Cytotoxic T-Lymphocyte Associated Protein-4 (Ctla-4) as Potential Drug Target for Cancer Therapeutics

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Abstract

Cancer is the second major cause of death after cardiovascular diseases and is the global threat to the human life. Over 60% of anticancer agents are derived from plants and have a diverse history in the treatment of cancer with significant effects. Current study was performed to investigate the biological action of the natural anticancer compounds having immune stimulating activities and to scrutinize the checkpoint inhibitor from natural sources. Initially 20 plants were screened out having anticancer and immune-stimulatory activities. Dataset of over 100 natural anticancer compounds retrieved from 20 potential plants were subjected to number of filters including ADMET properties, Lipinski rule of five and QSAR to pre-filter irrelevant compounds and screen out potential anticancer candidate that satisfy the drug properties. Using molecular docking approach, five (ascorbic acid, β-carotene, β-sitosterol, kaempferol and mivobulin) shortlisted natural anticancer compounds were docked with cytotoxic T-lymphocyte associated protein-4 (CTLA-4). The current analysis revealed good binding affinity of all compounds to the receptor protein CTLA-4 with high binding score. Among all tested compounds, ascorbic acid was completely buried into the active domain of CTLA-4 and showed strong binding interactions with high score function (-9.09kcal/mol). We concluded that our identified CTLA-4 inhibitor compound might be used as a potential drug candidate against cancer after thorough evaluation *in vitro*.

Key words: Cancer, CTLA-4, anticancer drug, medicinal plants, docking, ascorbic acid.

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Introduction

Globally, cancer is the second main cause of death after cardiovascular diseases (1). The etiology of cancer is still unknown because it is very complicated. However, cancer can be prevented by immune system because it can control the development of tumor cells by generating tumor specific antigen T lymphocytes. All cells of natural defense system work mutually against cancer, but T cells are the key mediators of antitumor immunity and immune surveillance is only regulated by them (2). T cells play a central role in the anticancer adaptive immunity. From the last few years, T cells were selected as target in immunotherapy (3) but in case of cancer checkpoint, inhibitor proteins basically stop the activity of T cells by cytotoxic T-lymphocyte associated protein-4 (CTLA-4) (4). CD28 and CTLA-4 are the earliest characterized receptors, which entirely express on the T helper cells and provide costimulatory and coinhibitory effects on the activation and proliferation of T cells. CTLA-4 acts as a negative regulator and it suppresses the activation of T cells through autonomous and non-autonomous pathways to maintain the self-tolerance and autoimmunity protection. Intracellular domain of CTLA-4 contains YVKM motif, which suppress the response of T cells by binding with P13K, PP2A and SHP-2 proteins and through dephosphorylation of TCR-proximal signaling proteins (5). Clinically CTLA-4 is the primary immune checkpoint inhibitor, and it maintains the regular immunologic homeostasis. To control cancer, the dendritic cells play basic function in the activation of T cells, but this is stopped by CTLA-4. The brakes on T cells at different stages are only released by inhibitor drugs (4). CTLA-4 has achieved significant benefit in various cancers by blocking immune-inhibitory signals (5).

Cancer being a worldwide threat to human life, needs prevention and its control and plants are the success approach in the prevention of cancer as well as in therapy. Plants give new bioactive molecules which prevent and reduce the effect of chemotherapy and other conventional treatments as they are easily available, economic and produce no toxic effects as compared to the allopathic drugs and chemotherapy. Plants are the excellent sources of bioactive components worldwide including the United States, where approximately 50-60% of cancer patients utilize natural anticancer agents. In rest of the world, a huge population in utilizing the plant

