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Insilico Analysis of Plumbagin and its synthetic analogues as potential Bcl-2 Inhibitors

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Abstract

Plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone), a quinonoid constituent isolated from roots of different Plumbaginales namely, *Plumbago rosea*, *P.zeylanica*, *P.capensis*, *P.capensis*. Potential role of Plumbagin as an anticancer effects have been reported in diverse cancer models such as prostate, lung, cervical, ovarian as well as melanoma. A recent report has showed that plumbagin induced cell cycle arrest and apoptosis in human melanoma cells by inhibition of Bcl-2 over expression. Bcl-2 is one of the most important proteins implicated in inhibition of apoptosis. Functional blockage of the above protein could restore the apoptotic pathway in tumor cells.In present study six different synthetic analogues of Plumbagin were analyzed for its activity against anti-apoptotic protein, Bcl-2. Molecular docking analysis using the Schrödinger Maestro software revealed benzoate plumbagin (CID: 46835862) showed more potent inhibitory activity against Bcl-2 protein, with a maximum interaction energy of -34.29 kcal/mol compared to that of plumbagin -23.39 Kcal/mol. The mode of interaction of all the six analogues was much stronger in contrast with standard plumbagin. This study provides new insights in understanding the six different analogues as potential candidates for cancer therapy.

Keywords: Bcl-2, Plumbagin, Glide, Epik

Contents

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1. Introduction

Cancer, one of the leading causes of death in both developing and developed countries. There is no proper cure till date. Apoptosis or programmed cell death can be defined as an evolutionarily conserved and highly regulated process, necessary for maintenance of cellular homogenesis. (Mc.Donald*et al.,* 2005, D.Hannahan*et al.,* 2000). A proper balance between cellular proliferation and attrition is needed for the normal tissue homeostasis. Any instability in this cellular balance would lead to defects in cell growth and ultimately in to cell death pathway . An notable feature of cancer is its ability to evade or ignore such physiological cues, which is governed by the dysregulation of intrinsic and extrinsic apoptotic mechanisms (Putzai*et al.,* 1996, K.C.Zimmermann*et al.*,2001&Danial*et al*., 2004). These intrinsic and extrinsic pathways are highly regulated by a family of proteins, Bcl-2 (B-cell lymphoma 2) which is composed of both proapoptotic and antiapoptotic members that cooperate through a complex series of protein - protein interactions.(Cory *et al.,* 2002, Borner*et al*., 2003, &*Van et al*., 2006)

Bcl-2 family of proteins are classified based on the presence of Bcl-2 homology domains (BH1-BH4). The anti-apoptotic proteins Bcl-2 and Bcl-xL possess a binding groove that accommodates the BH-3 domain of pro-apoptotic family members (Bad, Bik, Bid, Bim) preventing their oligomerization and the initiation of apoptotic cascade (Peteros*et al.*, 2000, and Huang *et al.,* 2002).

In normal cells Bcl-2, an anti-apoptotic 26 kDa protein, regulates the activity of pro-apoptotic proteins by direct binding and sequestration. (Youle*et al.,* 2002). Cancer cells frequently overexpress the prosurvival Bcl-2 family members so as to suppress the apoptotic signal in order to promote survival or confer resistance to chemotherapy.(Green, *et al*., 2002, Amundson*et al*.,. 2000). In addition to implementation of drug resistance they can also delay apoptosis in response to radiation therapy (Berghella*et al.,* 1998). The precise mechanism of Bcl-2 mediated apoptosis is still unclear (Chipuk,*et al*., 2008). The compounds specifically binding on the BH3-groove of the anti-apoptotic proteins have been demonstrated to act as efficient anti-cancer compounds (P.H. Bernado*et al.*, 2008, &D.Sivakumar*et al.,* 2011)

The crystal structure of Chimaeric Bcl-2/xL NMR and X-ray crystal structures have demonstrated that the binding groove is amenable to small-molecule intervention (Oltersdorf*et al*., 2007) and further studies have shown that such molecules are able to sensitise tumour cells to apoptosis.Therefore, the Inhibition of these antiapoptotic Bcl-2 family members should specifically target the abnormal cell death pathway found in these cancer cells and offers an attractive target for therapeutic intervention. Cancer chemotherapy has entered a new field of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs. (Seymore, 1999)

Research in the field of cancer therapy has led to the development of many modern medicines and therapeutics with promising anticancer activity. Among the many natural and synthetic compounds explored for anticancer potential, compounds with quinone containing moieties form a major part. Plumbagin (5-hydroxy-2-methyl-1, 4-naphtho quinone), a quinonoid constituent isolated from the roots of *Plumbago zeylanica*L. Plumbagin has a potent anticancer activity and is mediated by one of the following mechanism, by the inactivation anti-apoptotic, proliferative and angiogenic gene products (Sandur*et al*., 2006). Potential role of plumbagin, as an anti-cancer agent has been recognized (Parimala and Sachdanandam, 1993; Naresh*et al*., 1996; Sugie*et al*., 1998; Hazra*et al*., 2002) and its anti-cancer effects have been reported in diverse cancer models such as prostate (Powolny and Singh, 2008), lung (Hsu *et al.,* 2006; Gomathinayagam*et al*., 2008), cervical (Srinivas*et al*., 2004b; Nair *et al*., 2008), ovarian (Srinivas*et al.*, 2004a) as well as the melanoma (Wang *et al.,* 2008)

