IN-SILICO STUDIES OF MIF, MIEN 1, NAA40, RALB, AND PTGES PROTEINS INVOLVED IN COLORECTAL CANCER

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Abstract

Colorectal cancer is the second leading cause of cancer-related deaths, emerging as one of the major causes of morbidity and mortality worldwide. Our study intends to focus on 5 proteins; MIF, MIEN 1, NAA40, RALB, and PTGES that have been experimentally proven to play a key role in the progression of colorectal cancer. The selected proteins and their template sequences along with structures were retrieved from NCBI, UniProt, BLAST, and RCSB PDB followed by alignment analysis in Clustal Omega. The models of the proteins were generated and validated using Swiss-Model, SAVES v6.0, and ProSA. The active site was predicted with the employment of CASTp. Docking of the protein with the selected ligands was performed using Vina in PyRx and the most suitable confirmation of the ligand was selected via splitting the ligand using AutoDock vina. Further details of protein-ligand interactions were visualized in BioVia Discovery Studio. In silico analysis revealed the topological features of various binding mechanisms of the drug with respective proteins, reflecting the possible interactions that can reduce the progression of CRCs acting as targeted agents for suppressing tumor growth. However, further experimental analysis including in vivo, in vitro, and clinical trials are required to validate the possible outcome and determine its success rate.

Keywords: *Colorectal cancer, NCBI, PubChem, Anti-Colorectal cancer drugs.*

1. Introduction

Colorectal cancer (CRC) is a multi-factorial and heterogeneous complex of diseases that develops in the tissues of the colon or rectum and arises through a multistep carcinogenic process in which accumulation of genetic and epigenetic alterations occurs sequentially, targeting oncogenes, tumor suppressor genes and genes associated with DNA repair mechanisms. The survival rates vary at a global or regional level due to differences in access to diagnostic and treatment services. (Granados-Romero et al., 2017). Early CRC is usually asymptomatic, as the disease progresses, based on the anatomical and physiological functions of the colon and rectum, the signs and symptoms of tumors in different anatomical sites also differ. Common symptoms of colorectal cancer include abdominal pain, weight loss, change in appetite, discomfort or pain during bowel movements, rectal bleeding, etc. Most colorectal cancers begin as a growth on the inner lining of the large intestine called colon polyps. The probability of polyps transforming into cancer depends on the type of polyp. This gradual progression of normal epithelium to carcinoma, often supervened by dysplasia, transpires as a result of multiple sequential genetic mutations (Duan B, 2022).

Different diagnostic tools are used to detect colorectal cancer and determine the TNM staging for appropriate treatment. Where T indicates the extent of depth of invasion of the tumor in various layers of the bowel wall. N refers to the number of lymph nodes, the cancer has metastasized. M indicates the presence of distant metastasis (Kolligs, 2016).

These include sigmoidoscopy, colonoscopy, magnetic resonance imaging (MRI), transrectal ultrasonography, stool testing (GFOBT and FIT), double-contrast barium enema, computed tomographic (CT) colonography, contrast-enhanced CT (CECT) and many more (Świderska et al., 2014). Treatment strategies usually include a multimodal approach— surgery, chemotherapy, and radiation therapy— tailored to the disease's stage, patient health, and tumor extent. Emerging treatments, particularly in immunotherapy, show promise in targeting cancer cells more precisely based on individual tumor characteristics. (Duan B, 2022).

The macrophage migration inhibitory factor is an inflammatory mediator that triggers the MAPK and PI3K/AKT-dependent signalling pathways by regulating the expression of vascular endothelial growth factor-C (VEGF-C). (Osipyan et al., 2021). Migration and invasion enhancer 1 (MIEN 1) is a membrane-anchored protein acting as a chief regulator of filopodia formation and apoptosis inflating AnxA2 phosphorylation collectively stimulating cancer progression. (Kushwaha et al., 2019). N-alpha acetyltransferase 40 isoform 1 (NAA40) an epigenetic enzyme regulates apoptosis, and chromatin remodeling by specifically acetylating the alpha-amino group of serine 1 on histone H4 (N-acH4) and H2A (N-acH2A). Additionally, it regulates the One-Carbon-metabolic pathway conferring to 5-FU-based chemotherapy resistance. (Moreta-Moraleda et al., 2023). Ras-related protein RALB (RALB) is a multifunctional small G protein that modulates cellular functions such as tumor formation, vesicle trafficking, cytokinesis, filopodia formation, autophagy, apoptosis, and mitochondrial fission. (Richardson et al., 2022). Prostaglandin E synthase (PTGES) is an enzyme that produces prostaglandin E2 that plays a key role in activating pro-angiogenic factors, invasion, cell proliferation, and immune suppression within the tumor microenvironment by stimulating epithelial-mesenchymal transition and facilitating metastasis. (Murthy & Attri, 2023, Wang, 2005). Understanding the functions of these proteins is essential for unraveling the complexity of the disease and exploring potential therapeutic avenues, allocating a novel target for the treatment of colorectal cancer.

2. Materials and Methods

2.1 Homology Modeling

The target and template proteins with fewer than 250 amino acids that had been reviewed, and those with relevant publications were chosen using biological databases, such as NCBI, BLAST, PDB, ExPASy.