yielded natural products to deal the cancer and its complications. There is long list of herbal anticancer plants used around the globe but most important are *Allium sativum, Alpinia galangal, Andrographis paniculate, Angelica archangelica, Annona atemoya, Aralia elata, Asclepia scurassavica, Astragalus membranaceus, Azadirachta indica, Berberis vulgaris, Belamcanda chinensis, Boswellia serrata, Calligonum comosum, Camptotheca acuminate, Centella asiatica, Colchicum autumnale, Copaifera multijuga, Curcuma longa, Fagonia schweinfurthii, Garcinia indica, Gardenia jasminoides, Glycyrrhiza glabra, Hedyotis diffusa, Kaempferia parviflora, Lantana camara, Litchi chinensis, Menyanthes trifoliata, Morus alba, Morus nigra, Morinda citrifolia, Nigella sativa, Nitraria retusa, Oldenlandia diffusa, Paeonia lactiflora, Paeonia emodi, Perilla frutescens, Panax ginseng, Phyllanthus amarus, Platycodon grandifloras, Plumbago zeylanica, Podophyllum emodi, Podophyllum peltatum, Punica granatum, Rabdosia rubescens, Rhodamnia rubescens, Scutellaria barbata, Taxus baccata, Tinospora cordifolia, Tripterygium wilfordii, Viscum album, Wedelia chinensis, Withania somnifera, Ziziphus nummularia,* and *Zuojin wan* etc. These medicinal plants contain certain natural components, which illustrate the presence of antioxidants showing anticancer activities (6,7). For the development of natural drug research, Ethnobotany provides a big natural source from plant kingdom, and it became an urgent need to develop less toxic and more effective drugs. In clinical use more than 50% of all modern drugs are made from natural products. It has been recognized that many of these products have the ability of apoptosis in various cancer cells of human origin.

With the mean of identifying narrative agents of plant origin having antigrowth and effective apoptotic activities against various types of cancers, present study was conducted to investigate the biological action of the several common chemical entities from different plant sources and to identify the checkpoint inhibitor drugs from natural sources. The study aims the identification and characterize selected anticancer plant compounds, analogs and derivatives against cancer and to analyze the drug compounds that enhance immunity against cancerous cells.

Methodology

Plants Selection and Ligand Dataset

The current study involved the thorough literature survey and exploitation of computational databases to identify the most active anticancer plants. In current study 20 plants with profound and efficient anticancer activities were selected including *Nigella sativa, Viscum album, Ocimum tenuiflorum, Azadirachta indica, Morinda citrifolia, Glycyrrhiza glabra, Curcuma longa, Aloe vera, Panax ginseng, Podophyllum hexandrum, Withania somnifera, Lantana camara, Paeonia emodi, Oldenlandia diffusa, Plumbago zeylanica, Tinospora cordifolia, Psoralea corylifolia, Allium sativum, Andrographis* and *Berberis vulgaris.* Several hundreds of phytochemicals listed in supplementary Table were retrieved from selected plants by using the PubChem (http://www.ncbi.nlm.nih.gov/pccompound), Phytochemical/Ethnobotanical databases (8) and ZINC databases (9). AdmetSAR (10) program was used to calculate the ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the selected drug compounds using chemo informatics. The oral toxicity of selected ligand molecules was predicted through ProTox narrative approach (11) followed by energy minimization with Argus Lab.

Target protein selection and geometry optimization

Experimentally determined three-dimensional molecular structure of human CTLA-4 (PDB ID: 3OSK) was retrieved from protein data bank (http://www.rcsb.org/pdb/home) for the present study to investigate the putative binding inhibitors against CTLA-4. CTLA-4, receptor protein was optimized by removing nonstandard molecule and subjected to minimization using amber force field embedded in Chimer 1.7. (http://www.rbvi.ucsf.edu/chimera). After minimization polar hydrogen atom were assigned to receptor protein.

Molecular Docking

Molecular docking of selected natural compounds in the active binding pocket of CTLA-4 was performed by using Autodock vina (12) and PatchDock servers (13). Gasteiger partial charges were designated to ligand molecule and non-polar hydrogen atoms were merged. For receptor the values of X, Y and Z in active side residues were generated around 60×60×60Å grid points by AutoDock Vina at 0.375Å grid space (14). The Lamarckian genetic algorithm (LGA) and empirical free energy functions were

Table 1: QSAR description of the CTLA-4 inhibitor with final total docking energy in kcal/mol. M.W: Molecular weight, HBD: Hydrogen bond donor, HBA: Hydrogen bond accepter, CF: Chemical formula. BE: Binding Energy. GB: Gradient Boosting.

