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Molecular Docking and ADME Analysis of Substituted ThienopyrimidineMolecules on Colorectal Cancer

Mahalakshmi Suresha Biradar[1], Shachindra L Nargund[1], Shankar Thapa[1*]

[1]Department of Pharmaceutical Chemistry, Nargund College of Pharmacy, Bengaluru-560085, India
Corresponding Author: tshankar551@gmail.com
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Abstract: Colorectal cancer (CRC) is one of the leading cancer diseases, with 8% of death caused due to CRC worldwide. There are many pathways associated with the development of CRC and in that the Wnt/β-catenin pathway protein, which plays a major role in cell proliferation, is selected for the docking studies. Wnt protein binds to the receptor and causes accumulation of β-catenin in the cytosol. Thienopyrimidines are the fused pyrimidines along with five-membered hetero aromatic ring, which are the structural analog of biogenic purine. Thienopyrimidines are substituted with N-methyl piperazine and various anilines. This research is focused on to inhibit the Wnt protein from bind to the receptor using thienopyrimidine molecules as ligand by an in-silico approach (molecular docking, pharmacokinetics). The Wnt protein (PDB ID-4A0P) as a receptor and substituted thienopyrimidine molecules as a ligand are docked using PyRx software. The drug likeliness property is predicted by SwissADME. Based on the scoring function obtained by docking, the ligand TP02 is showing the highest binding energy of -9.0 Kcal/mol compared to the standard Bevacizumab Avastin -7.9Kcal/mol. Compounds TP04, TP07, TP08, TP17, and TP19 are also the best candidates and are not violating the criteria of Lipinski's rule and ADME (Absorption, Distribution, Metabolism, Excretion) properties. These results indicated that thienopyrimidine derivatives could be one of the leads for the treatment of colon cancer.

Keywords: *Colorectal Cancer, Wnt β-catenin, Thienopyrimidine, Molecular Docking, Drug likeness, ADME*

1. Introduction

Colorectal cancer is the uncontrolled growth of cells in the colon or rectum located at the lower end of the digestive tract. The symptoms of Colorectal Cancer (CRC) include a change in bowel movement & stool consistency, blood in stool, and abdominal discomfort caused due to obstructing the absorption of water, nutrients, and storage of waste in the body. The cause of CRC is consuming excess red meat, cold meat, and alcohol people are at high risk [1]. About 8% of death caused worldwide are due to CRC [2]. "Wnt/β-catenin, transforming growth factor-β (TGF-β)/SMAD, Sonic Hedgehog, EGFR (epidermal growth factor receptor) and Notch path-ways are the pathways associated with the

development of CRC" [3].

The wnt/β-catenin pathway is necessary for embryonic development which helps in the developmental process and various functions. Frizzled G protein and LRP (lipoprotein receptor-related protein) are the transmembrane receptors and they receive an extracellular signal in the form of Wnt proteins. The β-catenin molecule in the cytoplasm converts the signal and regulates the Wnt signal in the pathway [4]

.In absence of Wnt protein on the receptor, the signals are not imparted into the cell and β-catenin protein is phosphorylated by Casein kinase 1 (CK1) and Glycogen synthase kinase 3 (GSK3) protein [5].

Whereas in the presence of Wnt protein, the phosphorylation of β-catenins phosphorylation is blocked and they start to accumulate in the cytosol. Then they bind to the Lymphoid Enhancer Factor/ T-Cell Factor (LEF/TCF) proteins to move into the nucleus [6].

