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ORIGINAL ARTICLE **OPEN ACCESS**

Ligand based Lead Optimization and Pharmacophore Modeling For Identification of Noval 4-Methoxy-2-Biphenylcarbaldehyde Inhibitor against Colorectal Cancer Protein TP53

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ABSTRACT

Colorectal cancer is one of the leading causes of death around the world. It is 3rd major type of cancer and 4th biggest cause of death. It is estimated that in Pakistani population more than 80% of young population is at risk for colorectal cancer. Objectives: Present research work emphasized on the identification potent and selective target-based drug as well as provide effective herbal chemotherapy. Methodology: In the present study, Pharmacophore modeling approach, was followed by molecular docking and virtual screening. Molecular Docking was performed using and SwissDocker. Molecular Properties were predicted from SwissADME, Molinspiration. ACD/lab, EPSI-suite and ChemAxon databases/servers. The docked complex was analyzed by using Discovery-Studio. Findings: Pharmacophore modeling of four compounds present in medicinal plant dandelion were screened. The compound with highest Pharmacophore fit score Having Id NS_013645. To get highest Pharmacophore fit and best toxicity class derivatives of NS_013645 were selected. Compound 4'-Methoxy-2-biphenylcarbaldehyde showed the best estimate binding energy -6.84 and transformation value of 2.67. 2D interactions show direct interaction between amino acid Histidine (HIS-233) and oxygen of lead Pharmacophore with the distance of 2.31, 2.42 and 2.72 respectively. Collectively, we identified lead which indicates the best drug-likeness and potential to be best drug against P53 (R273H) in colorectal cancer.
Keywords: Colorectal cancer, TP53, CRCs Markers,

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INTRODUCTION

Colorectal cancer (CRC) is also known as bowel cancer and colon cancer. The cancer is developed into parts of large intestine colon or rectum. It is the third most common type of cancer around the globe and fourth major cause of death about 9% of all the cancers incidence colorectal cancer is involved. Over 1 million new cases recorded in 2002. It affects men and women equally [1]. Some common symptoms those are seen in colorectal cancer patients are rectal bleeding, abdominal pain, constipation or diarrhea —often named as 'change in bowel habit', loss of weight, and anemia [2]. p53 has a very essential role in preventing tumor development. Under oncogenic stress, wild-type p53 in most cases cause apoptotic cell death [1]. The p53 was found to make sure the quality and genomic stability of stem cells; therefore, it serves as restraint to CSCs formation. Its flawless performance is essential for the maintenance of healthy cells [3]. It is now known that some vital intracellular signaling pathways, like wnt/β-catenin signaling, RAS signaling, and p53 signaling are often dysregulated in colorectal cancer [4]. In tumor suppressor genes P53 is most often mutated gene. Unlike other tumor suppressor genes, the mutated p53 has point mutations. The mutation of somatic cell p53 is involved in about greater than 50% of human cancers. Mutated p53-273H/miR-27a/EGFR is known to play a crucial role in development of tumor [3]. The positions 273, 248,175 are most often mutated in p53 [4]. Even in the cancers which have normal/wild type P53 which is frequently not functioning because of changes in its regulators and mediators or just mediators [3]. The p53 mutant protein not just loses its tumor suppressor function but also acquires a novel oncogenic function and promote tumor formation. Mutant p53-expressing cell lines harbor larger

sub-populations with high expression of CSCs markers: ALDH1, DC44, and lgr5. The high expression is due to the binding of mutated p53 to their promotor sequences. Moreover, mutant p53-dependent chemotherapy resistance was found to be due to the involvement of ALDH1 [6]. Radiotherapy in rectal cancer patients showed increase in CSCs while ALDH1 predicts cancer recurrence. The mutation in p53 r273h results in enhanced survivability of cells and also resistance to anoikis [5]. Now derivatives of medicinal plants are playing vital role in treatment of tumors and cancers. This current study is going to demonstrate pharmacophore-based virtual screening to reveal novel inhibitors against p53 r273h colorectal cancer. Pharmacophore-based molecule libraries were screened search against reported compounds from the articles. The derivatives compounds were studied and suitable compounds with best properties were docked to get the best results. The inclusive in silico analysis may provide evidence for a reliable framework that could assist medicinal chemists for the design and development of novel molecules for potential drugs that target p53.

