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Research **IJMBR**

In-silico Molecular Docking of Indigofera Tinctoria Phytocompounds to Target EGFR/ERK and FGFR/FGF Pathway Proteins Involved in Prostate Cancer

Nidhi Premanand Honavar*

Abstract

Objective: *Prostate cancer being the second major cause of cancer related deaths in males, affects the small walnut shaped gland below the bladder called Prostate gland. The epidermal growth factor protein (EGFR), cascade protein (ERK) and the Fibroblast Growth Factor Proteins (FGFR and FGF) are involved in the different signalling pathways which leads to the formation of different products, that are responsible for the progression of the cancer and thus can be considered as the therapeutic targets. Indigofera tinctoria, a medicinal plant with proven anti-cancerous properties, was chosen for this study to investigate its different compound's therapeutic effect against the target proteins, which are responsible for the Prostate cancer.* ***Methods:*** *In this study, 20 different compounds were selected from the Indigofera tinctoria plant as ligands to check for their binding affinity against the Target proteins (EGFR, ERK, FGFR and FGF). The docking of the ligands with the target proteins were done by using PyRx Virtual tool. The computational investigation of all the ligands and the target proteins, such as its molecular structure, phytochemistry, their therapeutic actions and other data was carried out. The protein structure validation and the pharmacological evaluation of the ligands were done using the BIOVIA Discovery studio, PDB sum generate and ADMET lab 2.0 respectively.* ***Result:*** *The results of this study showed that the compounds, Pseudosemiglabrin, [(12S,15R,16R)-14,14-dimethyl-6-oxo-4-phenyl-3,11,13-trioxatetracyclo [8.6.0.02,7.012,16] hexadeca-1(10),2(7),4,8-tetraen-15-yl] acetate, Deguelin, Sumatrol and Rotenone showed good binding affinity with the target proteins and can be considered as a potential drug for the prostate cancer.* ***Conclusion:*** *The compounds selected were found to be active against the target proteins for the prostate cancer and thus can be utilised for the cancer suppression. The further invitro studies need to be done to support this study.*

Keywords: Prostate cancer, Molecular docking, *Indigofera tinctoria*, Epidermal Growth Factor Receptors, Fibroblast Growth Factor Receptors, extracellular-regulated kinase, ADMET lab 2.0,

INTRODUCTION

Prostate cancer, the second most common type of cancer found relevantly in males. It is the type of cancer which affects the small, soft, walnut shaped gland found below the bladder in men, which helps in secreting the seminal fluid and also in male reproduction. It also secretes few enzymes, lipids, amines and metal ions which are very much essential for the normal growth and function of spermatozoa. According to GLOBOCAN (Global Cancer Observatory), it was estimated that in 2018, 1,276,106 new cases of prostate cancer and about 358,989 deaths were reported worldwide with higher prevalence in the developed countries [1]. The chances of occurrence of prostate cancer increases after the age of 50. It also depends on the family history.

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EGFR (Epidermal Growth Factor Receptor) is an intercellular membrane protein belongs to a family of Receptor Tyrosine Kinases (RTKs) [2]. Previous researches have proved that the EGRF proteins are involved in the development of different types of cancers and also pathogenesis, thus is the most prominent target for the anti-cancer drugs [3, 4]. These trans-membrane proteins are activated by the peptide growth factors. Upon activation, EGFR proteins gets phosphorylated and leads to the network of signal transduction pathways with the help of other transmembrane receptor proteins [5, 6]. The activated EGFR protein further activates mitogen-activated protein kinase pathway (MAPK or MEK) and extracellular regulated kinase (ERK) pathway in prostate cancer.

FGF (Fibroblast Growth Factor), and the receptor FGFR (Fibroblast Growth Factor Receptor) has many different functions, such as embryonic development, differentiation, proliferation, survival, migration, and angiogenesis. The typical regulation of the FGF/FGFR system occurs in multiple human tumours, leading to the deregulated activation of ligand-dependent or -independent FGFR signalling [4]. FGFRs are widely expressed in developing and adult tissues and have various biological activities both *in vivo* and *in vitro*, including roles in angiogenesis, mitogenesis, cellular differentiation, cell migration and tissue-injury repair [7]. The FGF and FGFR proteins interact with each other on the cell surface with the mediations of Heparin Sulphate Proteoglycans (HSPGs) which is available on the cell surface, and forms the ternary complex HSPGD/FGF/FGFR, necessary for triggering the signal transduction pathways [5].

Indigofera tinctoria is a branching shrub, which belongs to Fabaceae family, widely distributed in tropical regions[8]. The Phytochemical constituents of *Indigofera tinctoria* are mainly responsible for its wide therapeutic actions such as in liver disease, heart palpitations, gout and also has laxative properties. It is used in traditional medicines, Ayurveda, Siddha and Unani Studies conducted on *Indigofera tinctoria* showed that it possesses cytotoxic effects, Anti-bacterial, Anti-oxidant, Anti-inflammatory, Anti hepatoprotective and Antidiabetic activity [9]. Some of the compounds found in the plant, such as Deguelin, pseudosemiglabrin, indirubin, tephrosin and kaempferol are proved to be having anti-cancerous properties and also help in curing various diseases [10–15].

METHODOLOGY

Retrieval of the Ligands

The ligands from the plant *Indigofera tinctoria* were selected for the study. For the retrieval of the ligands, the IMPPAT website was used (https://cb.imsc.res.in/imppat/home). The total of 20 ligands were selected. The PubChem ID and the canonical SMILES were noted from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The ligands were downloaded in 2D SDF format [16].

Retrieval of the Protein

The target proteins (4zzm, 5b7v, 2p23, 1m17) were searched in RCSB PDB website (https://www.rcsb.org/). The proteins were downloaded in pdb format. These proteins were added with their missing residues using Discovery BIOVIA studio [6].