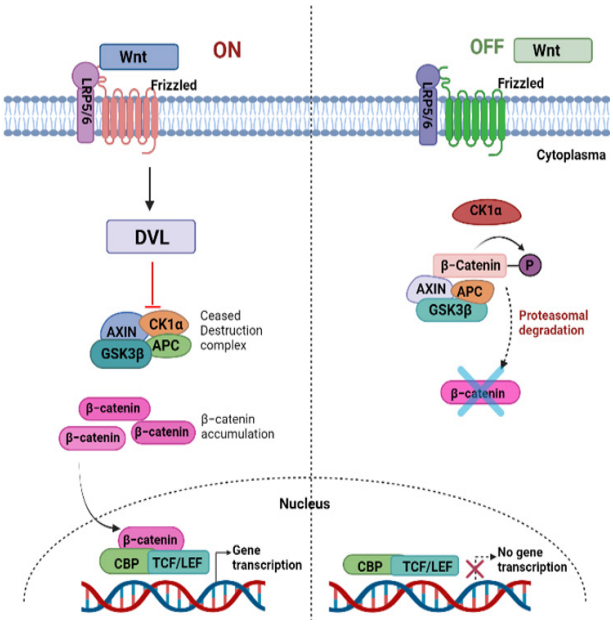


Figure 1. Wnt β -catenin pathway

Wnt genes are expressed by the activation of the Groucho complex. Wnt genes play role in the activation and deactivation of Wnt genes by regulating the degradation of the β-catenin molecule [7].

Thienopyrimidines are fused pyrimidine along with the five-membered hetero aromatic ring. It is the structural analogue of biogenic purine [8].

In the development of pharmaceutical compounds, the thienopyrimidine scaffold has become an interesting structural element. Thienopyrimidines used as potential anticancer [9], anti-inflammatory [10], antiviral [11], and antimicrobial[12] for the last two decades.

Thienopyrimidine molecule substituted with piperazine at 4th position exerts improved pharmacokinetic features and they play a key role in the bioavailability by increasing water solubility of the drug candidates due to the presence of nitrogen sites.[13]

Designing and developing new drugs maintaining the balance between pharmacodynamics and pharmacokinetic parameters is an important and challenging task in the pharmaceutical industry [14].

Various anilines and its derivatives are substituted at the 3rd position will form a variety of hydrogen-bonded complexes. They act as organic acids or base by interacting with oxygen and nitrogen as acceptors and donors. Aniline derivatives are used in various drug molecules and they serve as the best substituent for the

thienopyrimidine molecule [15].

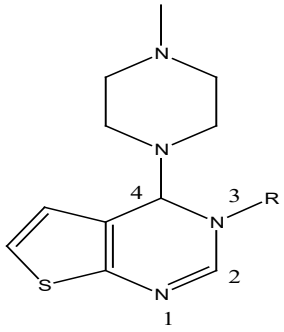
Computer-aided drug design (CADD) helps to optimize the lead compound and it is a fast process thereby we can save money and time. Computational approaches include drug design based upon structure, ligand, and fragments. Interaction between active compound and their target site(receptor, enzyme, and transporters) is achieved by CADD[16].

The present work is conducted to analyze the activity of thienopyrimidine substituted molecule to treat colon cancer by targeting Wnt protein with virtual tools (Molecular Docking and ADME Studies).

2. Methodology

2.1. Preparation of ligands

As thienopyrimidine has a purine ring and having an anticancer property. The various aniline and amines substituted with basic thienopyrimidine molecules were selected as ligands. The ligand's 2D (2 Dimension) & 3D (3 Dimension) structure was drawn in King Draw and Marvin's sketch. All the structures are saved in a '. mol2' file [17].



