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Molecular Docking Studies of Thymoquinone and its Analogues with Cyclooxygenase-2 (COX-2) for screening potential anti-colorectal cancer compounds.

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Abstract

Colorectal cancer (CRC) is the third most common type of cancer, with over one million cases and 700,000 deaths reported each year (Siegel et al., 2020). Although the complex disease aetiology of CRC is still unknown, it is clear that genetic mutations and chronic inflammation are two potential causative factors. Surgery, chemotherapy or radiotherapy, immunotherapy, hormone therapy, and pharmacology are all options for treating CRC, depending on the tumour site and stage of the disease. Researchers are particularly interested in screening herbal plants, isolating, identifying, and evaluating their secondary metabolites as potential drug leads. Thymoquinone is a phytochemical compound found in the Nigella sativa plant. It's also found in Monarda plants. By using computational docking studies, we were able to investigate the inhibitory action of Thymoquinone and its derivatives on colorectal cancer in this study.

Keywords: Colorectal cancer, Docking Studies, Thymoquinone, COX-2

Introduction

Colorectal cancer (CRC) is reported as the third most prevalent form of cancer accounting more than 1 million cases and 700,000 deaths reported each year (Siegel et al., 2020). The complex disease etiology of CRC is still not understood completely but, it is evident the Genetic mutations and chronic inflammation are two of the potential causative elements (Mármol et al., 2017). Colon cancer is caused by the spread of colorectal cancer cells to other areas of the body. Genetic changes, overexpression of Cytooxgenase-2 (COX-2), smoking, alcohol consumption, a poor diet, and a lack of physical activity are all contributing factors (Xing et al., 2008). Among those risk factors, overexpression of COX-2, which has been found in the majority of CRC has been associated to the development of cancer (Sinicrope & Gill, 2004). COX-2 mRNA and protein levels are higher in colorectal adenocarcinomas than in neighboring histologically normal mucosa in most cases.

The available treatment strategies of CRC include surgery, chemotherapy or radiotherapy, immunotherapy, hormonal therapy, and pharmacotherapy, focusing on the tumor site and stage of the disease (Hagan & Donovan, 2013). Apoptosis prevents damaged cells from developing out of balance under normal physiological conditions. But the secondary mutations in apoptosis-regulating genes affects these cells by circumventing the apoptosis regulatory systems (Zhang et al., 2021).

The commonly used chemo preventives include chemically synthesized or organic chemicals to prevent, reduce, or counteract carcinogenesis or the growth of metastatic (Zhang et al., n.d.). Almost all modern therapeutic medicines have their sources in herbal medicine, because they're either unaltered dietary supplements or their better synthetic analogues (Parmar et al., 2016). It is of significant interest for the researchers to screen herbal plants, isolate, identify, and evaluate their secondary metabolites as potential drug leads. Bioactive compounds have been modified to improve therapeutic effectiveness, bioavailability, specificity, as well as a variety of many features, including the implementation of some

potential chemotherapeutic agents (Newman & Cragg, 2007) (Sharma et al., n.d.). On the other hand, their high cost, negative side effects, drug interactions, and drug resistance difficulties spurred researchers to seek out less expensive and more effective alternatives. The identification of molecular targets related with cancer metabolism is currently required for the rational design of effective anticancer medicines.

Once the drug to be investigated has been established, investigations are performed off to assess & evaluate if the medication has the desired impact on cell culture and experimental animals. Following that, a significant majority of individuals should be enrolled in a clinical study, most side effects should be investigated, and certain standardizations should be followed. Budgets skyrocket during the clinical trials process. As a result, *in silico* pre-screening interactions were needed to identify potential targets which can be evaluated in the lab. This *in silico* process prior to the experiment significantly reduces the cost of the experiment (*Torjesen: Drug Development: The Journey of a Medicine... - Google Scholar*, n.d.; Wang et al., n.d.). The binding affinity of ligand molecules is computed via molecular docking, which is critical in understanding their metabolic functions. The initial step in the quest for new pharmacological substances is usually to identify the protein target and its regulator.

The main objective was by using molecular docking to identify proteins which could serve as chemotherapeutic agents in colorectal cancer, as well as to conduct a complete investigation to ensure that they were accurate and could contribute to new treatment strategies.