Purification of the Protein

The proteins were purified by removing the water molecules, Het atoms, ligand groups and additional chains apart from the A chain. For the proper binding of the protein with the ligand without the free energy disturbances from the water molecules. The protein purification was done using Discovery BIOVIA [17]. The purified proteins were subjected to PDB Sum generate(https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) where the secondary structure and the Ramachandran Plot of the proteins were obtained.

Molecular Docking and Visualization

The molecular docking of the ligands with the proteins were done one by one using PyRx software. These proteins were added as the macromolecules with the compounds from plants as ligands. The

energy minimization step was done, and the selected ligands were converted in to .pdbqt format [18]. These selected ligands were docked with all the selected proteins. The interactions of the ligands with the proteins were interpreted by taking their Binding energy scores into consideration. The binding energy scored corresponding to 0 RMSD (Root Mean Square Deviation) were taken into consideration as the best docking conformation. The top 6 conformations with least binding conformations from each protein were taken into consideration as the best complex for the target protein [19]. The .pdb files of the top 6 docked ligand structures were downloaded and visualised in BIOVIA Discovery studio.

The selected top ligands of the respective proteins in their respective conformations were downloaded in the .pdb format from the PyRx and visualised in the BIOVIA Discovery. The ligands were pasted in the purified protein structures. The 3D structure, 2D structure and the non-bond interactions were documented.

Pharmacological Studies

The pharmacological analysis of the ligands was done using ADMET lab 2.0 web server. It is an integrated online webtool for the prediction of the accurate Adsorption, Desorption, Metabolism, Elimination and Toxicity parameters of the compounds [20]. Physiochemical properties including molecular weight, lipophilicity, saturation etc. and the toxicity parameters were also tabulated and evaluated.

RESULTS

Selection of the Phytocompounds

The plant *Indigofera tinctoria* is known for its medicinal properties and the studies suggest that it has good anti-cancerous properties, effective against the colon cancer, lung cancer [17] and ovarian cancer. 20 ligands were selected from the IMPPAT website. The 2D structures of the ligands were downloaded from PubChem website in SDF format (Table 1). The structures were viewed using BIOVIA Discovery.

Table 1. Ligand name, PubChem ID and Canonical SMILES of the 20 selected compounds.

S.N.	Ligand	PubChem ID	Canonical SMILES
1	Indigo	10215	C1=CC=C2C(=C1)C(=C(N2)C3=NC4=CC=CC=C4C3=O)O
2	Indican	441564	C1=CC=C2C(=C1)C(=CN2)OC3C(C(C(C(O3)CO)O)O)O
3	Kaempferol	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
4	Indicine	73615	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1C(CC2)O)O
5	D-Galactose	6036	C(C1C(C(C(C(O1)O)O)O)O)O
6	Tephrosin	114909	CC1(C=CC2=C(O1)C=CC3=C2OC4COC5=CC(=C(C=C5C4(C3=O)O)OC)OC)C
7	Rotenone	6758	CC(=C)C1CC2=C(O1)C=CC3=C2OC4COC5=CC(=C(C=C5C4C3=O)OC)OC
8	Sumatrol	442824	CC(=C)C1CC2=C(O1)C=C(C3=C2OC4COC5=CC(=C(C=C5C4C3=O)OC)OC)O
9	Apigenin	5280443	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O
10	Luteolin	5280445	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O
11	indirubin	10177	C1=CC=C2C(=C1)C(=C(N2)O)C3=NC4=CC=CC=C4C3=O
12	dehydrodeguelin	3083803	CC1(C=CC2=C(O1)C=CC3=C2OC4=C(C3=O)C5=CC(=C(C=C5OC4)OC)OC)C
13	coumarin	323	C1=CC=C2C(=C1)C=CC(=O)O2
14	Deguelin	107935	CC1(C=CC2=C(O1)C=CC3=C2OC4COC5=CC(=C(C=C5

S.N.	Ligand	PubChem ID	Canonical SMILES
			C4C3=O)OC)OC)C
15	pseudosemiglabrin	156341	CC(=O)OC1C2C(OC3=C2C4=C(C=C3)C(=O)C=C(O4)C5=CC=CC=C5)OC1(C)C
16	1 H-indol-3-ol	50591	C1=CC=C2C(=C1)C(=CN2)O
17	[(12S,15R,16R)-14,14-dimethyl-6-oxo-4-phenyl-3,11,13-trioxatetracyclo[8.6.0.02,7.012,16]hexadeca-1(10),2(7),4,8-tetraen-15-yl] acetate	182678	CC(=O)OC1C2C(OC3=C2C4=C(C=C3)C(=O)C=C(O4)C5=CC=CC=C5)OC1(C)C
18	D-Mannose	18950	C(C1C(C(C(C(O1)O)O)O)O)O
19	Galactomannan	439336	C(C1C(C(C(C(O1)OCC2C(C(C(C(O2)O)O)O)O)OC3C(C(C(C(O3)CO)O)O)O)O)O)O)O
20	Rotenol	44257420	CC(=C)C1CC2=C(O1)C=CC(=C2O)C(=O)C3CCOC4=CC(=C(C=C34)OC)OC

Protein Structure Analysis

Ramachandran Plot and Ramachandran Plot Statistics

The Ramachandran plot of the proteins 1M17, 4ZZM, 5B7V and 2P23 were obtained with the help of 'PDB Sum Generate' website, as shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

1M17

The purified protein 1M17, when subjected to PDB sum generate, the results showed that 79.8% of the residues were in most favoured region, 18.1% of the residues in Additional favoured region, 0.3% in Generously favoured option. The 1.7% of the residues were found to be in the Disallowed regions (Figure 1).