2.2 Model Generation and Validation

The 3D models of the chosen proteins with the most accepted model reliability were generated using SWISS-MODEL, a platform for automated comparative modelling of 3D structures. The generated models were validated by SAVES v6.0, a comprehensive tool kit

featuring ERRAT, VERIFY3D, PROVE, PROCHECK-RAMACHANDRAN PLOTS, and WHAT CHECK for evaluating various stereochemical parameters of protein structures; and the Z scores were generated using PROSA, an interactive tool designed to detect errors and predict the overall quality of 3D protein structures.

2.3 Active Site Prediction

The active sites of the generated models were predicted using CASTp. CASTpFold is a continuation of CASTp that helps identify topological regions of proteins including their areas, volumes, surface pockets, and imprints.

2.4 Selection of Ligands

Three drugs 'isovitexin', 'baicalein', and 'celastrol' used to treat colorectal cancer were selected through a literature review in PubMed and then their 2D & 3D structures were obtained from PubChem, a public database providing data on chemical compounds and their biological interactions.

2.5 Docking of Ligands and Macromolecules

The three ligands selected were docked to each protein using Vina in PyRx, a virtual screening tool for the identification of lead compounds with desired biological functions and after the program was run to give the results containing the binding affinity and RMSD values were obtained. Autodock Vina was employed through the command prompt to split the ligands, while BIOVIA Discovery Studio Visualizer was used to visualize the interactions between the ligands and the protein.

3. Results and Discussion

3.1 Homology modelling

Selection of target protein: The following accession codes (**NP_002406.1, KAI4049203.1, NP_079047.2, NP_002872.1, NP_004869.1**) of the five desired proteins; **Macrophage migration inhibitory factor, Migration and invasion enhancer 1, N-alpha-acetyltransferase 40 isoform 1, Ras-related protein Ral-B, and Prostaglandin E synthase**, respectively were retrieved from NCBI. UniprotKB/Swiss-prot was accessed to obtain the FASTA sequence and gain insights into the functional and structural aspects, literature, and experiment evidence of the respective proteins.

Selection of template proteins: The BLAST search results of the five selected proteins were obtained. The results included the retrieval of the 4-letter accession code of the respective template proteins (*Homo sapiens);* **1MIF_A, 2LJK_A, 4U9W_A, 2KWI_A, and 3DWW_A** with query coverage > 70% and Per.identity > 27%. The 4-letter code was pasted in RCSB PDB and the template proteins were checked for the % of the unmodelled region (> 20%) in the 'sequence' section. The proteins which exhibited some to almost no entirely unmodelled regions, FASTA sequence as well as the PDB files of the respective template proteins meeting the required criteria were downloaded to proceed for homology modelling.

Multiple sequence alignment: Following the successful selection of the target and template sequence, the FASTA sequences of the target and template proteins retrieved were pasted in the 'Input sequence' section to perform the multiple sequence alignment using Clustal Omega, an automated tool that efficiently aligns two or more sequences.

The alignment file was generated with the output format set to "ClustalW with character counts". (Madeira et al., 2022).

3.2 Model Generation and Validation

Model generation: The FASTA sequence retrieved form UniProt was pasted in the 'Target Sequence' section available in the Swiss-Model to generate protein structure models of the target sequence by comparing the reference sequence to known protein structures available in the database and help in the selection of most suitable template as well as predict its 3D structure based on homology modelling.