The phytochemical substance thymoquinone is found in the Nigella sativa plant. It can also be found in planted Monarda fistulosa plants that can be steam distilled to make an essential oil. It's been labelled a pan-assay interference chemical since it binds to a wide range of proteins. Thymoquinone has clearly demonstrated its effectiveness as a hepatoprotective, anti-inflammatory, antioxidant, cytotoxic, and anti-cancer chemical, with mechanisms of action, supporting its classification as an emerging medicine. Thymoquinone has anticancer properties through a variety of mechanisms, including selective antioxidant (Entok et al., 2014), antibacterial (Kokoska et al., 2008), anti-inflammatory (Hajhashemi et al., 2004),

DNA structural interference (Khan et al., 2019), effects on carcinogenic signaling molecules/pathways (Khan et al., 2017) , and immunomodulation (Shaterzadeh-Yazdi et al., 2018) .

Materials and Methods

Hardware and software

This study was conducted on a Workstation (Dell) having 8 GB RAM & 1 TB hard storage capacity, installed with AutoDock 3.05 version. We also accessed web-based databases and tools online in this work.

Protein Preparation

The target structure of cyclooxygenase-2 (prostaglandin synthase-2) complexed with a selective inhibitor with accession ID: 6COX was extracted from the Protein Data Bank (PDB) (www.rcsb.org). It belongs to the classification of oxidoreductase proteins (Kurumbail et al., 1996).

Sequence Analyses

Physicochemical parameters of the 6COX protein including isoelectric point, instability index, hydropathicity, the atomic composition was computed using the ProtParam tool of ExPASy (Gasteiger et al., 2003).

Structural Analysis

The three-dimensional structure was determined using X-ray diffraction process with a resolution of 2.80 Å. The recovered protein structure was prepared using AutoDock

Methods. Water molecules and all non-standard residues (heteroatoms) have been excluded from the initial structure. Then, all missing hydrogens and kollman charges have been applied to the device; the prepared protein receptor was then saved as a .pdb format.

Ligand Preparation:

Five chemical derivatives of Thymoquinone of **Nigella sativa** (black seed herb) were drawn using JME Molecular Editor and transferred to PRODRG server, which takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg) (Schüttelkopf & van Aalten, 2004). In this analysis, the chirality, full charges, and energy minimization were added and PRODRG was run. Both ligands and receptor molecules were prepared in AutoDock software, to predict our small molecule to the target receptors by performing flexible docking. All analyses were performed using a standard protocol

Molecular docking studies:

Many docking algorithms becomes capable of constructing a wide range of possible structures, so they still need a means to score each structure to categories those of greatest interest. In the present study, the docking process was carried by employing the Autodock 3.05 method using the Lamrkian genetic algorithm as the score function was completed. A grid size of 60 Armstrong X, 62 Armstrong y, 60 Armstrong Z directions was chosen for the docking to accommodate any possible ligand-receptor complex in our flexible docking approach. The lower the value of ΔG indicates better the binding affinities between the target and the novel ligand molecule. The PyMOL molecular viewer (http:/www.pymol.org/) was used for the study of docked structures.

Results

Target protein sequence and structural analyses

We analyzed the physicochemical parameters of the 6COX protein sequence. The instability index of a protein provides an estimate of the stability of the protein in the test tube. Based on the weight value of different dipeptides, a protein with an instability index smaller than 40 is considered stable. Our results revealed the good stability of this protein complex with an instability index of 39.20 (Table 1).

Structure evaluation of natural ligand molecules

The chemical structures of our 5 potential anticancer Thymoquinone derivatives (A) TQ7, (B) TQ23, (C) TQ26, (D) TQ27, (E) TQA6, were drawn using JME editor and minimized in PRODRG server.

Structure evaluation and validation of 6COX

The 6COX is a protein with 587 amino acid residues. The experimentally determined crystal structure (X-Ray Diffraction) of our target complex with 2.80 Angstrom resolution was obtained from the (PDB ID: 6COX) (Figure 1).