MATERIAL AND METHODS

In this particular study compound selection, pharmacophore screening and its derivatives, docking and docking analysis were done. In the present work, we had selected colorectal cancer which was confirmed from paper subjected as disease selection, from gene card it was confirmed that the most highlighted gene involve for colorectal cancer was TP53. Further p53 gene was selected and hot spot mutation r273h was selected. The amino acid sequence was retrieved from UniProt with accession number P04637 [8]. The amino acid sequence was then inserted into an advance feature of PDB the sequence search. Human p53 core domain with hot spot mutation R273H (form I) was selected from PDB [9]. Both protein structures of mutated and non-mutated were downloaded in PDB format and visualized in chimera and Discovery Studio [9, 10 and 12]. Both the structures were superposed using the structure comparison (matchmaker) feature of chimera which has four chain 800 total residues each chain starts from 96th residues position to 288th [10]. Properties such as rotamers were checked from MolProbity [23].

The dandelion plant was taken for its anti-cancerous properties four chemical from dandelion is taken those are effective against colorectal cancer which was confirmed from paper. The dandelion study was done for different cancers and further the compounds were selected on base of articles those showed significant effects against only cancerous cells while not affecting normal body/somatic cells. The oral toxicity prediction of the compounds was found by using Protox server [15]. Four compounds were selected from constituents of dandelion because of their combined activity [7]. The compound was retrieved from Pubchem in SDF format which is beta-Amyrin having Pubchem CID number 73145 alpha-AmyrinwithPubChem CID number 73170, lupeol with CID number 259846 and Taraxasterol and Pubchem CID number 115250. The compounds were visualized in Chemdraw [25 and 26]. With the help of canonical smile which is retrieved from Pubchem and then inserted into Protox for oral toxicity [15]. Properties were checked from different databases such as SwissADME, molinspiration, chemicalize and ACD lab shown in table 3.

LigandScout software was used for generation of shared feature ligand base pharmacophore (in figure with pharmaco-fit score and different energy). Next, Compounds were screened against libraries of ligand scout (zinc, Princeton and drug) [11]. After screening, out of 45785 13056 compounds were screened in which from which top most scorer compound was NS_013645 and it was drawn in Chemdraw and in online Chemicalize tool from where structure in SDF format was retrieved [25]. The canonical smile was generated by using online SwissADME a Bioinformatics tool, the smile was inserted into Protox for oral toxicity prediction, the compound lies in 4th class [14 and 15].

The selected compound (NS_013645) was prepared for docking using chimera and was saved in mol2 format. Protein was prepared for docking preparation are performed by using chimera [10]. For docking online tools Patchdock and SwissADME were used result are taken by email, ten best structure was retrieved from FireDock which were then visualized in chimera some bumps were observed in compound structure [19, 20 and 27].

For removing the bumps and getting efficient toxicity class derivatives are taken from VICI which is an online bioinformatics tool. Out of 1000 derivative, about 200 random compounds were screened were taken and send for docking [24]. The results were taken by FireDock and visualized on chimera. The derivatives were screened on the basis of their interaction with protein and oral toxicity. 2D interaction of protein and chosen ligand was visualized in LigPot+ and Discovery Studio [12, 21 and 27].

RESULTS AND DISCUSSIONS

The goal of this research was to find a ligand from pharmacophore and its bioinformatics analysis for designing, identifying, and evaluating novel potential ligand/inhibitor for colorectal cancer. The main aim

of this study was to find a medicinal compound from the medicinal plants those have been traditionally known for being anti-cancerous they can cure colorectal cancer. The gene was chosen with high involvement in colorectal cancer that is p53/TP53 because it was found in different research articles that p53 mainly contributes in colorectal cancer and also has the highest value of GIFtS and GeneCards scores shown in table 1 [28].

Table 1 showing top 10 protein involve in colorectal cancer

Symbols	Description	GIFtS	GC ID	Scores
Tp53	Tumor protein p53	62	GC17M007661	178.42
BRCA1	DNA repair Associated	57	GC17M043044	173.10
APC	Wnt signaling pathway regulator	55	GC05P112707	166.13
MLH1	MutL Homologue 1	54	GC03P036993	162.73
MSH6	MutS Homologue 6	55	GC02P047695	152.02
MSH2	MutS Homologue 2	55	GC02P047402	146.80
BRCA2	DNA repair Associated	57	GC13P032315	134.72

Initially, the Dandelion root extract (DRE) was studied because of its strong anti-cancerous properties against colorectal cancer. Among the compounds from DRE the four compounds were proved to be the most effective against colorectal cancer. The combined effect of the four compounds was greater than the effect of individual compound. These four compounds were taken and their properties were studied from different databases and to check whether they can be drug-like.

Crystal structure of mutated(a) and normal protein/non-mutated(b) was downloaded from PDB and visualized in chimera figure 2.

The mutated and non-mutated protein structures were overlapped by chimer's tool which showed the difference of protein structures in figure 3. There was a visible structural difference found in them which appeared in the form of the loop difference shown in figure 3 [9 and 10].