Thienopyrimidine

Figure 2. Basic Thienopyrimidine molecule

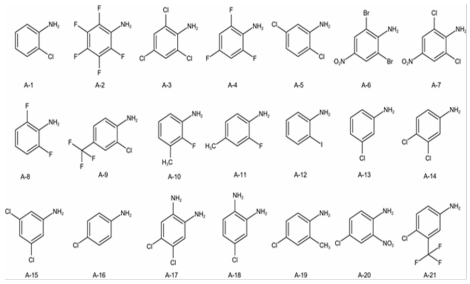


Figure 3.Substitution'R' = Ligands scaffold

2.2. Selection of protein

The protein is selected through NCBI (National Center for Biotechnology Information) Database. The targeting protein (β-catenin pathway) is searched in the NCBI. Homo sapiens(organism) is selected, and the amino acid sequence is read by selecting FASTA. Then RUN BLAST is chosen to get PDB (Protein Data Bank) ID. To get PDB ID the standard database protein data bank protein and program selection algorithm blastp (protein-protein BLAST) is selected before the blast. The PDB ID is obtained then it is filtered according to the E value (should be zero) and Query Cover (85%-100%). Those PDB IDs are searched in the RCSB (Research Collaboratory Structural Bioinformatics) database and checked for X-ray diffraction, Ram Chandran plot, Mutation, chain, and interacting ligand. The protein matching all the criteria is 4A0P and it is downloaded in '.pdb' format. Protein 4A0P is selected for docking studies[18].

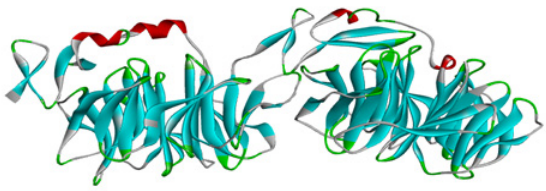


Figure 4.β-catenin pathway protein (PDBID:4A0P)

2.3. Receptor/protein preparation

The 4A0P protein is loaded into the discovery studio and a water molecule, hetero atom, ligand, and unwanted chain were deleted. The polar hydrogen is added to the protein structure and saved in '.pdb' format.

2.4. Prediction of Active Site

The binding pocket is determined by using the CASTp(Computed Atlas of Surface Topography of Proteins) server[19].

The following amino acid sequences are used as a binding pocket for docking.

GLU 697,HIS 698,VAL 699,VAL 700,GLU 701,PHE 702,GLY 703,LEU 704,ASP 705,TRP 721,GLY 725,THR 726,ASN 727,ARG 728,GLU 730,SER 732,ASP 735,GLY 736,GLN 737,HIS 738,ARG 739,GLN 740,VAL 741,TRP 744,LYS 745,LEU 747,HIS 902,PRO 917,ALA 918,TYR 920,ALA 931,PRO 932,THR 933,PHE 935,PHE 938,GLN 940,ALA 943,ASN 945,ARG 946,MET 947,VAL 948,ILE 949,ASP 950,GLN 953,SER 954,PRO 955,ASP 956,ILE 957,ILE 958,LEU 959,PRO 960,HIS 962,TYR 972,PRO 974,LYS 977,GLN 993,GLU 994,ASP 995,GLY 996,SER 997,TYR 1158,LYS 1169,THR 1179,LYS 1180,VAL 1181,GLN 1182,ALA 1183,ARG 1184,ILE 1185,ALA 1186,VAL 1194,LYS 1195,GLU 1196,LEU 119

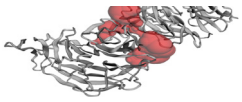


Figure 5. Binding Pocket

2.5. Procedure of Docking

Virtual screen (VS) based PyRx (combination of Autodock 4.2 and Autodock Vina) software was used for molecular docking of thienopyrimidine derivative with Beta-catenin pathway protein (PDB ID 4A0P).

This software also contains open babel for file format conversion. Discovery studio software was used for protein preparation and analysis. The prepared protein was loaded into PyRx software in the '.pdb' format and then converted into'. pdbqt' format by selecting the make molecule option. All the 3D structure was added one after the other.

The energy of the ligand was minimized and converted into'. pdbqt' format. All the ligands and proteins were selected and the protein active site is selected. The grid box was covering the binding pocket. The grid coordinate (X, Y, Z) was set to 68, 44, and 25 respectively. The CSV(Comma-Separated Values) file is saved so that it can be analysed for the best position and binding energy. The results were visualized by Discovery Studio[20].

2.6. Drug Likeliness

Swiss ADMET software was used for the determination of Drug Likeliness (Lipinski's Parameters). For drug likeliness, we considered the 2D

molecular descriptor like "Molecular weight (MW), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), Rotatable Bond (RT), Log P(lipophilicity) and Molecular Surface Area" etc[21].