4ZZM

Whereas, 86.4% of its residues of the protein 4ZZM fall under the most-favoured regions. The 8.3% of residues fall under Additional allowed regions. 3.0% in generously allowed regions And the remaining 2.3% of the residues in disallowed regions (Figure 2).

5B7V

Likewise, 5B7V protein had 87.3% of its residues in most favoured regions, 12.0% in Additional allowed regions and 0.6% in the Generously allowed regions (Figure 3).

2P23

However, the Ramachandran plot for the protein 2p23 showed that 92.2% of the residues fall under the most-favoured regions. The 6.9% of residues fall under Additional allowed regions. And the remaining 0.9% of the residues in Generously allowed regions (Figure 4).

These results suggests that the purified 3D models of the proteins are good quality models.

Secondary Structure

The PDB sum produced the secondary structures of the proteins namely 4zzm, 5b7v, 2p23, 1m17 respectively, as shown in Figure 1, Figure 2, Figure 3 and Figure 4 respectively.

1M17

The PDB findings for the secondary structure suggested that the protein has 4 sheets, 5 beta hairpins, 4 beta bulges, 11 strands, 15 helices, 11 helix-helix, 22 beta turns and 2 gamma turns. There are 324 residues in the structure

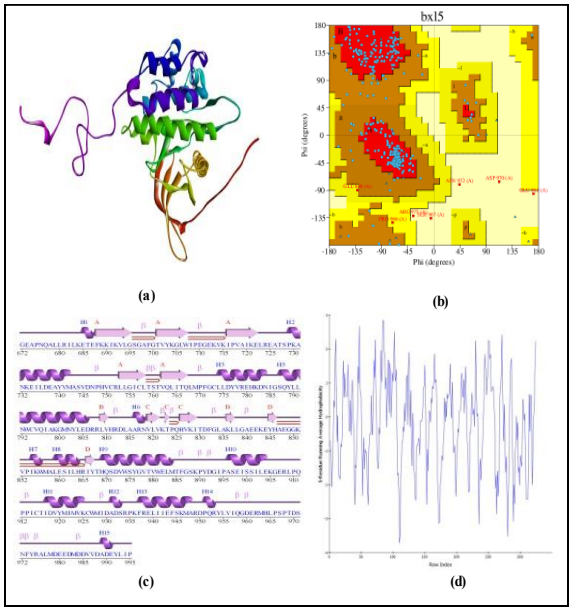

Figure 1: Structural analysis of 1M17 protein: (a) Refers to purified 1m17 protein structure. (b) Ramachandra plot, (c) Secondary structure of 4zzm protein and (d) Hydropathy plot.

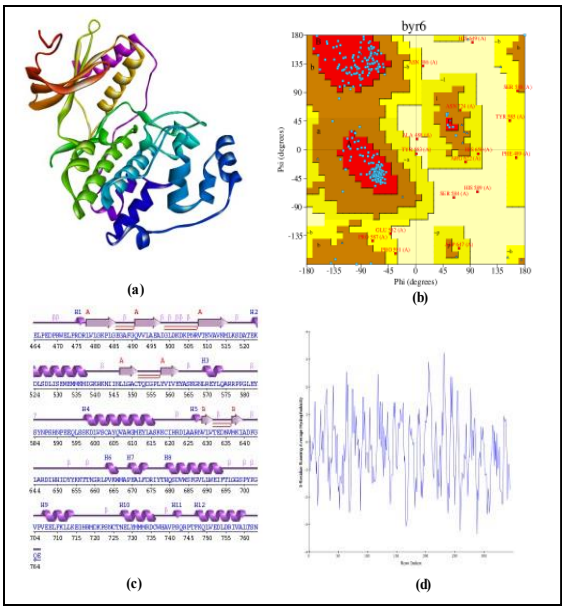

Figure 2: Structural analysis of 4ZZM protein: (a) Refers to purified 4zzm protein structure. (b) Ramachandra plot, (c) Secondary structure of 4zzm protein and (d) Hydropathy plot.

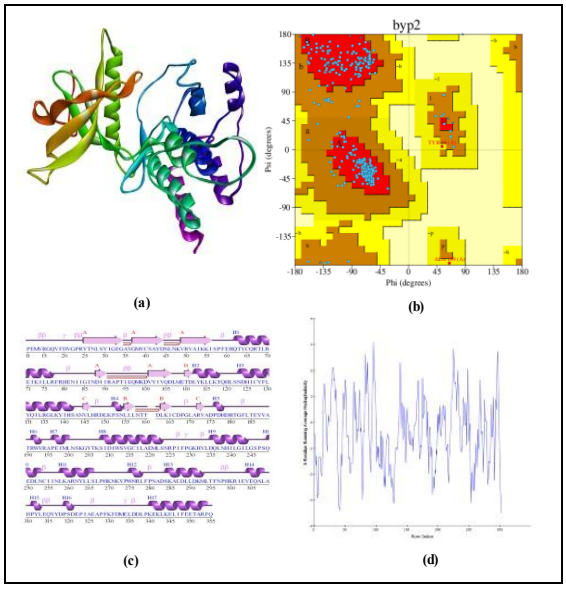

Figure 3: Structural analysis of 5B7V protein: (a) Refers to purified 5b7v protein structure. (b) Ramachandra plot, (c) Secondary structure of 4zzm protein and (d) Hydropathy plot.

