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HOMOLOGY MODELING OF TUBULIN β -1 CHAIN AND ITS DOCKING STUDY WITH COLCHICINE ANALOGUES

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ABSTRACT

Present study involves the homology modeling of human tubulin β -1 chain(TUBB1) using known protein structure of common pig tubulin. Its 3-D structure was evaluated and validated using PROCHECK comprising 96.8% amino acid residues in favored and allowed region of Ramachandran plot. Stability of the structure was confirmed by the program ERRAT having the overall quality factor as 83.476 and Verify_3D result with 83.85% residues having 3D-1D score > 0.2. With the structure of TUBB1 generated, flexible docking was performed using GLIDE. Results indicate that among 123 colchicine analogues, CID 5370577 was found as best interacting with TUBB1 having lowest dope score of -6.45. Particularly residues TYR208, SER172 and GLU181 of TUBB1 at bond length 2.272, 1.997 and 2.249 respectively form hydrogen bonds and showed strong nonbonding interaction with analogue (CID 5370577). Further the generation of different derivatives can be made by the modification in the moieties of CID 5370577. These derivatives can be used to develop effective drugs against acute gouty arthritis as well as cancer.

KeyWords: Colchicine, docking, drug design, molecular modeling, TUBB1.

INTRODUCTION

Identification of ligand-receptor interactions is important for drug design and the treatment of diseases. The formation and dissociation of specific noncovalent interactions between varieties of macromolecules play a crucial role in the function of biological systems.

Tubulin β -1 chain (TUBB1) is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the β chain and one at a non-exchangeable site on the α -chain. It is the complete sequence of about 451 residues having the molecular weight of 50328. Its major function is macromolecule (eg: protein etc) metabolism, organelle organization and biogenesis, protein polymerization. It also shows GTPase activity, hydrolase activity (acting on acid anhydrides, in phosphorus Containing anhydrides), nucleoside triphosphatase activity and pyrophosphatase activity. (<http://www.drugbank.ca/drugs/DB01394>).

Colchicine is a plant derivative, originally extracted from the bulb of *Colchicum autumnale*. It is an anti-inflammatory drug that has been in continuous use for more than 3000 years [1]. Its extract was first described as a treatment for gout in *De Materia Medica* by Pedanius Dioscorides in the first century. In August 2009, colchicine won Food and Drug Administration (FDA) approval in the United States as a stand-alone drug for the treatment of acute flares of gout and familial Mediterranean fever [2]. Colchicine has been demonstrated to dramatically abrogate the inflammatory response to urate crystals in humans. Colchicine by it for the treatment of acute attacks of gouty arthritis, before prophylaxis during the intercritical phase and in the late chronic tophaceous phase of the disease, before prophylaxis of Familial Mediterranean Fever attacks, and prevention of the development of amyloidosis in this disease. Colchicine has been showed to be effective in the treatment of articular, cutaneous and mucosal symptoms and sarcoidosis. Colchicine by it is a neutral and highly lipid-soluble compound at physiologic pH (measure of acid strength [pKa] = 12.8, molecular weight [MW] = 398), which enables its rapid passage into body tissues.

Tubulin-colchicine interaction has attracted a great deal of attention, probably because of its unusual chemical characteristics. The drug binds exceptionally slowly but noncovalently to tubulin and once formed the drug-protein complex is highly stable. [3]

In the present study structure modeling was performed by the program Modeler 9v3. Homology modeling relies on the identification of one or more known protein structures likely to resemble the structure of the query sequence, and on the production of an alignment that maps residues in the query sequence to residues in the template sequence [4]. The predicted model was validated with Procheck, What_Check, Verify_3d, Errat etc (<http://nihserver.mbi.ucla.edu/SAVES/>). These programs give the different information about the structural properties of modeled protein.

Schrodinger Inc docking software Glide 5.0 is frequently used to predict the binding orientation of small molecule drug to their protein targets in order to predict the affinity and activity of the small molecule. Docking plays an important role in the rational design of drugs. [5]

MATERIALS AND METHODS

Collection of sequences and Selection of template

Information about Colchicine, its various targets and the protein sequence of the target protein were retrieved from Drug bank, UniProt/SwissProt database (<http://www.uniprot.org/uniprot/Q9H4B7>). Template was selected by homology search of query protein (TUBB1) sequence against the databases available on PDB (<http://www.rcsb.org>) through BlastP [6]. Homologous structure of sequence having the lowest E-value, 50% and above identity, lower resolution was selected as template.

