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Ligand Based Virtual Screening to Identify Potential Anti Cancer Ligands Similar to Withaferin A Targeting Indoleamine 2,3-Dioxygenase

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For the treatment of cancer, Indoleamine 2,3-dioxygenase (IDO) is emerging as an important new therapeutic drug target characterized by pathological immune suppression. On the other hand, Withaferin A (WA) – active constituent of Withania somnifera ayurvedic herb has shown to be having a wide range of targeted anti cancer properties. Previously, we have elucidated the potential of WA in attenuating the Indoleamine 2,3-dioxygenase for immunotherapeutic tumor arresting activity using computational approaches. In this present study, a Ligand based virtual screening with a threshold of >50% similarity was performed, based on the structure of Withaferin A using ZINC database, to perform a structure based virtual screening on IDO, targeting key residues involved in its functionality. 33 compounds were identified as promising IDO inhibitors which are similar to the structure of WA based on free binding energy and ADME constraints, compared to Withaferin A.

Key words: Indoleamine 2,3-dioxygenase; Withaferin A; docking; free binding energy; Ligand based virtual screening; anti cancer immuno therapy.

Cancer is a genetic disease. For the treatment of Genetic cancers, the methodology of Target specific inhibition of individual proteins is involved in the cell environment[1]. Recently, for the treatment of cancer, Indoleamine 2,3-dioxygenase (IDO) (EC 1.13.11.17) is emerging as an important new therapeutic drug target characterized by pathological immune suppression. The tryptophan degradation is been boosted by rate-limiting enzyme IDO through kynurenine pathway leading to the halted growth of T cells which is diminishing the immunity[2-3]. The IDO presence on dentritic cells in tumor draining lymph nodes is gaining its importance as a promising novel anti cancer immuno therapy drug target as it is able to suppress the Tumor cells and activate T cells[4].

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Structural features of IDO

IDO is an oxygenase enzyme which catalyzes the oxygen (O2) containing molecule into its substrate and involved in the metabolism and synthesis of variety of biological substances. It is containing large and small distinct domains. The large domain is made up of two 3_{10} helices and 13 alpha-helices where four major helices run parallel to Heme plane and most of the helices are involved in hydrophobic interactions. And there is a Heme-binding pocket located at the above four major helices. Small domain is made up of 2 short beta-sheets, 6 alpha helices, and three 3_{10} helices. The widened substrate entrance site can be

observed. A long loop (residues 250– 267) connects the large domain with the small one above the distal side of the Heme cover the top of the Heme pocket. A flexible loop outside the Heme pocket at residues 360–380 was presumed in the structural coordinates[3].

Withaferin A (WA) – active constituent of Withania somnifera ayurvedic herb has shown to be having a wide range of targeted anti cancer properties[5-8]. Previously, we have elucidated the potential of WA in attenuating the Indoleamine 2,3-dioxygenase for immunotherapeutic tumor arresting activity using computational approaches [9]. In this present study, a Ligand based virtual screening with a threshold of >50% similarity was performed, based on the structure of Withaferin A using ZINC database, to perform a structure based virtual screening on IDO, targeting key residues involved in its functionality in order to identify the potential Ligand compounds with IDO target specific anti cancer activity with structural similarity with WA.

METHODS

Software and program

Schrodinger's maestro version 9.5 [10] is utilized to visualize the receptors, Ligand structures, hydrogen bonding network and to render images. The ZINC database[11] is a chemical compound database of commercially-available compounds, which contains over 21 million purchasable compounds in ready-to-dock, 3D formats, was used throughout this study to screen for potential inhibitors, based on structural similarities with the known IDO inhibitor Withaferin A, along with the molecular descriptor values. Arguslab 4.0.1 [12] was the primary docking program used in this work for the structure based virtual screening. All the docking runs were performed in Pentium(R) Dual-Core CPU @ 3.00 GHz, with 2 GB DDR RAM under Windows 7 operating system.

Preparation of IDO structures

The three-dimensional structures of IDO [PDB ID: 2DOT] were retrieved from the Protein Data Bank[13]. These structures were prepared by removing all bound crystal water molecules, ligands and hydrogen bonds were added. So obtained structures were saved in pdb files for further studies.

