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Author (s): Muhammad Maaz[1], Erum Dilshad[1], Suliman[1], Naeem Mehmood Ashraf[2]

Affiliation (s): [1]Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology (CUST), Islamabad Pakistan

[2]School of Biochemistry and Molecular Biology, University of Punjab Pakistan

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Identification of Anti-inflammatory Metabolites from *Trigonella foenum-greacum* using Computational Approaches

Muhammad Maaz[1], Erum Dilshad[1]*, Suliman[1], Naeem Mehmood Ashraf[2]
[1]Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology (CUST), Islamabad Pakistan

[2]School of Biochemistry and Molecular Biology, University of Punjab Pakistan

Abstract

Human disease prevalence has increased as a result of modern lifestyle, stress, and toxic waste. Worldwide, researchers aim to discover therapeutic compounds which may cure and prevent the onset of diseases. So, this study was planned to discover potential anti-inflammatory metabolites from *Trigonella foenum-graecum.* It is an annual plant within the family Fabaceae. This plant is used as a spice throughout the world and has many beneficial medicinal effects. It is commonly grown in Pakistan, India, and some Middle Eastern countries. Ten bioactive compounds representative of all classes, namely alkaloid, flavonoid, phytic acid, 4-hydroxy isoleucine, sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol were selected. Molecular docking of these ligands was carried out against drug targets namely cyclooxygenase-2, Human Neutrophil Elastase (HNE), microsomal PGES-2, and tyrosinase by using CB Dock and AMDock softwares. Further refining by screening filters produced sapogenin as the lead compound. All the visualization analysis and interaction studies were performed using PyMol molecular visualization tool and Ligplot^{+}. Celebrex was used as the standard for comparison. The comparison between sapogenin and celebrex showed that the former is much more active than the standard drug. This is a novel finding. So, it might be explored further as a drug candidate to treat chronic inflammatory diseases in the future.

Keywords: anti-inflammatory drugs, AMDock, CB Dock, celebrex, medicinal plants, *Trigonella foenum graecum*, sapogenin

*Corresponding author: dr.erum@cust.edu.pk

1. Introduction

Nature exemplifies the aspects required for coexistence, perfectly. Plants that contain natural compounds are used to cure a wide range of diseases [1]. Medicinal plants, used in herbal medicine, are an important source of medicines around the world. Some examples of the earliest herbal medicine systems include ayurvedic, unani, and Chinese traditional medicine [2]. Generally, medicinal plants have been used since the beginning of history. It can be said that ancient peoples used these plants for many purposes, such as fuel, clothing, shelter, and food [3]. Different parts of medicinal plants are used for the development of drugs, such as leaves, seeds, roots, and flowers. Mostly, medicinal plants contain bioactive compounds which have (direct or indirect) therapeutic effects and are used as medicinal agents, so these plants are used as complementary or alternative medicine, globally [4]. For the prevention and treatment of diseases, scientifically validated herbal medicines systematically use purified, standardized, and effective phytochemicals [5].

Trigonella foenum-graecum is an annual plant within the family Fabaceae. It is commonly known as Fenugreek, which is an aromatic herbaceous plant. Fenugreek is an important spice and is commonly used as traditional food and medicine [6]. It is one of the world's oldest cultivated spice crops, mostly grown in Pakistan, India, Turkey, Egypt, the United Kingdom, Canada, and the United States for medicinal purposes [7]. Its seeds are used in traditional medicine as an antibacterial, antidiabetic, and anti-inflammatory agent. It contains various classes of compounds, such as alkaloid, flavonoid, phytic acid, 4-hydroxy isoleucine, sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol. The edible part of the leaves contains moisture (86.1%), carbohydrates (6%), protein (4.4%), minerals (1.5%), fibre (1.1%), and fat (0.9%). Hence, this plant is a good source of proteins, fats, carbohydrates, minerals, and vitamins including vitamins A, B1, and C [8]. Moreover, it has antiviral, antimicrobial, antioxidant, and anti-inflammatory properties [9].

