European Journal of Biotechnology and Bioscience

Available online at www.biotechjournal.info

E J B B

European Journal of Biotechnology and Bioscience

ISSN: 2321-9122
EJBB 2014; 1 (6): 01-09
Received: 29-05-2014
Accepted: 11-06-2014

V. Ahire
School of Basic Medical Science. University of Pune. Pune. India

G R.Kulkarni
School of Basic Medical Science. University of Pune. Pune. India

Kaushala P Mishra
Nehru Gram Bharati University, Allahabad 211 002, India

S.A.H.Naqvi
Bio Discovery Group, Agra-600017.India

Correspondence:
S.A.H. Naqvi
Bio Discovery Group, Agra-600017.India

Stabilizing p53 mutant Y220C by 1-hydroxy-2-methylanthraquinone and its derivative: a virtual screening, molecular docking and ADMET study

V. Ahire, G. R. Kulkarni, Kaushala P. Mishra, S.A.H Naqvi

ABSTRACT
p53, as a transcription factor, plays an eminent role in tumor suppression. Y220C, a substitution mutation, which cause major structural changes in the protein, is evidenced to form a new protein cavity. This cavity is reckoned to accommodate small drug candidates, which may play a key role in cancer suppression. In the present, study we have tried to determine a drug candidate based on structural drug discovery. Docking simulation on mutated p53 was performed to determine the best drug candidate from the derivatives of 1-hydroxy-2-methylanthraquinon, a known anti-cancer agent. A total of 479 structures had been selected on the basis of molecular fingerprinting towards the 2D crystal structure of 1-hydroxy-2-methylanthraquinone. With a combination of tools and knowledge based method the cavity had been tested for identification of an accurate position vector for molecular docking studies. Molecular Docking was simulated and study has been carried out by using Lamarckian Genetic Algorithm (LGA), a novel conformational search strategy. MGL's Auto Dock 4 which have a free energy scoring function, based on linear regression and uses the AMBER force field for optimization, have been used for Docking studies. The post-docking studies had confirmed fluctuating binding energies along with a few cases (~14%) of structural damage and possible fragmentation. The minimum binding free energy had been recorded within a range between -15.09 and -4.88 kCal/mol. Our studies conform the binding of ligand at the active site cavity as hydrogen bonds and polar contacts between the ligand and the protein cavity.

Keywords: 1-hydroxy-2-methylanthraquinone, In silico screening, Molecular Docking, p53, Small molecule inhibitors, Y220C

1. Introduction

p53 mutation is the most common mutation in human cancer. p53 is triggered by genotoxic effects which lead to stabilization, modification of the post transcriptional processes and recruitment to chromatin. Being a transcription factor, it mediates change is gene expression that decided the cell fate for survival by arresting the cell cycle for repair or death via apoptosis. But mutations in p53 make the cell susceptible for with somatic or germ line cancer. Many of these mutant p53 proteins acquire oncogenic properties that enable them to promote proliferation as well as cell survival. Around 75% of p53 mutations are in the DNA binding domain and about 30% are in the hotspot codon regions [1]. These mutations are associated with the DNA binding domain thermo-stability to varying degrees [2]. Amino acids that are involved in the recognition of specific sequences of the DNA. Some mutations affect distant sites cause defects in the conformation of p53 rendering it inactive. If its native intact form is lost by mutations of single nucleotide, p53 activity is lost [2].

p53 is one of the ideal candidate for designing a drug for cancer therapy. The function of p53 can be restored by stabilizing the destabilized active conformation of mutants. This is achieved by identifying low molecular weight compounds by high throughput screening of chemical libraries [3]. These molecules can serve as lead compounds as novel efficient anti-cancer drugs. Since many tumors express elevated levels of p53, restoring the p53 wild-type function would help in killing tumor cells by inducing apoptotic pathways in tumors. Restoring p53 activity may not only lead to discovery of more potent analogs, but may also suggest new strategies for p53-targeting in tumor therapy. Also APR-246 which is an analog of PRIMA-1 is currently tested in a clinical trial [4].

In the past two decades, the identification of commonly mutated oncogenes and tumor suppressor genes has driven an unprecedented growth in our understanding of the genetic basis of human cancer. While cancer causing genes can help as drug targets which can be inactivated by small molecules, thereby causing a halt to cancer proliferation, the restoration of the activity of mutated tumor suppressor gene by such small molecules might be possible with a rational approach. However, there is a growing realization that many eukaryotic regulatory proteins are partially unfolded and such intrinsically disordered proteins acquire a folded structure after binding to their biological target.

A. Structure of p53

The 393 residue p53 tumor suppressor protein exists in a dynamic equilibrium to form tetramers. Each chain comprises well defined functional domains and natively folded regions (6-8). The N terminal part of the protein consists of the trans-activation domain (residues 1–63) followed by a proline rich region (64– 94). The central domain (p53 core domain) is accountable for binding. The C-terminal region of p53 contains the tetramerization domain (residues 325–356) and the negative regulatory domain at the extreme C terminus (363–393), which contains phosphorylation and acetylation sites and regulates the DNA binding activity of p53 (9-11).It has been found that p53 has a high affinity for genes involved in the cell cycle arrest and low affinity to genes involved in apoptosis (12, 13).