2. Materials and Method

2.1 Preparation of protein target structure

The three dimensional structure of protein was taken from the Protein data bank Database (PDB ID: 2W3L) (www.rcsb.org) and further modified for Glide docking calculations. The complex was imported to Maestro (Shrodinger), the Co-crystallized ligand was identified and removed from the protein. The final preparation of receptor model was generated using Protein Preparation tool (PPrep). Energy minimization of the protein structure was performed OPLS 2005 force field. Minimization were performed untill the average root mean square deviation reached 0.001 Kcal/mol.

2.2. Ligand preparation:

The ligand molecule structures of plumbagin, and its derivatives were taken from the PUBCHEM database. The three diamensional models of all the compounds were generated using 'LigPrep' module of Schrodinger, a ligand preparation tool interfaced with Maestro. The protonation state and tautomer search of the compounds was carried out by the Epik tool (Repasky, M.P., 2002) (Schrodinger Inc. NY).Each ligand structure were optimized by means of the OPLS 2005 FORCE FIELD using default setting.

2.3. Molecular Docking using GLIDE (Grid Based Ligand Docking with Energetics)

All the ligands were docked to the BH-3 domain of Bcl-2 receptor using Glide commercial software (R.A. Friesner*et al*., 2004). The receptor grid files were generated using grid - receptor generation program, by van der Waal scaling of the receptor after ensuring that protein and ligands are in correct form for docking. The default size was used for the bounding and enclosing boxes. The grid box was generated at the centroid of the Bcl-2, BH-3 domain. Glide searches for favorable interactions between one or more typically small ligand molecules and a typically larger receptor molecule usually a protein. The docking mode is selected as flexible since it automatically generates confirmation for each input ligand. The ligand poses pass generated by GLIDE passes through series of hierarchal filters which evaluates the ligand interaction with the receptor and final scoring is then carried out on energy minimization poses. The ligand poses that GLIDE generates pass through a series of hierarchical filters which evaluates the interaction of ligand molecule with the receptor. The initial filters test the spatial fit of the ligand to the

defined active site and at the final stages it evaluates and minimizes the grid approximation to the OPLS-AA non-bonded ligand receptor interaction energy. The ligands were docked initially using the "Standard precision" method and further refined using "Xtra precision" of the Glide algorithm. Of the different poses that were sampled, a minimum number of poses were selected and the structures having the minimal energy conformations were further evaluated for the favorable Glide docking score. A single best conformation for each ligand was considered for further analysis. The best ligand poses from the GLIDE-XP mode is selected for the Induced Fit docking . The Induced Fit Docking allows the receptor to alter its binding sites so that it more closely confirms to the shape and binding mode of ligand. Induced fit docking Protocol has been reported to be the robust and most accurate method for both receptor and ligand flexibility

Table 1: List of selected Ligands, its structure

S.No.	COMPOUND ID	LIGAND	STURUCTURE
1	46835862	BENZOATE PLUMBAGIN	
2	359908	3-BROMO, 5-HYDROXY 2-METHYL NAPTHAQUINONE	
3	46242847	BUTYRATE PLUMBAGIN	
4	46242849	CRONATE PLUMBAGIN	
5	7330512	ACETYL PLUMBAGIN	
6	46242953	PROPIONATE PLUMBAGIN	
7	10205	PLUMBAGIN	

3. Results and Discussion

Natural products derived from medicinal plants and their synthetic derivatives have been used as a good lead material in case of cancer treatment. Several natural compounds have been evaluated for their potential activity in diverse cancer models. Plumbagin, is a natural napthaquinone, possessing various pharmacological activities namely antimalarial, antimicrobial, anticancer, cardiotonic, antibiotic, and antineoplastic activities (Pillai*et al.,* 1981, R.Parimala*et al.*,1993 and M Krishnaswamy*et al*., 1980).

In the present study, plumbagin and its analogues were docked with the protein Bcl-2. The target protein BCL-2 a complex structure co-crystallized with the ligand DRO was taken for docking studies. As plumbagin (Docking score: -4.472467 & Glide Energy : -23.39928) and the available derivatives showed low scores and shared less interactions with active site residues of the protein, the effect of synthetic analogues of plumbagin were evaluated in the present study.

The analogues of the standard ligand molecule plumbagin were first docked in SP mode of GLIDE. Out of the different derivatives the best fit derivatives were chosen from the SP docking results and is docked with the target protein in XP mode and then based on the Glide energy, and the best ligand pose of each derivative were chosen for induced fit docking. Of the six different analogue molecules, Benzoate plumbagin and 3-bromo, 5-Hydroxy, 2-Methyl Napthaquinone exhibited a similar binding pattern as that of plumbagin. The compound 3-bromo, 5-Hydroxy, 2- Methyl Napthaquinone shared a very novel binding site with the protein sharing the interactions with the amino acid molecules ASN 102, ARG 105 & TYR 67.