Inflammation is a defence mechanism of the body that protects tissues from harmful stimuli. It is a complex biological reaction of vascular tissues against possibly harmful external and internal stimuli,

such as disease causing agents, chemicals, and foreign agents. The inflammatory action of *Trigonella foenum-graecum* is due to the inhibition of cyclooxygenase proteins (COX-1 and COX-2) and other novel enzymes, such as human neutrophil elastase (HNE), tyrosinase, and microsomal prostaglandin E synthase-2 (mPGES-2). Cyclooxygenase enzyme, also known as prostaglandins (PTGS), is responsible for the formation of prostanoids, together with arachidonic acid from prostaglandins, such as thromboxane and prostaglandin. There are two isoforms of cyclooxygenase usually known as Cox-1 and Cox-2. Cox-1 functions as a housekeeping isoform of cyclooxygenases and is mainly expressed to serve functions controlling urinary organ blood flow, stomach protection against ulcers, and preparation of prostaglandin E-2 [10]. On the other hand, cyclooxygenase-2 is associated with an inducible initial response, which is activated in response to genes and numerous extracellular or intracellular physiological stimuli. It is an important mediator of the inflammatory pathway. It could also be responsible for the high levels of PGs in abundant inflammatory conditions [11]. Microsomal prostaglandin E synthase-2 (mPGES-2) is an eicosanoid lipid mediator that significantly contributes to the pathogenesis of many inflammatory diseases [12]. HNE is a protease enzyme that belongs to the chymotrypsin-like family of serine proteins. These enzymes play a vital part in the pathogenesis of many infections, from chronic inflammatory diseases to infectious diseases. Melanin biosynthesis tyrosinase is a key enzyme that plays a significant role in skin inflammation. It was first identified by French chemist Gabriel Bertrand [13].

Molecular docking is an *in silico* method used to estimate the strength of the bond between a ligand and a target protein through a special scoring function in order to determine the correct structure of the ligand within the target binding site [14]. The purpose of molecular docking is to simulate the process of the molecular identification of target proteins and ligands. It also focuses on achieving the minimum independent energy of the whole system, which includes proteins and ligands with proper alignment [15]. The mechanism of the molecular docking of proteins can be performed between small ligands, protein peptides, protein proteins, and protein nucleotides. Mostly used

softwares for docking purposes are CB Dock, Autodock, Autodock vina, and AM dock [16]. Thus, this study aimed to find an effective and low-cost treatment for chronic inflammatory diseases which has fewer side effects, instead of using synthetic drugs. The objective of this research was to identify the various bioactive compounds of *Trigonella foenum-graecum* as potential inhibitors of cyclooxygenases, mPGES-2, HNE, and tyrosinase enzymes. It also aimed to analyze the binding conformation between targeted proteins and other inhibitors as standard anti-inflammatory agents. Furthermore, the objective was to compare the results of inhibitors or ligands with standard anti-inflammatory drugs and the selection of lead compounds.

2. Materials and Methods

2.1. Retrieval of 3D Structure of Target Proteins

The primary sequence of target proteins (Cox-2, mPGES-2, HNE, and tyrosinase) was taken in FASTA format from UniProt database under accession numbers P35354, Q9H7Z7, P08246, and P14679 and residues lengths 604, 377, 267, and 529, respectively [17]. The simplest template selection rule is to choose the structure that matches the modeled sequence. If possible, a template bound to the same or similar ligands as a model should be used. The 3D structure of the selected templates were taken from the Protein Data Bank (PDB).

ProtParam was used to determine the physicochemical properties of target protens [18]. Whereas, Interpro database was used to identify the functional domains of targeted proteins [19].

2.2. Retrieval of the Chemical Structure of Ligands

The chemical compounds of *Trigonella foenum-graecum* used as ligands were selected from the PubChem database. The selected ligands were refined through Chem Draw Ultra version 12.0.2 software. The selected compounds were then tested against the Lipinski's rule of five to check their likeliness to be used as active drugs in human beings. The $\log^P$ value, molecular weight, and the maximum number of H-bond acceptors and H-bond donors were determined. For more successful drug discovery, a lead needs to act more like a drug. Compounds were further screened based on drug score, drug likeliness, and toxicity. The potential success of a compound depends on its ADMET properties which were tested using

pkCSM, a tool that helps to find the ADMET properties of the compounds [20].