B. Strategy to overcome p53 mutation

p53 in normal cells is present in very small quantities with a half-life ranging in minutes. In majority of cancers its inactivation is found to be the major cause of carcinogenesis as mutant p53 can no longer carry outs functions as a tumor suppressor .This exerts and promotes the tumorigenesis. Its function to responding to stress is compromised. In cancers where this mutation can be reversed; it would help in restoring the function of p53 and thus leading debulking the tumor mass's altered p53 is majority of human cancers; functional restoration seems to be a logical target for cancer therapy. The wild- type function of p53 can be reactivated with the help of small molecule .One of such examples is that a carbazole derivative PhiKan083 found from the in silico databases. PhiKan083 binds to the mutated form of p53 and raises its melting temperature thereby reactivating the functioning of p53 [5].

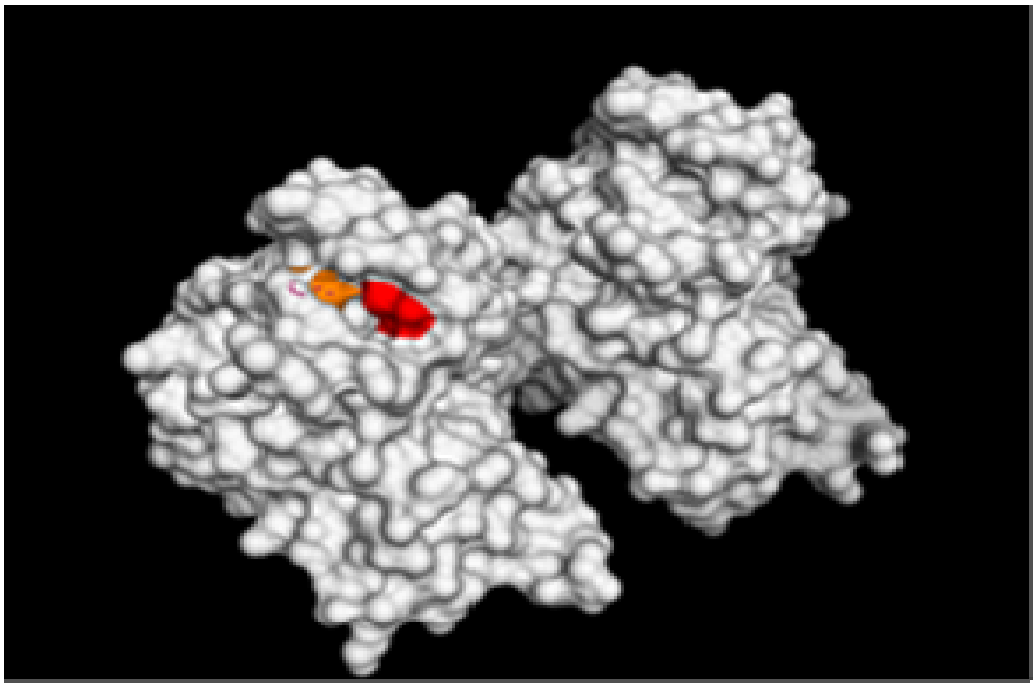


Fig 1: Protein Y220C Mutated p53 [PDB: 2X0W].Red represents V147, orange represents C220 (mutated).

2. Material and Methods

A. Ligand generation

2D equivalent structural derivatives of 1-hydroxy-2-methylanthraquinone were searched in ChemBank {release 2.1.8} and Pub Chem. By using this compound and inbuilt similarity fingerprinting search, molecules with minimum a 0.5 Tanimoto score had been taken. The search generates a total of 3000 ligands. Chem Sketch was used for sketching and generating MDL\Mol v2000 molecules with 2D coordinates. The ligands were converted into Protein Database (PDB) format by Open Babel.

B. Protein

p53 with Y220C mutation had been acquired from RCSB [6] with PDB ID: 2X0W.From the PDB format attached ligands were removed and energy minimization performed by standard optimization parameter of Swiss PDB Viewer [7].

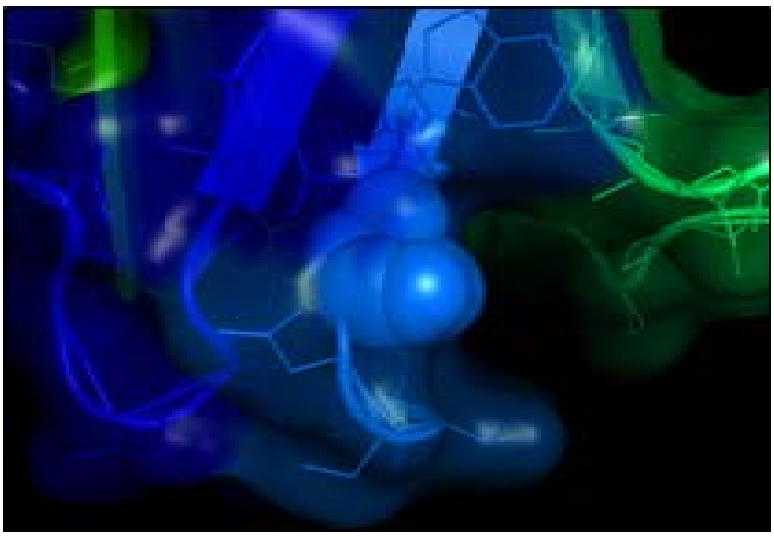
C. Active Site

A cavity near VAL147 is found to fulfill the conditions for the best DNA binding site which can be used as a druggable target. This was validation was confirmed with tools available on online servers like Site Hound and DoGSiteScorer.

