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PRECLUSION OF HYPOXIA: IDENTIFICATION OF POTENTIAL INHIBITOR AGAINST HIF-1 ALPHA PROTEIN THROUGH MOLECULAR DOCKING

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ABSTRACT: Low level of oxygen in tissues leads to hypoxia, and hypoxia-inducible factors (HIF) regulate the hypoxic and normoxic conditions in various tissues. During normoxia, HIF-1α binds to von Hippel-Lindau E3 ubiquitin ligase complex that targets HIF-1α to the ubiquitin-proteasome pathway for proteolytic destruction. But in hypoxia, HIF-1α move to the nucleus where it binds to CBP/p300 at CH1 domain. By considering this fact, the present study was conducted to search a suitable inhibitor that can bind to HIF-1α. The unliganded HIF-1α was docked and the best five docking solutions complex were selected and analyzed by Ligplot. The analysis showed that catechin, epicatechin, myricetin, dicarnoxide D, and pycnidione had the maximum potential to inhibit HIF-1α protein and may prove to be potential inhibitor for counterfeiting hypoxic conditions.

INTRODUCTION: Hypoxia acts on the vasculature directly conveying its damaging effects through disruption of the control of vascular tone, particularly in the coronary circulation, enhancement of inflammatory responses and activation of coagulation pathways. These effects could be particularly detrimental under pathological conditions such as obstructive sleep apnea and other breathing disorders [1]. Transcription factor, hypoxia-inducible factor-1α (HIF-1α), mediates the pathophysiological response caused due to hypoxia. HIF-1α is a heterodimeric protein, consist of α and β-subunits [2]. HIF-1α acts as a master regulator which induces the synthesis of proteins that promote metabolic changes in the cells of hypoxic tissues and controls angiogenesis, erythro-poiesis, and glycolysis *via* transcriptional activation of target genes under hypoxic conditions [3].

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In normoxic conditions, the HIF-1α is unstable and binds to the von Hippel-Lindau E3 ubiquitin ligase complex that targets HIF-1α to the ubiquitin-proteasome pathway for proteolytic destruction. Also HIF-1α does not interact with p300 in normoxic conditions instead oxygen-dependent process occurs in which hydroxylation of Asn803 [4], located within the C-TAD of HIF-1α takes place and prevent the interaction of HIF-1α with the CH1 domain of p300. However, in hypoxia condition the degradation rate of HIF-α protein is decreased so it travels to the nucleus; and dimerizes with HIF-β where it performs the protein-protein interaction with transcriptional co-activators such as the CH1 domain of p300 [5]. HIF-1α dimer/p300 complex binds to hypoxic response elements (HRE) on DNA and causes a plethora of downstream events via transcription mediation [6].

In the present work, various molecules have been studied which may have the potential to prevent protein-protein interaction between HIF1α/p300 at CH1 domain with the HIF-α CAD by using computer-aided drug designing. Appropriate set of ligands were taken from Pubchem database, the protein-ligand interactions were studied and the

lead molecule was selected on the basis of number of hydrogen bonds, binding affinity, inhibition constant, and validated by absorption, distribution, metabolism, and excretion (ADMET) studies.

MATERIALS AND METHODS:

Binding Site Prediction: The protein structure of HIF-1α (PDB ID - 1H2K) was retrieved from RCSB Protein Data Bank [7] (PDB) in Brookhaven's PDB format, and protein cleaning (removal of ligand and water molecules) was done using Autodock 4.2.6. [8] Binding sites of HIF-1α were predicted by using the software CASTp [9]. It is used to identify and measure the binding sites, active sites, surface structural pockets (accessible), interior cavities (inaccessible), shape (alpha complex and triangulation), area, and volume (solvent and molecular accessible surface) of each pocket and cavities of proteins. Docking of selected compounds was performed on a particular site of protein sequence from 786 to 826 [10].

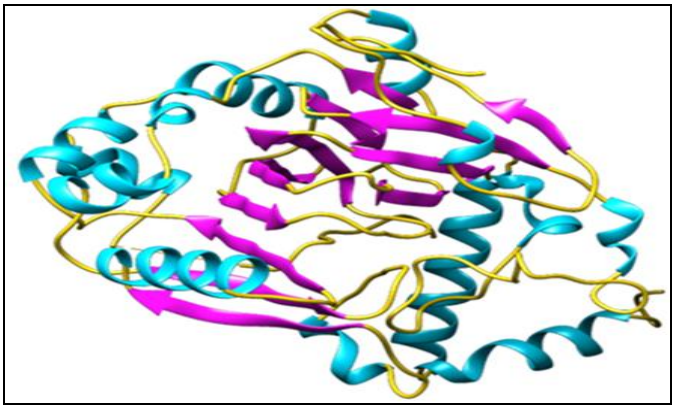

FIG. 1: STRUCTURE OF HIF-1α (ID-1H2K)

