Journal of Advanced Scientific Research
Available online through https://sciensage.info
ISSN 0976-9595
Research Article

ROLE OF COX-2 IN ORAL CANCER AND *IN-SILICO* DOCKING OF NATURAL FRAGMENTED AND DRUG BASED LIGANDS OF COX-2 FOR INHIBITION OF PROGRESSION OF ORAL CANCER

Arpita Maitra*[1], Sanjeet Kumar Das[2], R.R. Paul[3], Mousumi Pal[4]

[1]*Ph.D. Scholar, Department of Oral and Dental Sciences, JIS University, Kolkata, West Bengal, India, and Assistant Professor, Department of Oral Medicine and Radiology, Guru Nanak Institute of Dental Sciences and Research, Kolkata, West Bengal, India*

[2]*Ph. D Scholar, Department of Oral and Dental Sciences, JIS University, Kolkata, West Bengal, India, and Reader, Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research, Kolkata, West Bengal, India*

[3]*Department of Oral and Dental Sciences, JIS University, Kolkata, West Bengal, India*

[4]*Department of Oral and Maxillofacial Pathology, Guru Nanak Institute of Dental Sciences and Research, Kolkata, West Bengal, India*

**Corresponding author: arpita6795@gmail.com*

Received: 06-07-2023; Accepted: 04-08-2023; Published: 30-09-2023

 https://doi.org/10.55218/JASR.202314807

ABSTRACT

Oral cancer is known for its devastating effects which need to be addressed at the molecular level to achieve the best possible outcomes. An upsurge in the COX-2 levels amongst premalignant & malignant tissues may be attributed to increased transcription along with enhanced mRNA stability. Compelling research evidence is being shown with respect to complexes which have dual COX-2/COX-1 inhibitors are beneficial in chemotherapeutic procedures of cancer.

Amongst 15 compounds, we found the highest binding capacity to be -10.9 in rutaecarpine as per the ADV webserver and -11.2 in VX-809 (Lumacaftor) as per the ProdigY webserver. Highest hydrophobicity was seen in OSR, LMR & ECG weighing 389.33, 452.41 & 458.37 with the most common amino acids being Leu (338), Ser (339), Val (509), Val (335). In respect to the molar refractivity, OSR & LMR presented with 92.01 & 113.98 values, whereas the lipophilisity was found to be 2.46 & 3.08 respectively with toxicity score (assessed by toxi M score & ProTox), being 0.929 & 0.944 by ToxiM score & 4 in both compounds by ProTox class.

Characteristic properties such as the hydrophobicity, hydrophilicity, molecular weight, number of H-bond acceptors, Num of H-bond donors, molar refractivity, lipophilisity (ilogP), drug likeliness and toxicity score play an important role in understanding drug-drug interactions at the molecular level which helps in determining the pharmacological actions and its application in humans. Therefore, further research in the field is recommended to comprehend the drug reactions & their outcome.

Keywords: Oral cancer, Molar refractivity, Toxicity, Risk, Inflammation, Prostaglandins, NSAIDS.

1. INTRODUCTION

Oral cancer is the sixth most cause of cancer worldwide, with its devastating effects as well as its aftermaths being a major cause of concern for the individual as well as their closed ones as it hampers the quality of life to a large extent.

There has been enormous research being carried out to find preventive as well as treatment measures to counteract these situations but has not tasted much success till date even with the available data which makes this a diagnostic challenge of significance requiring attention.

Oral cancer is a multiphasic disease process with the presence of precursor lesions/conditions paving the way to the disease process, which can be counteracted with strict preventive measures to hinder its progression but has very little effect which may be attributable to many risk factors.

Human body has a well-fabricated defense system which attacks any untoward events especially infections which trigger an inflammatory reaction being acute in the initial stage, which slowly progresses to the chronic type. This inflammatory reaction leads to more damage at the site and is being postulated as one of the reasons for the

progression of the disease process.

Chronic diseases like oral cancer show severe signs of progressive chronic inflammation caused due to pro inflammatory mediators which includes but is not limited to interleukins and intracellular enzymes such as COX and LOX, all of which are responsible for an upsurge in the prostaglandin levels which is responsible for pain [1-3]. The metabolic activity of conversion of arachidonic acid to prostaglandins is catalyzed by COX. Prostaglandins play an important role of homeostasis across physiological activities [4-6].

There are two types of Cyclo-oxygenase: COX-1 & COX-2 [1]. In homeostasis, the primary COX enzyme for the production of essential prostaglandins during homeostasis is COX-1; which also forms the constitutive isozyme. On the other hand, COX-2 is more of a pathological constituent which is accountable for the high production of prostaglandins during the inflammatory process as well as pathogenic stimuli with risk of cancer progression [7-9].

It has been noted that COX-2 expression is induced by agents like pro-inflammatory cytokines (IL1b, TNFα), lipopolysaccharides, mitogens, and oncogenes (phorbol esters), growth factors (fibroblast growth factor, FGF; platelet-derived growth factor, PDGF; epidermal growth factor, EGF), hormones (luteinizing hormone, LH) and water-electrolyte imbalance, leading to augmented production of PG's in inflamed as well as neoplastic tissues [10].