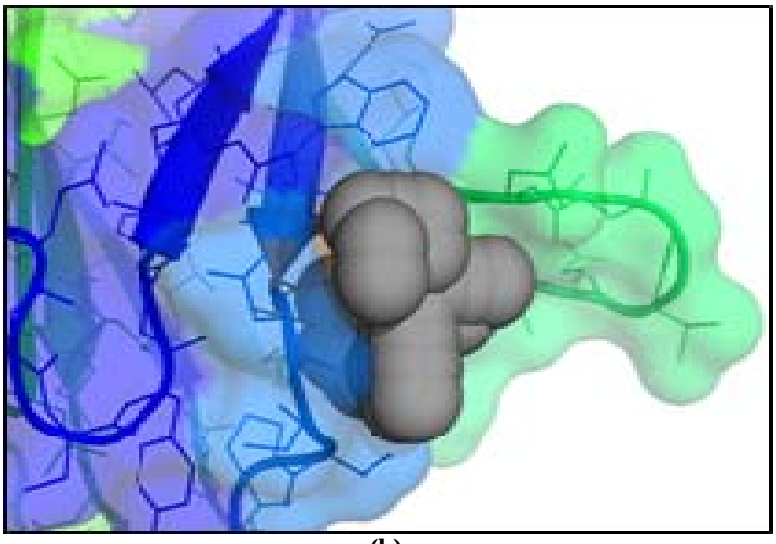
The protein cavity formed due to mutation, near valine 147, was tested by computational methods for drug binding site. Primarily, visualization software, PyMol was used to verify the cavity, followed by active site search by online tools like Site Hound and DoGSiteScorer [8, 9].

D. Docking Setup

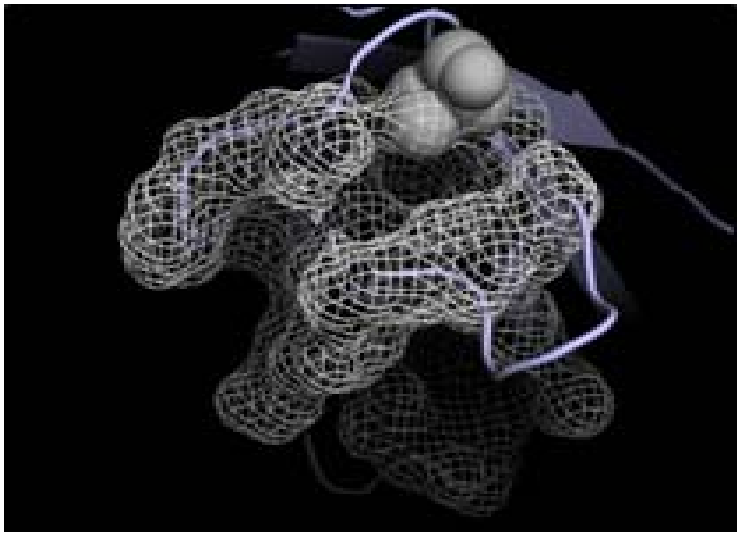
Protein-Ligand docking had been tested by Auto Dock 4.2 [10] which combines energy evaluation through grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein [5]. Docking involved the addition of polar hydrogen to the ligands using the Auto Dock hydrogen module and assigning the Kollam united atom partial charges. A standard docking procedure was used for the mutant p53 and ligands with LGA. It involved randomly placed individuals with a population size of 150. Maximum number of energy evaluations were 2.5 $*10^7$ and the mutation rate was 0.2 with crossing over rate of 0.80.Elitism was 1 for every generation. The results were clustered according to the 1.0 Å rmsd criterion with 10 independent docking runs for each ligand. Auto Grid was used to calculate the grid maps representing proteins. Grid size was set to 60*60*60 points with grid spacing of 0.375 Å. UCSF chimera was used to visualize the co-ordinates of the docked proteins to ligands within a region of 5 Å [11].



(a)



(b)



(c)

Fig 2: Protein Cavity
a) Direct Visualization. Blue sphere represents Valine 147
b) Site Hound search , cavity denoted by grey spheres
c) DoGSiteScorer search, Valine 147, denoted by grey spheres.