Some properties of protein related to rotamers are taken by moleinspiration wild-type protein having 3 poor rotamers which is about 1.15% and goal is less than 0.3%while favored rotameris 253 which are about 96.5% and goal is greater than 98% bad bonds are 0 out of 2558 and bad angles is one out of 3481. These and other properties of wild-type protein are shown in table 2

Table 2 properties of wild type p53 protein

All-Atom Contacts	Clashscore, all atoms:	1.04		100th percentile* (N=746, 1.92Å ± 0.25Å)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	3	1.15%	Goal: <0.3%
	Favored rotamers	253	96.56%	Goal: >98%
	Ramachandran outliers	0	0.00%	Goal: <0.05%
	Ramachandran favored	320	99.38%	Goal: >98%
	MolProbity score^	0.85		100th percentile* (N=11875, 1.92Å ± 0.25Å)
	Cβ deviations >0.25Å	0	0.00%	Goal: 0
	Bad bonds:	0 / 2558	0.00%	Goal: 0%
	Bad angles:	1 / 3481	0.03%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 16	0.00%	Expected: ≤1 per chain, or ≤5%

Table 3 properties of mutated p53 (R273H) protein

All-Atom Contacts	Clashscore, all atoms:	3.85		98th percentile* (N=839, 1.78Å ± 0.25Å)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	33	4.61%	Goal: <0.3%
	Favored rotamers	647	90.36%	Goal: >98%
	Ramachandran outliers	0	0.00%	Goal: <0.05%
	Ramachandran favored	758	98.83%	Goal: >98%
	MolProbity score^	1.68		85th percentile* (N=11266, 1.78Å ± 0.25Å)
	Cβ deviations >0.25Å	0	0.00%	Goal: 0
	Bad bonds:	0 / 6527	0.00%	Goal: 0%
	Bad angles:	0 / 8947	0.00%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	0 / 58	0.00%	Expected: ≤1 per chain, or ≤5%

In mutated p53(r273h) protein there are 33which are about 4.61% poor rotamers and favored rotamers are 647 which are about 90.36%. bad bonds are 0 out of 6527 and bad angles are 0 out of 8947 which shown in table 3 [23].

The dandelion plant's compounds in table 4 from articles have revealed to be effective against colorectal cancer. Their 2D structures are shown in figure 4. The properties of the four compounds are shown in table 4 [7].

Table 4 medicinal formation / medicinal compounds from dandelion and their properties

Compound Name	**Botanical Name**	**Local name**	**Family**	**Part used**	**Compound**
Dandelion	*Taraxacum officianale*	Ishkanagi	Asteraceae	Root extract	Alpha_Amyrin ,Beta_Amyrin,Lupeol, Taraxasterol.

Compound name	**IUPAC Name**	**Toxicity Class**	**ID**	**Half life**
Alpha_Amyrin	(3β)-Urs-12-en-3-ol [ACD/IUPAC	6	MolPort-003-892-645	0.084 Days (12-hr day; 1.5E6 OH/cm3) 1.010 Hrs
Beta_Amyrin	(3β)-Olean-12-en-3-ol ACD/IUPAC	6	MolPort-003-925-568	0.087 Days (12-hr day; 1.5E6 OH/cm3) 1.046 Hrs
Lupeol	(3β,18α,19α)-Urs-20(30)-en-3-ol ACD/IUPAC	4	MolPort-001-742-379	0.117 Days (12-hr day; 1.5E6 OH/cm3)/ 1.409 Hrs
Taraxasterol	3β)-Lup-20(29)-en-3-ol ACD/IUPAC	4	MolPort-027-835-585	0.114 Days (12-hr day; 1.5E6 OH/cm3) 1.370 Hrs
4'-Methoxy-2-biphenylcarbaldehyd	4'-Methoxy-2-biphenylcarbaldehyd	5	MolPort-008-150-389	0.313 Days (12-hr day; 1.5E6 OH/cm3) 3.755 Hrs