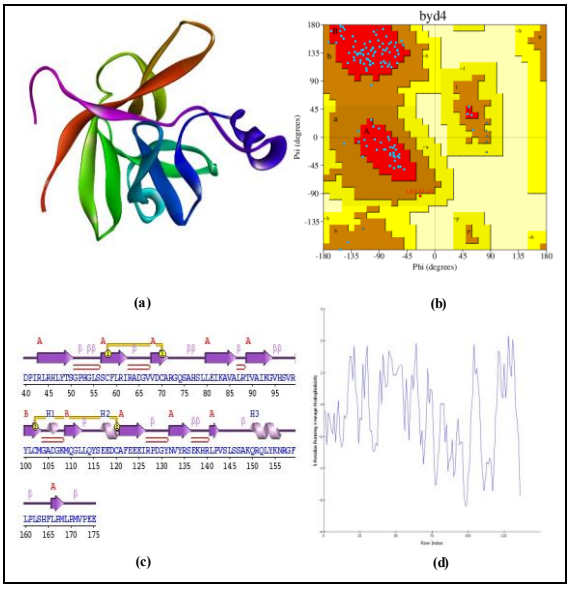

Figure 4: Structural analysis of 2P23 protein: (a) Refers to purified 2p23 protein structure. (b) Ramachandra plot, (c) Secondary structure of 4zzm protein and (d) Hydropathy plot.

4ZZM

In the case of4ZZM, the PDB sum result depicted are 2 sheets, 4 beta hairpins, 4 beta bulges, 7 strands, 12 helices, 15 helix-helix, 22 beta turns and 1 gamma turn. The protein contains 302 residues.

5B7V

However, for 5B7V, the PDB sum data showed 3 sheets, 4 beta hairpins, 5 beta bulges, 10 strands, 18 helices, 18 helix-helix, 24 beta turns, and 3 gamma turns. The structure consisted of 344 residues in total.

2P23

Finally, the PDB sum outputs of the 2P23 protein are 2 sheets, 6 beta hairpins, 5 beta bulges, 11 strands, 4 helices, 16 beta turns and 2 disulphides. The structure comprises of 136 residues in total.

Hydropathy Plots

The hydropathy plots of the proteins 1M17, 4ZZM, 5B7V AND 2P23 were investigated using the BIOVIA Discovery studio software, as shown in the Figure 1 Figure 2, Figure 3, Figure 4 respectively.

Molecular Docking and Visualization

The docking of the 20 ligands with the 4 protein targets were done using the PyRx Software. The ligands with the lowest binding energy with respect to zeroth value of the RMSD (Root Mean Square Deviation) were chosen as the best docking conformation of the protein targets.Once the docking of the ligand with the protein targets was done, the binding affinity and the RMSD/ub and RMSD/lb were documented. Among the 20 ligands screened, the top 6 ligands which had the lowest binding energy with the proteins namely 1m17 (Figure 5 and 6), 4zzm (Figure 7 and 8), 5b7v (Figure 9 and 10) and 2p23 (Figure 11 and 12) respectively were subjected to visualization using BIOVIA discovery Studio visualizer. The 3 dimensional and 2 dimensional models were acquired. Furthermore, the details of the non-bond interactions were also documented.

- Visualization of 1M17:
- Visualization of 4ZZM:
- Visualization of 5B7V:
- Visualization of 2P23:

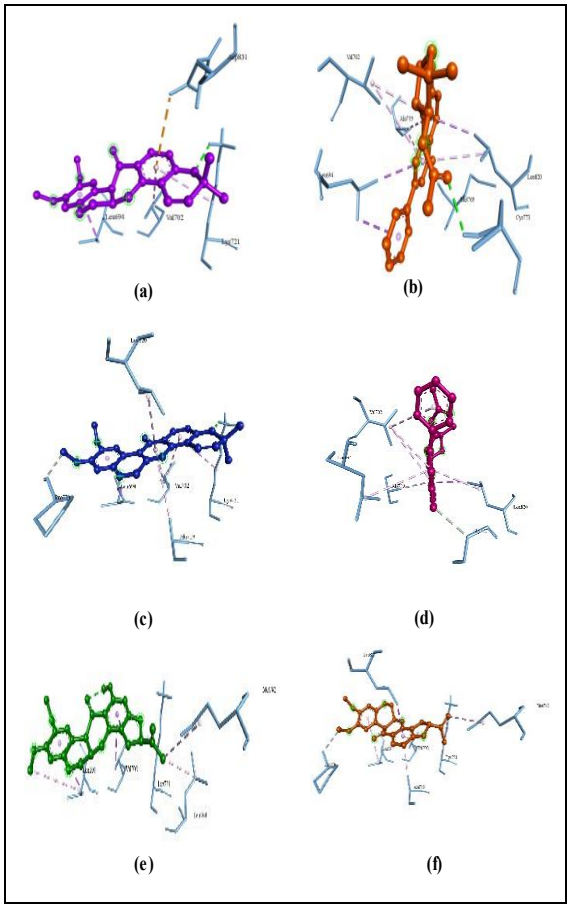

Figure 5: 3D interactions of top ligands interacting with 1M17 protein.

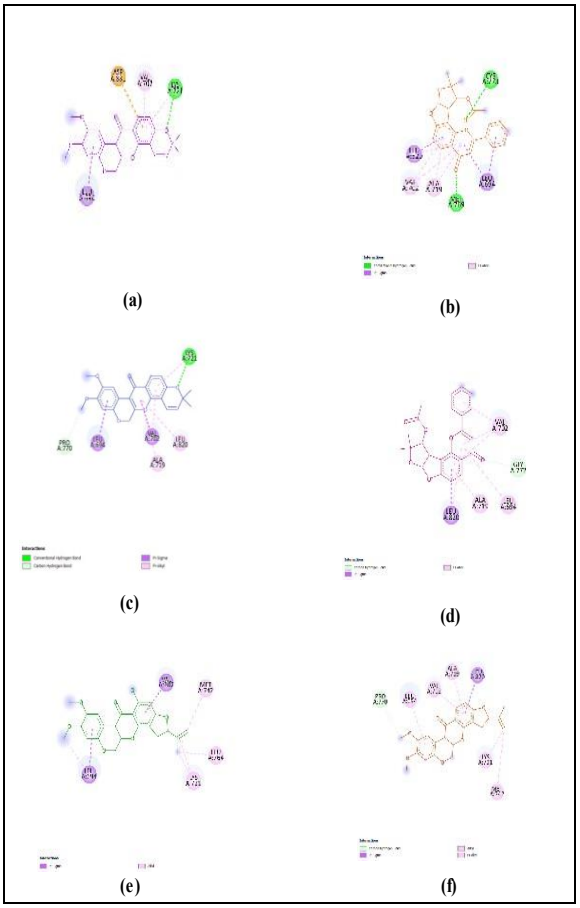

Figure 6: 2D interactions of top ligands interacting with 1M17 protein.