Generation of 3-D structure through homology modeling

Homology modeling was done using Modeler 9v3 [7, 8, 9]. This requires one sequence of known 3D structure with significant similarity with the target sequence and Python 2.5 script files containing Modeler commands. The co-ordinate file of template from PDB was used as such.

Evaluation and validation of the 3-D structure

All predicted 5 models were evaluated by Procheck [10], Errat [11] and Verify_3D [12]. Ramachandran plot statistics was used to evaluate the stability of the model. Gnuplot was finally used to plot the profiles generated by Modeler (<http://www.gnuplot.info>) to validate substantially the structure.

Virtual screening of flavopiridol analogues through molecular docking

Colchicine and its analogues were taken from NCBI Pub-chem in SDF format and converted to 3-D structure in PDB file format using weblab viewer lite program. Total 123 analogues were used in the study. The 3-D structure of tubulin beta-1 chain and colchicine 3-D analogues were used for molecular docking using GLIDE program [13].

Protein structure accession number

The refined homology model of 3D structure of human TUBB1 chain protein was submitted to PMDB [14] and the same was assigned the identifier PM0076390.

RESULTS AND DISCUSSION

Search for template for the protein sequence of human TUBB1 in Protein Data Bank through RCSB BLAST has generated 49 homologous structures. Most probable homologous proteins are listed in table-1. Among them NMR structure of tubulin protein of *Sus scrofa*, also known as common pig (PDB ID - 2HXF) was selected on the basis of its NMR structure, high identity (77%) and lowest E-Value (0) (Table-1).

Table No. 1: Homologs of tubulin beta-1 chain for selection of template.

S. No.	Templates	No. of Residues	Resolution	E value	Identity (%)
1.	1TVK	427	NMR Structure	0	78
2.	1TUB	427	NMR Structure	0	78
3.	1IA0	430	NMR Structure	0	77
4.	1JFF	430	NMR Structure	0	77
5.	2HXF	430	NMR Structure	0	77
6.	2HXH	430	NMR Structure	0	77

The protein sequences of Human TUBB1 containing α and β chain of tubulin along with the target, Kinesin-like protein KIF1A (PDB ID-2HXF) were aligned and shown in figure 1. The asterisk showed the identity of amino acids present in the three protein sequences. By using 2HXF as a template Modeler 9v3 has predicted five 3D models of our query protein TUBB1. The best model was selected according to its lowest Dope score (Table 2). Presence of maximum numbers of H-bonds, Helices, Strands and Turns of the model TBC1.B99990003.pdb also confirm that the selected structure was more compact than the other structure predicted. (Table 2). The 3D model of the protein has been depicted (Figure 2) in which α -helix, extended β -sheet, bridge β -turn, turns and coils are shown in purple, yellow, tan, cyan and white color.