Ligand-based virtual screening

Virtual screening[14] based on the structure of IDO inhibitor Withaferin A was carried out using the ZINC database. Two-dimensional structure of Withaferin A was used to search for similar compounds in the ZINC database, from the most common chemical suppliers (ChemBridge, ChemDiv, Ryan, Asinnex, MayBridge, Sigma-Aldrich, Comgenex, Otava and Specs), covering over 21 million chemical compounds. A total of 33 compounds with a threshold limit of 50% similarity, were identified to be structurally similar with Withaferin A. These 33 compounds were used in further molecular docking analysis.

Structure-based virtual screening

Arguslab 4.0.1 [11] was utilized to search for potential inhibitors amongst the 33 compounds found through Ligand based virtual screening, targeting IDO active sites. Docking between receptor and ligands database in .sdf format was performed using "Dock a Database" option of Arguslab 4.0.1 software. A spacing of 0.4 Å between the grid points was used. Lamarckian Genetic Algorithm (LGA) [15] was selected as docking engine with the default parameters. "Dock" was chosen as the calculation type, "Flexible" for the Ligand, and "A Score" was used as the scoring function.

RESULTS AND DISCUSSION

In this work, a Ligand based virtual screening approach was applied to virtually screen for potential inhibitors targeting IDO. As it is established that structurally similar compounds have same pharmacological features, we have performed a Ligand based virtual screening, based on the structure of Withaferin A using ZINC database, to identify its structurally similar compounds. 33 compounds thus found with the threshold of >50% similarity were used to perform structure based virtual screening on IDO, separately targeting the active residues involved in their binding activity using Arguslab 4.0.

Arguslab 4.0 was run to rank these 33 compounds based on their free binding energies, to screen for the molecules that potentially binds IDO proteins. Binding affinity calculations for all 33 compounds were carried out using Genetic algorithm implemented in Arguslab 4.0. Higher

Table 1. The Docking Simulation results of 33 ligands with IDO

S. No.	Ligand Id	Binding energy (Kcal/mol)	H-Bond forming residues	Hydrophobic interactions
1	8234189 (Withaferin A)	-9.01	SER167,LYS377, HEME	PHE163, ALA264, CYS129,PHE164, TYR126, VAL130,LEU230, LEU234, PHE226, ILE354, LEU384
2	71477799	-8.681	HEM404	ALA260, ALA264, TYR126,PHE163, VAL130, PHE226,LEU234, ILE234, ILE354,LEU384
3	165541	-7.484	HEM404	PHE226, ILE354, LEU384,PRO241, LEU234, PHE163,TYR126, PHE164, ALA264
4	16745397	-6.971	HEM404	PRO241, ILE232, LEU234,CYS129, PHE163, TYR126,PHE164, VAL130, ALA264,PHE226, ILE354, LEU384
5	11070744	-6.888	HEM404,LYS377	PRO241, ILE232, LEU234,CYS129, PHE163, TYR126,PHE164, VAL130, ALA264,PHE226, ILE354, LEU384
6	21574483	-6.7	HEM404	LEU834, PRO241, LEU230,LEU234, ALA264, PHE163,TYR126, VAL 130, PHE164,CYS129, PHE226, VAL350, ILE354
7	5315320	-6.562	HEM404	PRO241, ILE232, LEU234,CYS129, PHE163, TYR126,PHE164, VAL130, ALA264,PHE226, ILE354, LEU384
8	57519534	-6.296	HEM404	PRO241, LEU384, ALA264,CYS129, PHE163, TYR126,PHE164, VAL130, LEU234,LEU230, PHE226, VAL350, ILE354
9	10344751	-5.931	HEM404	PRO241, LEU234, ILE232,CYS129, PHE163, TYR126,PHE164, VAL130, ALA264,PHE226, ILE354, LEU384
10	23267120	-5.849	GLY262,LYS238, HEM404	ALA260,PRO241,PHE164,VAL130,ALA264,TYR126,PHE163, PHE226, ILE354, LEU234
11	10321754	-5.5	HEM404	PRO241, ILE232, PHE164,LEU234, CYS129, PHE163,TYR126, VAL130, ALA264,PHE226, ILE354, LEU384
12	10648050	-5.369	HEM404	PRO241, ILE232, PHE163,CYS129, VAL130, TYR126,PHE164, ALA264, PHE226,ILE354, LEU384
13	23266158	-5.339	HEM404	LEU234, ALA260, PHE291,PHE226, ILE354, TYR126,PHE163, VAL130, PHE164,ALA264
14	58443787	-5.299	ARG231,LYS377	PHE163, VAL130, ALA264,TYR126, CYS129, LEU234,PHE164, PHE291, PRO241,TRP92, ILE232, PHE226, LEU384, ILE354
15	5315323	-5.037	HEM404,LYS377,	LEU384, LEU234, PHE164,CYS129, VAL130, TYR126,PHE163, ILE354, ALA264,PHE226
16	60148725	-5.021	GLY236,GLY239, HEM404	PHE291, ALA260, VAL130,PHE163, TYR126, PHE164,ALA264, PHE226, LEU234,ILE354, LEU384, PRO241
17	23253886	-4.991	HEM404,LYS377	ALA264, VAL130, PHE164,TYR126, PHE163, LEU234,PHE226, ILE354, LEU384
18	21679027	-4.989	HEM404	LEU234, ALA264, PHE163,TYR126, PHE164, VAL130,PHE226, ILE354 , LEU384
19	70690364	-4.967	HEM404,LYS377	PHE163, VAL130, ALA264,TYR126, PHE164, LEU234,PHE226, ILE354, LEU384
20	5287384	-4.882	HEM404	PRO241, ILE232, LEU234,CYS129, PHE164,