2.3. Molecular Docking of Targeted Proteins

Cyclooxygenas-2, mPGES-2, HNE, and tyrosinase were used as target proteins and the selected ligands were alkaloid, flavonoid, phytic acid, 4-hydroxyisoleucin, sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol. CB dock is an online docking server that automatically identifies binding sites and is used to perform docking. It can simplify docking procedures and improve accuracy by predicting the target protein binding sites [21]. The first step in performing the docking process was to create ligand and target protein files in PDB and SDF formats. When input files were uploaded, CB Dock checked them and converted them to pdbqt format. Finally, CB dock provided us with five alternative poses and receptor models, from which the best one was chosen based on properties such as docking score, grid map, and cavity size. AMDock (Assisted Molecular Docking) is a user friendly graphical tool that assists in the docking of protein-ligand complexes using Autodock Vina and AutoDock4. AMDock integrates several external programs (Open Babel, PDB2PQR, AutoLigand, ADT scripts) to accurately prepare the input structure files and to optimally define the search space, offering several alternatives and different degrees of user supervision [22]. For the visualization of molecular structures, AMDock uses PyMOL, starting it automatically with several predefined visualization schemes to aid in setting up the box defining the search space and to visualize and analyze the docking results.

Protein-Ligand Interaction

After the docking process, the analysis of protein and ligand was performed using PyMOL (version 2.5) and their mutual interaction was measured for the interpretation of docking results. Using Ligplot^{+} (version v.1.4.5), the protein-ligand interaction was studied. This software automatically generates schematic diagram of the protein-ligand interaction of the given ligands in the PDB file [23]. The most active agonist was chosen as the lead compound after a detailed analysis of protein and ligand docking score, interaction, and ADMET studies.

Docking of the Standard Anti-Inflammatory Drug with Target Proteins

The docking results of 6 FDA approved drugs (celebrex, aspirin,

ibuprofen, paracetamol, naproxen and diclofenac) were compared with the docking results of 10 ligands, namely alkaloid, flavonoid, 4-hydroxy isoleucine, phytic-acid sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol. The docking of the selected drugs was performed against COX-2, mPGES-2, HNE, and tyrosinase using the online docking software CB Dock. Among all the selected drugs, it was determined that the most efficient standard drug was celebrex. It showed the best binding interactions and minimized score among all the selected drugs. According to its physiochemical properties, ADMET properties, and docking score, celebrex was selected as a standard for comparison with the lead compound.

3. Results and Discussion

It is possible to manage inflammatory diseases. The key factors involved are enzyme cyclooxygenases and other novel enzymes. These enzymes are involved in inflammatory pathways which play a key role in inhibiting inflammation. The enzymes involved in inflammatory pathways, such as Cox-2, mPGES-2, HNE, and tyrosinase, were selected as target proteins for this study [24].

3.1 Structure Modeling

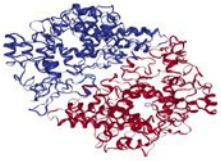
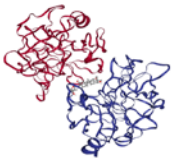
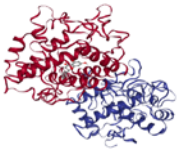
3.1.1 Primary Sequence Retrieval

Primary sequences of target proteins (Cox-2, mPGES-2, HNE, and tyrosinase) were taken in FASTA format from UniProt database under accession numbers P35354, Q9H7Z7, P08246, and P14679 and residues lengths 604, 377, 267, and 529, respectively.

3.1.2 Target Proteins Selection

Once a list of possible templates is obtained using search methods, it is important to select one or more templates that are particularly suitable for molecular docking. There are several factors to consider when choosing a template. The simplest template selection rule is to choose the structure that matches the modeled sequence. If possible, a template bound to the same or similar ligands should be used [25]. The structures of the selected templates were taken from the Protein Data Bank (PDB) and are listed in Table 3.1.

