J. Chosun Natural Sci.
Vol.15, No.4 (2022) pp.145-152
https://doi.org/10.13160/ricns.2022.15.4.145

In - Silico approach and validation of JNK1 Inhibitors for Colon Rectal Cancer Target

Bavya Chandrasekhar[1], Thirumurthy Madhavan[1*].

[1]*Department of Genetic Engineering, Computational Biology Lab, School of Bioengineering, SRM Institute of Science and Technology, Potheri, Kattankulathur 603203, Chengalpattu District, Tamilnadu, India.*

Abstract

Colon rectal cancer is one of the frequently diagnosed cancers worldwide. In recent times the drug discovery for colon cancer is challenging because of their speedy metastasis and morality of these patients. C-jun N-terminal kinase signaling pathway controls the cell cycle survival and apoptosis. Evidence has shown that JNK1 promotes the tumor progression in various types of cancers like colon cancer, breast cancer and lung cancer. Recent study has shown that inhibiting, JNK1 pathway is identified as one of the important cascades in drug discovery. One of the recent approaches in the field of drug discovery is drug repurposing. In drug repurposing approach we have virtually screened ChEMBL dataset against JNK1 protein and their interactions have been studied through Molecular docking. Cross docking was performed with the top compounds to be more specific with JNK1 comparing the affinity with JNK2 and JNK3.The drugs which exhibited higher binding were subjected to Conceptual - Density functional theory. The results showed mainly Entrectinib and Exatecan showed better binding to the target.

Keywords: Colon cancer; JNK-1; Docking; DFT

(**Recceived** November 22, 2022; **Revised** December 10, 2022; **Accepted** December 12, 2022)

1. Introduction

Colon cancer is one of the most common types of cancer all over the world.[1]The development of the tumour is generally seen in the inner lining of the rectum or the colon. The development in diagnosis and treatment for CRC has increased in the last few years, but the survival rate of CRC is poor due to cell metastasis.[2] C-Jun N-terminal Kinase (JNK1) is one of the 6 distinct groups of the Mitogen-activated protein kinase (MAPK) family.[3] JNK1, JNK2 is present ubiquitously, where other JNK3 is expressed in heart and brain [4].JNK1 is activated by different types of stimuli like epidermal growth factor, tumour necrosis factor, transition forming growth factor β, etc. The signalling varies depending upon the type and circumstances of the cell as in cancer development and it also regulates the progenitor cell proliferation and migration[4]. Targeting JNK1 in CRC has been lately characterized with data supporting up-regulation

† Corresponding author: thiru.murthyunom@gmail.com

of the protein. The signalling pathway of C-Jun N-terminal kinase1 regulates some important properties in cancer stem cells and it controls the cell cycle and apoptosis. JNK1 plays an important role in the cancer cell progression, cell differentiation, inflammation and apoptosis. The protein plays an important role in controlling expression based on cell death and survival.[5-7]JNK-1 has been implicated in a number of instances of colon cancer related to colitis and protein levels have been found to be high based on a study of JNK-1 in human colon cancer cell lines.[8-10]

Inhibition of JNK1 protein can suppress the cell proliferation of colorectal cancer.[11]This discovery has important implications for the development of new drugs that target this protein.[12]Previously JNK-1 inhibitors have been detailed to inhibit the JNK-1 function and suppress cell transformation. Conventional drug discovery is time consuming and costly whereas drug repurposing is the approach where the drugs that are already approved can also be used for other particular disease and disorders. This pathway could become a potential target for drug repurposing.[13] Drug repurposing is an action that involves the discovery of new signs for known drugs used in various fields.[14]Drug repurposing is a symptomatic approach to existing drugs.[15]In this study, For this approach Virtual screening, Molecular docking and DFT studies were carried out. We identified the inhibitors for colon cancer targeting JNK-1 protein using the ChEMBL database.[16]

2.Materials and Methods

2.1 Ligand and Receptor preparation

Virtual screening was performed for ChEMBL dataset. The drugs were downloaded from the database. The energy minimization was carried out in PyRx software [17]. The open Babel software is used to convert the all drugs from sdf format to pdbqt format. The Protein structure of the JNK1 (PDB ID:47LF)[18]was downloaded from a database such as RCSB with a resolution of 1.95 Å. The preparation of the receptor was done using AutoDock Mgl tools. The initial step of optimization was done by removing water molecules as it can interfere with the docking and the co-crystal structure. Kollman charges and Computer gasteiger charges, Polar hydrogen were added to protein. The atoms like Assign radii and Assign AD4 type was also added to the protein before performing the approach.