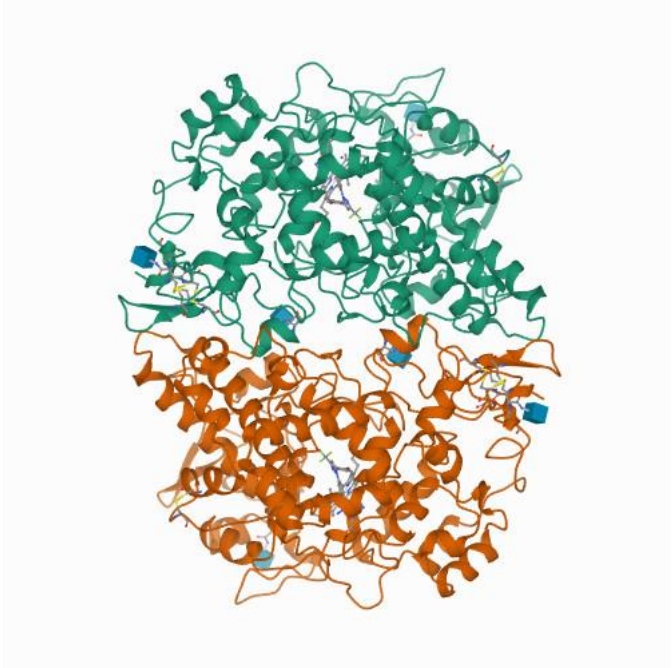


Figure 1: Target protein structure (3-D) of cyclooxygenase-2 (Prostaglandin synthase-2) complexed with a selective inhibitor (6COX)

To validate the structure, the energy minimization and structural refinement of the above structure of the target was done using YASARA Energy Minimization Server. We could optimize the energy of the structure from -300271.6 kJ/mol (score, -4.19) to -599113.6 kJ/mol (score, -1.204) in the refined model. The stereochemistry of the refined model of target was then subjected to ProCheck for stereochemical analysis. The results have been shown on the Ramachandran plot, where most residues (74.1%) were occupying the most favorable region (red), allowed zones (yellow) (22.5%) and the remaining 2.5% of residues were in the generously allowed region (light yellow) followed by only 0.8% residues falling in the most unfavorable zone of the disallowed region (white) (Figure 2).

We also analyzed our protein in the ProSA-web server for protein structure analysis where a good Z score of -8.86 (Figure 3 a and b) was obtained. The high accuracy of our structure was supported by Levitt-Gerstein's (LG) score of **7.638** and Maxus **-0.458** extracted in the Protein Quality Predictor (ProQ) tool. A ProQ LG score >4 suggests the extremely good

quality of the model structure. The quality factor for protein so obtained is 86.54 (Figure 4) in the ERRAT plot (which is used to evaluate and validate the crystal structure of a protein in which the error values are plotted as a function of the sliding 9-residue window location), further ensure the quality and reliability of structure as the higher quality score indicates higher quality. The regions of the structure that may be rejected at the 95% confidence level are represented as yellow bars. The outcomes of our work recommend the stability, quality and reliability of the target protein structure.

Docking analysis of 6COX with thymoquinone derivatives

Earlier studies on the nsp10-nsp16 complex of coronavirus suggest that this complex is crucial for replicating the virus in hosts.[20] In this study, we selected the most recent structure of the 6COX protein from PDB and performed the docking analysis of this target with 10 seleced compounds obtained from the natural origin showing inhibitory effects for viral infections. Our study using *in silico* docking tools also confirmed the findings inhibitory properties of 5 selected Thymoquinone derivatives against target protein in multiple conformations with the given range of binding energies (Table 2). The docking interactions profile of 5 selected natural (thymoquinone derivatives) compounds with 6COX protein complex can be easily understood by the interaction of their ligands with the active site residues of receptors by forming hydrogen bonds (Figure 5).

Table 1. Physicochemical parameters of 6COX

S. No.	Parameters	6COX
1	Mol. Weight	67239.94
2	No. of amino acids	587
3	Theoretical pI	6.86
4	Instability index (II)	39.20

5	No. of Negatively Charged Residues (Asp + Glu)	63
6	No. of Positively Charged Residues (Arg + Lys)	61
7	Aliphatic Index	79.20
8	Grand average of Hydropathicity (GRAVY)	-0.359
9	Atomic Composition	$C_{3049}H_{4669}N_{799}O_{870}S_{25}$

Table 2. Thymoquinone derivatives against target protein in multiple conformations with the given range of binding energies

Name of the Molecule	**Binding Energy (Kcal/mol)**	**Docking Energy (Kcal/mol)**	**No. of Hydrogen Bonds**	**Amino Acids**	**Distance A0**
TQ7	-9.35	-9.09	1	LEU352	1.6
TQ23	-0.42	-0.99	0	0	0
TQ26	-8.67	-1.74	1	HIS90	2.194
TQ27	-8.63	-9.99	1	LEU352	1.823
TQA6	-8.95	-9.14	1	HIS90	2.123