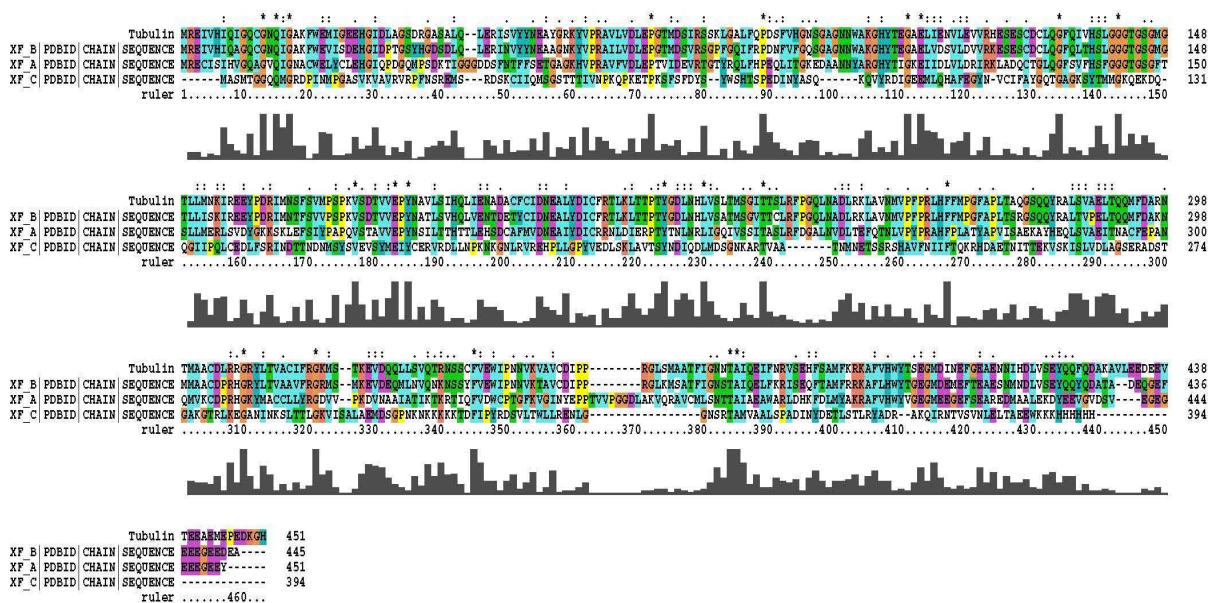


Figure 1 – MSA Result of human tubulin beta-1 chain with template sequence

Table No 2: Dope score and information about H-Bonds, Helices, Strands and Turns of the five models generated.

S. No.	Models	Dope score	H-Bonds	Helices	Strands	Turns
1.	TBC1.B99990001.pdb	-47431.36328	311	18	13	46
2.	TBC1.B99990002.pdb	-47310.61719	310	18	13	42
3.	TBC1.B99990003.pdb	-47660.28516	313	18	13	46
4.	TBC1.B99990004.pdb	-47345.20312	313	17	15	46
5.	TBC1.B99990005.pdb	-47410.92188	305	18	13	45

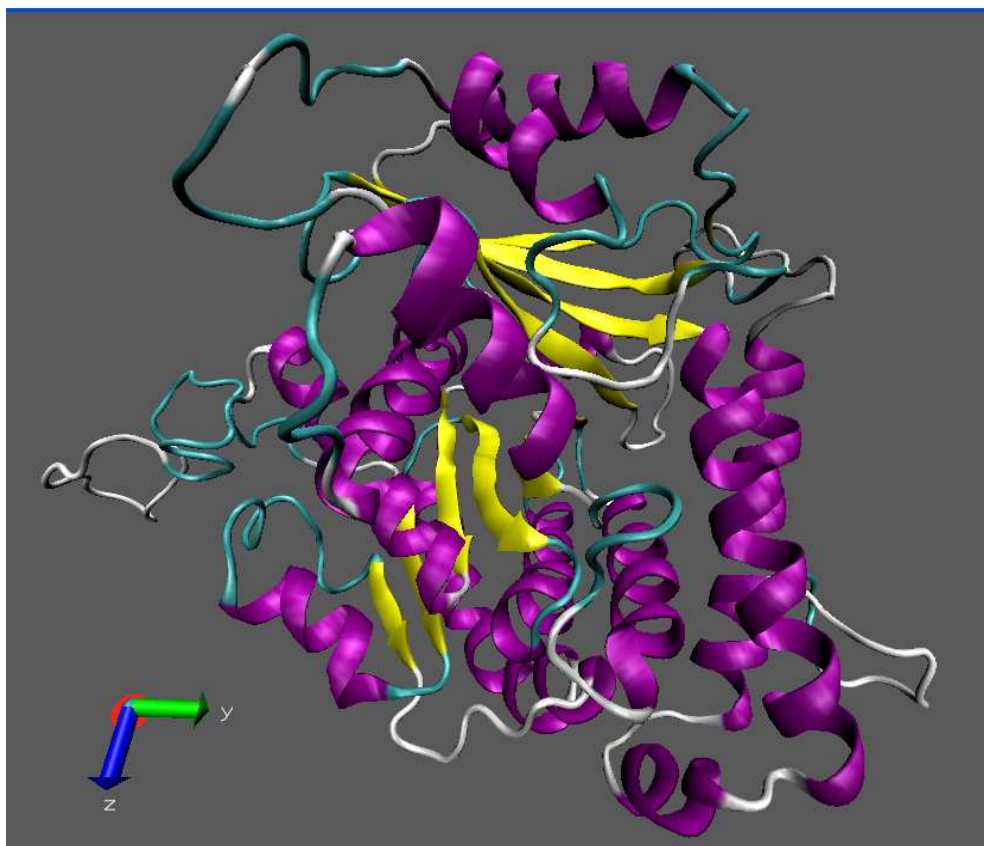


Figure 2-3D structure of the predicted protein TBC1.B99990003.pdb.