Literature data shows that there is an upsurge in the COX-2 levels amongst premalignant & malignant tissues, which may be attributed to increased transcription along with enhanced mRNA stability [11-13]. These in turn are promoted by oncogenes, cytokines, growth factors and tumor promoters. Increased transcription and decreased mRNA turnover have been noted in patients with colon cancer by overexpression of COX-2 [14].

The most postulated COX-2 action for cancer includes angiogenesis, xenobiotic metabolism, cell proliferation, immune function apoptosis and invasive nature of the tumor [10].

Research has made it evident that complexes which have dual COX-2/inhibitors are beneficial in chemotherapeutic procedures of cancer, as it helps downregulation of colorectal cancer progression by plummeting the aptitude for invasion as well as proliferation in cells of mouse colorectal cancer cell lines (CT26 cells) as well as human colorectal cancer (HCA7 cells), by supply of the PI3K/AKT pathway [15]. The amalgamation of celecoxib (COX-2 inhibitor) with MK886 (5-LOX inhibitor) can have been postulated to quash the progress of pancreatic tumor cells [16].

This dual action of drugs can be a game changer and is being studied with full extent as it can prevent as well as treat cancer by inhibiting inflammatory trajectory and suppress the progression of the disease process [1,17,18].

Non-steroidal anti-inflammatory drugs (NSAIDs) are well-known to treat symptoms like pain, redness, heat and swelling [19, 20]. The inhibitory activity of NSAIDS helps to prevent the biotransformation of arachidonic acid (AA) to end-products like prostaglandins (PGs), prostacyclin (PGI2), and thromboxane A2 (TXA2) via cyclooxygenase (COX) enzymes [9, 21].

The molecular docking technique is used to predict the binding geometry of the target molecules to calculate the enzymatic mechanisms interactions of NSAIDs and eugenol which COX in anti-inflammatory processes and in antitumor activity [1, 22, 23].

Hence, the use of docking technology to understand the geometric facial & spatial configuration with the binding effectiveness will provide us with an idea of the interactions and the capability to supersede the other drug which will help in creating or using the drugs of significance in prevention as well as treatment of oral cancer.

2. MATERIAL AND METHODS

The general functional form of the conformation-dependent part of the scoring function AutoDock Vina (Vina) is intended to work with summation over the pairs of atoms with the ability to move relative to each other with separation by 3 consecutive covalent bonds.

Each atom *i* is assigned a type *ti*, and together they form a symmetric set of interaction functions represented by *ftitj and* the interatomic distance *rij* should be defined. This can be seen as a summation of intermolecular and intramolecular contributions.

The optimization algorithm is designed to discover the global minimum of *c* as well as the other low-scoring conformations, which it then ranks (Inspired by X-Score & tuned using PDB bind).

Autodock as well as Vina use rectangular boxes for delineation of the binding site with the box providing explicit coordinates or describing a PyMOL selection like a reference ligand.

Mean coordinates of atoms are adjusted from PyMOL selection, and docking box is with the size and position adjusted as per the user's demands. Also two display

options with colour of the box frame can be adjusted. Also, binding site definitions can be transferred to input files for Autodock or Vina, represented in pdbqt format.
Prior calculation by autogrid program is performed and interaction energy between the ligand atom & receptor is calculated for entire binding site on a grid pattern, to ease and understand the interaction energies at each step of the docking process.
Two docking methods are followed

- AutoDock
- AutoDock Vina

AutoDock Vina is fast, effective, easy to use with minimal training and can perform docking experiments using well-tested default methods.
AutoDockVina uses a rigid receptor which reduces the size of the conformational space & provides a reliable search and forego scoring of each trial conformation.
The system significant receptor motion limitations can be overcome by

- Using receptor structures taken from receptor-ligand complexes
- Use of explicit receptor side chain flexibility during docking

2.1. The following software was used

- AutoDock 4.2.6
- Python 3.8.2
- Autodock Vina 4.2
- Chimera X
- MGLTools 1.5.4
- UCSF Chimera 1.12
- PyMOL 0.99

2.2. System requirements (as per software manual)

- Processor: i5-11300H @ 3.10GHz processor
- System memory: 8 GB RAM
- System type: 64-bit operating system
- Windows 10 Operating System

2.3. Ligand preparation

Three thousand (3000) natural drugs as well as compounds were collected over a literature survey with database retrieval from PubChem database. Thereafter, ligands of canonical Smiles were re-claimed and transformed into the protein data bank (PDB) form for docking, which served as standard values for comparison. The same transformation was done for many drugs and given the status of 'drug of choice' against the COX-2, which was also docked and equated.

2.4. Protein preparation

The 3D form of COX-2 for head and neck cancer was taken from the Protein Data Bank database (PDB Id: 3LNA). Protein was stored in as a complex with a peptide inhibitor. Further, water molecules, inhibitor & heteroatoms were retrieved from the protein for docking purposes. Finally, only A chain was maintained in pdb format. Thereafter, ligands were removed and crystallization of water molecules by the use of Chimera software was done. UCSF Chimera (Docking the Target Protein) is used for visualization & analysis of the molecular structures.