Figure 2 Ramachandran Plot

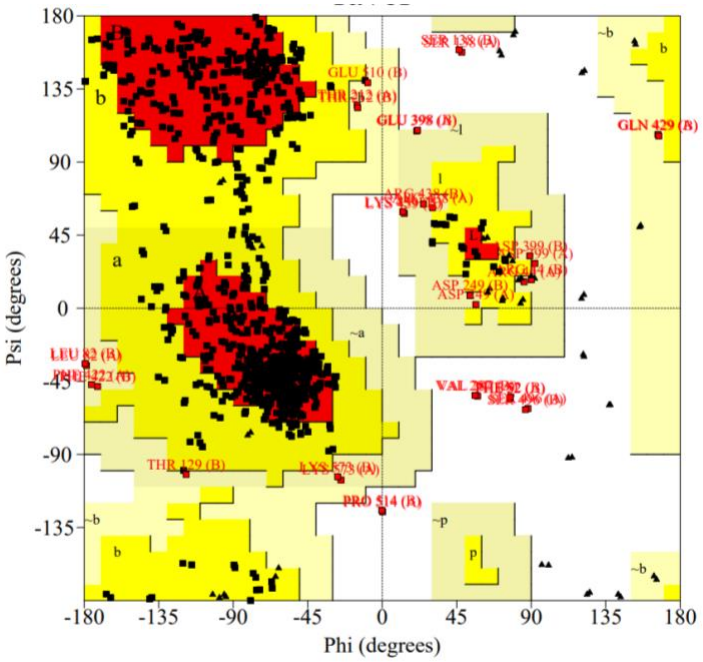


Figure 3 a and b – ProSA web results

a)	b)