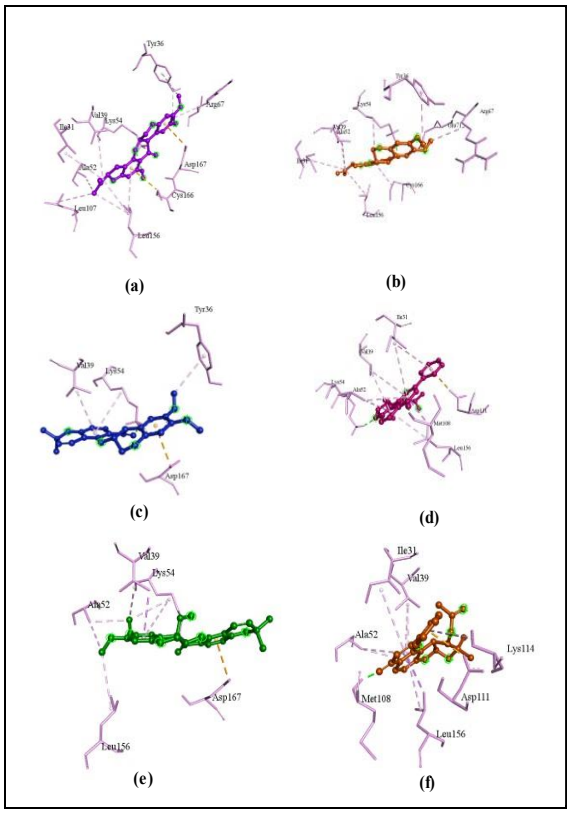

Figure 7. 3D interactions of top ligands interacting with 4ZZM protein.

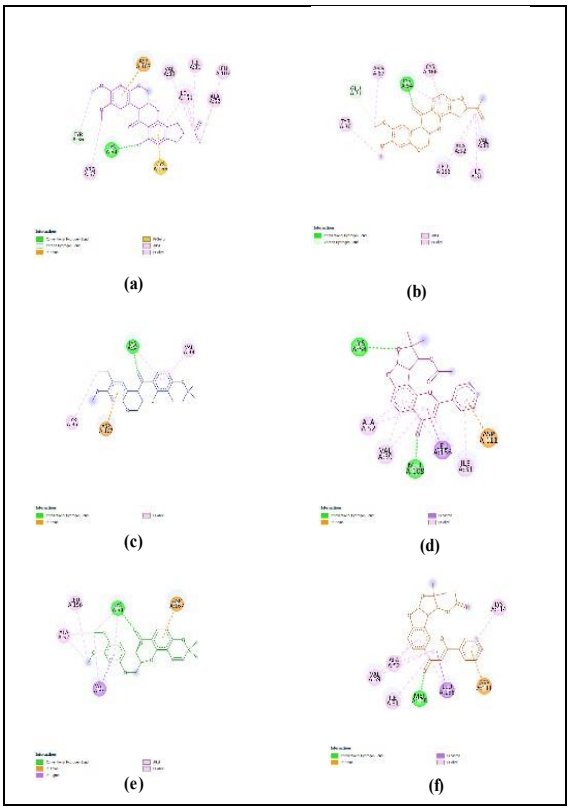

Figure 8. 2D interactions of top ligands interacting with 4ZZM protein.

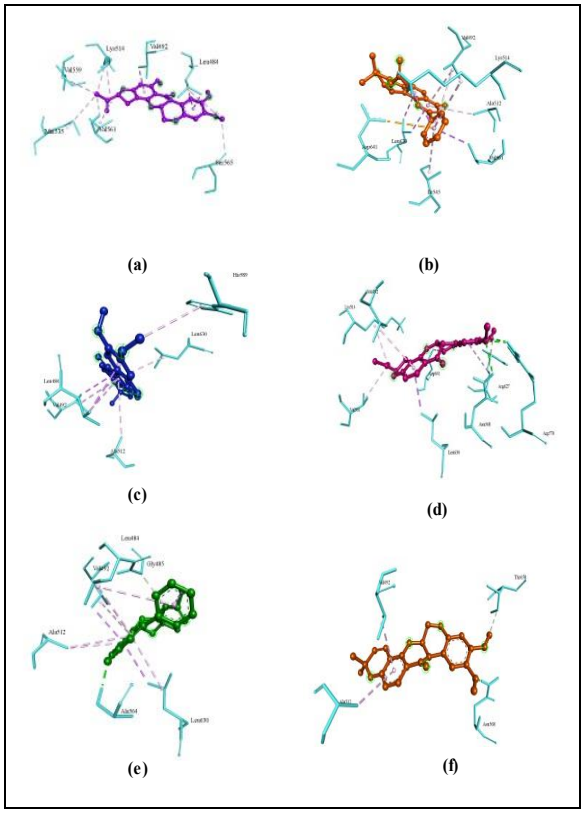

Figure 9. 3D interactions of top ligands interacting with 5B7V protein.