No.	Compounds	M.W.	HBD	HBA	HB	CF	BE kcal/mol	GB	Log p
1.	Salvinorin	432.469 g/mol	0	8	5	C23H28O8	-16.82	-53.17	2.21
2.	Picrocrocin	330.377 g/mol	4	7	4	C16H26O7	-11.54	-34.54	-0.5
3.	Kaempferol	286.239 g/mol	3	6	1	C15H10O6	-12.30	-43.78	2.0
4.	Brocalax	361.397 g/mol	0	5	7	C22H19NO4	-17.10	-57.28	3.37
5.	Anthracin	333.343 g/mol	0	4	4	C20H15NO4	-12.60	-53.53	3.1
6.	Diamicron	323.411 g/mol	2	4	3	C15H21N3O3S	-13.21	-49.16	1.5
7.	Shikonin	288.299 g/mol	3	5	3	C16H16O5	-15.30	-49.04	3.56
8.	Curcumin	368.385 g/mol	2	6	8	C21H20O6	-15.55	-47.82	3.29

applied by using the following parameters. A population of 150 randomly placed individuals were selected with a maximum number of 27,000 generations, a crossover rate of 0.80, mutation rate of 0.02, and number of energy evaluations was 2.5 x 10^6. Based on their R.M.S.D from one another (using the default threshold (2.0 Å R.M.S.D), the program automatically grouped potential receptor-ligand complex conformations into clusters. We selected the best docked ligand-protein complex and using DS visualizer, the interactions (hydrophobic and electrostatic) were measured.

Results

Screening of Natural Anticancer Compounds

Initially we identified and screened out 20 plants (*Nigella sativa, Viscum album, Ocimum tenuiflorum, Azadirachta indica, Morinda citrifolia, Glycyrrhiza glabra, Curcuma longa, Aloe vera, Panax ginseng, Podophyllum hexandrum, Withania somnifera, Lantana camara, Paeonia emodi, Oldenlandia diffusa, Plumbago zeylanica, Tinospora cordifolia, Psoralea corylifolia, Allium sativum, Andrographis* and *Berberis vulgaris*) exhibiting several hundreds of anticancer compounds (Supplementary Table) found efficient against cancer with profound anticancer and immunostimulatory properties.

Next the candidate compounds were screened and retrieved from PubChem, Phytochemical /Ethnobotanical and ZINC Databases for the selected plants. Among 20 selected plants, 8 small molecules (Table 1) were found common to all. The set of compounds were subjected to structure refinement, ADMET and Lipinski rule of five.

Analysis of Docking Interaction

After docking experiments, the best docked complexes were selected based on their minimum binding free energy and binding orientation within the binding pocket of CTLA-4. Information about binding pocket of CTLA-4 was gathered through literature as well as bound complexes of CTLA-4.

Docking analysis revealed high binding affinity of selected compounds within the binding pocket of CTLA-4 with number of hydrogen bonds, vander waals and electrostatic interactions (Table 1). Salvinorin showed four hydrogen bonds with CTLA-4 at energy -16.82kcal/mol, the length of hydrogen bonds was 4Å, 4.8Å, 5Å and 5.4Å, respectively. The Picrocrocin with CTLA-4 showed three hydrogen bonds with binding energy of -11.54 kcal/mol, whereas the lengths of hydrogen bonds were 5.2Å, 3.8Å and 4.4Å. Three hydrogen bonds with distance of 4.6Å, 3.7Å and 3.3Å were detected involved in the formation of Kaempferol-CTLA-4 complex at energy -12.30kcal/mol. Both Brocalax and Shikonin were interacted with CTLA-4 by two hydrogen bonds with bonds distance 4.5Å, 4.8Å and 4.5Å, 3.3Å respectively and with energy score -17.10kcal/mol and -15.30kcal/mol respectively. On the other side, both Anthracin and Diamicron interacted with CTLA-4 by one hydrogen bond with the bond distance 4.2Å and 6.3Å with energy -12.60kcal/mol and -13.21kcal/mol, respectively. Curcumin formed the complex with CTLA-4 by six hydrogen bonds with the bond distance of 2.4Å, 2.8Å, 3.9Å, 3.9Å, 4Å and 6Å with energy -15.55kcal/mol. Apart from strong hydrogen bonding other non-covalent interactions including ionic interaction, van der waals forces and hydrophobic interactions played important role in CTLA-4-ligands interactions as showed in Fig. 2.