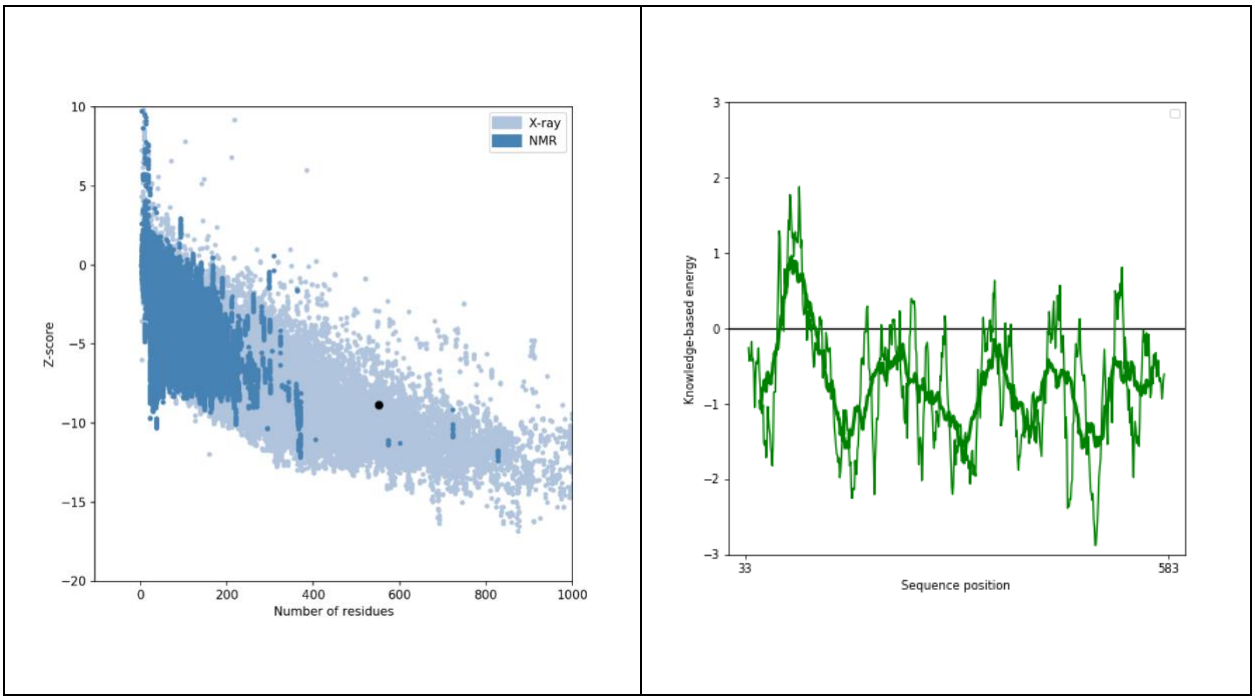


Figure 4 Errat plot

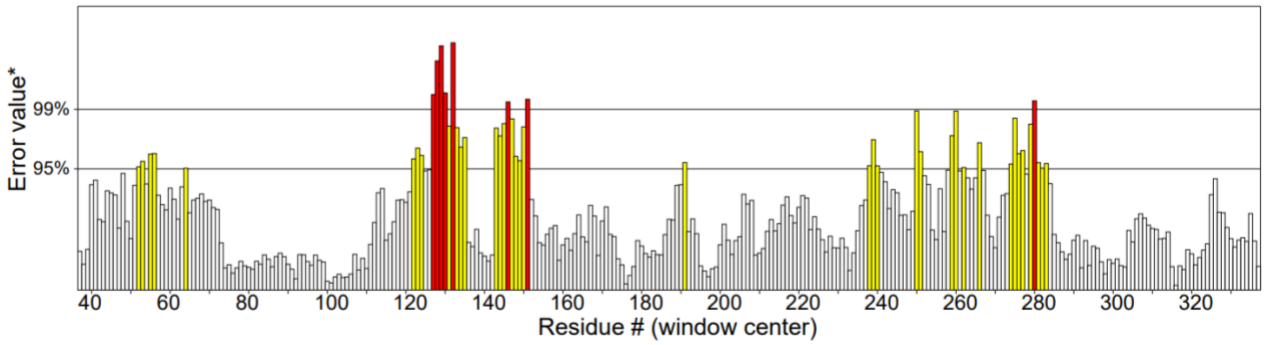