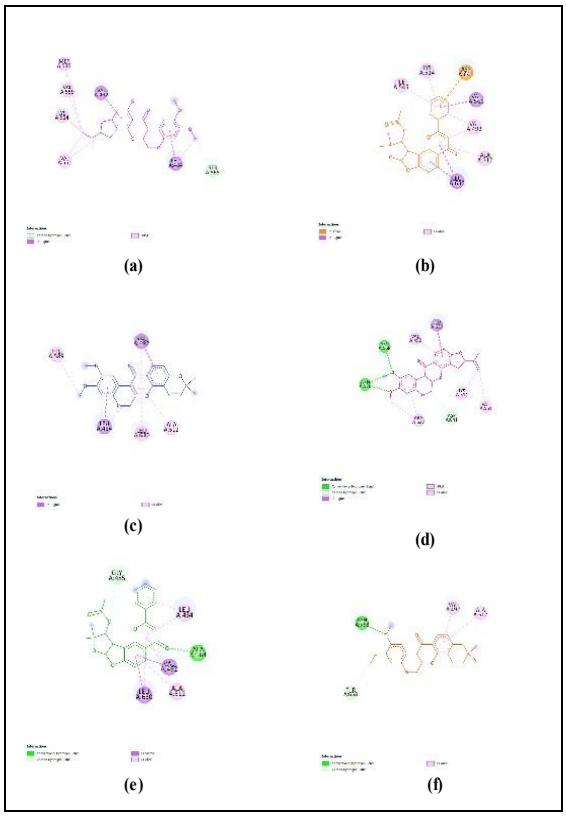

Figure 10. 2D interactions of top ligands interacting with 5B7V protein.

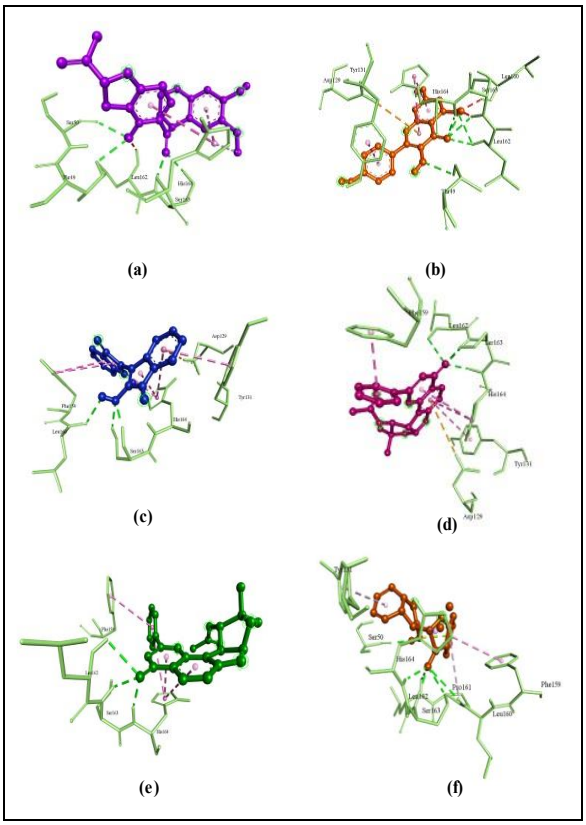

Figure 11. 3D interactions of top ligands interacting with 2P23 protein.

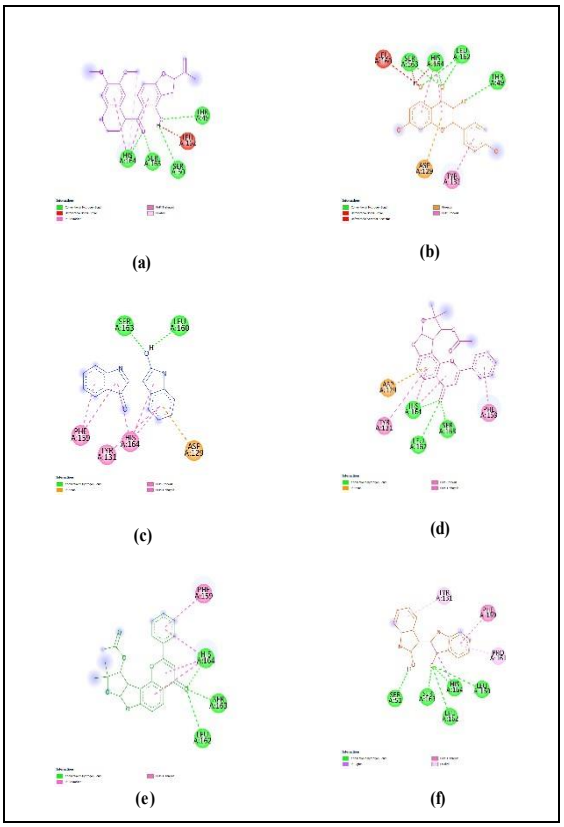

Figure 12. 2D interactions of top ligands interacting with 5B7V protein.

ADMET Analysis

The 6 ligands selected from the each of the four proteins were screened for their Adsorption, desorption, physiochemical properties, medicinal properties and their toxicity through ADMET lab 2.0 web tool. The results are depicted as follows (Tables 2–7).

Table 2. Physiochemical properties of the top 15 ligands.