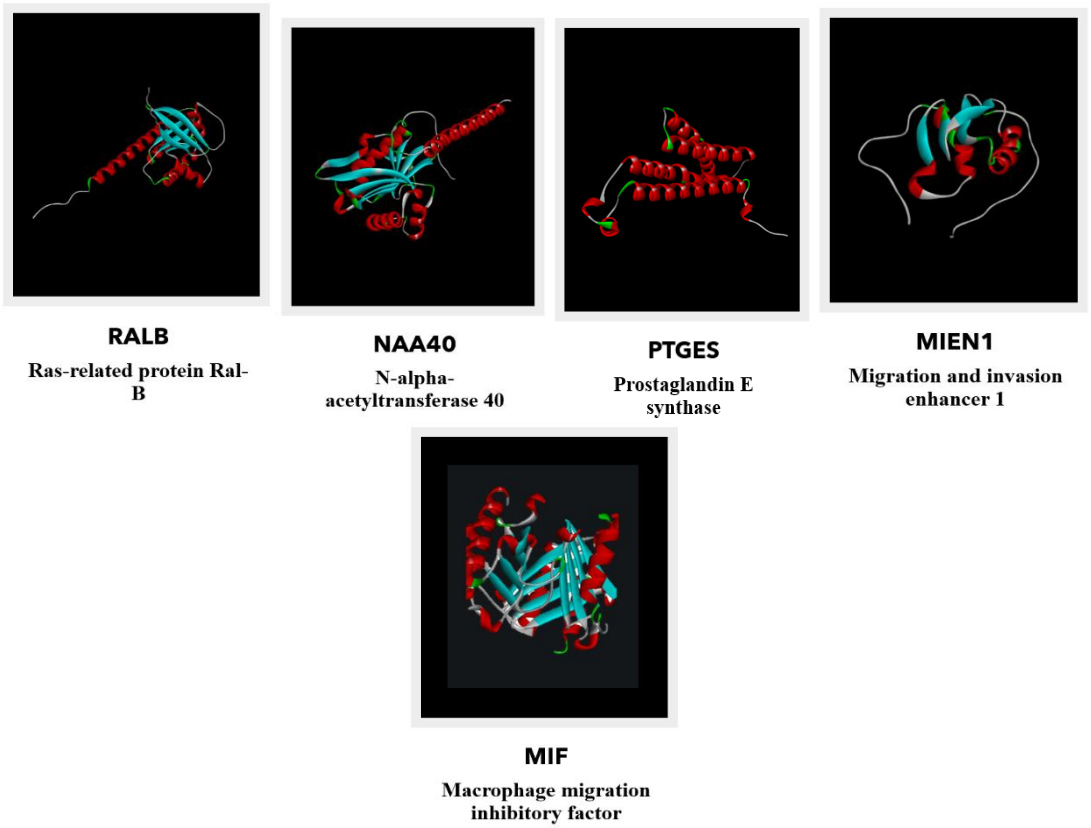


Figure 1: Structures of the Models Generated using Swiss-Model

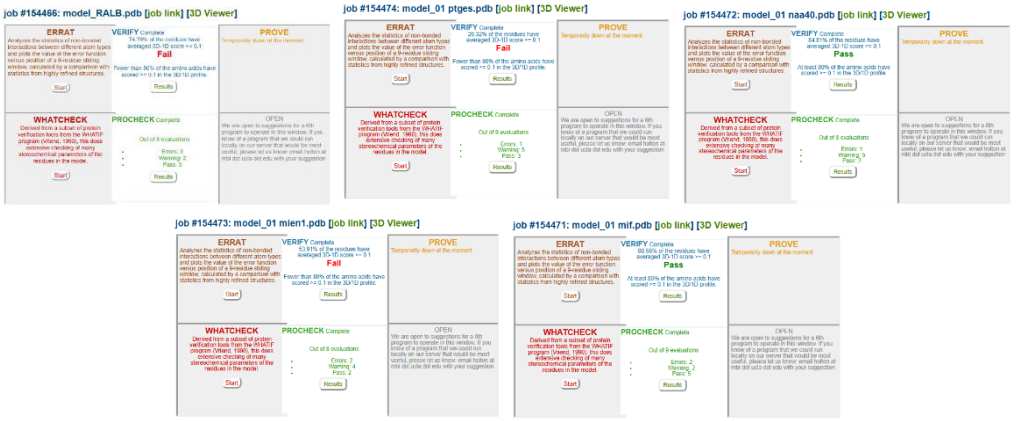
Model validation: It is performed by using SAVES v6.0 and the programs 'VERIFY 3D' and 'PROCHECK' were run for each of them. The scoring results from 'VERIFY 3D', PROCHECK SUMMARY, and Ramachandran plots having 93.4%, 83.3%, 89.7%, 89.9%, and 96.2% residues in the most favored region were obtained. **Verify 3D** is a method used to assess the quality of a protein model. A model is considered of good quality if over 80% of

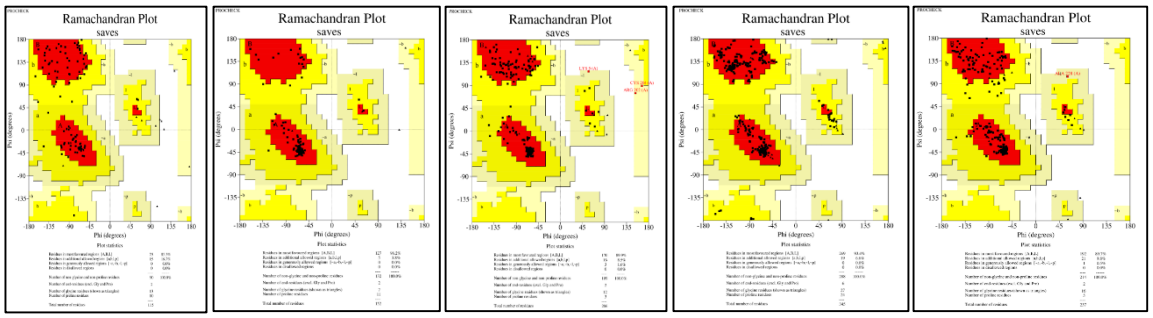
its amino acids achieve a score of ≥0.1 in the 3D/1D profile. However, verify 3D alone is insufficient for comprehensive validation, other parameters must also be examined. The models that failed the Verify 3D assessment were further evaluated using Procheck (Laskowski R A, 1993).

Further validation was performed by **ProSA**, which assesses the overall quality of the generated models which is represented in terms of a "Z score" that analyses the errors and indicates the extent of deviation of the given protein model from the expected energy distribution of native proteins. (Wiederstein & Sippl, 2007). The Z scores for the proteins MIF, MIEN 1, NAA40, RALB, and PTGES are -6.46, -5.47, -6.59, -5.84, and -4.59, respectively. The local model quality at window size 40, have negative values. Therefore, the models generated are validated by their negative Z-scores and predominantly negative energy plots.

3.3 Active-Site Prediction

CASTp was used to identify the surface pockets in each of the generated models, these pockets represent the spaces of conserved amino acids i.e., the amino acids that are critical for the protein's function. Furthermore, the protein and ligand interactions take place at these sites (Cammisa, 2013). The knowledge of which is significant for the in-silico studies of therapeutic targets. Using protein structures in PDB format and a probe radius (default 1.4 Å), the active site residues and cavity volumes for the proteins were predicted and tabulated based on pocket evaluation with CASTp.





