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Research Article

SYNTHESIS AND MOLECULAR DOCKING STUDIES OF 2 CHOLROMETHYL-3-METHYL-1-PHENYL SULFONYL-1H-INDOLE COMPOUND

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ABSTRACT

The p73 protein of the p53 family, has tumor suppressor activity. But its multiple isoforms possess different and sometimes opposing functions. It participates in the apoptotic response to DNA damage. It is phosphorylated in a cell cycle-dependent manner and negatively regulated by CDKs. When overproduced, it activates transcription from p53-responsive promoters and induces apoptosis.

The second increasing cause of the death after the cardiovascular diseases is cancer which is multifactorial, multifacitile, and multimechanistic disease. An attempt has been made to control such life threating diseases by synthesizing antitumor indole based compound and characterized through X ray diffraction methods. A synthesised compound was recognised as antitumor and antibacterial agent which have been studied by targeting p53Family using molecular docking studies.

Keywords: 2 Cholromethyl-3- methyl-1-phenyl sulfonyl-1H-indole, p53, molecular docking, targeted drug delivery

INTRODUCTION

The current scenario of the tumour and cancer, resulting in increase of mortality rate is alarming. According to WHO there is tremendous rise in the probability of prevalence of cancer in most of the tumours found in the breast, prostate, ulceritis, brain etc. Hence the management of the disease is a tedious process. But still the investigations reported the natural remedies and are under trial to stop this tumour cell cycle but yet there are no promising results. Keeping this in mind, this study has been planned. In this study 2 Cholromethyl-3- methyl-1-phenyl sulfonyl-1H-indole compound was studied at molecular level, pyrone and benzene rings play an important role in many areas of the medicine, X- ray diffraction studies were carried out to identify the details in intermolecular interaction.[1, 2] Current research on RNA dependent DNA polymerase enzyme is still at nascent stage. p73 family proteins were retrieved from the Protein Data Bank and the compound was subjected to docking for binding capacity confirmation studies [3]. Protein involved in this study are gene expression protein which allows cell cycle to mutate which is then turns to mass of cell collectively called as the tumour. Focus of the study is to be identify the binding capacity of the compound to associated targets so that future drug development can be planned out easily on these lines.

MATERIALS AND METHODS

Synthesis of the compounds

A mixture of 1-phenylsulfonyl-2,3-dimethylindole (5 g, 17.5 mmol) and finely powdered NCS (2.56 g, 19.17 mmol) in dry CCl_4 (80 ml) containing catalytic amount of benzoyl peroxide (0.1 g) was refluxed for 1 h and cooled. The floated Succinamide was filtered off and washed with CCl_4 (15 ml). The solvent was then removed completely under vacuo and recrystallized from $CDCl_3$

X ray diffraction analysis

Data was collected on a Bruker Kappa APEX II diffractometer using ω and φ scan mode with the range reflections $2.2 \leq \theta \leq 28.8^{\circ}$ using MoKα radiation. A total of 17616 reflections were collected, resulting in 3885 independent reflections of which 3201 had $I > 2\sigma(I)$. The intensities were corrected for Lorentz and polarization effects. The structure was solved by direct methods using SHELXS 97 program and final R-factor was 0.024. [1, 2]

COMPUTATIONAL TOOLS AND SERVERS

Physico-Chemical Parameters for protein prediction

The Physico-Chemical Parameters such as theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [4], half-life[5-8], instability index [9], aliphatic index [10] and grand average hydropathy (GRAVY) [11] amino acid composition by CLC bench were computed using the Expasy's Protparam (http://us.expasy.org/tools/protparam.html) prediction server and tabulated in **Table 1**,**2**

Table 1: Amino acid composition (in %) of desired proteins computed using CLC free Work Bench tool

Ala	Amino acids	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Tyr	trp	Val	Pyl	sec
6.6	5.0	3.9	4.3	1.7	6.1	5.4	7.7	3.8	3.8	7.4	3.6	3.0	3.0	9.1	9.8	6.1	0.6	3.1	5.8	0.0	0.0

Table 2: Parameters computed using Expasy's Prot Param tool

	Sequence Length	Mol. Wt	pI	-R	+R	EC	II	AI	GRAVY
P	635	69492.1	6.49	61	55	52425	6.41	67.12	-0.524

Secondary Structure Prediction

The tools SOPM, SOPMA [12] and SSCP (Secondary Structural Content Prediction) server [13] were used for the secondary structure prediction. **Table 3**