The compounds lie in toxicity class of 4 and 6 those are shown in figure 4. Next, we screened the libraries Princeton, Zinc, Drug after screening each library, compounds with the lowest pharmacophore scores (>50) were chosen out of 45785 compounds from Princeton library 10134 compounds were screened for shared feature pharmacophore generation from those we selected a compound from Princeton with highest Pharmacophore fit score of 74.86 with ID. NS_013645 Shown in table 6. The compound from Princeton library their IDs and Pharmacophore Fit score are shown in table 6. The compound lies in toxicity class of 5. We docked the ligand with protein P53 R273H mutated and founds some unfavorable bonds. Ligand is shown in figure 5. Then derivative compounds were found from VICI and then the derivative compounds are screened on the bases of oral toxicity [24]. The selected compound was obtained by series of filters and it lies in 5th class that is good for a potential drug shown in figure 5. We again docked the mutated p53 R273H protein using online server Patchdock and Swissdock to get the docking results with the derivative compound. The top 10 solutions/poses of the docking results were taken and studied. All the top ten poses shared the same pocket shown in figure 7. The pose showed very small binding energies that are required for stable complex. The 2D interaction of 4'-Methoxy-2-biphenylcarbaldehyde with protein is shown in figure 8 [19 and 20]. All the 2D interactions of top 10 poses of ligand are shown in figure 9. The properties of protein were found from MolProbity in table 3. In silico prediction of ADMET properties identifies the likeliness of compound to be a drug that can be used on human beings. The online tools used for predicting the ADME properties are admetSAR, SwissADME, pkCSM, and Protox. In silico prediction of ADMET properties identifies the likeliness of compound to be a drug that can be used on human beings. The water absorption, Caco2 permeability, Intestinal absorption (human), BBB permeability, Hepatotoxicity, AMES Toxicity, P-glycoprotein Substrate, P-glycoprotein Inhibitor, and Carcinogens are shown in table 5. 4'-Methoxy-2-biphenylcarbaldehyde fulfills all these requirements of being a drug-like compound [13, 14, 15,16].

Table 5: It covers the properties of the compound from absorption, Distribution, Metabolism, Excretion, Toxicity and the structural aspects of the compound for a candidate drug.

Structural Properties							
Rotatable bonds	**Polar surface area**	**Molar Refractivity**	**Lipophilicity/ Consensus Log Po/w**	**ClogP**			
3	26.30 Å^2	64.3±0.3 cm3	3.02	2.91			
LD50			**Density**				
2100mg/kg			1.1±0.1 g/cm3				
Absorption							
Solubility Water	**Intestinal absorption (human)**	**Permeability Of Cac02**	**Skin Permeability**	**P-glycoprotein substrate**	**P-glycoprotein I/II inhibitor**		
-3.57	Yes (1.00)	1.8261(LogPapp, cm/s)	-2.735 Numeric (log Kp)	No	No		
Distribution							
Blood-Brain Barrier	**Fraction unbound (human)**	**VDss (human)**	**CNS permeability**	**Subcellular localization**	**Carcinogens**	**Half-life**	
Yes (0.8108)	0.381 Numeric (Fu)	0.004 Numeric (log L/kg)	-1.351 Numeric (log PS)	Mitochondria 0.9539	Non	0.313 Days 12-hr day;1.5E6 OH/cm3	
Rules							
Lipinski	**Ghose**	**Veber**	**Egan**	**Muegge**	**Bioavailability Score**	**PAINS**	
No violation	No Violation	No Violation	No Violation	No Violation	0.55	0 alert	
Toxicity & Excretion							
Total Clearance	**Renal OCT2 substrate**	**Oral Toxicity class**	**Hepatotoxicity**	**Max. tolerated Dose (human)**	**hERG I & II inhibitor**	**T.Pyriformis toxicity**	**LOAEL**
0.261 log ml/min/kg	No	5	No	0.438 log mg/kg/day	No	0.285	9.59 og mg/kg_bw/day
Metabolism							
CYP3A4 inhibitior	**CYP2C9 inhibitior**	**CYP2D6 inhibitior**	**CYP2C19 inhibitior**	**CYP3A4 substrate**	**CYP2D6 substrate**	**Lead likleness**	
No	No	No	No	No	No	violation: MW<250	

Table 6 top ten compound ID and their Pharmacophore Fit Score

Compound id	Pharmacophore Fit Score
NS_013645	74.86
NS_005581	72.56
NS_007729	72.34
NS_020393	71.85
NS_013643	68.69
NS_005959	66.39
NS_007920	66.28
NS_012137	66.16
NS_012134	66.16
NS_012133	66.16

The results are shown in forest green colored protein and every ligand pose is colored differently and shown in ball and stick model in figure 7.

The docking results were analyzed in LigPlot+ and Discovery Studio for its 2D interaction with ligand and protein residues [12 and 21]. The best three pose shows the direct interaction of amino acid histidine oxygen of lead compound 4'-Methoxy-2-biphenylcarbaldehyde with the distance of 2.31, 2.42 and 2.72 Å respectively. The direct interaction was observed as the hydrogen bond between the ligand and protein residues. The common residues Val 218, Arg 202, Glu 221, Thr 231, Ile 232, His233, Glu 198, Glu 224, Asn 200, Pro 219, Thr 230, Tyr 220, Val 218, Gly 199, Val 197.

The compound shows great properties for being a candidate drug. The compound in docking complex has minimum binding energy which stands that this compound has great propensity to be a good candidate for the treatment of fatal disease colorectal cancer by targeting P53 R273H. These insilico strategies have simplified the process of designing a novel drug for diseases which has been previously very hard and expensive. It allows us to identify and investigate novel drug without wasting much time and money. Considering these findings, it concludes that selected compound is efficacious against colorectal cancer and further studies can be done to synthesis novel drugs against the fatal disease.