Compounds Selection and Preparation: According to the Lipinski rule of five several natural derivative compounds were filtered from the PubChem Database [11] and then screened 75 compounds against the HIF-1α protein. The structure of ligand was collected from PubChem (http://pubchem.ncbi.nlm.nih.gov) database. The ligands were retrieved in SDF format from the database and then converted to PDB format by using Open Babel GUI [12]. Ligands were prepared by the addition of hydrogen atoms, removal of the charge groups, and removal of other structures of the ligands with Autodock 4.2.6. Optimized structures of ligand and protein were ultimately used for molecular docking.

Molecular Docking: Virtual screening of the ligand-protein interaction for their binding affinity was carried out using AutoDock 4.2.6. Protein sequence from 786 to 826 was selected for docking. Input files were prepared by adding polar hydrogen, Kolloman charges and setting up the grid map. Rigid docking was performed using a Lamarckian Genetic Algorithm [13], and the runs were increased from 10 to 100 in order to search out the most preferred orientation of the ligand to the receptor, having the lowest binding energy. Finally, H-bonding and hydrophobic interactions were analyzed using Ligplot 1.4.5 [14], a program used to generate schematic diagrams of protein-ligand interactions. A small database of 75 compounds were formed on the basis of past findings, as showed in **Table 1**.

TABLE 1: LIST OF LIGANDS

S. no.	Name of compound	Compound ID	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Molecular Weight (g/mol)
1	Catechin	124203170	7	10	445.353
2	Epicatechin	124203168	7	10	445.353
3	Epigallocatechin	124203176	8	11	461.352
4	Gallocatechin	124203172	8	11	461.352
5	Proanthocyanidins	102115499	9	12	590.537
6	Theaflavins	102342127	17	28	1172.919
7	Thearubigins	100945367	13	22	902.723
8	Cyanidin	124203852	13	23	924.855
9	Delphinidin	102515282	12	25	872.691
10	Malvidin	118797967	8	14	655.585
11	Pelargonidin	102515511	9	17	726.64
12	Peonidin	122706400	5	11	461.399
13	Petunidin	102174359	12	21	917.843
14	Isorhamnetin	124202864	4	7	322.219
15	Kaempferol	123132000	11	19	740.664
16	Myricetin	102444976	8	12	464.379
17	Quercetin	123131991	6	12	476.39

18	Apigenin	122391238	9	13	534.47
19	Luteolin	123132010	10	15	580.495
20	Baicalein	102071505	1	5	270.24
21	Chrysin	102208339	7	13	562.524
22	Eriodictyol	122221847	8	11	450.396
23	Hesperetin	119058071	3	6	305.3
24	Naringenin	101242747	6	11	448.38
25	Daidzein	101736075	11	19	740.664
26	Genistein	122362400	3	5	678.954
27	Glycitein	124202362	5	11	460.391
28	Biochanin A	102463151	4	11	488.445
29	Formononetin	101220281	4	10	444.392
30	DicarnoxideA	16104921	0	4	408.623
31	DicarnoxideB	16104922	1	4	394.596
32	DicarnoxideC	16104923	0	4	408.623
33	DicarnoxideD	16104924	0	4	406.607
34	Sodwanone G	181445	1	6	498.66
35	Sodwanone H	177254	1	4	472.71
36	Sodwanone S	11540437	2	5	490.725
37	Sodwanone V	16099431	2	5	490.725
38	Sodwanone W	16099427	1	4	474.726
39	Sodwanone U	16099426	1	5	482.661
40	Sodwanone T	16099425	1	4	472.71
41	Sodwanone A	23427502	2	6	500.676
42	Sodwanone B	101675763	1	5	484.677
43	3-epi-sodwanone K	16099429	2	5	490.725
44	Sodwanone.B	21773186	1	5	484.677
45	Sodwanone.A	21773185	2	6	500.676
46	Sodwanone I	21585439	1	5	490.725
47	Sodwanone M	44566666	1	4	488.753
48	Sodwanone P	100964660	1	5	490.725
49	Sodwanone R	15513433	0	4	470.694
50	Sodwanone Q	15513432	1	3	456.711
51	Sodwanone O	15513430	1	5	488.709
52	3-epi-sodwanone K 3-acetate	16099424	1	6	532.762
53	Yardenone	15378863	0	5	488.709
54	Yardenone A	637861	1	6	504.708
55	Furospongolide	21637526	0	3	328.452
56	2-[methyl11C]Methoxyestradiol	16750137	2	3	301.414
57	(R)-Bakuchiol	49836433	1	1	256.389
58	Deferoxamine	2973	6	9	560.693
59	Desferriexochelin 772MS	49852345	5	13	719.789
60	Ciclopirox Olamine	38911	3	4	268.357
61	N-Oxalylglycine	3080614	3	5	147.086
62	DMOG	560326	1	5	175.14
63	N-Oxalyl-L-alanine	14985588	3	5	161.113
64	Alahopcin	163341	4	8	261.234
65	Dealanylalahopcin	101813531	4	6	190.155
66	Indirubin	5359405	2	3	262.268
67	N-(S-Nitroso-N-acetyl-D,L-penicillamine)	45040110	2	14	549.548
68	S-nitrosoglutathione	104858	5	10	336.319
69	Nonoate	5461016	0	2	157.233
70	Vinblastine	13342	3	12	810.989
71	Colchicine	6167	1	6	399.443
72	Pycnidione	10370280	3	7	548.676
73	Dihydrotestosterone	10635	1	2	290.447
74	Androgen Methyltestosterone	6010	1	2	302.458
75	Phorbol12myristate 13-acetate	27924	3	8	616.836