Further the predicted model was verified through a series of programs like Procheck [10], Errat [11] and Verify_3D [12]. Procheck was used to perform full geometric analysis as well as stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry [10]. After running the Procheck, Ramachandran plot shows that for model TBC1.B99990003.pdb, 85.2% residues were in the favored region, 11.6% in the additional allowed region, 2.9% in the generously allowed region and 0.9% of the residues in the disallowed region, (Figure 3) which made this model more acceptable compared to other predicted models.

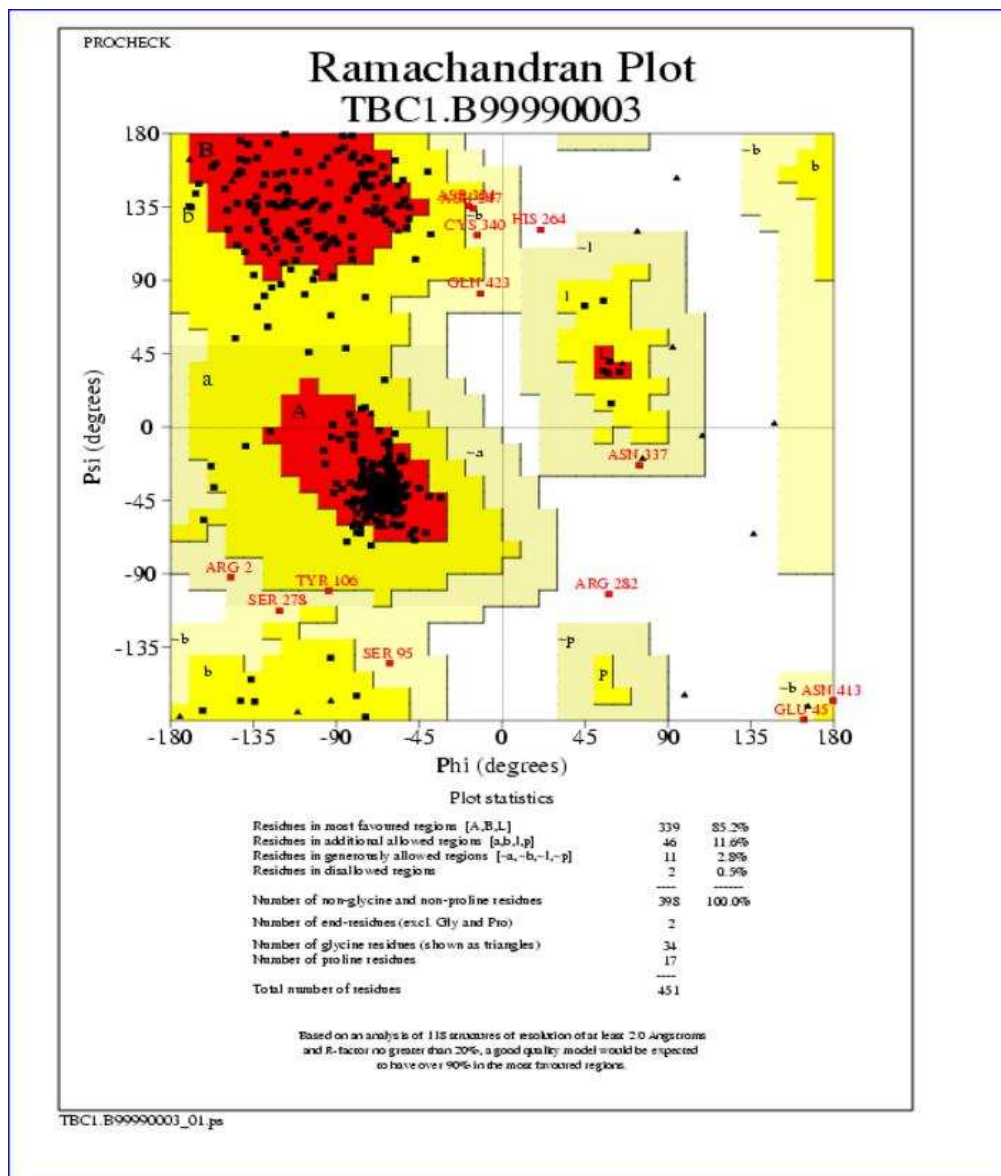


Figure 3: Ramachandran Plot for the Model TBC1.B99990003

Errat program was used to analyze the statistics of non-bonded interactions between different atom types and provide the overall quality factor of the predicted models [11]. For model TBC1.B99990003.pdb overall quality factor was found 83.476, and it was the highest among the five models.

Verify_3D program was used to determine the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop,