The most critical residues of CTLA-4 for interaction with small molecule inhibitor are; VAL:34, ARG:40, THR:44, GLN:45, THR:47, THR: 53, TYR:54 ASN: 58, GLU: 59, THR:61 SER:72, GLY:74, ASN:75, GLY:83, ARG: 85, ASP:88, LYS:95 and GLU:98, while ARG 40, THR 47 and TYR 54 showed strong hydrogen bonding interaction and rest of the binding pocket residues were involved in electrostatic interaction to place candidate inhibitors inside the active site of CTLA-4. Our molecular docking results clearly demonstrate strong binding affinities of shortlisted putative anticancer compounds to CTLA-4 with high scoring functions.

The analyses of binding profile and molecular interactions of the current study strongly supported those natural phytochemicals are suitable drug compounds against human CTLA-4 in cancerous cells to inactivate the downward pathway. We concluded that these phytochemicals are best choice towards cancer therapeutics. (Figure 1).

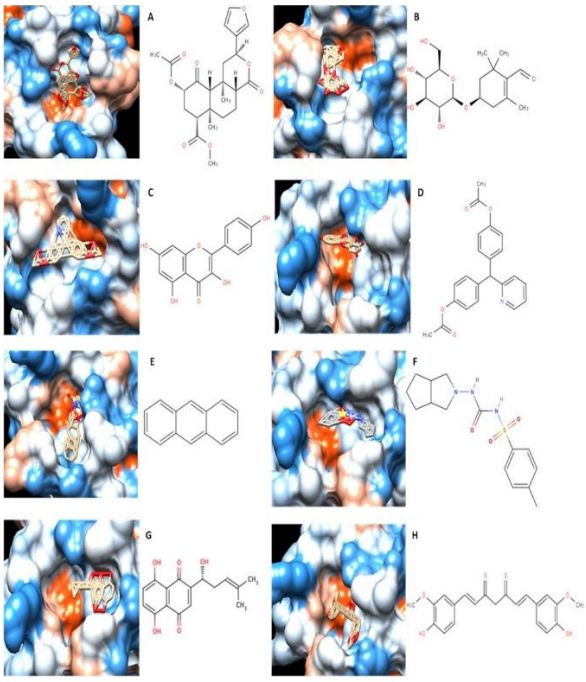

Fig. 1. A-H. In pharmacophore model, surface and sticks are representing binding pockets and compounds respectively. A) Salvinorin, B) Picrocrocin, C) Kaempferol D) Brocalax, E) Anthracin, F) Diamicron, G) Shikonin and H) Curcumin showed buried effects in the binding pocket of CTLA-4. Orange and blue surface of binding pockets indicate the hydrophobic interaction with all these compounds.