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Prediction: ADMET properties of the best ligands and HIF-1α were predicted by using online an admetSAR server [15]. Various properties of chemical compounds such as blood-brain barrier, human intestinal absorption, AMES toxicity, carcinogenicity, and bio-degradation were calculated using the server.

RESULTS AND DISCUSSION:

Binding site Analysis: PDB structure of HIF-1α contains 821 amino acids residues having 2 chains (A, S) and 2.15 Å resolutions **Fig. 1**. The pocket contain 44 amino acids in which ASP799, CYS800, GLU801, VAL802, ASN803, ALA804, PRO805, ILE806, GLN814 were more useful for the ligand binding.

Molecular Docking Analysis: After the docking of listed compounds **Table 1** only 11 were selected for the inhibition as showed in **Table 2** and further 5 compounds were selected on the basis of number of hydrogen bond, maximum negative binding affinity, and maximum inhibition constant as shown in **Table 3**.

Ligplot shows hydrogen bonds between catechin, epicatechin, myricetin, dicarnoxide D & pycnidione with the amino acids that were present in the targeted site as showed in **Fig. 2**. After 100 run catechin and epicatechin bind with five amino acids of targeted active site of protein structure and it's indicated by dark color in **Table 3**.

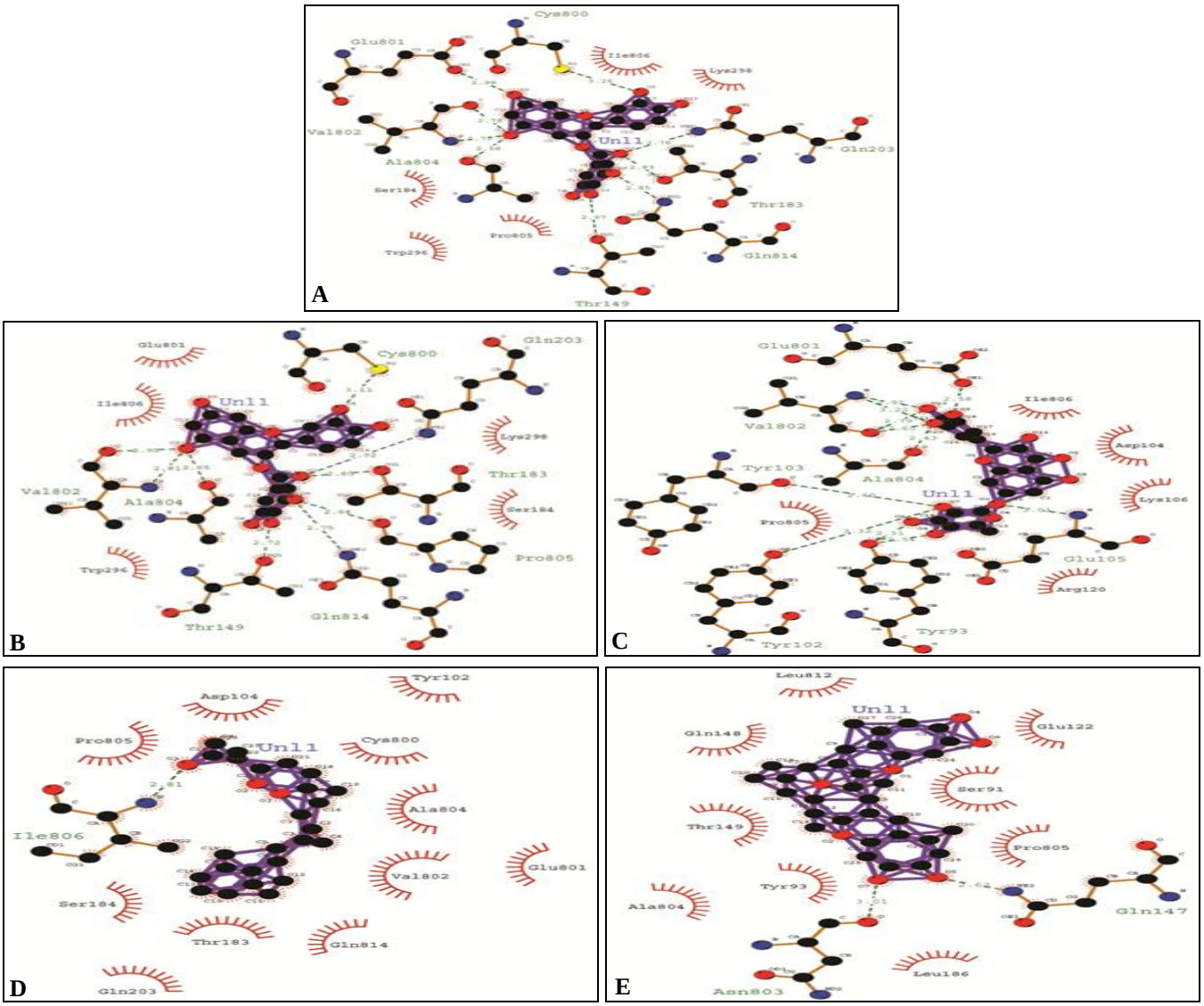

FIG. 2: LIGPLOT SHOWS THE INTERACTION OF CATECHIN (A), EPICATECHIN (B), MYRICETIN (C), DICARNOXIDED (D) AND PYCNIDIONE (E) WITH THE HIF-1α PROTEIN

TABLE 2: SHOWS THE BINDING ENERGY & INHIBITION CONSTANT OF COMPOUNDS (FOR 10 RUNS)