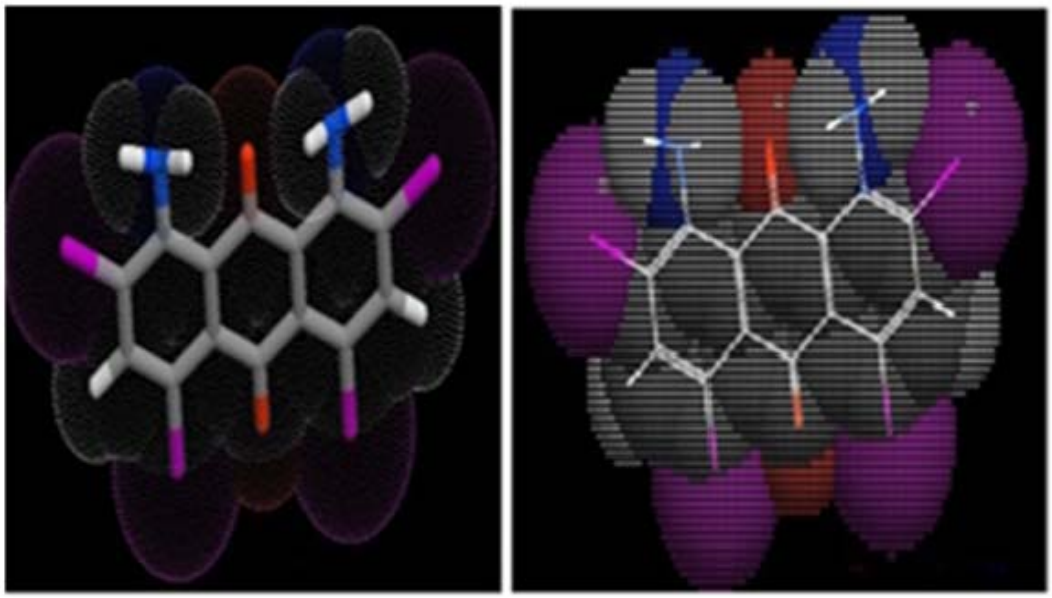


Fig 3a: Structure of SK 3

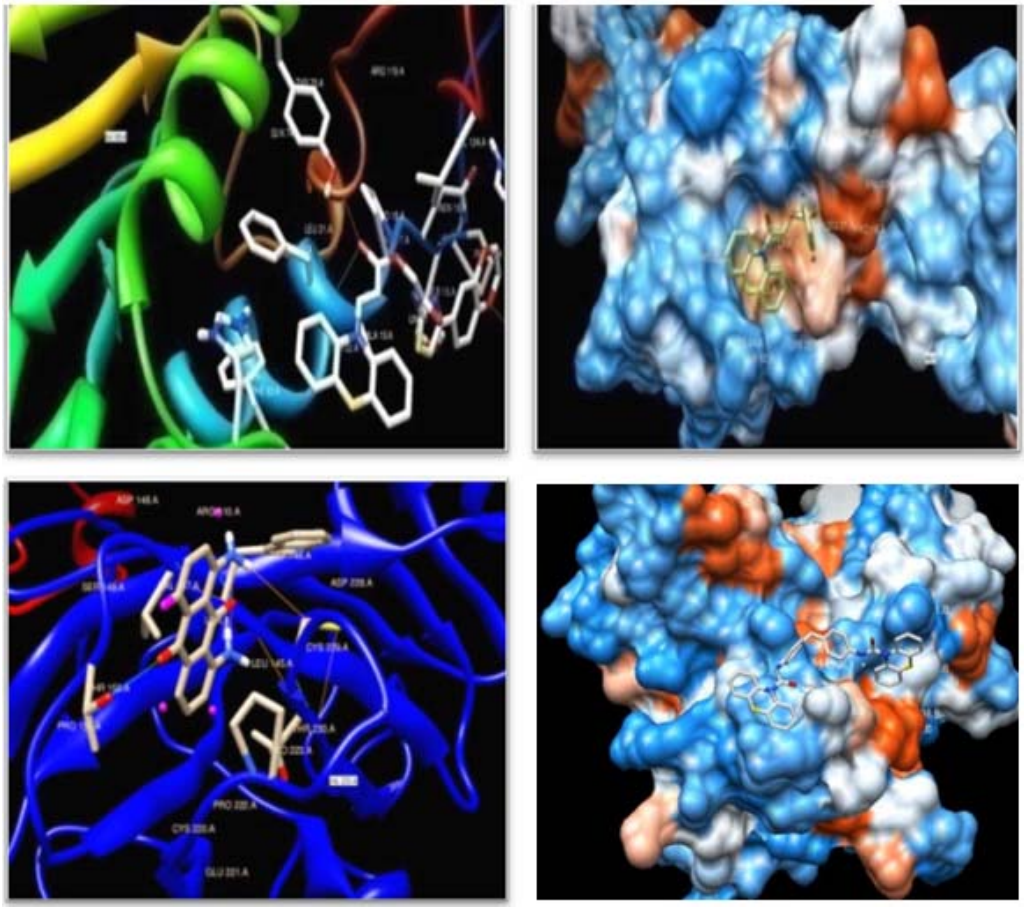


Fig 3b: 3D conformation of H-Bond Interaction

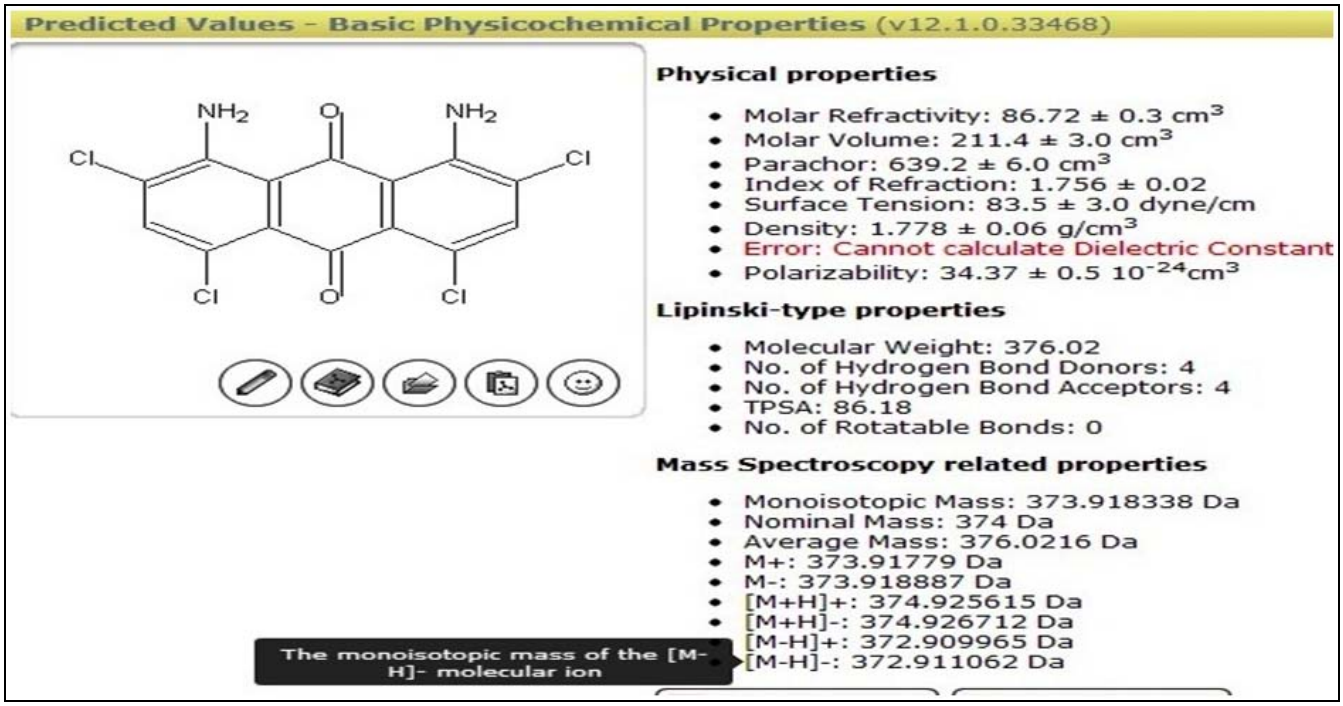
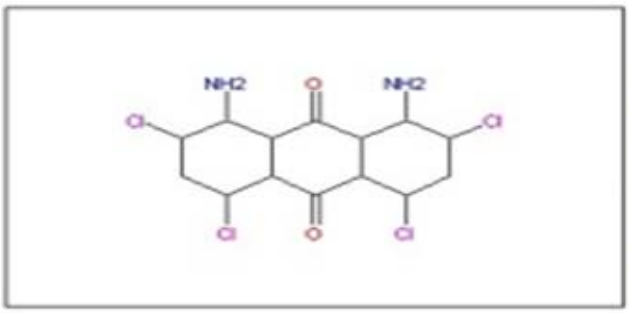


Fig 4a: Physiochemical Properties of SK3



Molecular formula: C14 H18 Cl4 N2 O2
Molecular weight: 386.01
Number of HBA: 4
Number of HBD: 4
MolLogP : 2.79
MolLogS : -4.00 (in Log(moles/L)) 38.16 (in mg/L)
MolPSA : 67.98 A^2
MolVol : 312.60 A^3
Number of stereo centers: 10