2.5. Protocol

- Click on the file and fetch by ID
- Input the PDB ID of the protein (3LNA)
- When the protein is fetched, the PDB file can be downloaded beforehand and opened thorough File > open.
- Displays the structure retrieved in UCSF Chimera
- Create a working directory for the docking project with easy access points such as Users/Desktop/ Docking/.
- Start saving all your prepared files.

2.5.1. Preparing the Target Protein for Docking

- Optimization of protein for docking
  - Click on Tools > Structure Editing > Dock Prep
  - Chimera contains all required dock prep tools within the structure editing file menu.
  - Within dock prep box, select all options except "Delete non-complexed ions" and proceed.
- Add hydrogen to the proteins
- Use Gasteiger charges to assign charges to the protein
- Save the file as preped_3LNA.PDB.

2.5.2. Determination of active site

The active site is primarily determined for enzyme inhibition and this active site of the protease was determined using the available literature.
Later, the processed protein data bank file without heteroatoms was uploaded and the best of 8 (z-score) potential ligand-binding sites was selected for docking.
The predicted amino acid residues were then equated against the amino acids at the active site of the celecoxib-COX-2 co-crystallized complex.

2.5.3. Preparing the Ligand for Docking

Fetched through the software with correct Pubchem compound ID (CID)

Structure Editing > Build Structure > PubChem CID or you can even insert the simplified molecular-input line-entry system (SMILES) of the novel compound being used.

- PubChem CID is entered
- The ligand as well as the protein is optimized
- Tools > Structure Editing > Dock Prep, and repeat steps followed for preparing the protein (solvents removal, followed by addition of hydrogens and determine charge.
- Ligand saved as prep_pubchemid.pdb file in working directory

2.5.4. Docking

- Tools > Surface or Binding Analysis >Autodock Vina
- Grid box value at the active site is set up (at the site of previous inhibitor)
- In case an inhibitor was absent, the literature provides that site as well as the active site
- Output file was saved as PUBCHEM ID_out. pdbqt in same directory. Eg 52034.pdbqt
- Inhibitor molecule attached to the original 3D structure was deleted
- Actions > Atoms and Bonds >Delete
- Removal of the inhibitor was important to easily visualize the docking results
- PDB needed to be saved again as preped_3LNA. PDB
- Receptor as the protein from drop-down menu & ligand were chosen
- Important to set the right receptor and ligand
- Local path was designated as to where the installed version of Autodock Vina was placed.

2.5.5. Outcome of Docking

After the successful run of Autodock Vina, the score, root-mean-square deviation (RMSD) lower bound, and RMSD upper bound was obtained.

2.5.6. Molecular docking using AutoDock 4.2.6

AutoDock 4.2.6 was downloaded from 'The Scripps Research Institute' official website (http:// autodock. scripps.edu/) with other supporting software viz., Python 3.8.2 and MGLTools 1.5.4. Docking of ligands and COX-2 was done indigenously by docking 'one ligand at a time to the protein' manually using AutoDock 4.2.6.

2.5.7. Initializing and preparation of PDBQT files

- Prior to docking, the required folder was selected
- The processed protein molecule was imported into the AutoDock 4.2.6 workspace.
- Addition of polar hydrogen atoms
- Kollman and Gasteiger charges for the protein were computed.
- The protein was then saved in PDBQT format.
- The ligand was imported into the workstation with the torsion tree being defined by choosing the root with identification of rotatable bonds & saved in PDBQT format.
- The ligand and protein transferred into the workspace for further simulation.

2.5.8. Grid parameters

As stated, the projected active site was in harmony with PyMOL co-crystallized protease with a peptide inhibitor which ensured exact binding of ligand exactly binds to the active site of the protease.

2.5.9. Gridparameters

- Center grid box values - x¼ =30, y¼= -23, and z¼=-18
- Offset values - 19, 17, and 18 respectively.
- File format - grid parameter file (GPF).

2.5.10. Running AutoGrid and AutoDock

AutoGrid executable & GPF files were used as input and changed into grid log file (GLG) & launched.

Genetic algorithm was set to default

i) Number of GA runs: 10
ii) Population size: 150
iii) Number of energy evaluations:2.5 million (2.0 Å clustered tolerance)
iv) Number of generations: 27000

The Lamarckian genetic algorithm followed & saved as docking parameter file (DPF) file format. AutoDock was transformed to the docking log file (DLG) and docking was done.

DLG file contains top 8 free binding energy energies for every run and inhibitory constant with

result saved in PDBQT format & lowest binding energy complex in PDB format.

2.5.11. Visualizing interactions

UCSF Chimera 1.12, PyMOL 0.99 and PBDsum web server were used to visualize and study the 2-dimensional and 3-dimensional, and surface annotation of ligand interaction with the protein.