Docking scores, Glide energy and intermolecular interactions were recorded for the standard compound plumbagin, and its available derivatives. The results were tabulated in Table.2. The glide energy and docking scores of the analogue Benzoate Plumbagin (CID : 46835862) was about -34.291330 kcal/mol and -5.3817311 was more effective compared with the standard molecule plumbagin. The higher negative interaction energy of the inhibitor molecules substantiate their inhibitory potential against the protein. The above results conclude that these synthetic analogues of plumbagin can be synthesized and checked *invitro* and *invivo* for enhanced activity against cancer. These analogue molecules could be furtherdeveloped as an effective lead molecule for the therapeutictreatment of cancer

Table2: Induced fit docking results of best compounds (GLIDE)

S. No.	Compound	GLIDE energy (Kcal/mol)	GLIDE Score	Interaction D-H…A	Distance Between Donor and Acceptor (Å)
1.	Plumbagin (CID:10205)	-23.39928	-4.472467	(ASN102) NH…0	2.984
				(ARG105)NH…O	2.844
				(TYR 67) OH…O	2.983
2.	Benzoate Plumbagin	-34.291330	-5.817311	(ARG105)NH…O	2.964
	(CID:46835862)			(ARG105)NH…O	3.288
				(TYR 67) OH…O	2.810
3.	3-Bromo,5-Hydroxy,2-	-32.493824	-5.528491	(ARG105)NH…O	2.500
	Methyl Napthaquinone			(ARG105)NH…O	2.848
	(CID: 359908)			(TYR 67) OH…O	2.640
				(ASN102) NH…0	2.666
4.	Butyrate plumbagin	-30.67610	-3.621890	(ARG 88) NH…0	2.731
	(CID:46242847)			(ARG 88) NH…0	2.973
5	Cronate plumbagin	-30.507160	-4.009083	(TYR 67) NH…0	2.839
	(CID:46242849)			(TYR 161)NH…0	2.859
6	Acetyl plumbagin	-30.501531	-4.301627	(ARG105)NH…O	2.787
	(CID:7330512)			(TYR 67) NH…0	2.874
7	Propionate plumbagin	-29.61956	-3.372949	(ARG105)NH…O	2.914
	(CID : 46242953)			(TYR 67) NH…0	2.114
				(ARG105)NH…O	3.151

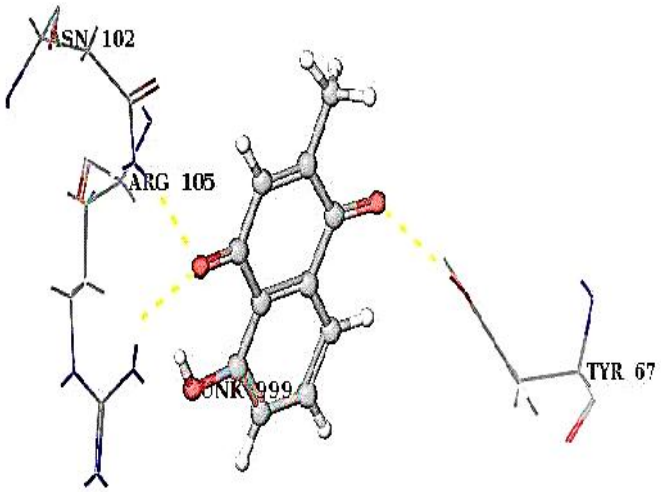

Figure 1: Induced fit interaction between Plumbagin and Bcl-2 protein

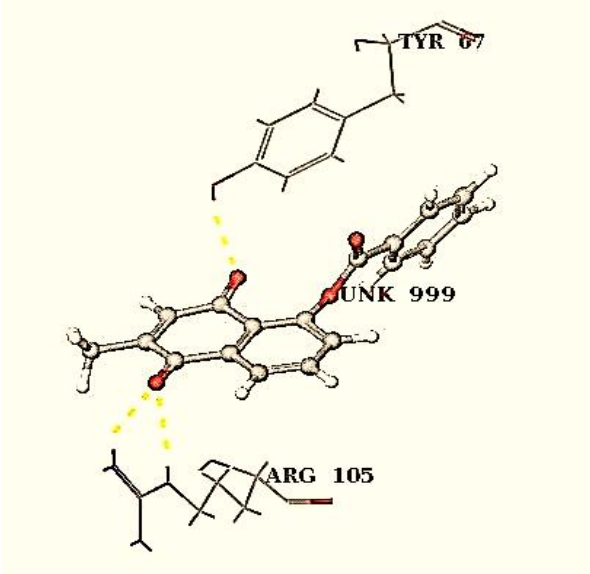

Figure 2: Induced fit interaction between analogue Benzoate Plumbagin and BCL-2 protein

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