3. Result and Discussion

3.1. Docking result analysis

Docking studies and drug likeliness results of ligand and standard bevacizumab were listed in the Table1. The 2D structure of the ligand is indicated in Figure 3. The highest binding energy is shown by the compound TP02 having ΔG = -9.0 kcal/mol and formingfour hydrogen bonds with the protein (VAL699, ARG739, 2 H bond with HIS 738) which have a bond distance of 2.79 Å, 3.0 Å, 2.83A0, 2.30 Å respectively. This binding score is better than reference compound ΔG = -7.9 kcal/mol and it is also forming a 4 H bond with the protein. Compound TP19 is showing binding energy of ΔG= -8.6 kcal/mol with five pi-alkyl bonds with the amino acids (ILE 957, ARG739, PRO960, VAL700, and ALA918) from a distance of 5.41 Å, 5.28 Å, 4.28 Å, 5.05 Å, and 5.32 Å respectively. Two conventional H-bond asl observed between the compound TP19 and protein.

Units: MW = grams/mole, TPSA = ng/mL, LogK= cm/h, Binding Energy = kcal/mol

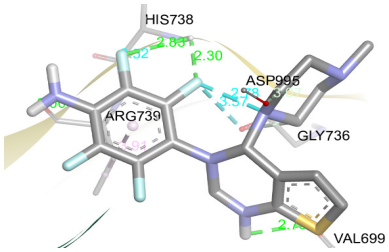
The binding energy of ΔG = -8.5 kcal/mol is shown by compound A-8 along

Table 1. Drug likeness, Docking score, and ADME properties

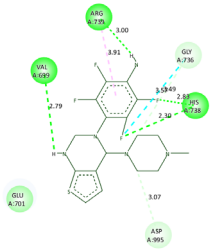
Code (R=Scaffold)	Lipinski's RO5/drug-likeness						ADME					Binding Energy
	MW	RB	HBA	HBD	TPSA	LogP	LogS	GI Absor -ption	BBB Permeability	P-gp substrate	LogK	
TP01 (A-1)	327.45	2	3	1	76.34	2.93	-3.23	High	No	Yes	-7.01	-7.5
TP02 (A-2)	399.41	2	7	1	76.34	2.98	-3.88	High	Yes	Yes	-7.16	-9
TP03 (A-3)	396.34	2	3	1	76.34	3.34	-4.42	High	Yes	Yes	-6.54	-8
TP04 (A-4)	363.43	2	5	1	76.34	3.03	-3.56	High	No	Yes	-7.08	-8.4
TP05 (A-5)	361.89	2	3	1	76.34	3.11	-3.83	High	No	Yes	-6.78	-8.1
TP06 (A-6)	451.34	3	5	1	122.16	2.85	-4.22	High	No	Yes	-7.39	-8.2
TP07 (A-7)	406.89	3	5	1	122.16	2.76	-3.90	High	No	Yes	-7.17	-8.4
TP08 (A-8)	345.55	2	4	1	76.34	3.01	-3.40	High	No	Yes	-7.04	-8.5
TP09 (A-9)	395.45	3	6	1	76.34	3.14	-4.10	High	Yes	Yes	-6.80	-7.7
TP10 (A-10)	341.47	2	3	1	76.34	3.03	-3.54	High	No	Yes	-6.84	-7.5
TP11 (A-11)	341.47	2	3	1	76.34	3.14	-3.54	High	No	Yes	-6.84	-7.8
TP12 (A-12)	327.45	2	3	1	76.34	2.80	-3.23	High	No	Yes	-7.01	-7.9
TP13 (A-13)	327.45	2	3	1	76.34	2.80	-3.23	High	No	Yes	-7.01	-8.3
TP14 (A-14)	361.89	2	3	1	76.34	2.89	-3.83	High	No	Yes	-6.87	-7.9
TP15 (A-15)	361.89	2	3	1	76.34	3.07	-3.83	High	No	Yes	-6.78	-7.9
TP16 (A-16)	327.45	2	3	1	76.34	2.80	-3.23	High	No	Yes	-7.01	-8
TP17 (A-17)	376.91	2	3	2	102.36	2.76	-3.48	High	No	Yes	-7.35	-8.4
TP18 (A-18)	342.46	2	3	2	102.36	2.32	-2.88	High	No	Yes	-7.59	-8.1
TP19 (A-19)	341.47	2	3	1	76.34	3.01	-3.54	High	No	Yes	-6.84	-8.6
TP20 (A-20)	372.443	3	5	1	122.16	2.13	-3.65	High	No	Yes	-7.01	-8.2