polar, non-polar etc) and compare the results to good structures [12]. The results showed that model-3 has passed this verification and 83.85% of the residues have an averaged 3D-1D score > 0.2. Further process is proceeded by taking the model generated by the modeler with lowest dope score i.e. TBC1.B99990003.pdb. GNUPLOT was used to plot the residues wise energy profiles of TBC1.B99990003.pdb and the template 2HXF. In this graph, the refined conformation of the loop between residues 220 and 250 forms the other half of the active site and the latter part is well resolved in the template and probably correctly modeled in the target structure (Figure 4).

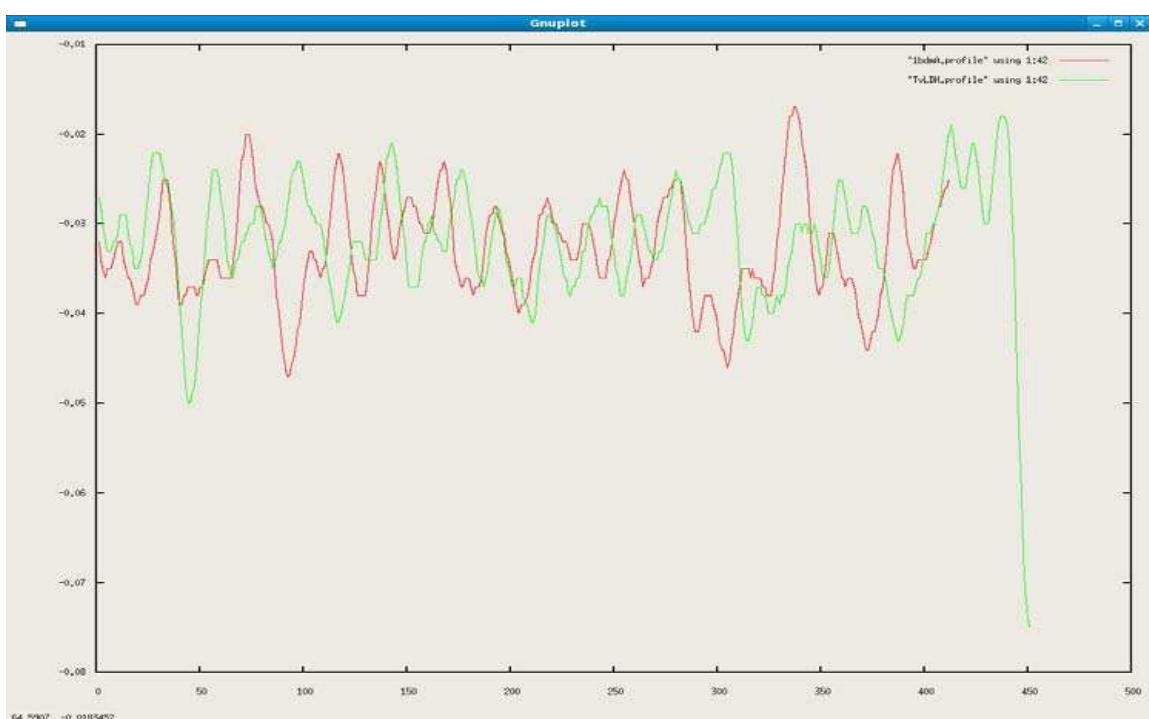


Figure 4: Gnuplot between the energy profile of generated model and the template

In order to make more comprehensive study of Colchicine as more potent drug for gouty arthritis, docking was performed with its 123 analogues and target TUBB1. For this the sitemap was generated (Data not shown). In the docking process, energy of all ligands in the dataset were minimized and docked with the target. The conformation with the lowest Glide score was chosen as the optimally docked ligand. Among all the analogs, analog with compound ID 5370577 (7-acetamido-1, 3, 10-trimethoxy9-oxo-6, 7-dihydro-5H-