Drug-likeness model score: -0.95

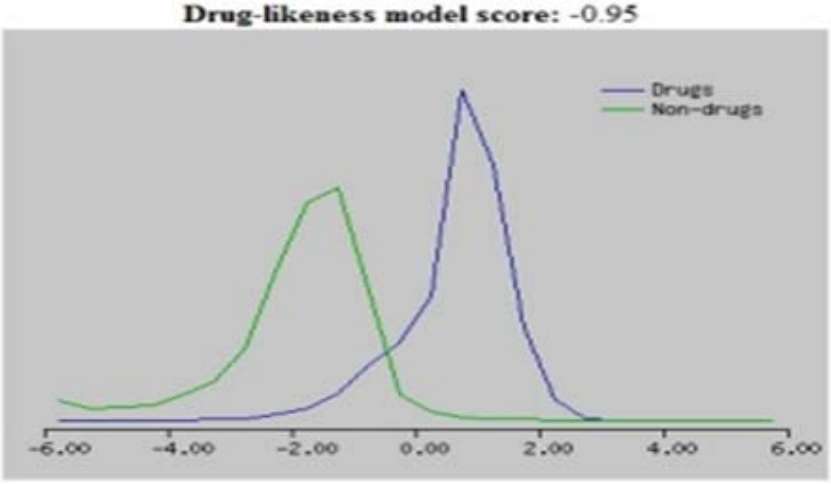
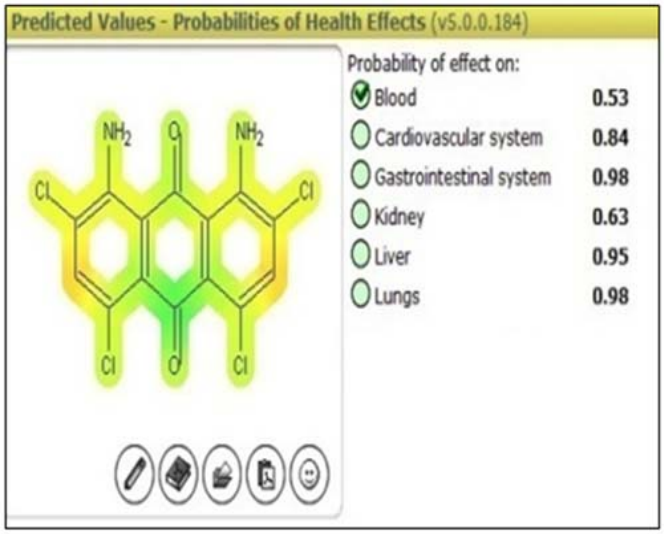
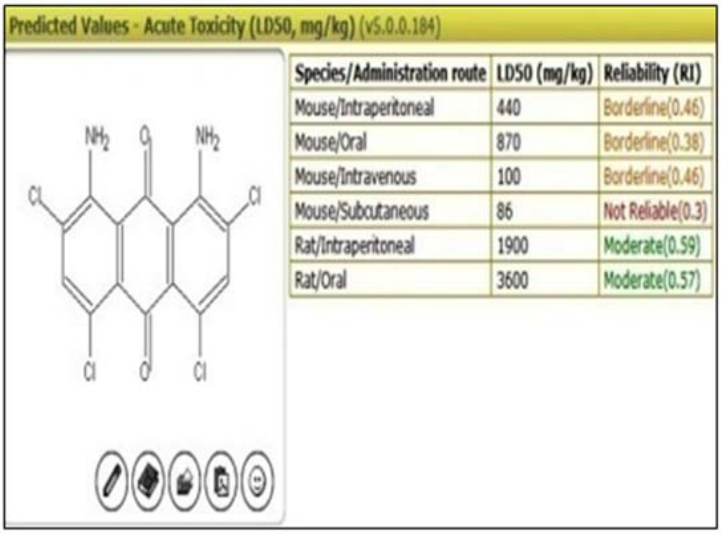


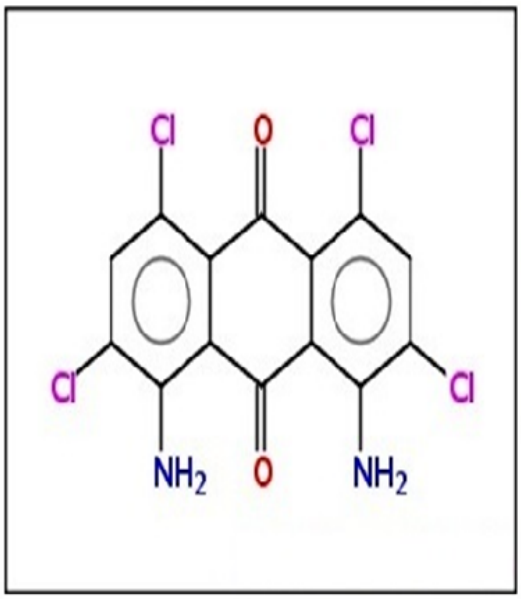
Fig 4b: Molecular Properties and Drug likeliness of molecule SK-3





Species/Administration route	LD50 (mg/kg)	Reliability (RI)
Mouse/Intraperitoneal	440	Borderline(0.46)
Mouse/Oral	870	Borderline(0.38)
Mouse/Intravenous	100	Borderline(0.46)
Mouse/Subcutaneous	86	Not Reliable(0.3)
Rat/Intraperitoneal	1900	Moderate(0.59)
Rat/Oral	3600	Moderate(0.57)

Fig 5a: Health Effects & LD 50 of SK 3



GPCR ligand	-0.06
Ion channel modulator	-0.06
Kinase inhibitor	0.03
Nuclear receptor ligand	-0.33
Protease inhibitor	-0.19
Enzyme inhibitor	0.06