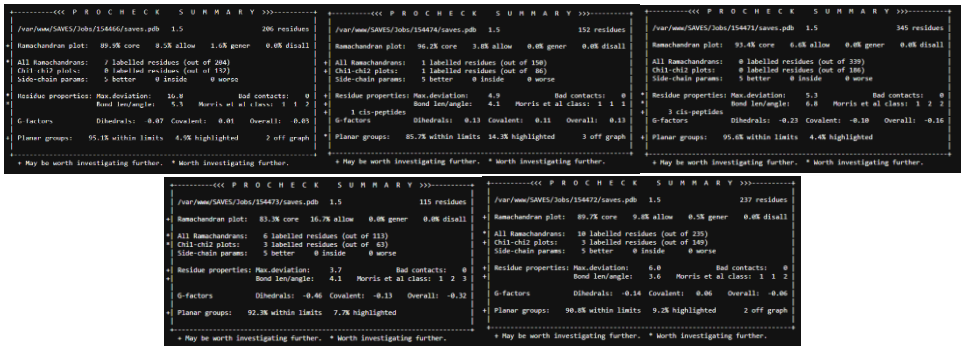


Figure 2: A) Results of Model Validation from Saves v6.0. B) Result Summaries of Procheck. C) Ramachandran Plots from Procheck.

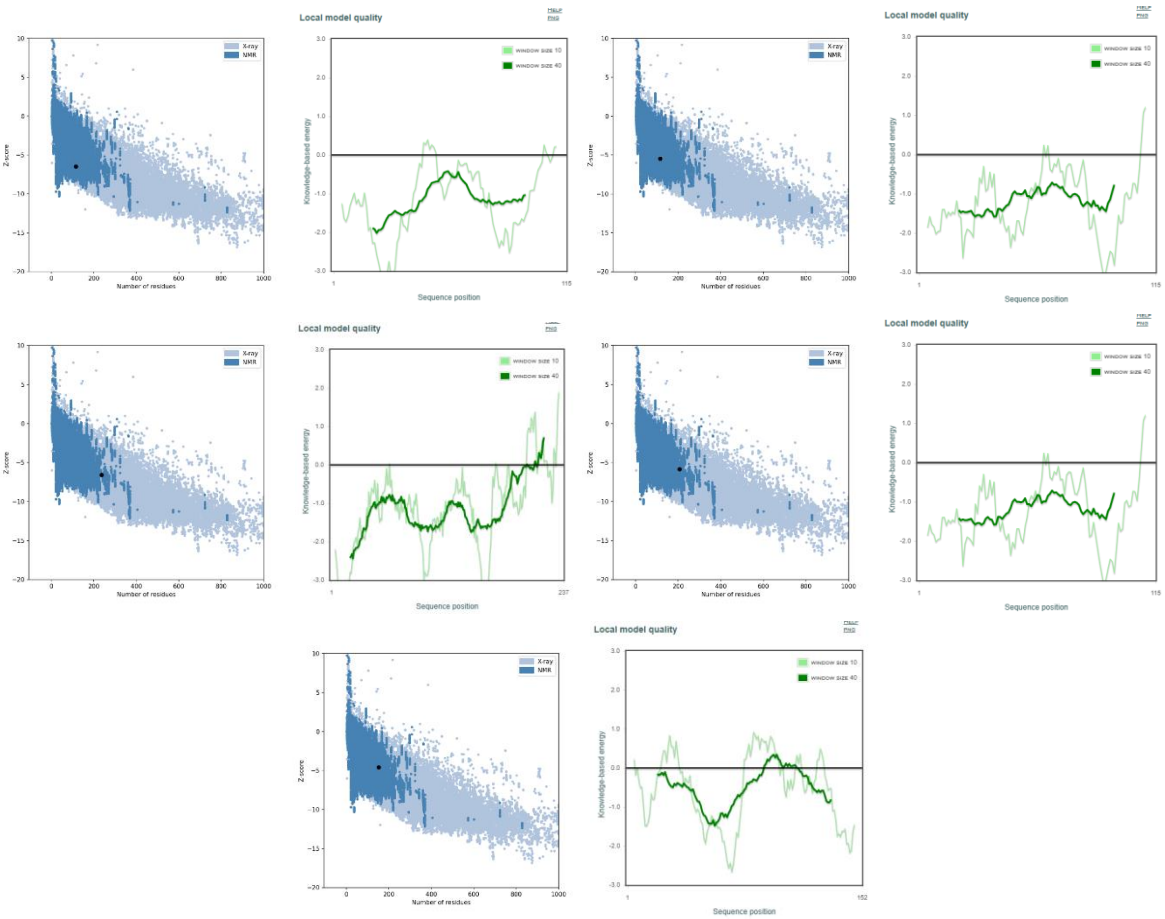
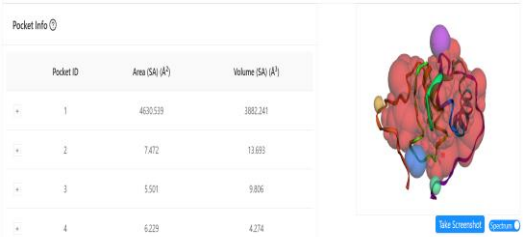
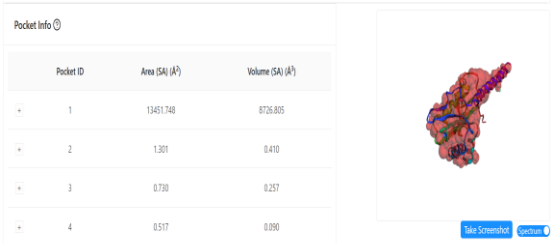