2.5.12. Docking validation

The docking procedure was validated using two methods:

1. Celecoxib inhibitor from the COX-2 was detached and inserted into active site with AutoDock 4.2 by manual co-crystallized complex opening in a notepad & removal of inhibitor heteroatoms from COX-2 & adding onto a new notepad in PDB file format (Open Babel web server). The same protocol was followed to ensure the precise binding of inhibitor to the active site cleft in comparison to actual co-crystallized complex when superimposed (using PyMOL 0.99). The root mean square deviation was assessed with superimposition of 2-dimensional image of the amino acid residues by Chimera-X software.
2. Decoy ligands analogous to celecoxib peptide inhibitor were acquired and docked alongside the active site of COX-2. This enhances ligand enrichment & is essential to validate docking.

2.5.13. Pharmacokinetic properties and Lipinski's rule of 5

Pharmacokinetic properties were determined using Swiss ADME (http://www.swissadme.ch/).

Lipinski's oral drug likeliness properties were anticipated using PubChem database which has

i) Molecular weight
ii) Number of hydrogen bond donors - <5
iii) Number of hydrogen bond acceptors - <10
iv) Log P - <5
v) Molar refractivity - <140

Ligands toxicity was assessed using ProTox-II, Toxi-M.

The top 8 ligands that showed the best binding energy are being investigated for a potential cure against head and neck carcinoma.

In total, 550 compounds (natural, fragmented compounds as well as drugs) were vetted by molecular docking which showed displayed moderate score in comparison with existing substrate as well as inhibitor bound crystal structures for docking score.

3. RESULTS

On analysis of 15 compounds (Table 1) via the webservers (ADV & ProdigY), we found the highest binding capacity to be -10.9 in rutaecarpine as per the ADV webserver and -11.2 in VX-809 (Lumacaftor) as per the ProdigY webserver. On a combined analysis, the highest energy as per both the servers was found to be in VX-809 compound & the lowest was in Indirubin.

Table 2 shows that the highest hydrophobicity as per the amino acids in the 8 compounds to be with respect to OSR, LMR & ECG respectively and the most common amino acids showing hydrophobicity across the 8 compounds to beLeu(338), Ser(339),Val(509),Val (335).

A detailed chemical analysis of the 8 compounds was done wherein the highest molecular weight was observed in OSR, LMR & ECG weighing 389.33, 452.41 &458.37 respectively. Amongst the 8 compounds the ECG was the compound with the capability of 11 NHBA & 8 NHBD, which was more than that of the acceptable range in either case, however OSR & LMR, recorded to have similar values (8 - NHBA, 2 - NHBD).

Further, with respect to the molar refractivity, OSR & LMR presented with 92.01 & 113.98 values, whereas the lipophilisity was found to be 2.46 &3.08 respectively. In terms of toxicity score (assessed by toxi M score &ProTox), we found that OSR & LMR presented with almost identical values (0.929 & 0.944 by ToxiM score; 4 in both compounds by ProTox class) making them equal contenders as compounds of significance.

4. DISCUSSION

Pharmacological line of treatment forms an essential component of any treatment protocol irrespective of the disease process. Therefore, enormous research is done in the hunt for better drugs which can provide maximum beneficial outcome, whilst minimizing damage or complications caused by them. The mechanism of action is dependent on the characteristics of each drug with the cell as well as other pharmacological drugs which help to determine their application in patients.

Pain relief is the most common reason a patient approaches the physician, which requires efficacious result. It is imperative to understand the pharmacokinetics of the drug in relation to its interaction with the patient in terms of absorption, uptake, binding, cell-drug interaction, drug-drug interaction & its excretion.

COX is known to be the core enzyme responsible for transformation of arachidonic acid to prostaglandins, which is critical in arbitrating the homeostatic functions across various physiological systems [4-6]. Therefore, it is important to understand the basis of the cell-drug interaction at the molecular level.

COX consists of two isoforms; COX-1& COX-2. COX-1 is the constitutive isozyme, whose inherent role is basal fabrication of essential PGs during the process of homeostasis and is considered to be more prevalent in physiological conditions. In contrast COX-2 is found to be higher in pathologic/non-physiologic conditions with miniscule amounts observed in normal physiologic conditions. COX-2 plays an imperative role during inflammatory activity (during infection and/or cancer) leading to increase in prostaglandin levels [7-9].

On the other hand, Lipoxygenases (LOXs) forms a heterogeneous class of enzymes which helps initiates the peroxidation activity of polyinsaturated fatty acids. Amongst these, 5-LOX enzyme is a lipoxygenase isoform in relation with inflammation, bronchoconstriction, hypersensitivity, anaphylaxis, and asthma [8, 19, 24]. Ca^{2+} targeted membrane binding and phosphorylation at specific serine residues helps to regulate the LOX-5 activity [8, 24].

Our study analysis earmarked the evaluation of the top 15 compounds which formed a complex with COX-2. They were assessed & recorded as per the autodock vina RMSD score as well as the prodigy webserver in relation to celecoxib. Celecoxib was used for evaluation in our study as it has a binding energy of -12.5kcal/mol, which is considered as the gold standard for evaluation in terms of comparative analysis with other drugs.

The 15 docked conformations generated in our study during the docking with ADV of each ligand with their highest binding energy conformation was selected for 2D visualization of interactions, after which we segregated 8 compounds. These 8 compounds were selected based on the values obtained by autodock vina score of -9 and above with a prodigy value of -10.