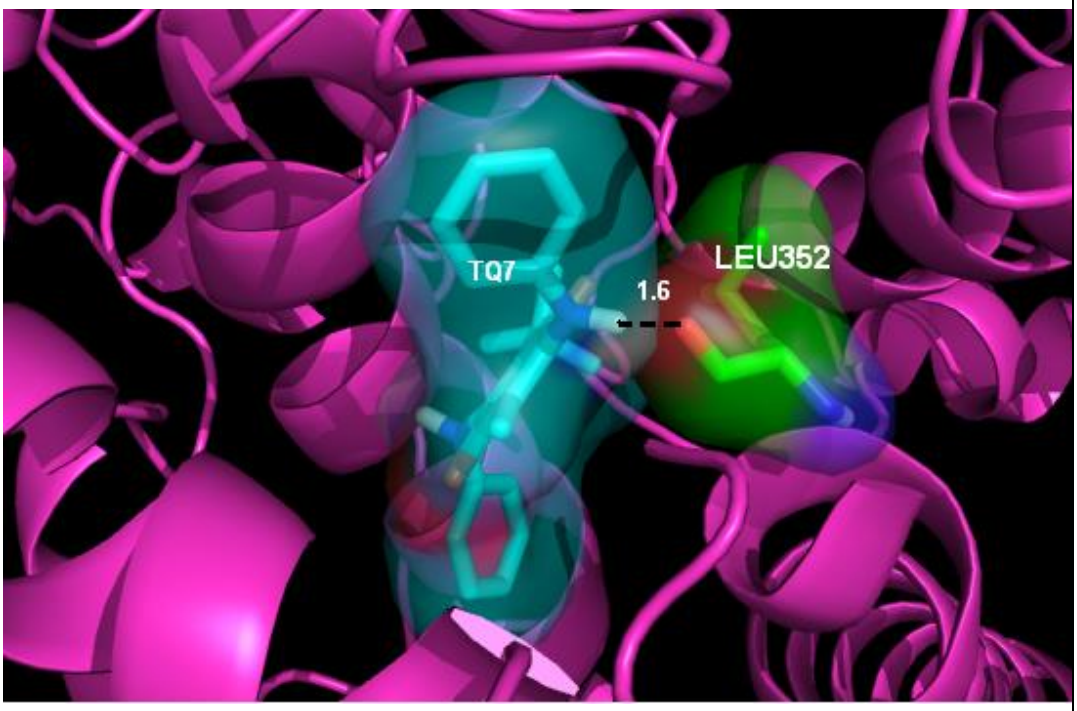
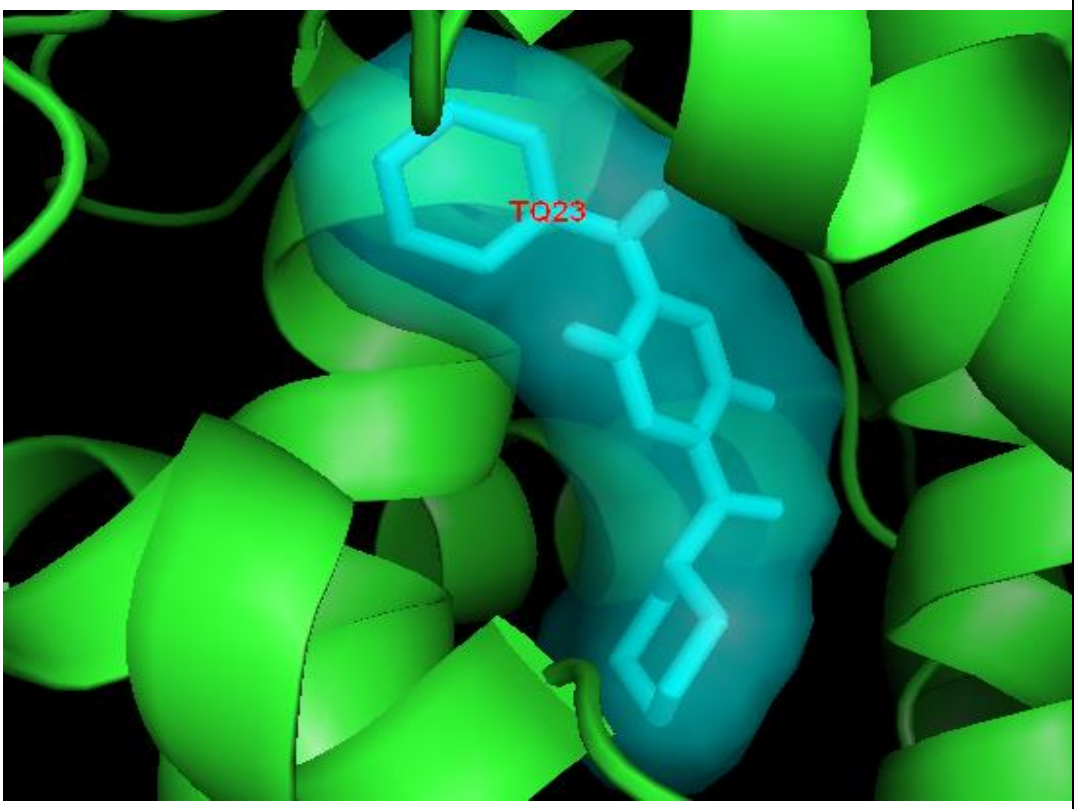