Secondary structure prediction of the p73 family protein

```
        10        20        30        40        50        60        70
        |         |         |         |         |         |         |
AQSTATSPDGGTTFEHLWSSLEPDSTYFDLPQSSRGNNEVVGGTDSSMDVFHLEGMTTSVMAQFNLLSST
hhettccccchhhhhhhhhhhcttccccccccccccceeeeccccccceeeeeeccccccchhhhhhhhhh
MDQMSSRAASASPYTPEHAASVPTHSPYAQPSSTFDTMSPAPVIPSNTDYPGPHHFEVTFQQSSTAKSAT
hhhhhhhccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccce
WTYSPLLKKLYCQIAKTCPIQIKVSTPPPPGTAIRAMPVYKKAEHVTDVVKRCPNHELGRDFNEGQSAPA
eecchhhhhhhhhhhttccceeeeeccccccccceeeeccceecchhhhhhhhhhhccccccccccccccccc
SHLIRVEGNNLSQYVDDPVTGRQSVVVPYEPPQVGTEFTTILYNFMCNSSCVGGMNRRPILIIITLEMRD
ceeeeccccccheeeeccccccceeeeeccccccccchhhhhhhhhhhhccccccccccccceeeeeeecctt
GQVLGRRSFEGRICACPGRDRKADEDHYREQQALNESSAKNGAASKRAFKQSPPAVPALGAGVKKRRHGD
ccceeccceeeeeeeccttccccchhhhhhhhcccccccccccccccccccccccccccchhhhccccctt
EDTYYLQVRGRENFEILMKLKESLELMELVPQPLVDSYRQQQQLLQRPSHLQPPSYGPVLSPMNKVHGGM
cceeeeeettccchhhhhhhhhhhhhhhhhcctthhhhhhhhhhhhhcccccccccccchhccccccchh
NKLPSVNQLVGQPPPHSSAATPNLGPVGPGMLNNHGHAVPANGEMSSSHSAQSMVSGSHCTPPPPYHADP
ccccccccccccccccccccccccccccccccccceeccccccccccccccccccccccccccc
SLVSFLTGLGCPNCIEYFTSQGLQSIYHLQNLTIEDLGALKIPEQYRMTIWRGLQDLKQGHDYSTAQQLL
hhhhhhhhttccchhhhhhhhttchhhhhhhhccchhhhhhhhccccccchhhhhhhhhhhttcccccchhh
RSSNAATISIGGSGELQRQRVMEAVHFRVRHTITIPNRGGPGGGPDEWADFGFDLPDCKARKQPIKEEFT
hhccccceeeccccccccthhhhhhheeccceeeeccccccccccccchheeeeccccccccccchhhhhh
EAEIHhhhhh
```

Sequence length: 635

Table 3:Secondary structure prediction using SOPMA

Alpha helix (Hh)	:173 is 27.24%
3_{10} helix (Gg)	: 0 is 0.00%
Pi helix (Ii)	: 0 is 0.00%
Beta bridge (Bb)	: 0 is 0.00%
Extended strand (Ee)	: 82 is 12.91%
Beta turn (Tt)	: 23 is 3.62%
Bend region (Ss)	: 0 is 0.00%
Random coil (Cc)	: 357 is 56.22%
Ambigous states (?)	: 0 is 0.00%
Other states	: 0 is 0.00%

Identification of Transmembrane region

The SOSUI server [14] performed the identification of transmembrane regions the predicted transmembrane helices were visualized and analysed using helical wheel plots generated by the program Pepwheel [15] included in the EMBOSS 2.7 suite. Showed in **(Fig.2, 3)**

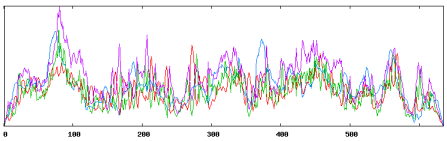


Figure 1: Graphical representation of Amino acid position

Presence of SS bonds

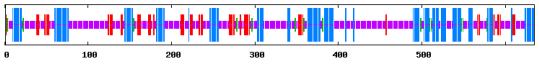


Figure 2: Transmembrane regions identified by SOSUI server.