				TYR126,VAL163, PHE163, ALA264,PHE226, ILE354, LEU384
21	10413210	-4.869	HEM404	PRO241, leu234, PHE164,TYR126, PHE163, ALA264,ILE354, LEU384
22	16680446	-4.673	HEM404,ARG231, LYS238	LEU234, PHE163, VAL130,PHE164, TYR126, ALA264,ILE354, PHE226
23	56649344	-4.546	HEM404,LYS377	PRO241, ILE232, PHE226,LEU230, LEU234, ALA264,CYS129, PHE164, VAL130,TYR126, PHE163, LEU384,ILE354
24	71167047	-4.543	HEM404,LYS377	LEU384, PRO241, ILE232,LEU230, LEU234, PHE291,CYS129, PHE164, TYR126,VAL130, ALA234, PHE163, PHE226, ILE354, PHE227
25	21574482	-4.519	HEM404,LYS377	PHE163, ALA264, CYS129,PHE164, TYR126, VAL130,ILE232, LEU234, LEU230,PHE226, VAL350, ILE354, LEU384
26	71477945	-4.232	HEM404,ALA264	ALA264, PHE214, PHE164,VAL130, PHE163, TYR126,LEU234, PHE226, CYS129,LEU230, ILE232, ILE354, LEU384
27	12444955	-4.056	HEM404	LEU384, ALA260, PHE291,MET295, ILE232, LEU234,LEU230, PHE226, PHE160,CYS129, PHE164, VAL130, ALA364, TYR126, ILE354
28	66575620	-3.8	HEM404,ALA264	PHE291, ILE232, PHE163,VAL130, TYR126, PHE164,CYS129, PHE226, LEU234,LEU230, VAL350, ALA264, ILE354, LEU384, PHE227
29	10051187	-3.62	SER235	PRO241, ALA260, LEU384,LEU230, PHE226, ILE354,PHE227, PHE164, LEU234,PHE163, ALA254, VAL130,CYS129, TYR126, ILE232
30	10413139	-3.292	HEM404	ALA260, LEU234, PHE164,TYR126, PHE163, LEU230,ALA264, LEU384, ILE354,PHE226, PHE387, PHE291
31	10457436	-3.056	LYS377	LEU384, PHE291, PHE163,ALA264, ILE232, PHE226,ILE354, LEU230, LEU234,PRO241
32	10161347	-3.046	LYS377	PRO241, LEU234, VAL130,PHE164, CYS129, TYR126,PHE163, ALA264, PHE226,ILE354, LEU354, LEU384
33	49864537	-2.776	HEM404	ILE354, LEU384, PRO241,ILE232, LEU234, PHE291,PHE226, LEU230, PHE163,PHE164, CYS129, VAL130,ALA264, TYR126,
34	58443792	-1.849	GLY262	PHE291, PHE287, LEU230,LEU234, PHE163, ALA264,PHE226, ILE354, LEU384