4'-Methoxy-2-biphenylcarbaldehyde has the above 95% of human intestinal absorption which should be above 30% to be able to get absorbed through the human intestine. Its BBB (Blood Brain Barrier) value is 0.8108 which should be greater than 0.3 to cross the blood-brain barrier. It shows no violation of Lipinski rule of five, Ghose, Veber, Egan and Muegge rules. The bioavailability of our compound is 0.55. The compound doesn't show hepatotoxicity or AMES toxicity. It is not a P-glycoprotein Substrate and also it is a non-inhibitor of P-glycoprotein Inhibitor I and II. The topological polar surface area TPSA of compound should be greater than 20Å and less than 130Å and the TPSA of our compound is 26Å. Our compound is moderately soluble in water at 25 C. LD50 value of our compound is 2000 compound [13, 14, 16 and 17].

CONCLUSION

In the present study, the analysis suggests that the selected drug and novel compound is efficacious in the treatment of acute colorectal cancer. The plant derivative compound is selected because of its binding results with mutated protein ADME properties, QSAR study of compound its properties of Absorption, Distribution, Metabolism, and excretion showed best results for being a great drug candidate. In concluding that 4-Methoxy-2-biphenylcarbaldehyde may be a good option for the treatment of colorectal cancer. Considering these findings, further studies and synthesis of these novel compounds may result in similar response rates and help to cure colorectal cancer.

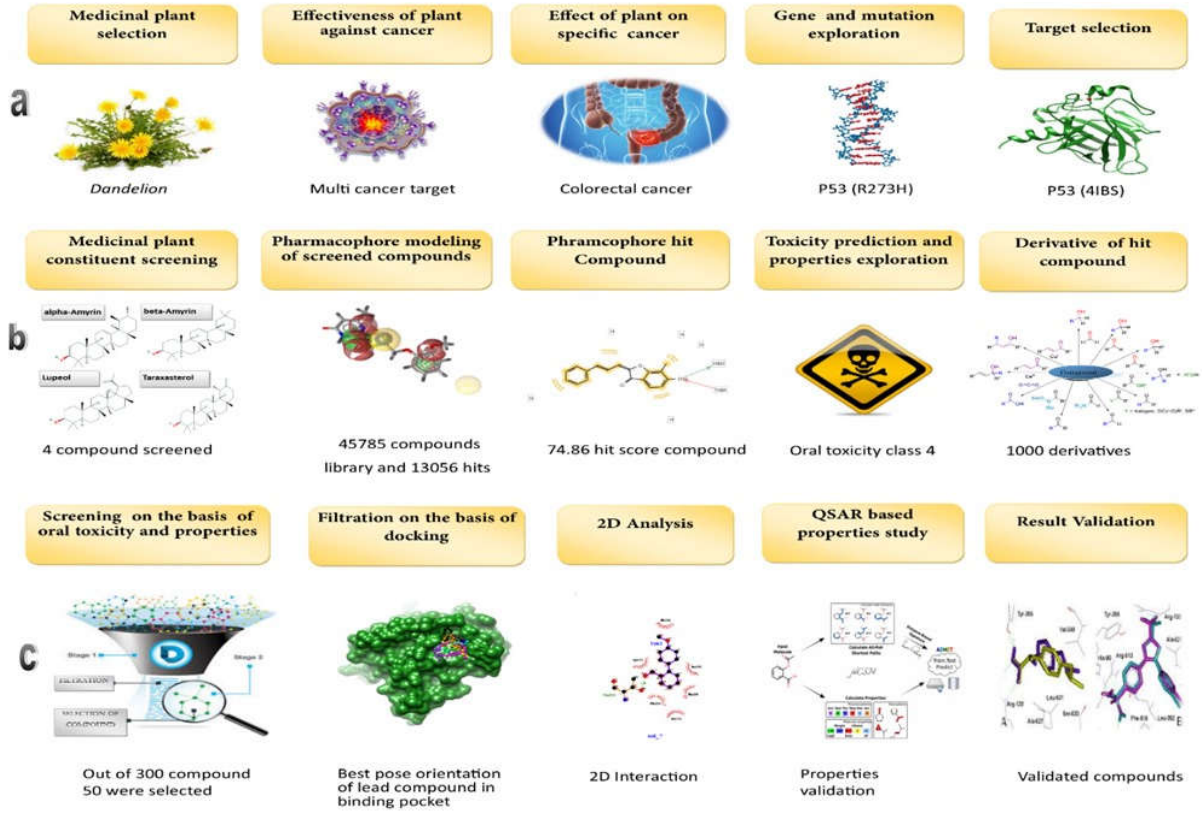

Figure 2 (A to C) Bioinformatics workflow of the overall methodology. (a) Target optimization of potent compound and receptor protein. (b) Lead screening. (c) Scheme describing structure-based in-silico molecular docking studies