PubChem ID	MW	Vol	nHD	nHA	nRot	nRing	nHet	fChar	Flex	TPSA	LogS
442824	410.14	404.022	1	7	3	5	7	0	0.111	83.45	-5.114
107935	394.14	395.232	0	6	2	5	6	0	0.074	63.22	-4.469
156341	392.13	392.595	0	6	3	5	6	0	0.111	74.97	-4.504
182678	392.13	392.595	0	6	3	5	6	0	0.111	74.97	-4.504
3083803	392.13	392.595	0	6	2	5	6	0	0.074	67.13	-5.227
44257420	396.16	403.788	1	6	5	4	6	0	0.217	74.22	-5.296
6758	394.14	395.232	0	6	3	5	6	0	0.111	63.22	-5.497
10177	262.07	266.912	2	4	1	4	4	0	0.048	65.45	-4.653
114909	410.14	404.022	1	7	2	5	7	0	0.074	83.45	-4.623
5280445	286.05	273.977	4	6	1	3	6	0	0.056	111.13	-3.629
10215	262.07	266.912	2	4	1	4	4	0	0.048	65.45	-4.271
5280863	286.05	273.977	4	6	1	3	6	0	0.056	111.13	-3.624
5280443	270.05	265.186	3	5	1	3	5	0	0.056	90.9	-3.606
439336	504.17	434.858	11	16	7	3	16	0	0.389	268.68	0.414
441564	295.11	278.223	5	7	3	3	7	0	0.188	115.17	-2.172

Table 3. Medicinal chemistry of the top 15 ligands

PubChem CID	QED	Synth	Fsp3	Lipinski	PAINS
442824	0.777	3.999	0.348	Accepted	0
107935	0.767	3.698	0.348	Accepted	0
156341	0.614	3.773	0.304	Accepted	0
182678	0.614	3.773	0.304	Accepted	0
3083803	0.637	3.056	0.261	Accepted	0
44257420	0.607	3.595	0.348	Accepted	0
6758	0.740	3.868	0.348	Accepted	0
10177	0.707	2.512	0	Accepted	0
114909	0.814	3.853	0.348	Accepted	0
5280445	0.511	2.420	0	Accepted	1
10215	0.707	2.487	0	Accepted	0
5280863	0.546	2.375	0	Accepted	0
5280443	0.632	2.253	0	Accepted	0
439336	0.154	4.839	1	Rejected	0
441564	0.514	3.514	0.429	Accepted	0

Table 4. Absorption properties of the top 15 ligands

PubChem CID	Caco-2	MDCK	Pgp-inh	Pgp-sub	HIA	F (20%)
442824	-4.807	1.78E-05	0.274	0.002	0.021	0.002
107935	-4.952	2.42E-05	0.999	0	0.009	0.009
156341	-4.725	4.36E-05	0.999	0	0.006	0.005
182678	-4.725	4.36E-05	0.999	0	0.006	0.005
3083803	-4.869	2.85E-05	0.998	0.001	0.010	0.003
44257420	-4.760	2.22E-05	0.351	0.006	0.005	0.003
6758	-4.860	2.80E-05	0.872	0.001	0.007	0.002
10177	-4.947	1.58E-05	0.006	0	0.010	0.015
114909	-4.819	2.24E-05	0.997	0	0.014	0.008
5280445	-5.028	1.00E-05	0.004	0.274	0.047	0.998
10215	-5.093	1.43E-05	0.045	0	0.007	0.004
5280863	-4.974	9.07E-06	0.004	0.011	0.008	0.856
5280443	-4.847	1.16E-05	0.004	0.820	0.015	0.995
439336	-6.235	0.000687522	0.001	0.890	0.999	0.980
441564	-5.230	4.06E-05	0	0.093	0.798	0.018

Table 5. Distribution properties of the top 15 ligands

PUBChem CID	PPB	VDss	BBB	Fu
442824	97.56%	0.529	0.014	2.39%
107935	99.55%	0.561	0.063	2.41%
156341	89.03%	1.580	0.048	8.09%
182678	89.03%	1.580	0.048	8.09%
3083803	89.69%	0.629	0.048	8.18%
44257420	99.75%	0.509	0.029	1.24%
6758	97.78%	0.594	0.094	2.41%
10177	98.74%	0.367	0.348	1.16%
114909	93.53%	0.945	0.197	8.26%
5280445	95.44%	0.533	0.009	5.98%

PUBChem CID	PPB	VDss	BBB	Fu
10215	99.50%	0.384	0.359	1.16%
5280863	97.86%	0.522	0.009	4.41%
5280443	97.25%	0.510	0.012	3.67%
439336	10.43%	0.189	0.417	59.63%
441564	36.23%	0.652	0.616	57.69%

Table 6. Metabolism and excretion properties of the top 15 ligands

PubChem CID	CYP1A2-inh	CYP1A2-sub	CYP3A4-inh	CYP3A4-sub	CL	T12
442824	0.098	0.843	0.865	0.708	7.89	0.074
107935	0.269	0.882	0.947	0.750	3.417	0.084
156341	0.121	0.213	0.152	0.228	1.647	0.087
182678	0.121	0.213	0.152	0.228	1.647	0.087
3083803	0.363	0.963	0.553	0.566	2.561	0.237
44257420	0.233	0.961	0.789	0.836	9.560	0.136
6758	0.083	0.918	0.904	0.797	8.285	0.069
10177	0.982	0.808	0.448	0.355	0.795	0.135
114909	0.223	0.756	0.857	0.884	3.703	0.118
5280445	0.981	0.154	0.549	0.092	8.146	0.898
10215	0.978	0.871	0.448	0.596	0.625	0.155
5280863	0.972	0.110	0.697	0.080	6.868	0.905
5280443	0.988	0.145	0.833	0.126	7.022	0.856
439336	0	0.006	0.001	0	0.767	0.537
441564	0.072	0.076	0.009	0.039	2.257	0.565