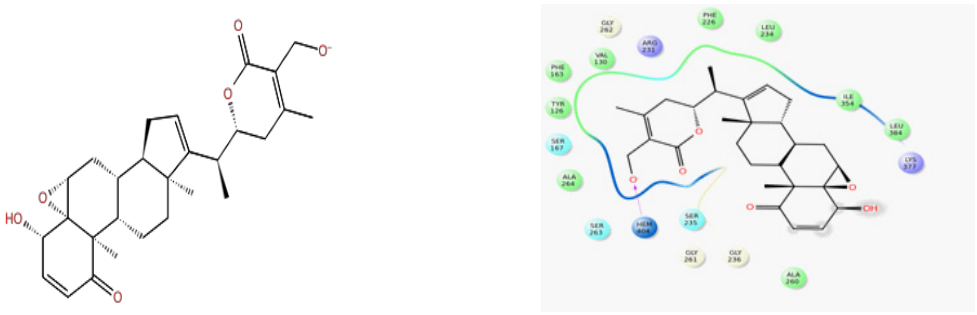
binding affinity compared to Withaferin A with good pharmacological properties is the basis for selecting these 33 compounds. Only these 33 compounds results will be discussed in further for IDO complex, in detail.

Structure based virtual screening

One of the possible modes of action we hypothesized for these compounds is attenuating the IDO activity, thus inhibiting the tumor growth in subsequent steps. In order to explore the possibility of these selected compounds to attenuate this IDO, we carried out structure based virtual screening for IDO, targeting the key residues involved in its activity.

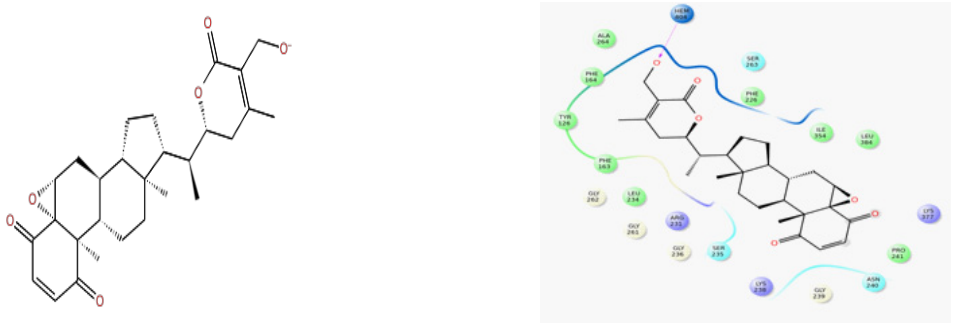
Inhibitors bound to IDO active site

Among 33 compounds obtained from the ZINC database were virtually screened onto the IDO targeting its active residues. All the compounds analyzed were successfully docked with a binding energy range of -8.6 to -1.8 Kcal/mol, whereas compounds, ZINC71477799 and ZINC58443792, showed the highest and least binding affinity respectively. A total of 33 compounds with near to binding affinities for IDO that of Withaferin A