2.2Molecular docking

Molecular Docking is an In-Silico method to find the binding energy of the ligands to the preferred target protein. Docking were carried out using Autodock4.2.[19] The grid box was generated in the active site of the protein. The active site is the region where the biochemical reaction and the ligand binding takes place. The grid box was generated in the active site of the protein. The grid parameter file has three dimensions X, Y, Z, the parameter file was saved. The dimension of the protein was at the site of x = -3.626, y = 52.342, z = 4.2, the grid box size was size x = 40, y = 40, z = 40. Autogrid and Autodock were taken place.

2.3Cross docking

Cross docking was performed to be even more specific about the drug that has been

used against JNK1 and JNK2, JNK3. The structure of JNK-2 and JNK-3 were downloaded from RCSB. The proteins were also optimised by following all the steps in preparation of the receptor, the compounds were docked against these proteins. The results are tabulated to check the binding affinity of these protein comparing with JNK1.

2.4 Density functional theory

DFT has become a widely used technique in recent years for determining molecular structure. DFT is used to take a look at the electronic structure of molecules, atoms and solids. In this current study,the gaussian 09 software was used to optimize the structures[20-21]. A number of Density functional methods like B3LYP, B3LYP, B3PW91, BH, and H, BH, and HYLP are available. B3LYP is a popular method.It represents the hybrid becke 3-Lee-Yang-parr correlation function. The properties of the ten best compounds were set with the help of Gaussian 09 based on 6-31G (d, p) using the becke 3-Lee-Yang-paar (B3LYP) method. The parameters used in this theory include HOMO and LUMO energies [22], Electron interaction, Electrophilicity index and chemical potential, absolute hardness.

3.Results and Discussion

3.1 Molecular docking

In Silico drug repurposing approaches include virtual screening plays act as a filter to choose the compounds. The virtual screening was done for JNK-1 protein against ChEMBL database, from which top 10 hits were selected based upon their high binding affinity. Top hits of the output from virtual screening were further studied using AutoDock 4.2 docking tool. The selection of lead compounds is based on the evaluation of their binding interactions between the protein and the ligands. This process is performed manually by analyzing the binding interactions between a drug and a protein.

Molecular docking used to recognize what is the behavior of the particular small molecule when it has interacted with the target protein. Molecular docking involves two major steps, the first step in the process is the prediction of the ligand's structure in the protein's pocket. The second step involves the estimation of the binding affinity. All ligands were docked into JNK-1's binding pockets. Irinotecan, Lifitegrast, and Ponatinib have been shown similar binding affinity scores (-12.5kcal/mol) also nandrolone-phenylpropionate, nilotinib, paliperidone has been shown similar binding affinity score (-11.6kcal/mol), moreover Exatecan, ledipasvir,