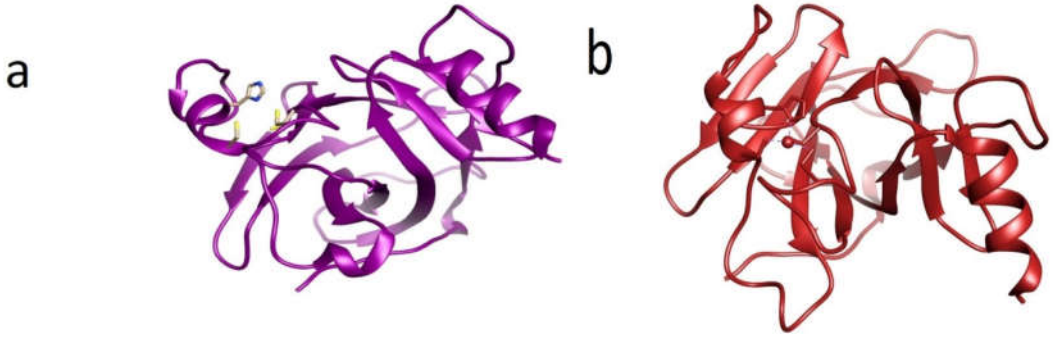

Figure 3 (a) wild type p53 proteins (2OCJ). (b) Mutated p53 r273h (4IBS)

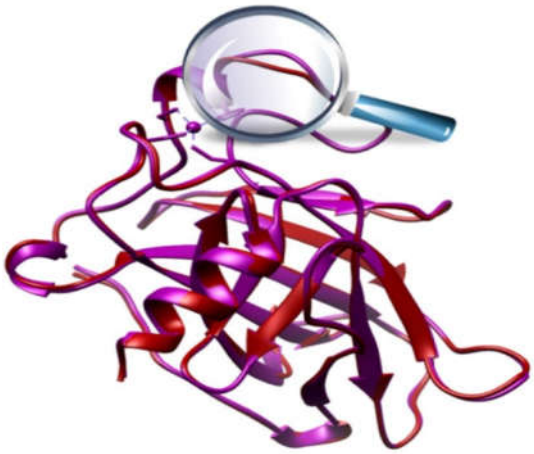

Figure 4 superimposed protein 4ibs & 2ocj showing difference in only one loop

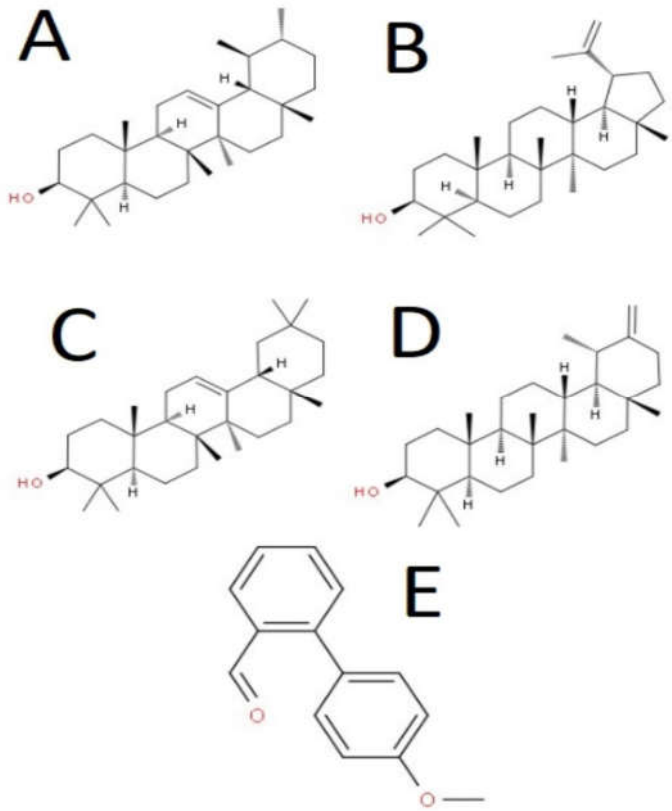

Figure 5 (A-E):(A)alpha-amyrin, (B)beta-Amyrin,(C)lupeol,(D)tarexesterol,(E) 4'-Methoxy-2-biphenylcarbaldehyde

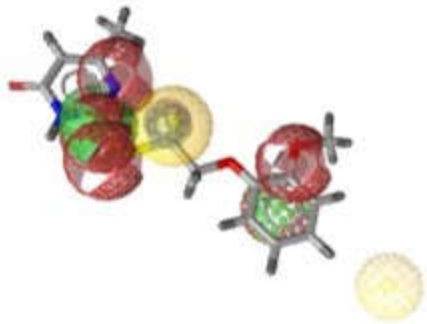

Figure 6 The top-ranked chemical feature-based pharmacophore model developed using the LigandScout program. The pharmacophore includes one aromatic rings (blue), four hydrophobes (yellow), three hydrogen acceptor feature (red) and one hydrogen donor (green). Distance is given in Angstrom.