Name of compound	PubChem CID	Binding Energy (kcal/mol)	K_i (nm)	H Bond	Name of amino acids
Catechin	124203170	-9.40	128.22	7	Thr183, Cys800,Asp799, Val 802, Asp104,Tyr102
Epicatechin	124203168	-9.71	75.93	7	Tyr102, Tyr103, Ala804, Val802, Arg238
Isorhamnetin	124202864	-9.02	243.01	4	Cys800, Glu801, Tyr102, Arg238
Kaempferol	123132000	-10.66	15.42	5	Val802, Tyr102, Tyr93, Ile806
Myricetin	102444976	-11.89	1.93	5	Arg238, Glu801,Cys800,Thr183
Chrysin	102208339	-9.20	181.15	4	Thr102,Glu105, Tyr93,Ile806
Glycitein	124202362	-8.42	670.27	3	Tyr93, Glu801
Biochanin A	102463151	-9.28	156.70	5	Val802, Ala804, Tyr102
Dicarnoxide D	16104924	-10.28	29.20	1	Ile806
Sodwanone Q	15513432	-9.79	66.37	3	Thr149, Glu817, Glu181
Pycnidione	10370280	-11.37	4.66	2	Gln147, Asn803

TABLE 3: SHOWS THE BINDING ENERGY & INHIBITION CONSTANT OF COMPOUNDS (FOR 100 RUNS)

Name	PubChem CID	Binding Energy (kcal/mol)	K_i (nm)	H Bond	Name of amino acids
Catechin	124203170	-11.41	4.35	9	Gln203, Cys800, Glu801, Val802, Thr183,Gln814, Thr149, Ala804
Epicatechin	124203168	-11.22	5.99	9	Cys800, Glu203, Thr183, Pro805, Glu814, Val802, Ala804,Thr149
Myricetin	102444976	-11.94	1.78	11	Glu801,Val802, Ala804, Tyr103, Glu105, Tyr102, Tyr93
Dicarnoxide D	16104924	-10.00	46.97	1	Ile806
Pycnidione	10370280	-13.01	0.29	2	Gln147, Asn803

ADMET Analysis: The results obtained from the ADMET server showed in **Table 4** and **5**. Compounds like catechin, epicatechin, myricetin, showed inability to cross CNS while dicarnoxide D, pycnidione were able to cross CNS. HIA+ value means all compounds will be easily absorbed by the human intestine.