Figure 3: Graphs Obtained from ProSA Depicting Z-Scores and Showing Overall and Local Model Quality of MIF, MIEN 1, NAA40, RALB, PTGES, Respectively

POCKET ID	VOLUME (SA)	AMINO ACIDS
1.	12452.024	MET, PRO, PHE, ILE, VAL, ASN, THR, ARG, SER, ALA, ASP, GLY, LEU, GLN, LYS, TYR, HIS, CYS
2.	1.255	PRO, GLY,
3.	1.250	ASP, LEU, ARG
4.	0.405	GLN
5.	0.378	GLN, LEU

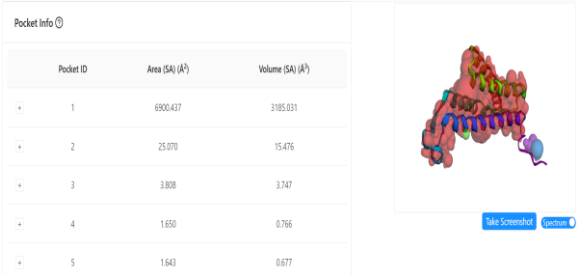


POCKET ID	VOLUME (SA)	AMINO ACIDS
1.	3882.241	GLY, GLU, PRO, THR, GLN, VAL, ALA, SER, ARG, ILE, TYR, CYS, PHE, THR, LEU, LYS, ASN, ASP,
2.	13.693	ARG, GLU
3.	9.806	LEU, GLY, CYC, VAL, ILE
4.	4.274	MET, SER, GLY, PHE
5.	3.888	THR, ASN, SER



POCKET ID	VOLUME (SA)	AMINO ACIDS
1.	8726.805	MET, GLY, ARG, LYS, SER, ALA, GLU, GLN, LEU, ASP, VAL, CYS, ASN, PRO, PHE, TYR, ILE, THR, TRP,
2.	0.410	GLU, SER, GLN
3.	0.257	ASN, SER
4.	0.090	LYS, ARG
5.	0.067	SER, HIS

POCKET ID	VOLUME (SA)	AMINO ACIDS
1.	6579.355	SER, LEU, ALA, HIS, VAL, ILE, MET, GLY, THR, GLN, PHE, TYR, ASP, GLU, PRO, ARG, LYS,
2.	26.691	PHE, SRG, CYS
3.	2.875	LEU
4.	2.759	CYS, LEU
5.	0.562	ASN, LYS



POCKET ID	VOLUME (SA)	AMINO ACIDS
1.	3185.031	LEU, VAL, MET, SER, PRO, ALA, PHE, CYS, THR, ILE, LYS, TYR, GLY, GLN, ARG, ASN, GLU, HIS,
2.	15.476	HIS, SER
3.	3.747	PRO, ALA, MET
4.	0.766	GLN, LEU
5.	0.677	THR, LEU

Figure 4: A) CASTpfold Results of MIF, MIEN 1, NAA40, RALB, and PTGES Proteins, Respectively. B) Summary of the Pockets and their Constituent Amino Acids

3.4 Selection of Ligands

Iso-Vitexin, Baicalein, Celastrol are potential drugs that can be used to treat colorectal cancer based on the experimental literature obtained from PubMed. The SDF formats of the drugs were downloaded from PubChem that were opened in Discovery Studio and saved in the PDB format to dock them with the proteins to study their interactions.

3.5 Docking of Ligand and Macromolecule

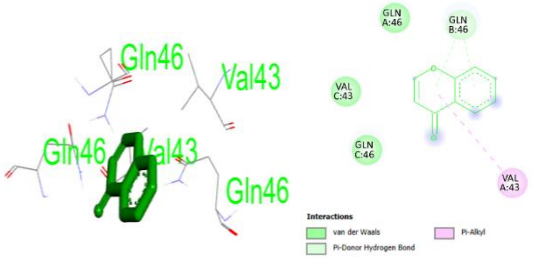
Virtual screening of potential drugs against a target protein can be achieved by molecular docking using PyRx Vina. It utilizes the AutoDock Vina algorithm to predict the orientation of the ligand as well as ligand-protein interactions indicating how well the ligand will fit into the protein's binding site. (Pawar & Rohane, 2021). RMSD (Root Mean Square Deviation) represents the extent of deviation of the docked ligand molecule's position with respect to the reference position, determining how well the predicted binding site aligns with the known crystal structure. The confirmation with the lowest binding energy and RMSD values of zero are selected. This implies that there is no difference between the atomic positions of the two structures being compared when superimposed on each other, representing a close match (Carugo & Pongor, 2001) were accurately predicted by Vina.

Splitting of ligand files: This operation was performed using Vina software and Command Prompt. AutoDock Vina is a software that can be utilized to split the ligand files in their PDBQT format based on their rmsd values and binding affinities. The most desirable ligand conformation (lowest binding energy and 0 RMSD values) is used in the final step for visualizing the interactions between the drug and proteins.

3.6 Visualization of Interactions between Ligands and Proteins

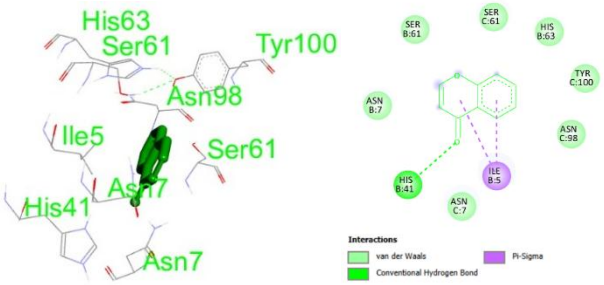
The interactions between the drugs and each protein were visualized in Discover Studio in the form of 2D and 3D structures.