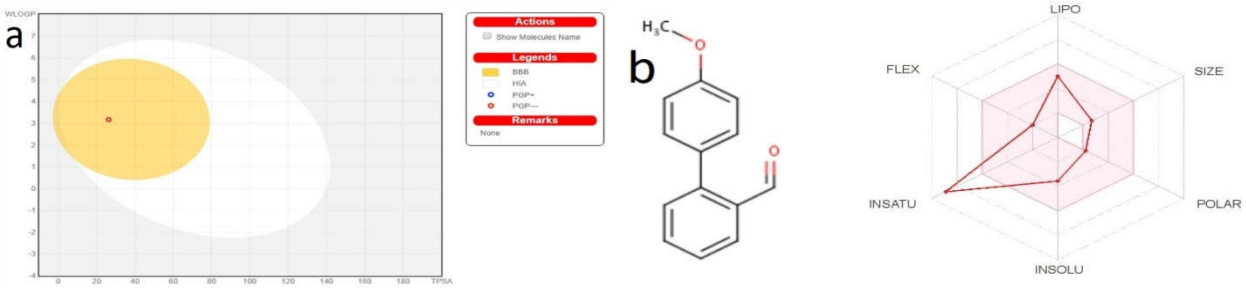

Figure 7 (a) Boiled egg representation of the compound 4-Methoxy-2-biphenylcarbaldehyde. The BOILED EGG model delivers a rapid, intuitive, easily reproducible yet statistically unprecedented robust method to predict the passive gastrointestinal absorption and brain access of small molecules useful for drug discovery and development. (b) Which shows the overall drug-likeleness of the compound.

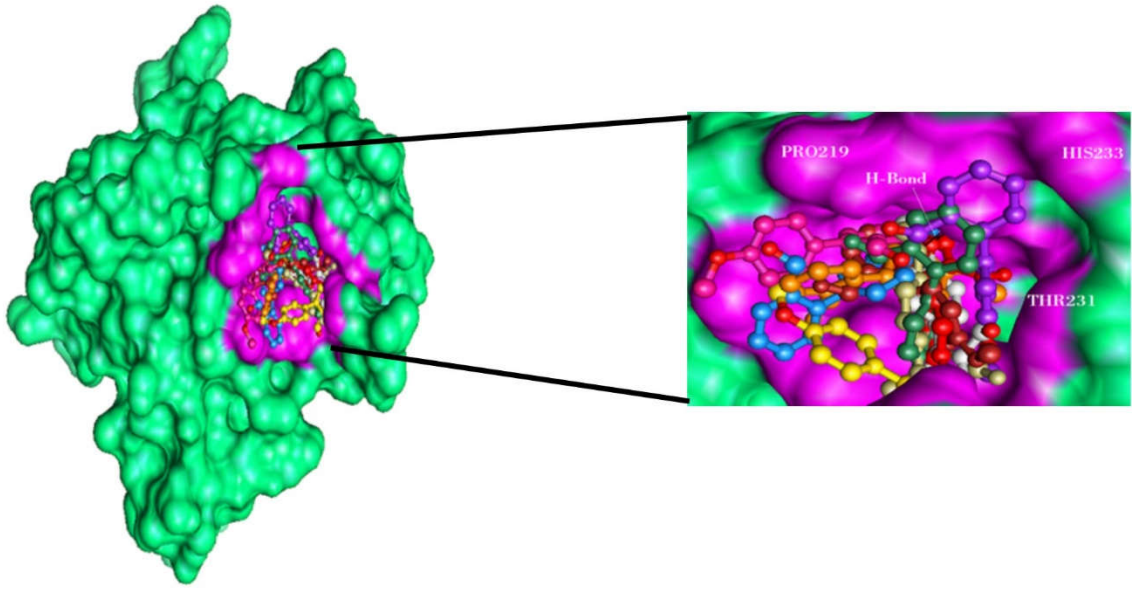

Figure 8 hydrophobic surface of receptor showing all compound in ball and stick model with the best pose in the binding cavity