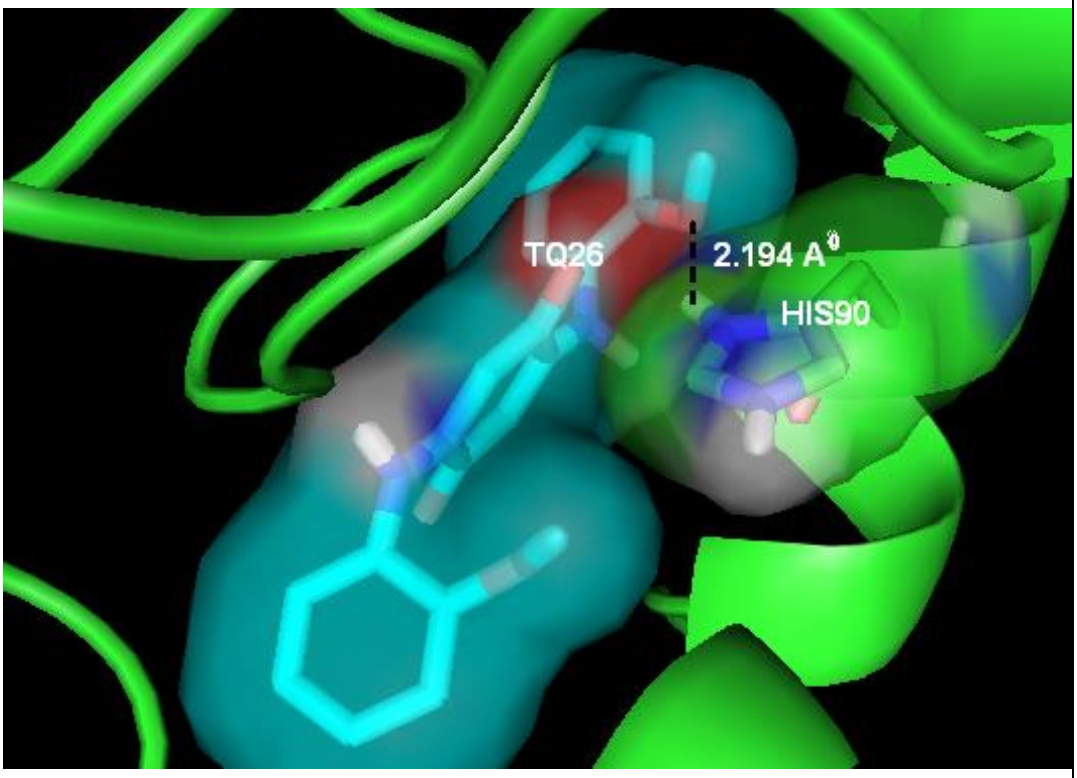
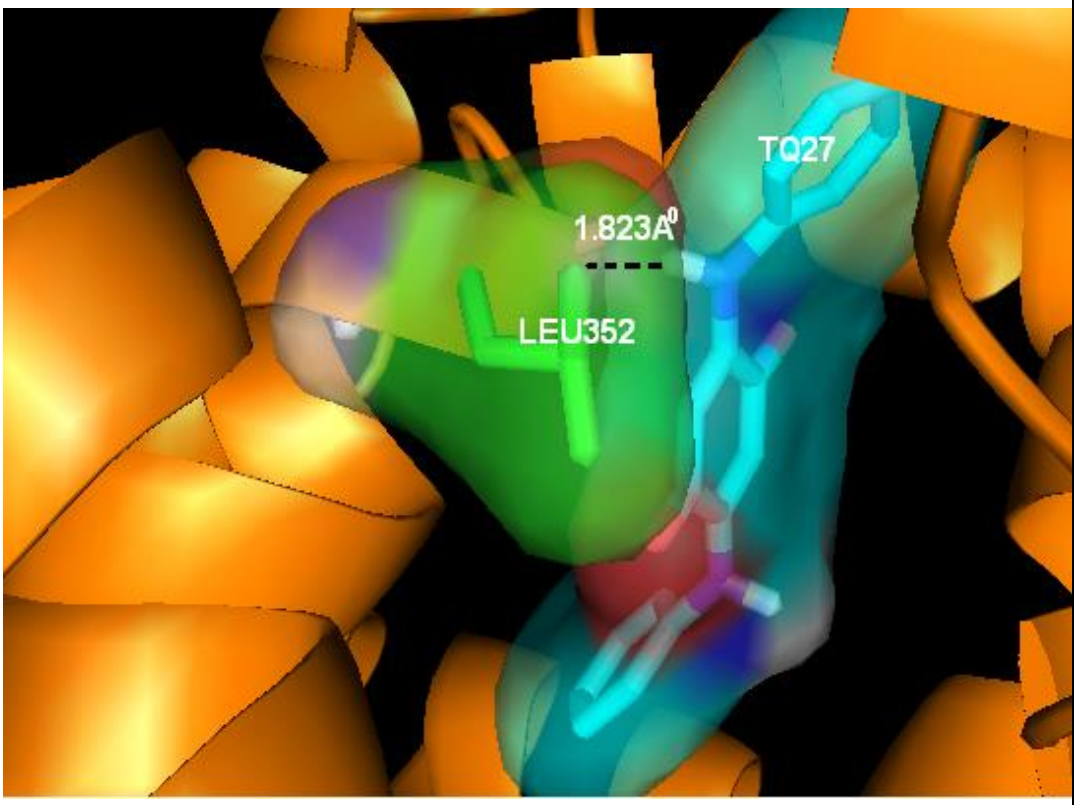