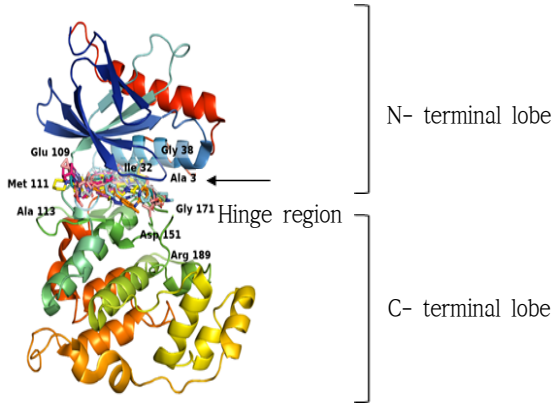


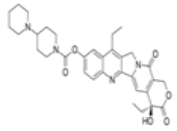
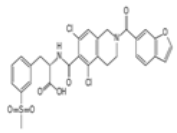
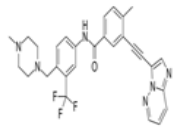
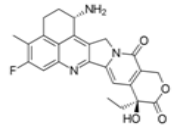
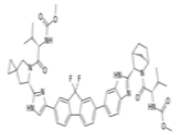
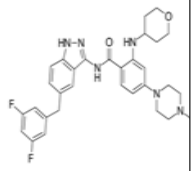
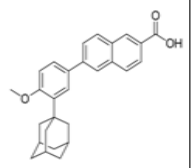
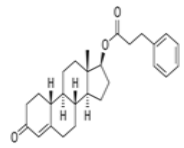
Figure 1. Three dimensional structure of JNK-1and main structural elements are coloured in Cyan (Hinge region), Marine (ATP binding loop), Lime green (Activation loop), Green (DGF motif).

Entrectinib, Adapalene also shows a good binding affinity. Table 1 lists the hydrogen bonding and hydrophobic interactions between

ligands and proteins with their binding affinities.

The results of molecular docking showed a relationship between proteins and ligands also shows hydrogen and hydrophobic bonding interactions with residues and ligands. Figure 1 Provides a graphic picture of a protein and the top ten ligands.

Table1: Binding affinity of top hits and their Hydrogen and Hydrophobic interactions

Compounds	Structure	Binding affinity (kcal/mol)	Hydrogen bond Interactions	Hydrophobic Interactions
Irinotecan		-12.5	Lys 55,Gln 37, Arg 69,Asn 156	Ile 32,Val l40,Leu 57,Leu110,Met 111,Ala 113,Val 158,Leu 168
Lifitegrast		-12.5	Lys 55,Gln 37, Arg 69,Lys 153, Ser 34	Ile 32,Ala 36,Val 40,Ala 53,Met 108,Leu 110,Met 111,Ala 113,Val 158,Leu 168,Leu 172,Val 187
Ponatinib		-12.5	Ser 34	Ile 32,Val 40,Ala 53,Met 108,Leu 110,Met 111,Val 158,Leu 168
Exatecan		-12.4	Gly 37,Lys 55, Arg 69, Asn 156	Ile 32,Val 40,Ala 53,Leu 57,Leu 110,Met 111,Val 118 Leu 168
Ledipasvir		-12.2	Gly 35, Val 186, Asp -112	Ile 32,Ala 36,Val 40,Ala 42,Leu 110,Met 111,Ala 113,Leu 168,Leu 172,Val 186,Val 187
Entrectinib		-12.1	Asn 156	Ile 32,Ala 36,Val 40,Ile 86,Leu 110,Met 111,Val 158,Leu 168,Leu 172,Val 187
Adapalene		-11.8	Lys 55	Ile 32,Val 40,Ala 42,Ala 53,Met 108,Leu 110,Met 111,Ala 113,Leu 168
Nandrolone phenylpropionate		-11.6	Asn 114	Ile 32,Val 40,Ala 53,Met 108,Leu 110,Met 111,Val 158,Leu 168

3.2 Cross docking

Cross docking was done to validate the results with JNK2 and JNK3, the top 10 hits from the Molecular docking results were docked against these molecules. The binding affinity of JNK2 and JNK3 where compared with JNK1 and it is found that binding affinity of JNK1 is relatively higher.

3.3 Density function theory

DFT operates on the principles of quantum interpretation and mechanics and has gradually become popular in the field of computational analysis.Density functional theory calculated for hit compounds that have good binding affinity .Density functional theory has ten descriptors such as Molecular dipole moment(Debye), E_{HOMO}(eV), E_{LUMO}(eV), HOMO/LUMO gap (ΔE), Total energy (E_Y)(in eV),Absolute hardness(η), Global softness(σ), Electronegativity (χ), Chemical potential(μ), Electrophilicity index (ω)(table 2). Every compound has different