TP21 (A-21)	395.45	3	6	1	76.34	2.94	-4.10	High	Yes	Yes	-6.80	-7.1
Reference	275.34	4	3	1	47.56	2.75	-2.90	High	Yes	Yes	-6.41	-7.9

with the three pi-alkyl bonds with the amino acids PRO960, VAL700, ARG739 with a distance of 4.19 Å, 5.48 Å, 5.09 Å. Compounds TP04, TP07, and TP17 show the same binding energy of ΔG = -8.4 kcal/mol but their interactions with the binding pocket are different. Compounds TP12, TP14, and TP15 show similar binding energy as a reference which is ΔG = -7.9 kcal/mol but their interaction pattern is different.

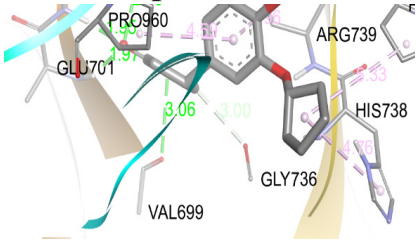


3D interaction

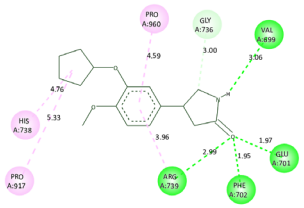


2D Interaction

Figure 6. Interaction of TP02 with protein



3D Interaction



2D Interaction

Figure 7. Interaction of Standard (Bevacizumab Avastin)

3.2.Drug Likeness and ADME properties analysis

The drug likeliness property (Lipinski's rule) and Absorption, Distribution,

Metabolism, and Excretion (ADME) properties estimated are listed in table 1. Lipinski's rule of five for orally active drugs states that MW<500, logP<5, HBD<5, HBA<10 the drug which is meeting these criteria can be used for oral administration, number of rotatable bonds<10, topological polar surface area (TPSA<140 Å) is also considered. None of the compounds violated the criteria.
ADMET properties like LogS (water solubility), GI absorption, BBB permeability, P-gp substrate, and LogK (skin permeation) are considered. Some of the compoundsare slightly soluble (-2 to -4) and some are insoluble (<-4) in water. All the compounds are highly absorbed in the GI tract. Only a few compounds are permeable through the BBB andthe compound showing the highest binding energy is one of them. The p-gp substrate is necessary for removing the drug from the intestine, gut, liver, and kidney into bile and urine. All of the compounds are showing a positive result for the P-gp substrate. Skin permeation value for the drug candidates should be in the range of -0.769 to -5.218cm/h but the compounds are showing less value so none of the compounds is skin permeable.

4. Conclusion

The Docking studies of the thienopyrimidine substituted molecule with Wnt protein showed the highest binding energy compared to standard Bevacizumab Avastin. Compound TP02, TP08, TP04, TP07, and TP17 showexcellent binding energy and they obey Lipinski's rule and ADME properties. The results of the present study showed good binding energy, ADME properties, and docking value along with electrostatic, Vander Waals forces of attraction, and desolvation energies, which play an important role in molecular interaction with the receptor protein. This study concludes that the thienopyrimidine derivatives can be the best choice for colorectal cancer but the in-vitro analysis is necessary to validate the data.

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6. Conflict of interest

All the authors declare there is no conflict of interest.

7. Funding

Funding was not provided for this project.

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