Table 3.1. Targated Protein Structures Retrieved from PDB with PDB ID and Structural Resolution

S #	Templates	Resolution	PDB ID	Structure
1	Complexed with selective inhibitors, cyclooxygenase-2 (Prostaglandin Synthase-2), SC-558 In space group I222.	2.80 Å	6COX	
2	Microsomal prostaglandin E synthase type-2.	2.60 Å	1Z9H	
3	Extremely glycosylated human leukocyte elastase crystal structure complex with thiazolidinedione inhibitor.	2.70 Å	6F5M	
4	The copper-depleted and copper-bound pro-tyrosinase crystals structures.	2.05Å	3W6Q	

3.1.3 Functional Domains Identification

Proteins contain one or more active domains that perform different functions. Cyclooxygenase contains two functional domains namely epidermal growth factor and an-peroxidase domain belonging to the Cox-2 family [26]. The first domain starts from the residue number 21 and ends at 53, while the second domain starts from residue 201 and ends at 560, as

shown in Fig 3.1. Microsomal Prostaglandin E synthase-2 (mPGES-2) contains a functional domain by the name of Glutathione S-transferase, while N-terminal domain belongs to PGES-2 family, starting from the residue number 104 and ending at 172 [27], as shown in Fig 3.2. Human Neutrophil Elastase (HNE) contains a functional domain by the name of trypsin belonging to Peptidase-S1A family, starting from residue number 30 and ending at 242 [28], as represented in Fig 3.3. Tyrosinase contains a functional domain, namely the central domain of tyrosinase. It belongs to the copper protein family, starting from the residue number 170 and ending at 403, as represented in Fig 3.4.