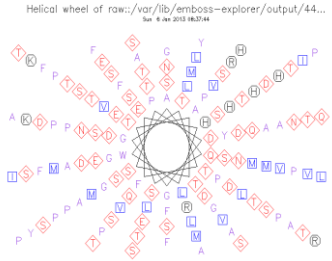


Figure 3: Helical wheel representation of predicted helix of p73 protein.

The presence of SS bond and their bonding patterns were predicted by CYS_REC [16] and RASMOL server. CYS_REC (http://linux1.softberry.com/berry.phtml) identified the position of a cystiene, total number of cystiene presented along with the most probable SS bond pairs in the protein sequences (Table 4).

Disulphide (SS) bond pattern of pairs predicted, by CYS_REC (using primary structure) and identified by RASMOL (using 3D structure modelled).

CYS	167	SS-bounded	Score=21721920.0
CYS	173	SS-bounded	Score=21721916.0
CYS	212	SS-bounded	Score=21721868.0
CYS	282	SS-bounded	Score=21721916.0
CYS	287	SS-bounded	Score=21721946.0
CYS	323	SS-bounded	Score=21721958.0
CYS	325	SS-bounded	Score=21721960.0
CYS	528	SS-bounded	Score=21721942.0
CYS	551	SS-bounded	Score=21721932.0
CYS	554	SS-bounded	Score=21721928.0
CYS	679	SS-bounded	Score=21721916.0

Pocket finding

In addition to protein characterization, protein retrieved from the database was analyzed for pockets before docking studies, to ensure the possible number of binding sites of protein and ligand. The presence of amino acid position has been analysed by the web tool Pocket finder (Table 5).

Possible pocket sites in given protein

Min. Coords: (-9, 49, 72)
Max Coords: (7, 69, 96)
Predicted site 1
Site Volume: 719 Å^3
Protein Volume: 83487 Å^3

X ray Diffraction analysis

CRYSTAL AND EXPERIMENTAL DATA	
EMPIRICAL FORMULA	$C_{16}H_{14}ClNO_2S$
TEMPERATURE	295 K
FORMULA WEIGHT	319.79
WAVELENGTH	0.71073 Å
CRYSTAL SYSTEM	Monoclinic
SPACE GROUP	$P2_1/c$
UNIT CELL DIMENSIONS (Å)	a= 7.9769(6)
	b=10.8064(9)
	c=17.3418(12)
UNIT CELL DIMENSIONS	β=97.500(2)°
VOLUME Å^3	1482.1(2)° Å^3
Z	4
CALCULATED DENSITY	1.433mg/m^3
ABSORPTION COEFFICIENT	0.40mm^{-1}
REFINEMENT METHOD	Full matrix
F(000)	664
CRYSTAL SIZE	0.30X0.28X0.26 mm
θ – RANGE FOR DATA COLLECTION	2.2 to 28.8°
GOODNESS-OF-FIT ON F^2	1.04
REFLECTIONS COLLECTED/UNIQUE	17616/3201
R-FACTOR	0.024

Computational molecular docking studies

Crystallographic structures of protein p73 family were retrieved from the RCSB database with PDB ID, computational analysis was done to compute ligand protein binding affinity of the compound. Docking calculations were carried out using Docking Server [17]. The MMFF94 force field [18] was used for energy minimization of ligand molecule (indole2) using Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined.

Docking calculations were carried out on p73 protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. Affinity (grid) maps of 20×20×20 Å grid points and 0.375 Å spacing were generated using the Autogrid program [19]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic termsrespectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [20]. Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

RESULTS

Molecular docking Studies

The structures of the ligands were drawn using tool Chembiodraw 11.0. Fig.1 and converted into PDB format using Molecular conversion tool VCC lab online server[9, 10]. The Crystallographic structures of protein associated p73 family were retrieved from the RCSB database with PDB ID 2WQJ were docked with ligand using Autodock 4.0 with authenticated Lamarckian genetic algorithm. This is clearly depicted in the docking (Fig.2, 3). The interaction is showing efficient docked score viz.,-6.51 kcal/mol which is considered as a good score in ligand- protein interactions.