Fig 5b: Bioactivity of SK3

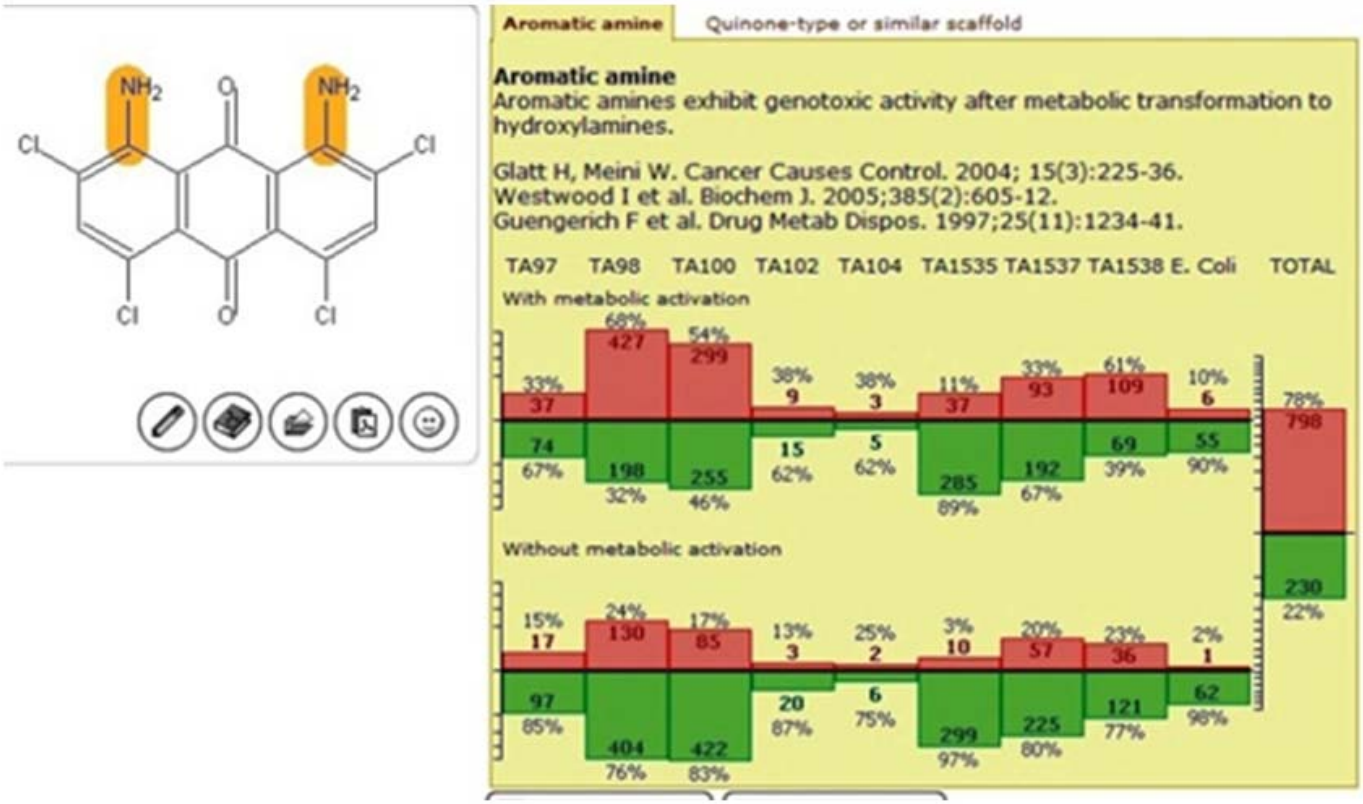


Fig 5c: Geno-toxicity Hazards of SK3

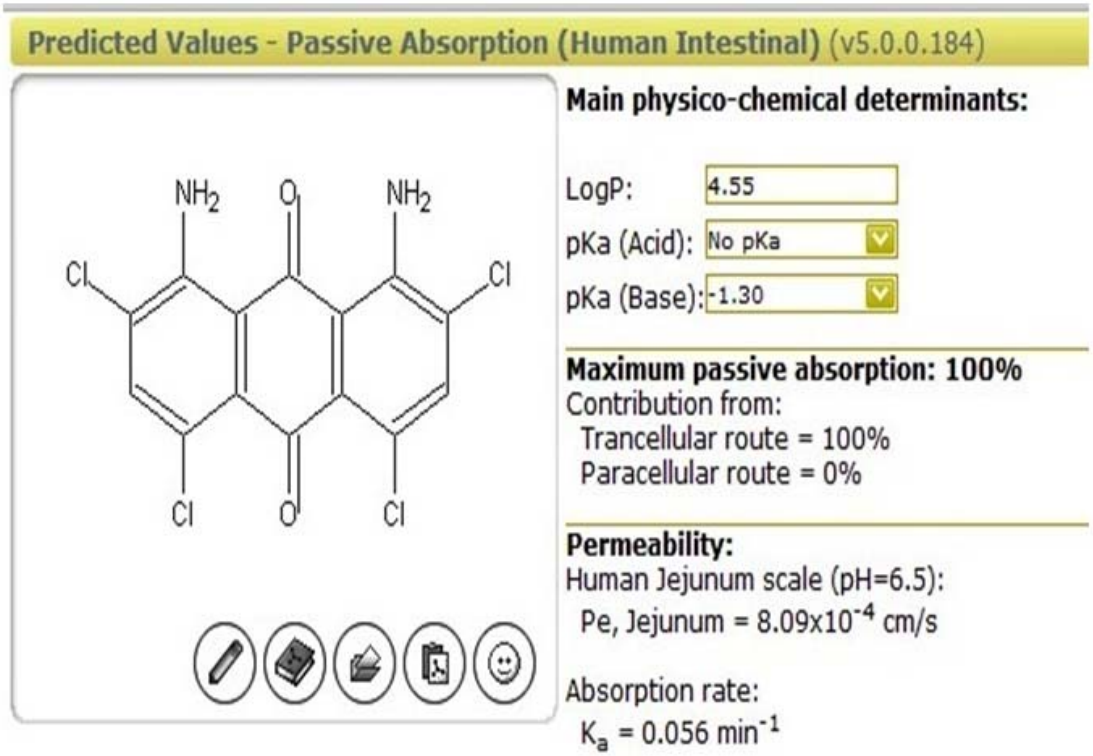


Fig 5d: Passive Absorption of SK3

3. Results and Discussion

A molecular docking approach using Lamarckian Genetic Algorithm was carried towards different derivatives of 1-hydroxy-2-methylanthraquinone. Merging a novel algorithm for speedy binding site identification and assessment with easy-to-use property picturing tools, the software has provided an effectual means to discover and better exploit the characteristics of cavity. Total of 479 molecules were virtually screened on the basis of the structural similarity of 1-hydroxy-2-methylanthraquinone. Molecules like VA-6, SK-5, SK-12, SK-8 and SK-3 which showed H- Bonds with active site residue and promising ADMET results.SK 3 was found to be most potent amongst all. Figure 3a and b depicts the structure of SK3 and its conformation of H-bond interaction. The molecule exhibited drug likeliness score as of -0.95 with Mol PSA as 67.98 A^2 and MolVol as 312.60 A^3. The MolLogS was -4.00 (in Log (moles/L)) 38.16 (in mg/L).Figure 5c presents the geno-toxic hazards of SK3 and figure 5b reveals its partial absorption. The molecule exhibited no indication for mutagenicity, & tumorigenicity. Also, no indication for irritating & reproductive effects found. The LD_{50} calculated for Rat/Intra-peritoneal as 0.59(moderate) and Rat/Oral as 0.57(moderate) is shown in Figure 5c and d. lastly, figure 5a and b show health effects, LD_{50} and bioactivity of SK3. The maximum passive absorption (Human Intestinal), transcellular route is 100 %. Permeability for Human Jejunum scale (pH 6.5) is $8.09X13^{-4}$ cm/s and absorption rate, Ka is 0.056 min^{-1}.Further *in vitro* and *in vivo* study is required on this molecule to design new derivatives with higher potency and specificity.

4. Conclusions

Based on the Molecular Docking & ADMET study of 479 molecules we found that 1 molecule SK-3 showed better affinities with the active site residue of p53 core domain mutant Y220C. The molecule follows Lipinski's Rule of 5 and showed promising Drug Likeness score.