These 8 compounds were redocked using autodock 4.2 and were enlisted based on their Ki value scores, 3 digit codes and 2-D structures.

The 8 selected compounds as per the two servers were Rutaecarpine (RTP), TanshinoneI (TSN), Ostarine (GTx-024, MK-2866) (OSR), VX-809 (Lumacaftor) (LMR), Apigenin (AGN), Tanshinone IIA (TSA), Epigallocatechin Gallate (ECG) & Baicalein (BCN).

The application of the moleculardynamics simulation is the most widely accepted approach for predicting the protein–ligand complex's stability. The 100 ns atomistic MD simulation is performed to explore the dynamic property of each identified protein-ligand complex and compared with the dynamic behavior of the ligand-free protein (LFP) co-crystalline inhibitor bound protein. This helps to understand the underlying molecular dynamics along with their interactions to form complexes which helps in understanding the inhibitory effect [1, 10, 25].

Amongst all the parameters, hydrophobicity & hydrophilicity of the compound are the most important parameters of significance which have to be understood to comprehend drug-drug interactions as well as their outcome. The hydrophobic/hydrophilic properties of the drug in respect to their binding site which are observed over the surface are important in determining as to whether the drug is hydrophobic or hydrophilic in nature. The interactions are observed in terms of their stacking potential which plays a pivotal role to grade/rank the molecular docking; in addition to assessing and confiding the relationship amongst the target as well as drugs. It is these properties, which ultimately help to estimate the surface properties as well as stacking on the membrane collateral to the molecular dynamics run as well as features of interface amid the membrane and membrane-binding molecule [25].

The amino acids with residual atoms and their number of these compounds are enlisted in the table for a better comprehensive understanding of the drug. Also, the hydrophobic or hydrophilic natures of the compound with respect to the amino acid were assessed & enlisted using the pdbsum webserver was added.

Proteins are considered the back-bone/ building blocks of life as each & every cell in the human body is made of protein, with the structure of a protein being fabricated by the chain of amino acids. The role of amino acids cannot be ignored and plays a detrimental role in regulation and maintenance of activities of the body as they are the basis for even pharmacokinetics involving drug interactions.

Along with docking score, interaction with crucial amino acid residues may be other important criteria in the selection of potential inhibitors. The most common amino acids with residual atoms which showed hydrophobicity across these 8 compounds was found to be Leu(338), Ser(339), Val(509), Val (335). Our study analysis also showed that the maximum amount of hydrophobicity as well as hydrophilicity in amino acids (with residual atoms) was recorded in His (75), Phe (504), Tyr (314).

We also found that amongst these 8 compounds, the maximum amount of hydrophobicity with OSR, LMR & ECG respectively, as per the list of amino acids we observed.

A detailed analysis done with respect to the properties of the 8 selected compounds using SWISSAdme in terms of the molecular weight, number of H-bond acceptors,

Num of H-bond donors, molar refractivity, lipophilisity (ilogP), drug likeliness and toxicity score using Protox and Toxi-M server was assessed as these are important to understand drug-drug interactions

As hydrophobicity is more favorable for selection of drug the three most common compounds that is OSR, LMR & ECG was of more significance. All the 8 compounds were found to be within the acceptable molecular range of ≤500 with the highest molecular weight to the lowest molecular weight being ECG - 458.37, LMR - 452.41, OSR - 389.33, TSA - 294.94, RTP - 287.32, TSN - 276.29, AGN & BCN - 270.24 g/mol respectively.

For a drug-like molecule, the molar refractivity should be between 40 and 130. The highest molar refractivity amongst the 8 compounds was found to be of LMR (113.98), followed by ECG (112.06), OSR (92.01), RTP (87.41), TSA (84.7), TSN (80.24), AGN &BCN (73.99) respectively. Further assessment of hydrogen bond acceptors & donors found OSR & LMR to be in acceptable range, whilst ECG seen to be otherwise in the acceptable range.

Amongst all the 8 compounds, both AGN & BCN showed characteristic similarity in terms of MW, MR, NHBA, NHBD, drug likeness & Protox class, with the Toxi M score almost approximating each other. However, ilogp was not similar with AGN recording a score of 1.89 & BCN recording it to be 2.43.

As per the molar refractivity, lipophilisity and toxicity score assessed by toxi M score &ProTox was almost similar making the most common preferable compounds against all the 8 compounds. Amongst all the drugs, the highest Toxi M score was recorded in TSN, followed by AGN, RTP, BCN, TSA, LMR, OSR and the least in ECG. However, it was only AGN & BCN which were classified as Protox class 5, whereas all other class of drugs was classified into Protox class 4.

Amongst the 8 compounds assessed, we found OSR & LMR to have the most optimistic properties with acceptable range of characteristics and toxicity, which forms the basis of the clinical research at the initial stages, before progression of the compound for further assessment at the clinical level in patients.