benzo[a]heptalen-2-yl) (E)-3-(4-methylphenyl) prop-2-enoate) (figure 5) showed the lowest glide score - 6.45 (figure 6) and minimal RMSD (RMSD=1.015 Å). Various residues like ASP209, CYS244, TYR208, VAL175, GLU205, SER 172, SER176, PRO220, THR221, LEU225, ASN204, THR178, GLU181 and MET170 were found in the interaction region of the protein (figure 7). Various physical and chemical characteristics of this analog have been tabulated in table 3 for the understanding of the nature of the analog.

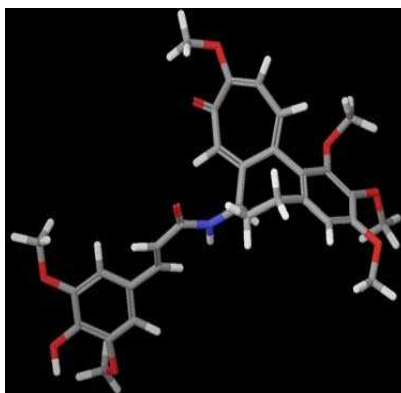


Figure 5: Analog with compound ID 5370577

Rank	Title	GSscore	vdW	Coul	Emodel	CvdW	Intern	Conf#	Pose#
1	5370577	-6.45	-44.2	-7.0	-66.0	-51.2	14.3	1	41
2	537229	-5.58	-33.6	-10.9	-64.3	-44.5	0.8	1	1
3	5370577	-5.45	-41.6	-6.7	-65.4	-48.3	10.4	1	10
4	5370577	-5.20	-38.0	-6.6	-70.5	-44.6	8.1	1	1
5	537229	-5.05	-36.8	-8.1	-60.4	-45.0	4.0	1	51
6	53457	-4.95	-39.2	-8.5	-48.0	-47.7	9.6	1	41
7	626968	-4.70	-35.0	-13.3	-58.1	-48.3	5.2	1	2
8	329990	-4.38	-33.4	-12.4	-63.0	-45.8	10.1	1	2
9	435445	-4.16	-42.4	-6.3	-65.0	-48.8	8.8	1	3
10	84038	-4.15	-35.1	-6.8	-58.7	-41.9	1.4	1	23
11	629638	-3.98	-40.7	-6.2	-55.2	-46.9	3.2	1	104
12	435445	-3.97	-43.3	-5.8	-58.8	-49.2	23.3	1	1
13	629638	-3.86	-38.2	-4.9	-55.7	-43.1	2.8	1	98
14	629638	-3.82	-36.8	-11.9	-60.9	-48.7	7.4	1	1
15	17650	-3.64	-30.0	-5.7	-52.1	-35.7	5.1	1	1
16	329990	-3.62	-39.9	-9.7	-59.4	-49.6	11.4	1	13
17	5375858	-3.58	-29.5	-25.9	-75.8	-55.4	4.2	1	2
18	3994623	-3.50	-41.2	-5.8	-58.8	-47.0	6.9	1	21
19	628637	-3.46	-38.4	-2.9	-51.8	-41.3	8.7	1	16
20	73627	-3.44	-28.6	-6.5	-47.9	-35.0	0.3	1	31
21	84038	-3.40	-37.2	-8.1	-59.5	-45.3	7.9	1	28
22	84038	-3.37	-36.5	-8.6	-56.2	-45.1	12.6	1	34
23	629638	-3.30	-35.1	-6.6	-56.0	-41.8	0.6	1	13
24	626968	-3.26	-29.2	-10.6	-58.5	-39.7	1.1	1	1
25	626968	-3.25	-30.6	-10.2	-54.0	-40.8	10.5	1	20
26	17650	-3.24	-39.8	-0.5	-55.3	-40.3	1.6	1	3
27	635302	-3.18	-31.8	-4.0	-42.0	-35.8	3.0	1	82
28	84038	-3.08	-37.0	-6.0	-58.3	-43.0	9.7	1	5
29	10833149	-3.07	-30.4	-5.7	-48.7	-36.2	4.6	1	1
30	6330029	-3.06	-27.6	-20.5	-68.0	-48.1	1.7	1	222
31	44566	-3.06	-33.9	-1.5	-37.2	-35.5	4.2	1	114
32	635301	-3.06	-33.9	-1.5	-37.2	-35.5	4.2	1	114
33	6420667	-3.02	-29.3	-4.5	-48.6	-33.8	4.7	1	4
34	161898	-2.97	-29.6	-0.9	-31.7	-30.5	5.4	1	175
35	44566	-2.96	-31.4	-1.5	-43.3	-32.9	3.3	1	1
36	635301	-2.96	-31.4	-1.5	-43.3	-32.9	3.3	1	1
37	10833148	-2.95	-29.9	-5.3	-47.6	-35.2	3.8	1	1
38	110937	-2.93	-35.1	-2.2	-49.9	-37.3	1.2	1	1

