy of −61.02 Kcal/mol in case of sonic Hh which is comparable to standard inhibitor robotinikinin and thus it seems to have good modulating ability for Hh as well as gli proteins. Solasonine **(4)** and tylophorine **(5)** are the other compounds which have shown binding energy better than standard drugs in case of Gli protein thus seems mono-targeted compounds against CSCs. The interaction pattern and pose of best docked lead compounds and standard drug molecules are shown in Figs. 3–5.

In-silico study indicated that compounds **(1–5)** may be explored further as targeting molecules against CSCs and hence as anticancer agent. The possibility of investigating these compounds as targeting agents against CSCs can be supported by multiple supportive facts established through experimental events. Compound (1) was found effective against multiple cancer cell lines which include U937, A549-s, CCRFCEM, HL-60, rat hepatocytes and CEM/ADR5000 (Akinboye and Bakare, 2011). Possible role of compound (1) against CSCs can be further rationalized by its inhibitory effect on hypoxia induced factor-1 (HIF-1). HIF-1 is directly involved in multiple signaling cascades which are responsible for maintaining stem like characters in cancer cells (Akinboye and Bakare 2011; Keith and Simon 2007). Anticancer potential of compound **(2)** is also well documented in literature (Shenvi et al., 2014). The possible mechanism by which compound **(2)** mediate its anticancer potential is by inhibiting angiogenesis (Sarfaraj et al., 2012). CSCs are demonstrated to be significantly

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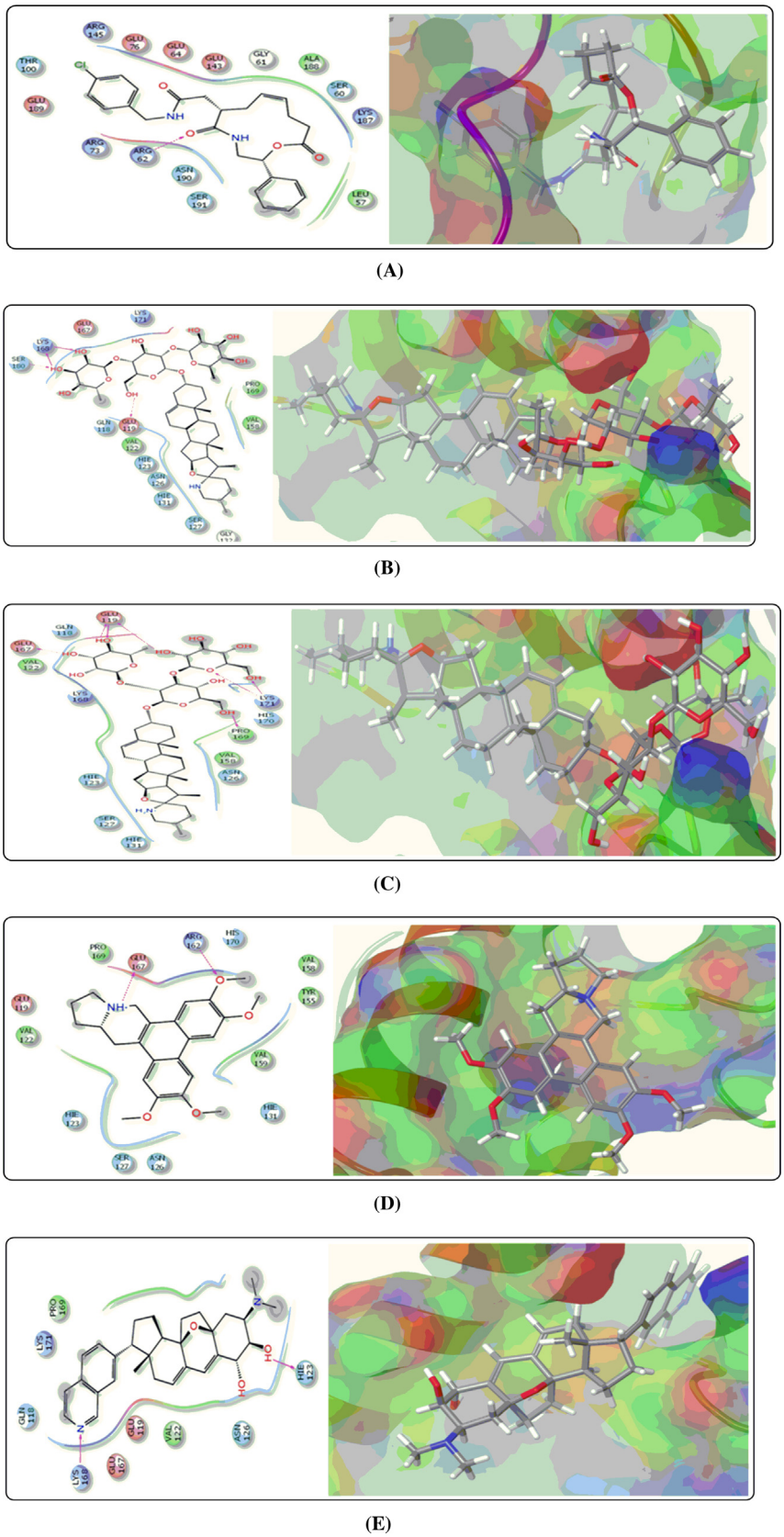