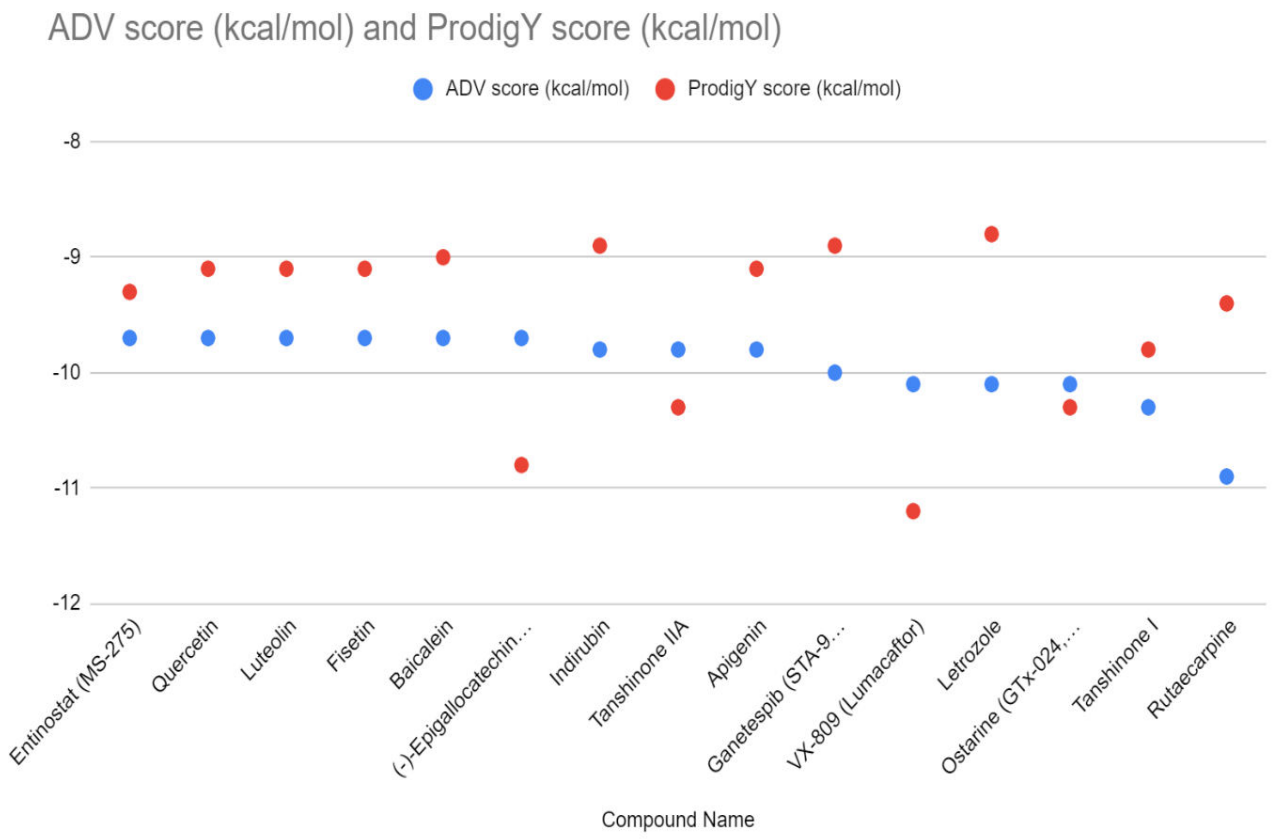
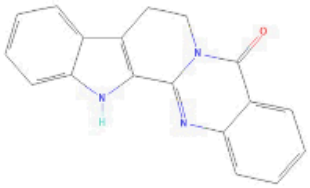
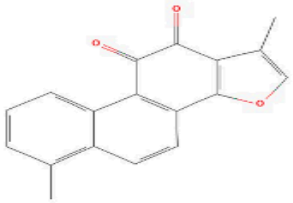
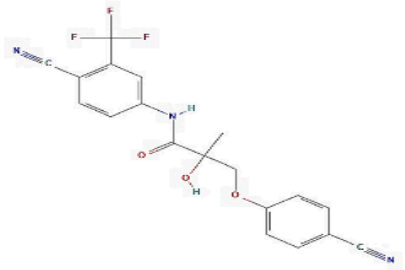
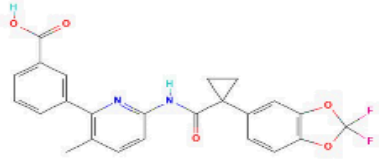


Fig. 1: Top 15 compounds that produce complex with cox-2, have high binding energy based on autodock vina rmsd score and prodigy serve in relation to celecoxib that has binding energy of -12.5kcal/mol.

Table 1: Top 15 compounds that produce complex with cox-2, have high binding energy based on autodock vina rmsd score and prodigy server n relation to celecoxib that has binding energy of -12.5kcal/mol.)