Figure 6: Screen-shot of the docking report generated by GLIDE 5.0

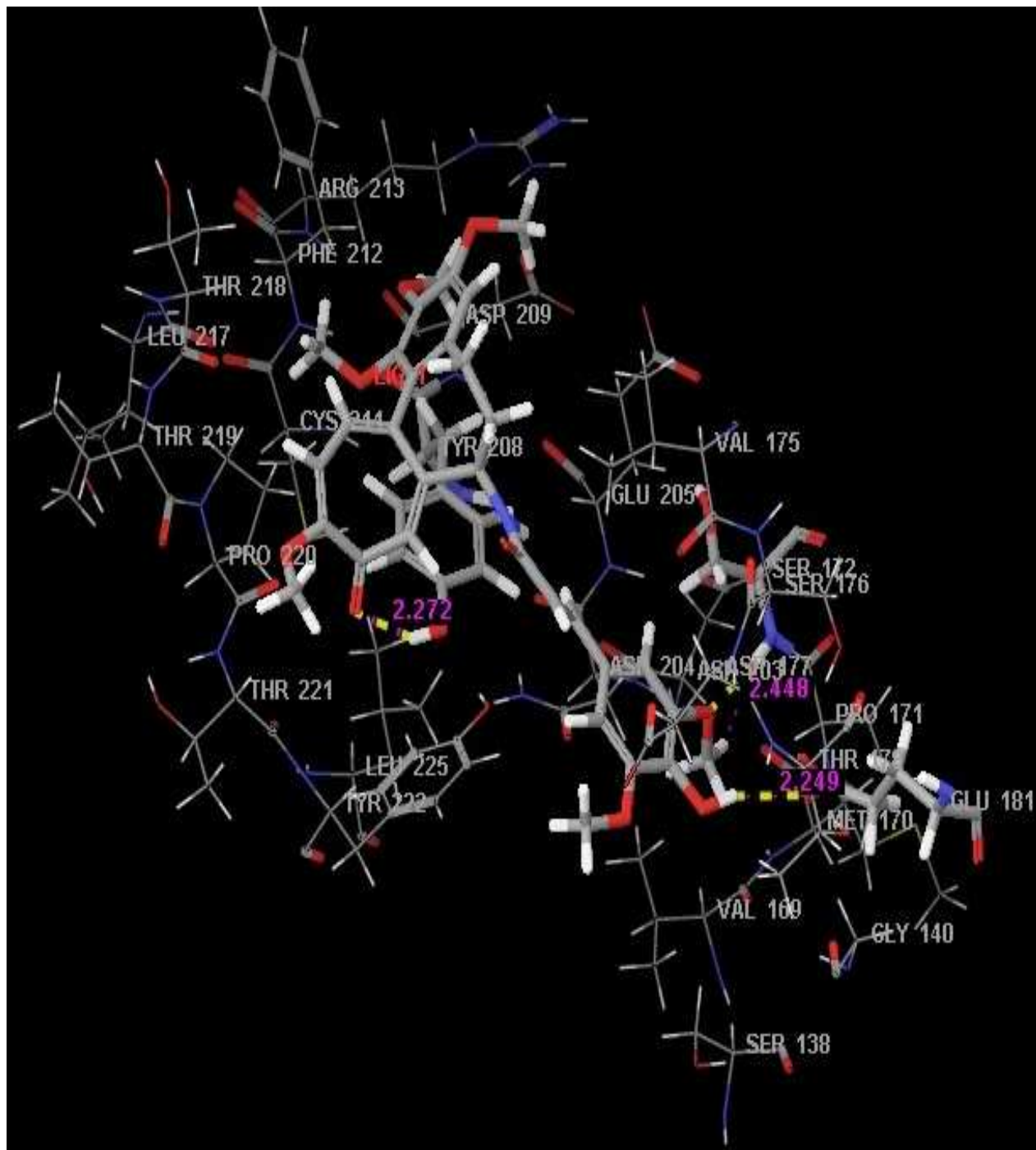


Figure 7: docked structure with hydrogen bond information at 5Å distance

Table 3: Physical and chemical characteristics of analog CID 5370577 at 5Å distance

Analog related information	Values
Molecular Weight	529.58034 [g/mol]
Molecular Formula	C ₃₁ H ₃₁ NO ₇
H-Bond Donor	17
H-Bond Acceptor	8
Rotatable Bond Count	2
MonoIsotopic Mass	529.210052
Topological Polar Surface Area	100
Heavy Atom Count	39
Formal Charge	0

Total three hydrogen bonds were formed between analog 5370577 and target TUBB1 with the residues TYR208, SER172 and GLU181 at bond length 2.272, 1.997 and 2.249 respectively. (Figure 8).

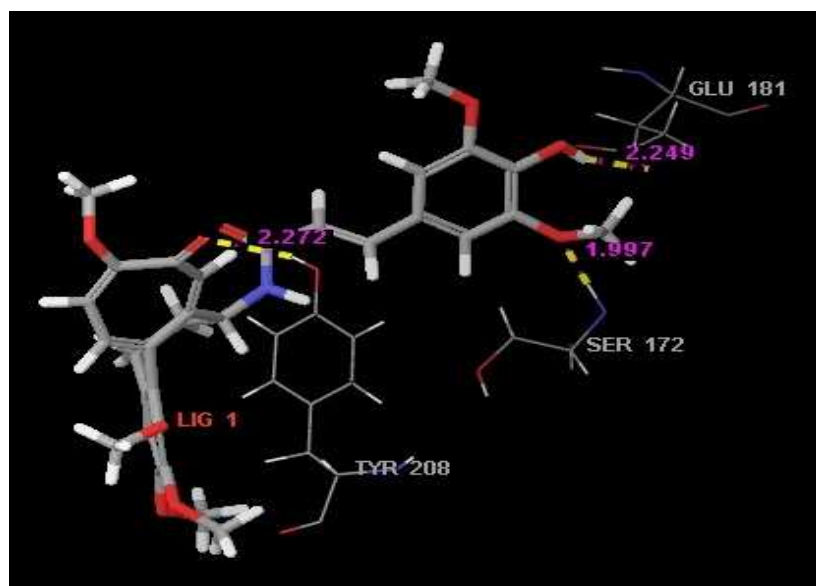


Figure 8: docked structure of the protein and the analog CID 5370577 showing interactions and active site residues

Predicting energetics of protein-ligand binding and searching space of possible poses and conformations are the major challenges in finding new drugs. Further several variants of the analog CID 5370577 can be designed by modifying its moieties at different positions in the compound to find out better drug than the existing one. This ligand has diverse and interesting biological activities, and it should be particularly useful as lead compound for drug development. Better understanding of the interactions between tubulin β -1 chain and ligand CID 5370577 will be crucial for the treatment of acute gouty arthritis, familial Mediterranean fever and cancer as well as useful for pharmaceutical and functional food industries. Hence the selected analogue CID_5370577 can be used as a replacement of the existing drug Colchicine and may be subject to laboratory confirmation and clinical trial.

CONCLUSION

3-D structure of TUBB1 protein of human was constructed with modeler9v3 and the virtual screening of drug was performed to search suitable analog among 123 analogs of drug Colchicine. Compound ID_5370577 was found more potent one comprising highest binding affinity with modeled TUBB1 protein. The present investigation may provide new insight to control superfluous use of drugs *in vitro*. The 3-D structure of tubulin β -1 chain of human and Colchicine analogue CID_5370577 would needed to be verified experimentally. Further various variants of this analog can be designed and docked to find out more potent drug than the suggested Colchicine (CID_5370577).

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