Table 2: Binding affinity of JNK2 and JNK3 with top hits comparing with JNK1

Binding affinity (kcal/mol)			
Compounds	JNK-1	JNK-2	JNK-3
Irinotecan	-12.5	-7.8	-9.4
Lifitegrast	-12.5	-7.5	-8.4
Ponatinib	-12.5	-7.3	-8.9
Exatecan	-12.4	-7.5	-8.8
Ledipasvir	-12.2	-8.3	-9.7
Entrectinib	-12.1	-7.2	-9.1
Adapalene	-11.8	-7.3	-8.5
Nandilolone-phenpropinate	-11.6	-6.1	-9.0
Nilotinib	-11.6	-7.6	-9.4
Paliperidone	-11.6	-7.4	-8.6

optimized structures. The red and blue colors represent HOMO and LUMO states. The energy gap is calculated by using the difference between E_{HOMO} and E_{LUMO}.Exatecan(0.45), Ledipasvir(0.33), Nilotinib(0.35) have a low energy gap (ΔE).The low energy compounds are strong and stable. All 3 compounds have low strength spacing, resulting in a higher reactivity of the inhibitors to the protein. Electronegativity is a chemical effect that represents the course of molecules and draws electrons [40]. The electronegativity value should be higher because then only it can inhibit the activity of the particular protein.when compare the ten compounds electronegativity value Irinotecan(-4.14), Lifitegrast (-4.57), Ponatinib(-3.77), Exatecan((-5.325), Ledipasvir (-1.85), Entrectinib (-3.61), Adapalene(-3.72), Nandrolone _phenyl propionate(-3.65), Nilotinib(-4.055), Paliperidene(-3.71). Irinotecan has a high electronegativity value (-5.325). The molecular dipole moment for the ligands was found using DFT /B3LYP/6-31G calculation. Exatecan was found to have high dipole moment (10.98) and Adapalene has low dipole moment (3.17). The DFT optimized structure is given in Figure 2.

4. Conclusion

Conventional drug development process involves elaborate and time-consuming protocols, and they seldom produce drugs on demand. Drug repurposing is one the most convenient approach. The Top compounds that bind to JNK1 (PDBID: 47LF) were identified based on high binding affinity of drugs. Among them Irinotecan, Lifetegrast, Ponatinib and Exatecan has the good binding affinity and their interaction with the protein. When the drugs were further analysed in density functional theory, Exatecan had the best Electronegativity value. Upon further study of those drugs in density functional theory it was found that Exatecan and

Irinotecan had excellent electronegativity value and this drug also had good binding affinity. Irinotecan had already been used as combinational chemotherapy drug which can also be used for colon cancer. Lifetegrast is used in the treatment of the dry eyes and Ponatinib is used for chronic myeloid leukaemia. Through In-silico analysis these drugs have been shown good results in all the approach we carried out.