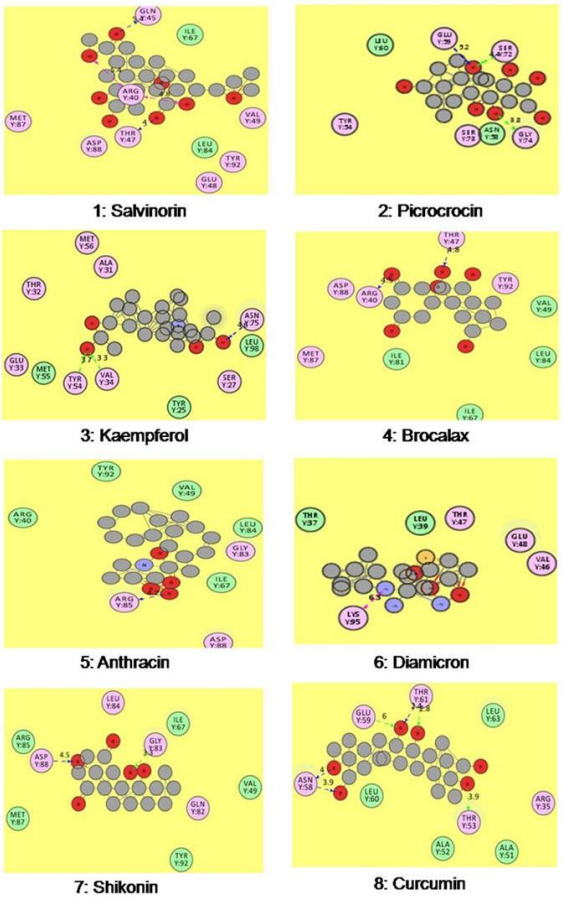

Fig. 2: Binding characterization of selected hits within the binding pockets of CTLA-4. 1) Shikonin, 2) Picrocrocin, 3) Kaempferol, 4) Brocalax, 5) Anthracin, 6) Diamicron, 7) Salvinorin, 8) Curcumin. Ligand is shown in grey ball and stick representation. Interacting residues with their type of interactions are shown in their respective color e.g Hydrogen bonding (pink), Vander Waals, π-alkyl and π-sigma interactions (cyan).

Discussion

T cells are key player in the anticancer

adaptive immunity (3), surprisingly on the onset of cancer, the checkpoint inhibitor proteins mostly stop the activity of T cells by cytotoxic T-lymphocyte associated protein-4 (CTLA-4) (4). CTLA-4 expresses entirely on the surface of T helper cells and provides costimulatory and coinhibitory effect on the function, activation and proliferation of T cell. This distinctive feature makes CTLA-4 a discriminating target for the development of cancer-specific small molecule drugs.

Since ancient times natural plant products have been empirically used and their uses are increasing gradually due to their potency. Novel potential molecules become a demand for pharmaceutical industry because the drug candidates could not be synthesized due to lack of model of a natural template (15). In the process of drug designing, the correct structural information in proper manner is most important to investigate the interactions between drug and protein target and to study the mode of action of drugs. Through investigation of structural constraint of CTLA-4 with putative anticancer compounds; Curcumin, Anthracin, Shikonin, Salvinorin, Diamicron, Brocalax, Picrocrocin and Kaempferol (CASSDBPK) demonstrated that all inhibitors were docked within the binding pocket with the involvement of number of forces put forwarded by critical binding residues. Overall, strikingly dominant, and consistent behavior of VAL:34, ARG:40, THR:44, GLN:45, THR:47, TYR:54 ASN: 58, SER:72, GLY:74, GLY:83, ARG: 85, LYS:95 and GLU:98 were observed throughout the docking experiments. In the development of drug process evaluation of ADMET profiles of lead compounds becomes a major challenge (16) because due to poor toxicity and pharmacokinetic properties, most of the drug compounds failed in the development of drug process. In the process of drug compounds selection of ADMET properties is the earliest phase in the discovery of affective lead compounds (17). Optimization in drug discovery is one of the key parameters and it is achieved by the knowledge about the diffusion of drugs through Blood Brain Barrier (BBB) (18). To cross the BBB, permeability of drug candidates is based on several factors such as molecular size, HB, lipophilicity, dissolved potential and pka values (19), however CASSDBPK physiologically, physiochemically and certain biopharmaceutical obeyed the rule of ADMET properties, hence these factors

markedly influenced the bioavailability of oral medicine (20).

In view of dynamic behavior of selected inhibitor with crucial swing of interaction determining key residues of binding pocket, we are confident that CTLA-4 is a credible drug target in cancer prevention and further investigations may open a new therapeutic perspective.

Conclusion

The current investigations signify the identification of natural compounds with profound anticancer activity against CTLA-4. The eight candidate drug compounds Curcumin, Anthracin, Shikonin, Salvinorin, Diamicron, Brocalax, Picrocrocin and Kaempferol (CASSDBPK) have shown high anticancer activities and proved them as more effective for cancer targeted therapy. Further experimental validation of these compounds to address the worthiness of our data and clinical trials are required to see their potential and efficacy in controlling various cancers.

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