Table 7. Toxicity properties of the top 15 ligands

PubChem CID	hERG	H-HT	DILI	Ames	Carcinogenicity	Respiratory	IGC50	LC50
442824	0.049	0.242	0.628	0.122	0.156	0.791	4.963	7.644
107935	0.111	0.807	0.659	0.229	0.769	0.869	4.820	7.135
156341	0.012	0.663	0.966	0.800	0.787	0.328	4.788	7.279
182678	0.012	0.663	0.966	0.800	0.787	0.328	4.788	7.279
3083803	0.017	0.995	0.983	0.684	0.883	0.859	4.624	6.952
44257420	0.048	0.556	0.613	0.246	0.326	0.775	5.025	7.149
6758	0.072	0.249	0.722	0.283	0.280	0.785	5.004	7.915
10177	0.050	0.699	0.986	0.341	0.539	0.652	4.550	5.211
114909	0.285	0.833	0.132	0.230	0.897	0.534	4.219	6.090
5280445	0.064	0.084	0.905	0.536	0.095	0.220	4.432	5.222
10215	0.023	0.468	0.973	0.617	0.501	0.882	4.709	5.364
5280863	0.070	0.098	0.979	0.672	0.097	0.090	4.386	5.223
5280443	0.057	0.072	0.854	0.475	0.277	0.266	4.588	5.208
439336	0.054	0.053	0.103	0.105	0.008	0.005	1.945	0.324
441564	0.024	0.067	0.656	0.272	0.155	0.251	2.883	3.256

DISCUSSION

Prostate cancer is one of the most common types of malignancies, diagnosed in men. It results from the different environmental factors, diet, age and family history of the disease [21, 22]. The diagnosis of this cancer can be done by blood test such as Prostate Specific Antigen (PSA) test, digital rectal exam, biopsy etc. The treatment of the prostate cancer depends on the extent of its spread. There are no proven medicines that can help in curing the disease yet, but surgery and radiation therapy can be done

to improve the survival rate of the person [23]. There are different pathway proteins that are involved in the progression of the prostate cancer such as, epidermal growth factors (EGFR) and fibroblast growth factors (FGFR) [24]. These proteins have series of signalling pathways that are involved in the progression of cancer. Thus, targeting these proteins for the anti-cancer treatment can be promising.

In this study, 4 proteins, (EGFR, ERK, FGFR and FGF) were taken as target proteins. The structures were viewed using BIOVIA Discovery. The purified proteins were subjected to pdb sum generate. The Ramachandran plot results suggested that all the 4 selected proteins were good quality models, and had most of their residues come under allowed regions. The secondary structure prediction of the all the proteins were also done for the target proteins which had 324, 302, 344 and 136 residues respectively.

In Molecular Docking and Visualization among the 20 ligands screened, the top 6 ligands which had the lowest binding energy with the proteins namely 1M17, 4ZZM, 5B7V and 2P23 respectively were selected. The visualization results showed the implication of Leu 694 and Val702 were the most prevalent amino-acids involved in the binding with the ligand in 1M17 protein. Val A.39 was common in binding with all the top ligands in 4ZZM protein, and Leu 162 was the common amino acid in 2P23 protein. As shown in the Figure 6, Figures 8 and Figures 12 respectively.

ADMET Analysis results showed that, Pseudosemiglabrin and [(12S,15R,16R)-14,14-dimethyl-6-oxo-4-phenyl-3,11,13-trioxatetracyclo [8.6.0.02,7.012,16] hexadeca-1(10),2(7),4,8-tetraen-15-yl] acetate compound were found to be having best binding affinity with all the 4 target proteins that is, -9.3 and -9.1 respectively with the protein 1M17, -8.5 and -8.4 with 4ZZM protein, -10.8 and -10.1 respectively with 5B7V protein and -8.2 with the 2P23 protein. These 2 compounds have 0 Lipinski rules violation, 0 PAINS alert (Table 3). The pharmacokinetic properties suggested that these compounds have high GI absorption, Plasma protein binding was found to be less than 90% and thus can be used as a therapeutic drug (Table 5).

Apart from these 2 compounds, the other compounds such as Sumatrol (PubChem ID: 442824), Deguelin (PubChem ID: 107935), and Rotenol (PubChem ID: 44257420) showed least binding affinity with the 3 target proteins, they are 1m17,4zzm, and 5b7v. These compounds had 0 Lipinski's rule violation, 0 PAINS alert, and were moderately soluble in water. The compound Sumatrol had high GI absorption and no Blood brain penetration. The other 2 compounds, Deguelin and Rotenol showed High GI absorption and blood brain penetration (Table 6). The results of this study showed that the compounds, Pseudosemiglabrin, [(12S,15R,16R)-14,14-dimethyl-6-oxo-4-phenyl-3,11,13-trioxatetracyclo [8.6.0.02,7.012,16] hexadeca-1(10),2(7),4,8-tetraen-15-yl] acetate, Deguelin, Sumatrol and Rotenone showed good binding affinity with the target proteins and can be considered as a potential drug for the prostate cancer.

CONCLUSION

As the plant derived compounds (Herbal medicines) are being the trend, because of its long history of improving human health and being the cure for many diseases, *Indigofera tinctoria* proves to be a promising plant with its anti-cancerous properties, can be used as a remedy for prostate cancer. The 4 different pathway proteins involved in the disease were selected, and docked against the 20 ligands from the plant. top 6 ligands selected for each protein, with the least binding affinity, and were visualized. The ADMET analysis suggested that the top selected compounds have 0 Lipinski rule violation and also showed good results as an anti-cancer drug. The compounds such as Pseudosemiglabrin, Deguelin and Rotenol have already been studies for its anti-cancerous properties but their implication in Prostate cancer can be helpful in the treatment of the disease.

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Abbrevations

EGFR: Epidermal Growth Factor Receptor
ERK: Extracellular-signal regulated kinase
FGFR: Fibroblast Growth Factor Receptor
FGF: Fibroblast Growth Factor
PDB: Protein Data Bank
GLOBOCAN: Global Cancer Observatory
RTK: Receptor Tyrosine Kinases
MAPK: Mitogen-Activated Protein Kinase
HSPG: Heparin Sulphate Proteoglycans
IMMPAT**: Indian Medicinal Plants, Phytochemistry And Therapeutics**
RMSD: Root Mean Square Deviation

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