Fig 2: Electron density maps of HOMO and LUMO of top hits

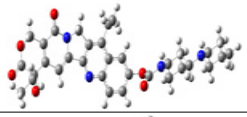
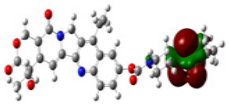
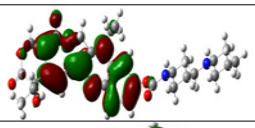
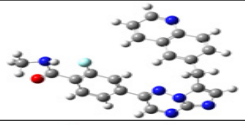
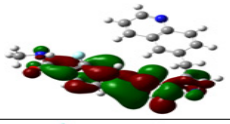
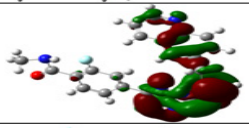
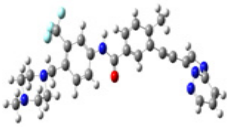
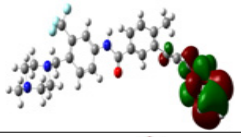
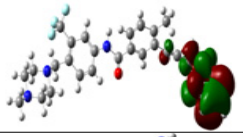
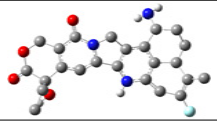
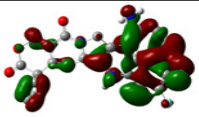
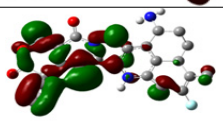
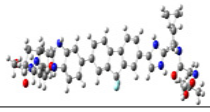
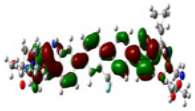
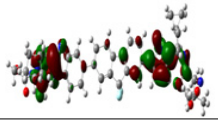
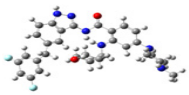
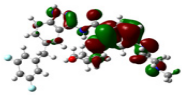
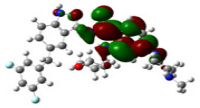
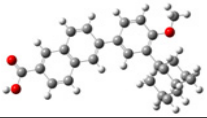
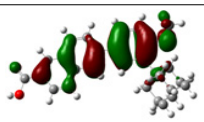
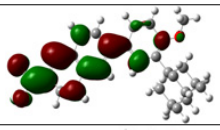
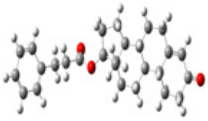
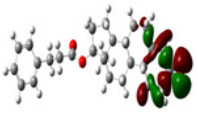
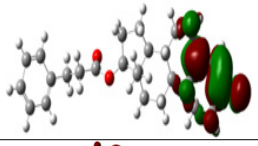
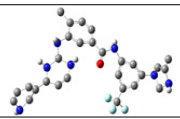
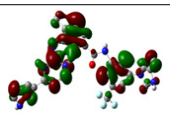
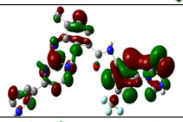
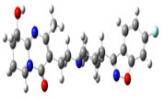
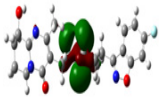
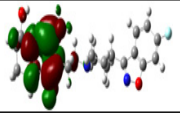
Compound	DFT optimized structure	HOMO	LUMO
Irinotecan			
Lifitegrast			
Ponatinib			
Exatecan			
Ledipasvir			
Entrectinib			
Adapalene			
Nandrolone phenpropionate			
Nilotinib			
Paliperidone			

Table 3: Statistics of DFT based molecular descriptors of top hits.

Compounds	(E γ) (in eV)	(Debye)	EHOMO	ELUMO	(ΔE)	(η)	(σ)	(χ)	(μ)	(ω)
Irinotecan	-53083.1	8.59	-6.04	-2.25	3.79	1.89	0.26	-4.14	4.14	1.09
Lifitegrast	-1390.13	3.89	-6.41	-2.74	3.67	1.83	0.27	-4.57	4.57	5.70
Ponatinib	-49645.6	4.14	-5.63	-1.91	3.72	1.86	0.268	-3.77	3.77	3.82
Exatecan	-1480.6	10.98	-5.55	-5.10	0.45	0.22	2.22	-5.32	5.32	63.01
Ledipasvir	-81337.2	10.39	-2.01	-1.68	0.33	0.16	2.98	-1.85	1.85	5.52
Entrectinib	-51163.6	5.77	-5.97	-1.25	-4.71	-2.35	-0.21	-3.61	3.61	-4.6
Adapalene	-35632.9	3.17	-5.71	-1.73	3.98	1.99	0.25	-3.72	-3.72	3.47
Nandrolone phenylpropionate	-34695.1	5.107	-6.15	-1.15	5	2.5	0.2	-3.65	3.65	2.66
Nilotinib	-1826.6	6.43	-4.23	-3.88	0.35	0.17	2.85	-4.05	4.05	46.98
Paliperidone	-39106.7	4.79	-5.99	-1.43	4.56	2.28	0.21	-3.71	3.71	3.01

Total Energy(E γ)(in eV),Molecular dipole moment (Debye), HOMO/LUMO Gap(ΔE), Absolute Hardness (η), Global Softness(σ), Electronegativity(χ), Chemical potential(μ), Electrophilicity index (ω)

References

[1] Ba Q, Hao M, Huang H, et al, "Iron deprivation suppresses hepatocellular carcinoma growth in experimental studies", Clin Cancer Res., Vol. 17, pp. 7625-7633, 2011.

[2] Wan ML, Wang Y, Zeng Z, et al, "Colorectal cancer (CRC) as a multifactorial disease and its causal correlations with multiple signaling pathways", Bioscience Reports., Vol. 40, pp. 1-15, 2020.