Sr. No.	Compound Name	Code	ADV score (kcal/mol)	ProdigY score (kcal/mol)
1	Rutaecarpine	RTP	-10.9	-9.4
2	Tanshinone I	TSN	-10.3	-9.8
3	Ostarine (GTx-024, MK-2866)	OSR	-10.1	-10.3
4	Letrozole	LTZ	-10.1	-8.8
5	VX-809 (Lumacaftor)	LMR	-10.1	-11.2
6	Ganetespib (STA-9090)	GTB	-10	-8.9
7	Apigenin	AGN	-9.8	-9.1
8	Tanshinone IIA	TSA	-9.8	-10.3
9	Indirubin	IRN	-9.8	-8.9
10	(-)-Epigallocatechin Gallate	ECG	-9.7	-10.8
11	Baicalein	BCN	-9.7	-9
12	Fisetin	FSN	-9.7	-9.1
13	Luteolin	LTN	-9.7	-9.1
14	Quercetin	QRN	-9.7	-9.1
15	Entinostat (MS-275)	ETS	-9.7	-9.3

Table 2: Based on the plot diagram the compounds with autodock vina score -9 and above are selected and prodigy value of -10 and above are selected. 8 compounds fulfil both the criterias. Hence, the top 8 were redocked using autodock 4.2 and enlisted based on their Ki value scores, 3 digit codes and 2-D structures

Compound Name	Code	Structure	AD 4.0 Ki
Rutaecarpine	RTP		69.28 nM
Tanshinone I	TSN		128.90 nM
Ostarine (GTx-024, MK-2866)	OSR		131.47 nM
VX-809 (Lumacaftor)	LMR		41.10 nM

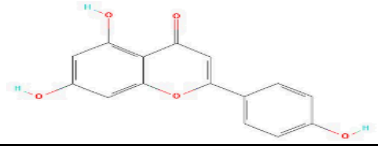
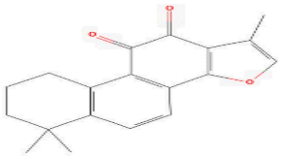
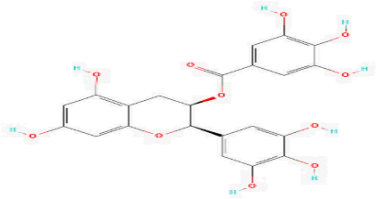
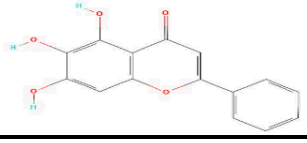
Apigenin	AGN		3.01 uM
Tanshinone IIA	TSA		62.65 nM
(-)-Epigallocatechin Gallate	ECG		6.99 uM
Baicalein	BCN		3.29 uM

Table 3: The amino acids with residual atoms and their number is enlisted. Along with that if they are hydrophobic or hydrophilic is enlisted using pdbsum webserver

Name of AA With residue number		RTP	TSN	OSR	LMR	AGN	TSA	ECG	BCN
His	75	HP	HP	HP	H	H	HP	H	H
Ile	503		HP			H		HP	H
Phe	504	HP	HP	HP	HP	H	HP	HP	H
Tyr	371			H		H		H	HP
Ser	516		HP	H		H		HP	HP
Leu	338	HP	HP	HP	HP	HP	HP	HP	HP
Ser	339	HP	HP	HP	HP	HP	HP	HP	HP
Val	509	HP	HP	HP	HP	HP	HP	HP	HP
Tyr	341	HP	H	HP	H	HP	H	H	HP
Gln	178	HP	HP	HP	HP	HP	HP		HP
Ala	502	HP		HP	HP	HP			HP
Val	335	HP	HP	HP	HP	HP	HP	HP	HP
Trp	373			HP		HP		HP	HP
Arg	106				H		HP	H	
Met	99				HP			HP	
Val	102				HP			HP	
Leu	345				HP			HP	
Ala	513	HP	HP	HP	HP		HP	HP	
Tyr	334							HP	
Gly	512			HP				HP	
Leu	517	HP	HP		HP		HP	HP	
Tyr	101				HP				
Leu	78				HP				
Val	74				HP				
Leu	370			HP					
Met	508			HP					
Ile	503	HP		HP			HP		
Ala	502						HP		
Arg	499		HP				HP		

Table 4: Using SWISSAdme, the molecular weight, number of H-bond acceptors, Num of H-bond donors, molar refractivity, lipophilisity(ilogP), drug likeliness and toxicity score using Protox and Toxi-M server is analysed of these top 8 elements.

Parameters	RTP	TSN	OSR	LMR	AGN	TSA	ECG	BCN
MW	287.32 g/mol	276.29 g/mol	389.33 g/mol	452.41 g/mol	270.24 g/mol	294.34g/ mol	458.37 g/mol	270.24 g/mol
Acceptable range	≤ 500	≤ 500	≤ 500	≤ 500	≤ 500	≤ 500	≤ 500	≤ 500
NHBA	2	3	8	8	5	3	11	5
Acceptable range	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
NHBD	1	0	2	2	3	0	8	3
Acceptable range	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5
MR	87.41	80.24	92.01	113.98	73.99	84.7	112.06	73.99
Acceptable range	40–130	40–130	40–130	40–130	40–130	40–130	40–130	40–130
ilogp	2.51	2.44	2.46	3.08	1.89	2.79	1.53	2.43
Acceptable range	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5	≤ 5
Druglikeness	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)	Yes (0 Violation)
Toxi M score	0.98	0.986	0.929	0.944	0.985	0.948	0.926	0.957
ProTox class	4	4	4	4	5	4	4	5