Analysis of toxicity properties showed that compounds were non-carcinogenic and non-AMES toxic. Acute oral toxicity shows III, IV phase of oral toxicity means small values (between 300 to 2000 mg/kg) of compounds will be not toxic for humans.

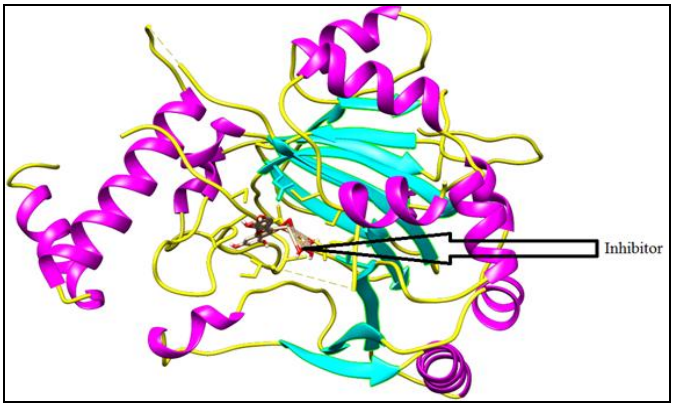

FIG. 3: SHOWS INTERACTION BETWEEN HIF-1α PROTEIN AMINO ACID AND CATECHIN PREPARED BY CHIMERA[16]

TABLE 4: ADME PROPERTIES OF COMPOUND USING THE ADMETSAR

PubChem Id	Blood-brain Barrier (BBB)		Human intestinal absorption (HIA)		Caco-2 Permeability		Renal organic cation transporter		CYP Inhibitory Promiscuity	
	Result	P	Result	P	Result	P	Result	P	Result	P
124203170 Catechin	BBB-	0.604	HIA+	0.886	Caco2-	0.895	Non-inhibitor	0.933	Low CYP Inhibitory Promiscuity	0.806
124203168 Epicatechin	BBB-	0.604	HIA+	0.886	Caco2-	0.895	Non-inhibitor	0.933	Low CYP Inhibitory Promiscuity	0.806
102444976 Myricetin	BBB-	0.756	HIA+	0.905	Caco2-	0.749	Non-inhibitor	0.939	Low CYP Inhibitory Promiscuity	0.564
16104924 Dicarnoxide D	BBB+	0.895	HIA+	0.924	Caco2+	0.625	Non-inhibitor	0.791	Low CYP Inhibitory Promiscuity	0.672
10370280 Pycnidione	BBB+	0.767	HIA+	0.972	Caco2+	0.606	Non-inhibitor	0.919	Low CYP Inhibitory Promiscuity	0.933

TABLE 5: TOXICITY PROPERTIES OF COMPOUNDS USING ADMETSAR

PubChem Id	AMES toxicity		Carcinogen		Biodegradation		Acute oral Toxicity	
	Result	P	Result	P	Result	P	Result	P
124203170 Catechin	Non AMES toxic	0.904	Non-carcinogens	0.961	Not ready biodegradable	0.609	IV	0.376
124203168 Epicatechin	Non-AMES toxic	0.904	Non-carcinogens	0.961	Not ready biodegradable	0.609	IV	0.376
102444976 Myricetin	Non-AMES toxic	0.931	Non-carcinogens	0.946	Not ready biodegradable	0.907	III	0.518
16104924 Dicarnoxide D	Non AMES toxic	0.686	Non-carcinogens	0.826	Not ready biodegradable	0.878	III	0.494
10370280 Pycnidione	Non-AMES toxic	0.683	Non-carcinogens	0.909	Not ready biodegradable	1.00	III	0.405

CONCLUSION: The docking of protein with a ligand is a significant method in structural biology for searching a potential inhibitor. The goal of docking software is to predict the predominant binding mode (s) of a ligand with a protein of known 3-D structures. Screening studies of these 75 ligands obtained from Pubchem database were docked against HIF-1α on binding site of CBP/p300 using Autodock 4.2.6. The present study concludes that the catechin, epicatechin, myricetin, dicarnoxide D, pycnidione were found to be active against HIF-1α protein as showed in **Table 3** and catechin, epicatechin may be used as an inhibitor for preventing hypoxic condition in human because both compounds bind with the maximum number of amino acids of an interesting site **Fig. 3** and may be restricted binding of CBP/p300 on HIF-1α.

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