1. Interaction of Iso-vitexin with MIF Protein



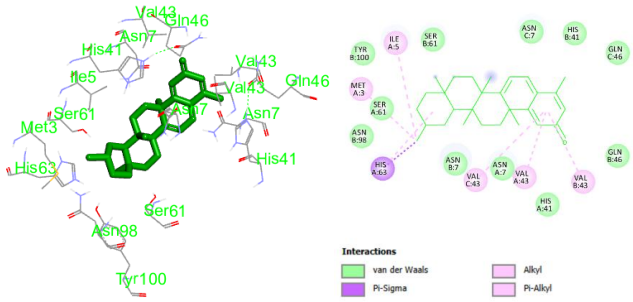
Name	Distance	Interactions
B:GLN46:HE21 - :UNK0	2.87179	Hydrogen Bond (Pi-Donor Hydrogen Bond)
B:GLN46:HE21 - :UNK0	2.7296	Hydrogen Bond (Pi-Donor Hydrogen Bond)
:UNK0 - A:VAL43	5.37465	Hydrophobic (Pi-Alkyl)

Interaction of Baicalein with MIF protein



Name	Distance	Interactions
B:HIS41:HD1 - :UNK0:O5	2.70275	Conventional Hydrogen Bond
B:ILE5:CD1 - :UNK0	3.67916	Hydrophobic (Pi-Sigma)
B:ILE5:CD1 - :UNK0	3.73697	Hydrophobic (Pi-Sigma)

Interaction of Celastrol with MIF protein

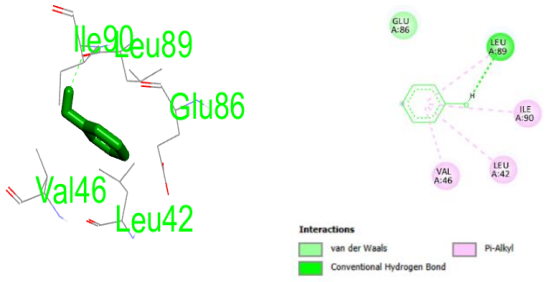


Name	Distance	Interactions
:UNK0:C25 - A:HIS63	3.72139	Hydrophobic (Pi-Sigma)
:UNK0:C25 - A:MET3	4.13422	Hydrophobic (Alkyl)
:UNK0:C25 - A:ILE5	5.05294	Hydrophobic (Alkyl)
:UNK0 - A:VAL43	5.1364	Hydrophobic (Pi-Alkyl)
:UNK0 - B:VAL43	4.61232	Hydrophobic (Pi-Alkyl)

Figure 5: A) 3D & 2D Structures. B) Interactions Summaries of MIF

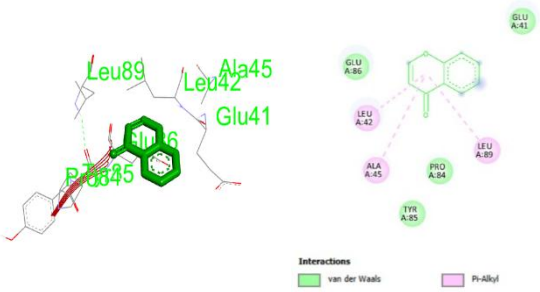
For all three ligands, interactions involving hydrogen bonds and hydrophobic bonds with the specific protein residues are observed. Hydrogen bonds are one of the most important non-covalent interactions that play a crucial role in stabilizing the ligand-protein complex. This suggests that baicalein and iso-vitexin drugs may effectively bind to MIF protein, which could influence its function and downstream signaling pathways associated with colorectal cancer progression. The presence of hydrophobic bonds, such as alkyl, pi-alkyl, pi-sigma, and van der Waals interactions may influence the localization and orientation of the drug within the binding pocket of MIF, affecting its inhibitory effects on colorectal cancer cell growth and metastasis.

2. Interaction of Iso-vitexin with MIEN 1 Protein



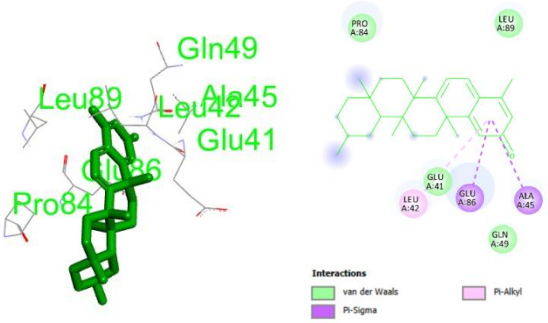
Name	Distance	Interactions
:UNK0:H51 - A:LEU89:O	2.48688	Conventional Hydrogen Bond
:UNK0 - A:LEU42	5.38231	Hydrophobic (Pi-Alkyl)
:UNK0 - A:VAL46	4.87864	Hydrophobic (Pi-Alkyl)
:UNK0 - A:LEU89	4.93028	Hydrophobic (Pi-Alkyl)
:UNK0 - A:ILE90	5.05932	Hydrophobic (Pi-Alkyl)