Human Oral Bioavailability of the compound SK-3 is between 30% and 70% and Probability that compound has: %F(Oral) > 30%: 0.884and %F(Oral) > 70%: 0.432. Probability of positive Ames test: 0.32 and Volume of distribution (Vd): 4.33 L/kg was Hydrophobic neutral drug (no acid groups with pKa<7.5, no base groups with pKa>=7, logP>1). Most of the drugs in this group have moderate Vd values. (90% of values are in the range of 0.5-10 L/kg). Vd can be larger for very hydrophobic drugs.

The extent of the work stretches to the in silico approach for determining the binding mode. Further there is need to generate *in vitro* and *in vivo* activity of the generated data to synthesize and test so to design new drugs with better specificity and metabolism.

5. Acknowledgement

We thank UGC, Government of India for the grant of Junior Research Fellowship (JRF) to Ms. Vidhula Ahire.

6. Conflict of Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

7. References

1. Petitjean A, Mathe E, Kato S *et al*. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. Hum Mutat 2007; 28:622-629.
2. Bullock AN, Fersht AR. Rescuing the function of mutant p53.Nat Rev Cancer 2001; 1:68-76.
3. Wang W, Rastinejad F, El-Deiry WS. Restoring p53-

dependent tumor suppression. Cancer Biol Ther. 2003; 2(4 Suppl 1):S55-63.
4. Lane DP, Hupp TR. Drug discovery and p53. Drug Discov Today. 2003; 15:8(8):347-55.
5. Boeckler FM, Joerger AC, Jaggi G, Rutherford TJ, Veprintsev DB, Fersht AR. "Targeted rescue of a estabilized mutant of p53 by an in silico screened drug," Proceedings of the National Academy of Sciences of the United States of America, 2008; 105(30)10360–10365,
6. http://www.rcsb.org/
7. Guex N. Peitsch MC. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling, 1997.
8. Volkamer A *et al*. Analyzing the topology of active sites: on the prediction of pockets and subpockets. J. Chem. Inf. Model. 2010; 50(11):2041-52
9. Volkamer A *et al*. Combining global and local measures for structure-based druggability predictions. J. Chem. Inf. Model. 2012; 52:360-372
10. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS *et al.,* Auto dock 4 and Auto DockTools4: automated docking with selective receptor flexiblity. J Computational Chemistry 2009; 16:2785-91.
11. http://www.cgl.ucsf.edu/chimera