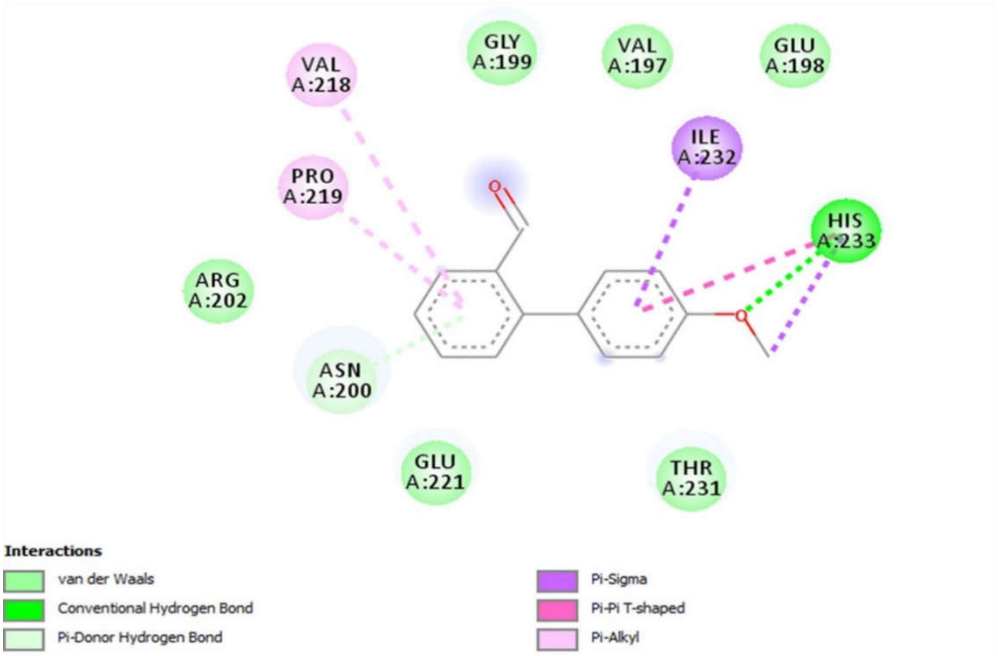

Figure 9: Docking interactions of short-listed candidate inhibitors of p53 4IBS representing different features: dotted lines represent H-bond, pink color represent electrostatic force of attractions and green represents Vander Val forces. The ligand interacts with His233

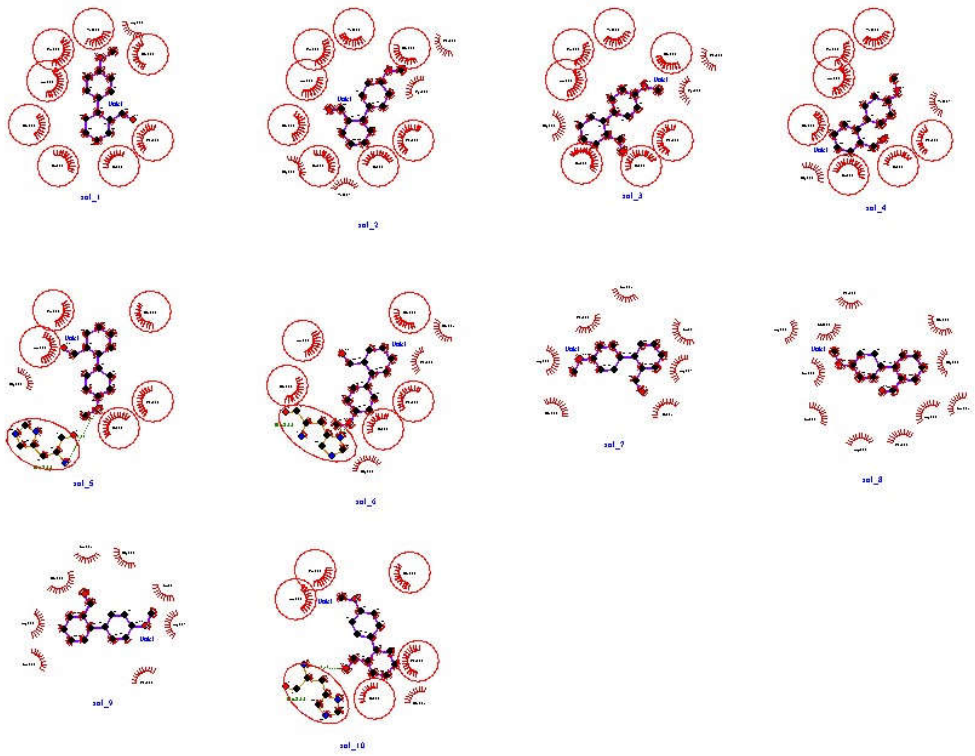

Figure 10 isolated view of 2D interactions of docking complexes

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