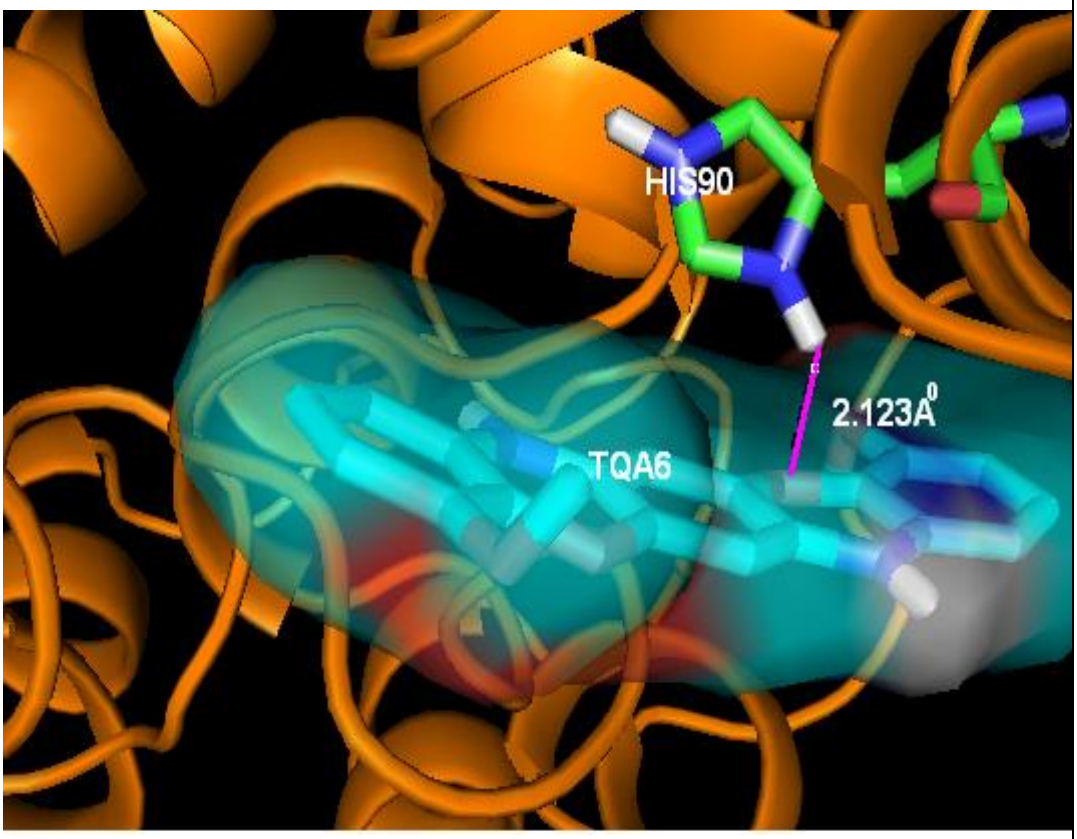
Figure 5 Docking studies

Compound	Docking
TQ7	

TQ23	

TQ26	

TQ27	

TQA6	

Discussion

Colorectal cancer is a serious global concern with a significant mortality rate. The disease has a complex etiology due to which it becomes difficult to adapt appropriate treatment strategies and therapeutics. In this study, we have explored the known Thymoquinone anti-cancer compound and its derivates as potential therapeutics for colorectal cancer using in silico studies. We docked COX-2 target with the five ligands (TQ7, TQ23, TQ26, TQ27 and TQA6). The binding affinity for the COX-2 was observed highest in the case of TQ7 (-9.35 kcal/mol) for LEU357. The five thymoquinone derivatives compounds that fall in the acceptable range of binding energy are TQ7 (-9.35), TQA7 (-8.95), TQ26 (-8.67), and TQ27 (-8.63). Our molecular docking studies confirmed that the phytochemical present in black seed, Thymoquinone and its derivatives can potentially inhibit colorectal cancer.

Conclusion

In this study, thymoquinone analogues were found to be selective COX-2 inhibitors. Docking tests indicated good Van der Waals contact and hydrogen bonding towards COX-2. TQ7, the first compound, had the best electrostatic and steric interaction with 6COX and formed the tightest hydrogen bond network with the active site. This compound has the potential to be the most effective therapeutic molecule or active inhibitor in the treatment of cancer and tumors. Our findings indicate that our newly synthesized classes of Thymoquinone analogues could be effective as cancer chemo preventive and therapeutic agents.

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