Interaction of Baicalein with MIEN 1 Protein



Name	Distance	Interactions
:UNK0 - A:LEU42	4.76645	Hydrophobic (Pi-Alkyl)
:UNK0 - A:ALA45	5.27707	Hydrophobic (Pi-Alkyl)
:UNK0 - A:LEU89	5.36701	Hydrophobic (Pi-Alkyl)

Interaction of Celastrol with MIEN 1 Protein

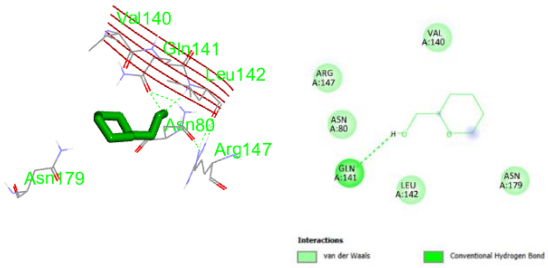


Name	Distance	Interactions
A:ALA45:CB - :UNK0	3.9388	Hydrophobic (Pi-Sigma)
A:GLU86:CG - :UNK0	3.96837	Hydrophobic (Pi-Sigma)
:UNK0 - A:LEU42	5.40251	Hydrophobic (Pi-Alkyl)

Figure 6: A) 3D & 2D Structures. B) Interactions Summaries of MIEN 1.

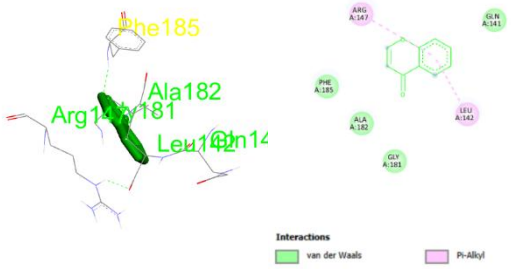
Strong hydrogen bond interaction between Iso-vitexin and LEU89 amino acid of pocket 1 of MIEN 1 protein, indicates favorable binding interactions that can stabilize the drug-protein complex. These interactions are crucial for disrupting the functions of MIEN1, which is often dysregulated in cancer cells. By targeting key residues involved in these interactions in regard to the specificity of the hydrogen bond interaction, the drug may exert its inhibitory effects on MIEN 1, leading to anti-cancer effects in colorectal tumors.

3. Interaction of Iso-vitexin with NAA40 Protein



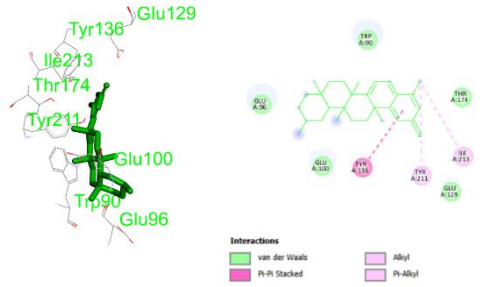
Name	Distance	Interactions
:UNK0:H43 - A:GLN141:OE1	2.27182	Conventional Hydrogen Bond
A:ALA182 - :UNK0	4.70586	Hydrophobic (Alkyl)

Interaction of Baicalein with NAA40 Protein



Name	Distance	Interactions
:UNK0 - A:LEU142	5.12022	Hydrophobic (Pi-Alkyl).
:UNK0 - A:ARG147	5.44639	Hydrophobic (Pi-Alkyl).

Interaction of Celastrol with NAA40 Protein

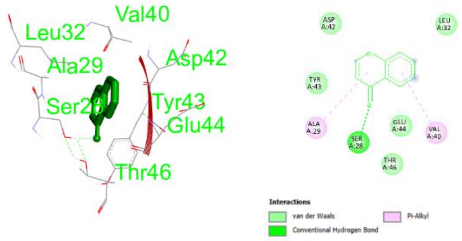


Name	Distance	Interactions
:UNK0 - A:TYR136	5.45924	Hydrophobic (Pi-Pi Stacked)
:UNK0:C33 - A:ILE213	4.83694	Hydrophobic (Alkyl)
A:TYR211 - :UNK0:C33	4.89063	Hydrophobic (Pi-Alkyl)

Figure 7: A) 3D & 2D Structures. B) Interactions Summaries of NAA40

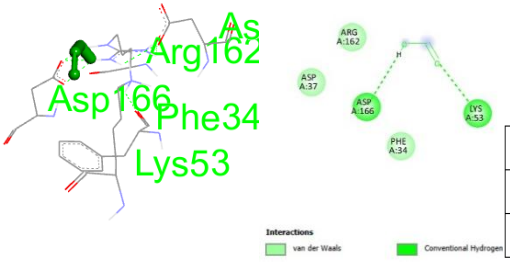
The presence of hydrogen bond and electrostatic interaction between the Iso-vitexin drug and GLN141 amino acid residue of the protein, suggest potential binding affinity and stabilization of the ligand-protein complex.