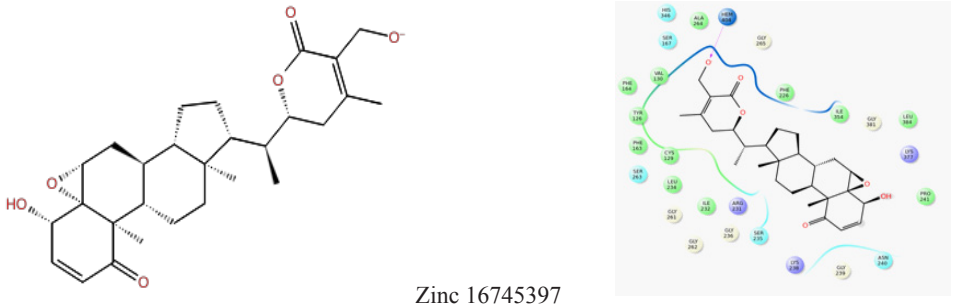
Zinc 71477799

Fig. 1(a). Ligand structures of the Best Binders for IDO



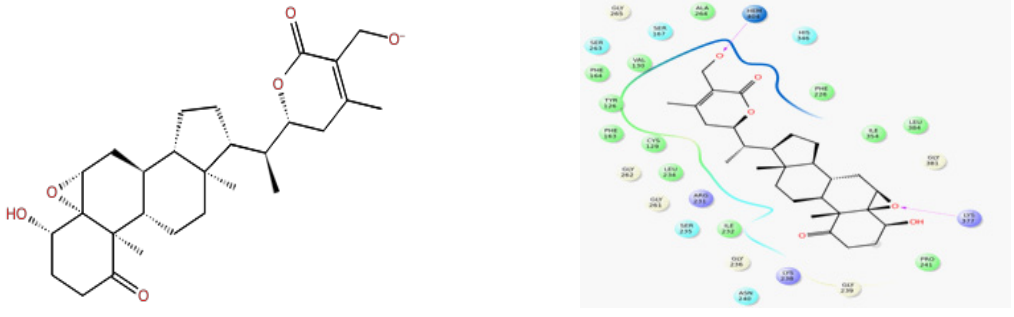
Zinc 165541

Fig. 1(b). Ligand structures of the Best Binders for IDO



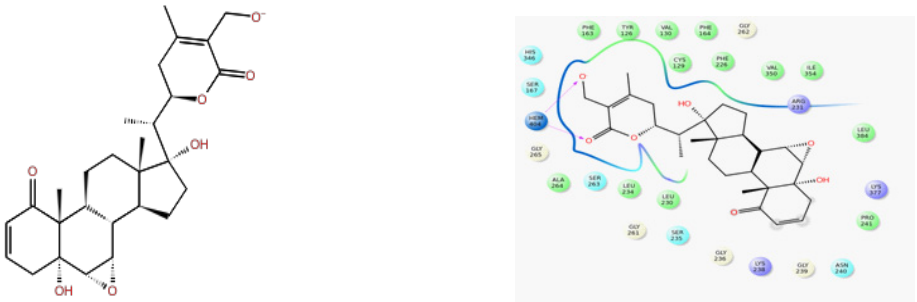
Zinc 16745397

Fig. 1(c). Ligand structures of the Best Binders for IDO



Zinc 11070744

Fig. 1(d) Ligand structures of the Best Binders for IDO



Zinc 21574483

Fig. 1(e). Ligand structures of the Best Binders for IDO

were tabulated (Table 1). Top three compounds with the strongest affinity for the IDO binding site were ZINC 71477799, ZINC 165541 and ZINC 16745397, which showed binding energy of -8.681, -7.484 and -6.971 Kcal/mol, respectively. The best binder was compound ZINC 71477799 (-8.681 Kcal/mol). This compound (Fig 1a) formed one hydrogen bond with Heme active site and several hydrophobic interactions with residues inside the IDO binding site. Several hydrophobic interactions formed between compound ZINC 71477799 and residues PHE163; ALA264; CYS129; PHE164; TYR126; VAL130; LEU230; LEU234; PHE226; ILE354 and LEU384. Top five best binders with their structure and interactions were represented in Fig 1a, Fig 1b, Fig 1c, Fig 1d, Fig 1e.

CONCLUSION

In the present study several WA structurally similar compounds were identified near to binding affinity of WA. The interaction revealed in the present is of high importance in designing novel IDO target specific drug compounds. Compound ZINC71477799 was identified as the promising structurally similar compound to WA of worth considering for further investigation towards its activity to act as immunotherapeutic anti cancer compound targeting IDO in specific.

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