[3] Attene-Ramos MS, Wagner ED, Plewa MJ, Gaskins HR , "Evidence that hydrogen sulfide is a genotoxic agent", Mol Cancer Res., Vol.4 , pp. 9-14 , 2006.

[4] Wagner, E. F. & Nebreda, A. R. "Signal integration by JNK and p38 MAPK pathways in cancer development", Nat Rev Cancer., Vol. 9, pp. 537-549, 2009.

[5] Semba T, Sammons R, Wang X, Xie X, Dalby KN, Ueno NT, "JNK Signaling in Stem Cell Self-Renewal and Differentiation", Int J Mol Sci., Vol. 21, pp. 1-19, 2020.

[6] Hibi M, Lin A, Smeal T, Minden A, Karin M, "Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain", Genes and Development., Vol. 7, pp. 2135-2148, 1993.

[7] Davis RJ, " Signal transduction by the JNK group of MAP kinases", Cell., Vol.103, pp. 239-252, 2000.

[8] Prause M, Mayer CM, Brorsson C, et al, " JNK1 Deficient Insulin-Producing Cells Are Protected against Interleukin-1 ? -Induced Apoptosis Associated with

Abrogated Myc Expression", J Diabetes Res., Vol. 2016, 2015.

[9] Itzkowitz SH, Yio X ," Inflammation and cancer - IV. Colorectal cancer in inflammatory bowel disease: The role of inflammation", American Journal of Physiology - Gastrointestinal and Liver Physiology., Vol. 287, pp.7-17, 2004.

[10] Hasselblatt P, Gresh L, Kudo H, Guinea-Viniegra J, Wagner EF, "The role of the transcription factor AP-1 in colitis-associated and β-catenin-dependent intestinal tumorigenesis in mice", Oncogene., Vol. 27, pp. 6102-6109, 2008.

[11] Mahalingam D, Keane M, Pirianov G, Mehmet H, Samali A, Szegezdi E, "Differential activation of JNK1 isoforms by TRAIL receptors modulate apoptosis of colon cancer cell lines", British Journal of Cancer., Vol. 100, pp. 1415-1424, 2009.

[12] Zhengxiang Z, Yunxiang T, Zhiping L, Zhimin Y, " KNTC1 knockdown suppresses cell proliferation of colon cancer", 3 Biotech., Vol. 11, pp. 262, 2021.

[13] Liu Z, Xia Y, Zhang X, et al, " Roles of the MST1-JNK signaling pathway in apoptosis of colorectal cancer cells induced by Taurine", Libyan Journal of Medicine., Vol. 13, 2018.

[14] Greenman C, Stephens P, Smith R, et al, "Patterns of somatic mutation in human cancer genomes", Nature., Vol. 446, pp.153-158, 2009.

[15] Knapp S, "New opportunities for kinase drug repurposing and target discovery editorial", British Journal of Cancer., Vol. 118, pp. 936-937, 2018.

[16] Pushpakom S, Iorio F, Eyers PA, et al, " Drug repurposing: Progress, challenges and recommendations", Nature Reviews Drug Discovery., Vol. 18, pp. 41-58, 2018.

[17] Welcome to the PyRx Website.

[18] Zardecki C, Dutta S, Goodsell DS, Voigt M, Burley SK. RCSB Protein Data Bank: " A Resource for Chemical, Biochemical, and Structural Explorations of Large and Small Biomolecules", Journal of Chemical Education., Vol. 93, pp. 569-575, 2016.

[19] Morris GM, Goodsell DS, Pique ME, et al, User Guide AutoDock Version 4.2 Updated for Version 4.2.6 Automated Docking of Flexible Ligands to Flexible Receptors.: 1991. http://autodock.scripps.edu/

[20] Kurth S, Marques MAL, Gross EKU, Density-Functional Theory. Encyclopedia of Condensed Matter Physics, pp. 395-402, 2005.

[21] Noureddine O, Issaoui N, Al-Dossary O, "DFT and molecular docking study of chloroquine derivatives as antiviral to coronavirus COVID-19", Journal of King Saud University - Science., Vol. 33, pp. 101248, 2021.

[22] Gupta VP. Principles and applications of quantum chemistry.