4. Interaction of Iso-vitexin with RALB Protein



Name	Distance	Interactions
A:SER28:HG - :UNK0:O9	1.88726	Conventional Hydrogen Bond
:UNK0 - A:ALA29	4.9115	Hydrophobic (Pi-Alkyl)
:UNK0 - A:VAL40	4.86181	Hydrophobic (Pi-Alkyl)

Interaction of Celastrol with RALB Protein

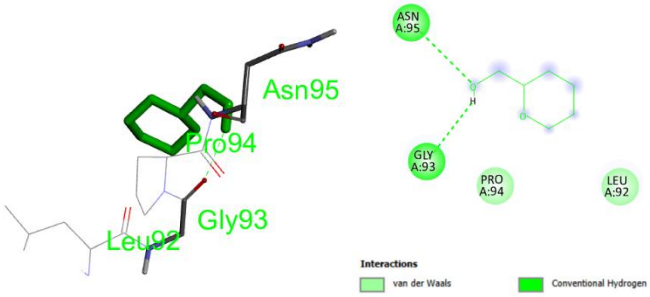


Name	Distance	Interactions
:UNK0:H67 - A:ASP166:OD1	2.59244	Conventional Hydrogen Bond
A:LYS53:HZ2 - :UNK0:O2	2.76427	Conventional Hydrogen Bond

Figure 8: A) 3D & 2D Structures. B) Interactions Summaries of RALB

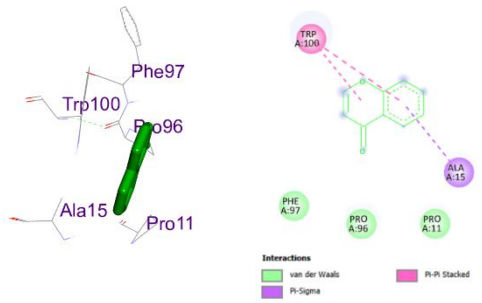
The presence of conventional hydrogen bonds between SER28 amino acid of pocket 1 of the protein with iso-vitexin drug and ASP166, and LYS53 amino acids of pocket 1 of the protein with Celastrol drug contribute to the stability of the drug-protein complex. The hydrophobic interactions like Pi-Alkyl bonds between the ALA29 and VAL40 amino acid residues with iso-vitexin drug influence the binding affinity and conformational stability of the RALB and potentially modulate its activity in colorectal cancer cells. Targeting RALB with molecules that form strong hydrogen bonds and electrostatic interactions could disrupt RALB signalling pathways that drive cancer cell proliferation and survival.

5. Interaction of Iso-vitexin with PTGES Protein:



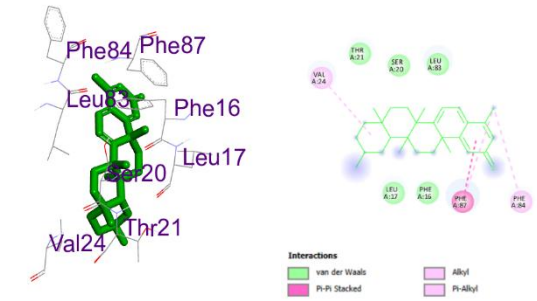
Name	Distance	Interactions
:UNK0:H43 - A:GLY93:O	2.26871	Conventional Hydrogen Bond
A:ASN95:HN - :UNK0:O5	2.83742	Conventional Hydrogen Bond

Interaction of Baicalein with PTGES Protein



Name	Distance	Interactions
A:ALA15:CB - :UNK0	3.86593	Hydrophobic (Pi-Sigma)
A:TRP100 - :UNK0	4.86929	Hydrophobic (Pi-Pi Stacked)
A:TRP100 - :UNK0	3.73381	Hydrophobic (Pi-Pi Stacked)
A:TRP100 - :UNK0	4.6173	Hydrophobic Pi-Pi Stacked)

Interaction of Celastrol with PTGES Protein



Name	Distance	Interactions
:UNK0 - A:PHE87	3.73972	Hydrophobic (Pi-Pi Stacked)
A:VAL24 - :UNK0	4.97471	Hydrophobic (Alkyl)
A:PHE84 - :UNK0:C33	4.92685	Hydrophobic (Pi-Alkyl)
A:PHE84 - :UNK0:C33	4.54887	Hydrophobic (Pi-Alkyl)

Figure 9: A) 3D & 2D Structures. B) Interactions Summaries of PTGES

The conventional hydrogen bonds and electrostatic interactions between the drug Iso-vitexin and specific amino acid residues; GLY93 and ASN95 present in pocket 1 of PTGES protein suggest potential binding affinity and stabilization of the ligand-protein complex,

which may influence its function and downstream signalling pathways associated with colorectal cancer progression. These ligand-protein interactions between different drugs and proteins may contribute to understanding the efficacy of the drugs in modulating the activity of the particular protein, potentially impacting colorectal cancer cell proliferation and survival.

Conclusion

Colorectal cancer is a devastating disease imposing the victims with less than 5% survival rate. Even in the present rapidly advancing decade, there are still no efficient treatment strategies for the disease. In the above context, our study is focused on elucidating the interactions between key proteins implicated in colorectal cancer-such as MIF, MIEN 1, NAA40, RALB, and PTGES, and drugs like Iso-vitexin, Baicalien, and Celastrol. By understanding the various interactions such as hydrogen bonding and hydrophobic bonds that play a significant role in the specific binding of the drug to the target and in maintaining its stability, the development of better-targeted therapies is possible. The interactions between the ligands and the various protein residues provide insights into potential mechanisms by which these ligands could modulate the protein's activity and signalling pathways implicated in colorectal cancer. The findings highlight specific amino acid residues within proteins such as GLN46 and HIS41 in MIF; LEU89 in MIEN 1; GLN141 in NAA40; GLY93 and ASN95 in PTGES. Targeting these binding sites on the protein residues could disrupt its function, and inhibit tumor growth, paving the way for the development of novel drugs offering hope for improved patient outcomes. However, further experimental validation and studies are needed to confirm the significance of these interactions in the context of colorectal cancer therapy.

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