Fig. 5. Interaction pattern and pose of standard drug GANT61 **(A),** solamargine **(B),** solasonine **(C),** tylophorine **(D)** and cortistatin **(E)** with gli protein.

involved in tumor related angiogenesis and associated mechanisms which indicate that there is direct link between CSCs and tumor angiogenesis process (Eyler and Rich, 2008). Thus, considering the in-silico observations as well as several other factors, compound **(2)** may be explored further as a lead molecule against CSCs. Compound **(3)** which has shown good binding ability

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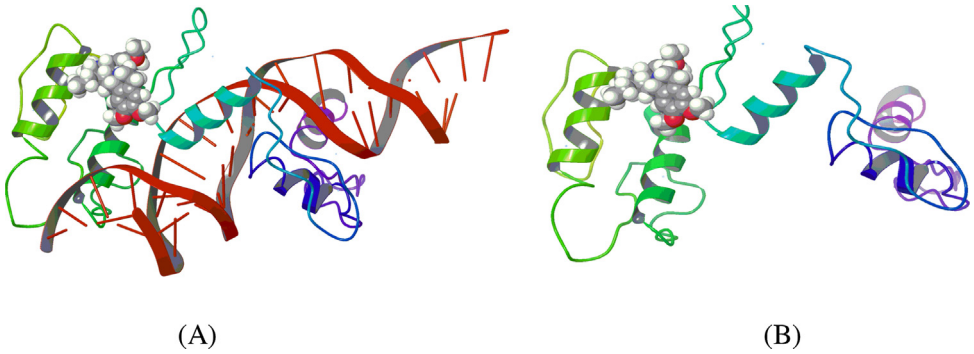

Fig. 6. Binding pattern of emetine with 2GLI in the presence of DNA molecule (A) and with DNA molecule deleted (B). Binding cavity is situated distinctly apart from DNA binding site of gli protein.

towards Hh as well as Gli is also a promising anticancer compound as demonstrated in several experiments. CSCs are highly resistant to apoptosis which is partially due to overexpression of anti-apoptotic protein Bcl-2. Compounds showing Bcl-2 inhibitory potential or apoptosis inducing abilities may provide a way to trigger apoptosis in CSCs (Kuo et al., 2000; Lagadinou et al., 2013). Compound **(3)** has shown significant apoptosis inducing ability which in fully or partially mediated through Bcl-2 related activity and thus seems to be a potent targeting agent against CSCs. Furthermore TNFs which are significantly involved in epithelial-mescenchymal process and thus critical in CSCs generation as demonstrated in breast cancer cells are significantly modulated by compound **(3)** and thus may prove good anti CSC agent (Asiedu et al., 2011; Liu et al., 2004). Compound **(4)** which has shown great binding ability towards Gli protein, also produced immense anticancer effects against multiple cell lines (Koduru et al., 2007). Anticancer activities on HeLa, MCF7 and HT29 cancer cell lines indicated that it inhibits cancer cell growth by blocking cell cycle in G_0/G_1 phase (Koduru et al., 2007). Little is known about the mechanistic prospective of anticancer potential associated with compound **(4)** but good binding energy towards Gli protein indicating its possibility for being developed as anti-CSCs agent. Compound **(5)** has also shown significant anticancer potential in several studies. Study conducted on HepG2, HONE-1, and NUGC-3 carcinoma cells indicated that anticancer potential of compound **(5)** is mediated through downregulatiom of cyclin A2 mediated activity (Wu et al., 2009). Moreover, compound **(5)** and its analogs have shown inhibitory effect on AMP response elements, activator protein-1 sites, or nuclear NF-κB binding site-mediated transcriptions which provide a unique mechanism against tumor cells (Gao et al., 2004). Good binding energy score towards Gli protein further indicating its inbuilt ability to modulate Hh pathway which may be actively involved in maintenance of CSCs. Furthermore, Gli is a transcription factor which binds to DNA and regulate transcription of several genes (Kasper et al., 2006). Critically analyzing the binding pattern of Gli with emetine (Fig. 6) and other docked compounds indicate that the docked site of Gli is present significantly away from DNA binding site of the Gli protein. This available extra binding cavity (EBC) seems to be quite useful in drug design towards CSCs. Compounds targeting towards EBC of Gli may interfere with transportation of Gli protein through nuclear membrane and/or its binding towards DNA molecule which is usefull in targeting CSC. Furthermore, CSCs are generally well equipped with multi drug resistance proteins (MDR) and thus directly targeting it with anticancer agents is very challenging (Donnenberg and Donnenberg 2005). Linking DNA targeting anticancer agents with compounds having binding ability towards EBC of Gli protein may be an useful strategy to saturate CSC with anticancer agents. Thus, compound **1–5** with best binding ability towards Gli can be useful in several way in treating cancer by targeting CSCs.