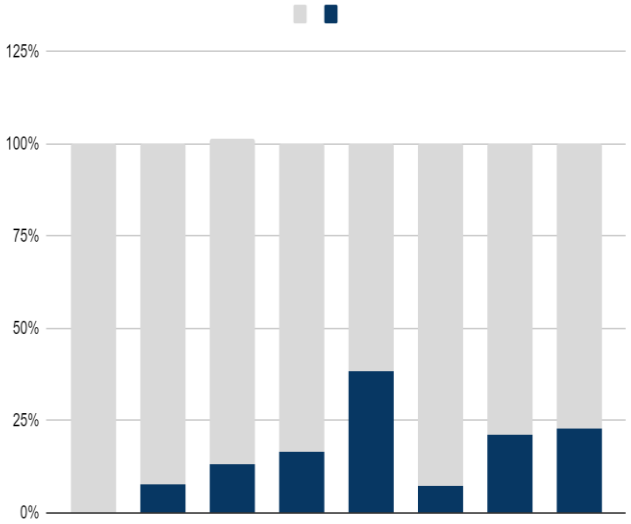


Fig. 2: Based on the plot diagram the compounds with autodock vina score -9 and above are selected and prodigy value of -10 and above are selected. 8 compounds fulfil both the criterias. Hence, the top 8 were redocked using autodock 4.2 and enlisted based on their Ki value scores, 3 digit codes and 2-D structures.

5. CONCLUSION

The characteristic properties of the compounds, such as the hydrophobicity, hydrophilicity, molecular weight, number of H-bond acceptors, Num of H-bond donors, molar refractivity, lipophilisity (ilogP), drug likeliness and toxicity score play an important role in understanding drug-drug interactions at the molecular level which helps in determining the pharmacological actions and its application in humans.

6. ACKNOWLEDGEMENT

My sincere thanks to the Department of Oral and Maxillofacial Pathology for guiding me in planning and executing the review. I thank my senior colleagues in the Department and the Post Graduate students for active help and support in the attempt.

Conflicts of interest

Nil

7. REFERENCES

1. das Chagas Pereira de Andrade F, Mendes AN. *Scientific Reports*, 2020; **10:**16204
2. de Araújo Lopes A et al. *Oxid. Med. Cell. Longev*. 2018, 8194849.
3. Amy IY et al. Cell Rep., 2020; **31:**107471.
4. Lee K, Lee SH, Kim TH. *Int. J. Mol. Sci.,* 2020; **21:**1851.
5. Piper K, Garelnabi M. *J. Clin. Transl. Endocrinol.,* 2020; **19:**100216.
6. Gomes FI, Cunha FQ, Cunha TM. *Biochem Pharmacol.,* 2020.
7. Famitafreshi H, Karimian M. *Degener Neurol Neuromuscul. Dis.*, 2020; **10:**1–13.
8. da Silva-Souza HA et al. *Biochim. Biophys. Acta Biomembr.,* 2014; **1838:**1967-1977.

9. Costa-Junior HM et al. P*rostaglandins Other Lipid Mediat.*, 2009; **88:**51-61.
10. Zarghi A, Arfaei S. *Iranian Journal of Pharmaceutical Research,* 2011; **10(4):**655-683
11. Dannenberg AJ. *Lancet Oncol.*, 2001; **2:**544-51
12. Shao J, Sheng H, Inoue H, Morrow JD, Dubois RN. *J Biol Chem.*, 2000; **275:**33951-33956.
13. Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. *J Biol Chem.*, 2000; **275:** 11750-11757.
14. Subbaramaiah K, Dannenberg AJ. *Trends Pharmacol. Sci.*, 2003; **2:**96-101.
15. Chang J et al. *Biochem. Biophys. Res. Commun.*, 2019; **17:**1-7.
16. Ding X, Zhu C, Qiang H, Zhou X, Zhou G. *Biomed Pharmacother.*, 2011; **65:** 486-490.
17. Manju SL, Ethiraj KR, Elias G. *Eur J Pharm Sci.*, 2018; **121:**356-381.
18. Sethi G, Shanmugam MK, Ramachandran L, Kumar AP, Tergaonkar V. *Biosci Rep.*, 2012; **32:**1-15.
19. Lamie PF, Philoppes JN, Rárová L. *Arch. Pharm.,* 2018; **351:**1-11.
20. Oniga SD et al. *Molecules*, 2017; **22:**1-15.
21. Almeida EJ et al. *CiêncAgrotec,* 2010; **34:**1658-63.
22. Trott O, Olson AJ. *J Comput Chem.*, 2010; **31:**455-61.
23. Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock Vina with AutoDockTools: A Tutorial, 2012.
24. Gilbert NC et al. *FASEB J.*, 2012; **26:**3222-3229.
25. Muhammad S, Fatima N. *Pharmacogn Mag.*, 2015 May; **11(Suppl 1):**S123-S126.