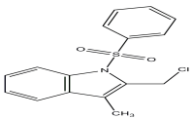
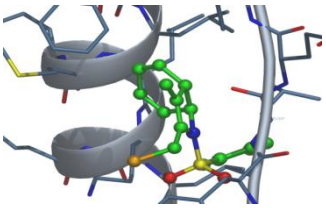
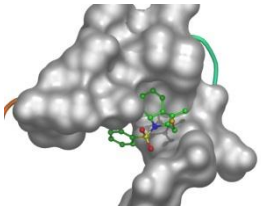
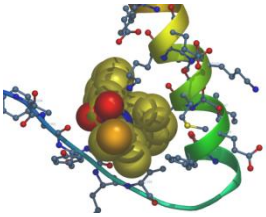


Figure1: Ligand (2 Cholromethyl-3- methyl-1-phenylsulfonyl-1 H-indole compound)







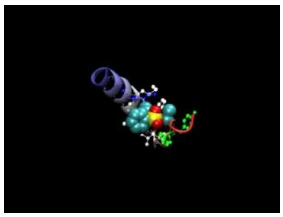


Table 4:Energy values of docked 2 Cholromethyl-3- methyl-1-phenylsulfonyl-1H-indole compound ligand with tumour suppressor receptors with maximum poses.

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1.	-6.51 kcal/mol	16.99 uM	-7.39 kcal/mol	-0.09 kcal/mol	-7.48 kcal/mol	60%	643.553
2.	-6.23 kcal/mol	27.19 uM	-7.29 kcal/mol	-0.09 kcal/mol	-7.38 kcal/mol	10%	636.53
3.	-5.78 kcal/mol	57.56 uM	-6.76 kcal/mol	+0.01 kcal/mol	-6.75 kcal/mol	20%	643.018
4.	-5.09 kcal/mol	185.30 uM	-6.05 kcal/mol	-0.12 kcal/mol	-6.17 kcal/mol	10%	645.183

Table 5: possible interaction of the polar and non polar amino acid

Interaction Table

polar			hydrophobic			other		
O1 (*14*) [*3.64*]	–	LYS372 (*NZ*)	C1 (*1*) [*3.43*]	–	LEU357 (*CB*)	N1 (*5*) [*3.11*]	–	LEU357 (*CB*)
			C2 (*2*) [*3.61*]	–	LEU357 (*CB*)	S1 (*13*) [*3.83*]	–	LEU357 (*CB*)
			C3 (*3*) [*3.54*]	–	LEU357 (*CB, CD2*)	O2 (*15*) [*3.56*]	–	LEU357 (*CB*)
			C4 (*4*) [*3.43*]	–	LEU357 (*CB, CD2*)	C15 (*20*) [*3.76*]	–	LYS372 (*CB, CD, CG*)
			C12 (*17*) [*3.31*]	–	LEU357 (*CB, CD2, CG*)	C16 (*21*) [*3.18*]	–	LYS372 (*CD, CG*)
			C13 (*18*) [*3.59*]	–	LEU357 (*CD2*)	O1 (*14*) [*3.49*]	–	LYS372 (*CD*)
			C3 (*3*) [*3.33*]	–	VAL359 (*CG2*)			
			C5 (*6*) [*3.45*]	–	VAL359 (*CG2*)			
			C5 (*6*) [*3.27*]	–	PHE365 (*CD1, CE1*)			
			C6 (*7*) [*3.63*]	–	PHE365 (*CD1, CE1*)			
			C9 (*10*) [*3.73*]	–	PHE365 (*CD1, CE1*)			
			C5 (*6*) [*3.77*]	–	LEU368 (*CB*)			
			C6 (*7*) [*3.32*]	–	LEU368 (*CB*)			
			C7 (*8*) [*3.66*]	–	LEU368 (*CB*)			
			C6 (*7*) [*3.52*]	–	MET369 (*CG*)			
			C7 (*8*) [*3.65*]	–	MET369 (*CG*)			

CONCLUSION

In recent years, there has been considerable emphasis on the anti-carcinogenic agents, laboratory based synthesized compound have more access towards drug development. Antitumor properties of indole based compounds has been already published, Docking studies are the presently promising tool towards the drug development. In the present study, protein of p53 family expresses and induce the tumor which is known to be tumor suppressor as well as playing the significant role in apoptotic response in DNA damages, such a serious life threatening protein p73 has been widely studied and ligand showing good poses after structure based interaction studies. This interaction plays a significant role in structure based drug designing. The study of protein nature its composition and elaborative models provides targeting in docking. Current score surely explains positive correlation between the docked ligand and receptor by perfect score. To explore more research Insilco it is to be concluded that the synthesized compound was found effective and need more validation using dynamic studies for proper validation of the drug. Also wide open frame drug research can be suggested by this investigation.

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