3.2. Pharmacokinetic profile of lead compounds

Pharmacodynamics results indicated that compounds **(1–5)** may prove good lead to get rid of cancer by targeting CSCs. But pharmacokinetic properties of drug like molecules are also an important aspect to be taken in case of drug molecules. The pharmacokinetic parameters of all five lead molecules have been studied along with other studied alkaloids using QikProp

Table 3
Predicted ADME properties of lead molecules

S.no	Compound	Q P log Po/w[a]	Q P log HERG[b]	QPP Caco (nm/sec)[c]	Q P log BB[d]	QPP MDCK (nm/sec)[e]	Q Plog Kp[f]	Q P log Khsa[g]	% HumanOral Absorption[h]
		(−2.0 to 6.5)	(acceptable range: > −5.0)	< 25−poor and >500−great	(−3 to 1.2)	<25poor >500great	(−8.0 to −0.1)	(Acceptable range: −1.5 to 1.5).	>80 %−High, >25 %−Poor
1	Emetine	5.123	6.827	319.749	0.619	176.55	−5.18	1.231	88.8
2	Cortistatin	4.114	−6.529	298.53	−0.206	148.171	−4.165	0.876	95.3
3	Solamargine	0.128	−5.845	6.845	−2.881	2.503	−7.059	−0.685	3.774
4	Solasonine	−0.754	−6.646	1.165	−4.317	0.403	−8.242	−1.02	0
5	Tylophorine	4.392	−5.708	2250.9	0.679	1315.5	−2.861	0.693	100

[a] Predicted octanol/water partition co-efficient log *p*.
[b] Predicted IC50 values to block HERG K+ channels.
[c] Predicted Caco-2 cell permeability. It represents the ability of drug to cross gut blood barrier.
[d] Predicted brain/blood partition coefficient.
[e] Predicted apparent MDCK cell permeability. It represents the ability of drug to cross blood-brain barrier.
[f] Predicted skin permeability.
[g] Predicted binding to human serum albumin.
[h] Predicted oral absorption of drug in percentage term.

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application available with Maestro 9.6. Lead compound **(1)**,**(2)** and **(5)** was seems to be acceptable with respect to all the major ADME parameters (Table 3).

Compounds **(3)** and **(4)** found poorer with respect to their ADME profile. Both the compounds seem to have poorer oral absorption which restricts its usage as oral dose formulations. Furthermore, other parameters such as skin permeability, brain/blood partition coefficient, permeability towards blood-brain barrier as well as solubility factors are less favorable in case of both the compounds.

Thus for lead compound emetine, cortistatin and tylophorine, along with pharmacodynamics profile also seems to have ideal pharmacokinetic profile also. Furthermore lead molecules, Solamargine and solasonine need structural modification to improve its ADME properties.

4. Conclusions

Resistance towards cancer chemotherapy can be overcome by targeting CSCs which are the main factors behind resistance and relapse of disease. Current targeting molecules towards CSC are mono-targeted drugs and resistance may occur due to mutations in their protein targets. Thus including multi-targeted strategy to eliminate CSCs seems to be a good strategy to overcome cancer related resistances. Lead molecule emetine and cortistatin have shown good binding ability towards three well established CSC target proteins, Hh, Smo and Gli of Hh pathway. Multitargeting ability of both the compounds can be thus be explored as a measure to overcome cancer related resistances. Predicted ADME profile as well as documented mechanistic aspects of both the compounds further clarify that both the compounds have ability to be included in CSCs based cancer chemotherapy. Solamargine alkaloid has also shown good binding ability towards sonic Hh and gli protein which clearly indicates that it has pharmacophores with multi-targeting ability. Thus identifying and simplifying pharmacophores of solamargine may improve ADME profile and make it a good CSCs based anticancer agent. Other compounds solasonine as well as tylophorine appears to have good Gli modulating ability and can be further explored as CSC based anticancer agents. The pharmacokinetics profile of tylophorine is good but solasonine require structure simplification for improving its ADME features. Thus all the five lead molecules seem to have ability to target CSCs and may be included in CSCs based anticancer agents with suitably modifying the structure. Furthermore we have explored an unique drug binding cavity and termed it as extra binding cavity (EBC). This EBC seems to be an important addition in CSCs targeting strategies and can be used is several ways to target CSCs and thus cancer.

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