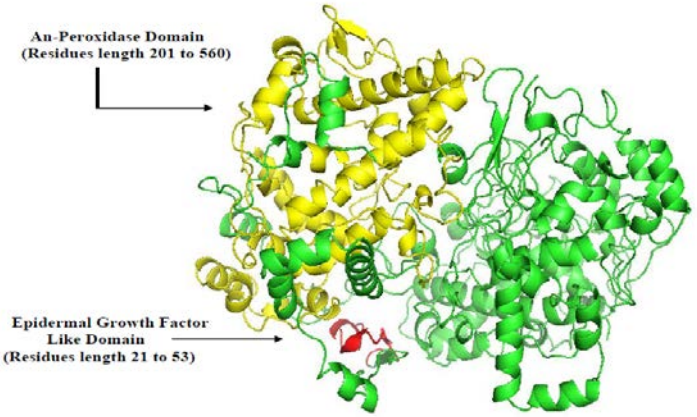


Figure 3.1. Functional Domains of Cox-2

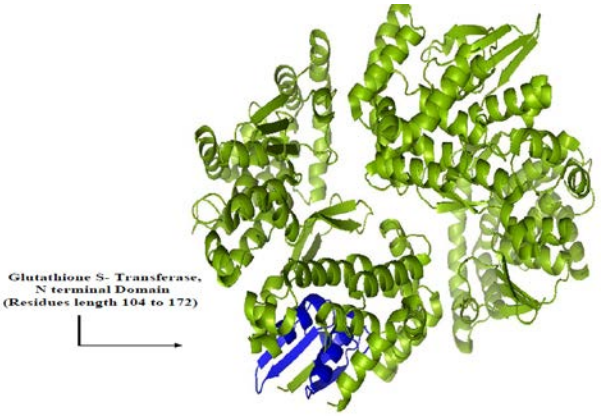


Figure 3.2. Functional Domains of mPGES-2

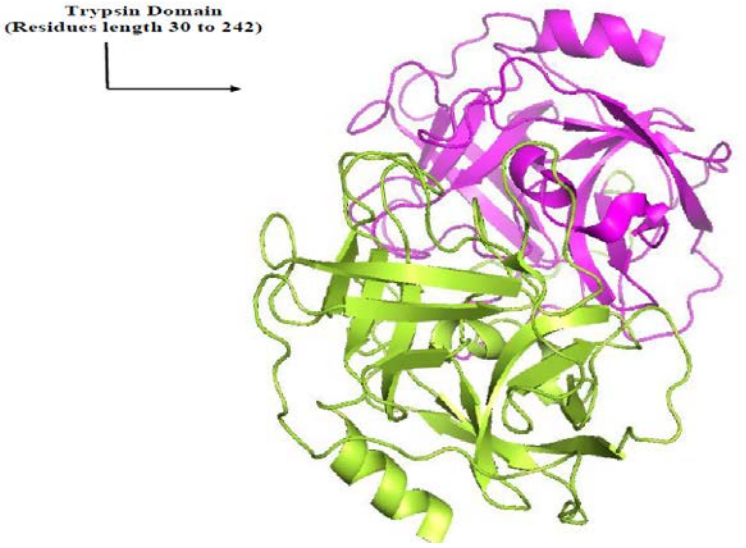


Figure 3.3. Functional Domains of HNE

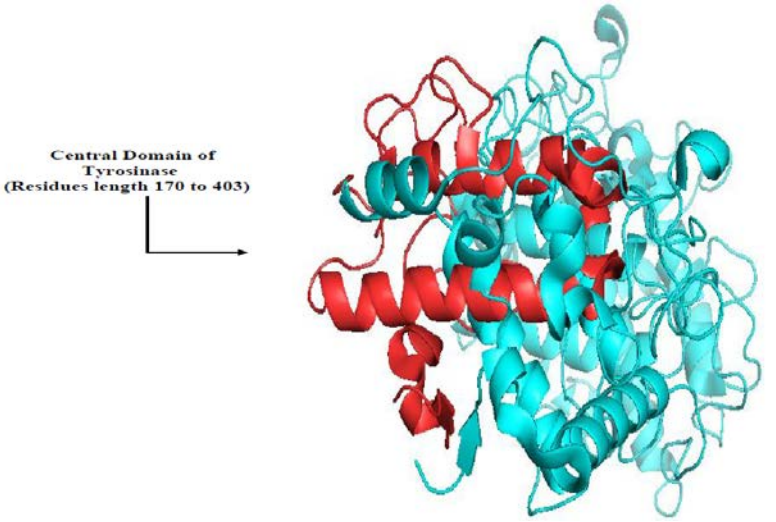


Figure 3.4. Functional Domains of Tyrosinase

3.1.4 Physiochemical Properties of Selected Proteins

The physicochemical properties of COX-2, mPGES-2, HNE, and tyrosinase were calculated using the ProtParam online tool. This online tool was used to calculate different physical and chemical parameters of the targeted proteins. The computed parameters included theoretical PI, amino acid composition (positive and negative charge), atomic compound, extinction coefficient, molecular weight, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) [29]. Theoretical PI greater than 7 indicates the basic nature of the proteins, while PI less than 7 shows the acidic nature of the targeted proteins. The extinction coefficient shows the absorption of light. An instability index of less than 40 shows the stability of proteins, while an instability index greater than 40 shows the instability of proteins [30]. All physicochemical parameters of targeted proteins are presented in tables 3.2, 3.3, 3.4 and 3.5, respectively.

Table 3.2. Physiochemical Properties of Cyclooxygenase-2 (Cox-2)

MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability Index	Aliphatic Index	GRAVY
68996.12	7.02	62	61	73980	73230	37.67	80.70	-0.287

Table 3.3. Physiochemical Properties of Microsomal Prostaglandin E synthase 2 (mPGES-2)

MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability Index	Aliphatic Index	GRAVY
39964.88	9.23	36	44	67965	67840	44.87	87.08	-0.236

Table 3.4. Physiochemical Properties of Human Neutrophil Elastase (HNE)

MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability Index	Aliphatic Index	GRAVY
28518.06	9.71	13	24	20105	19480	47.88	102.25	0.237

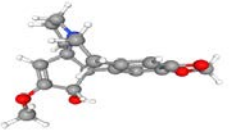
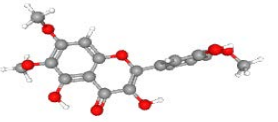
Table 3. 5. Physiochemical Properties of Tyrosinase

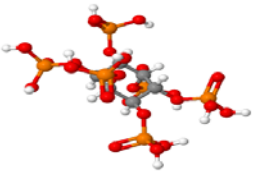
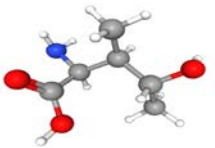
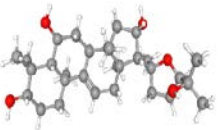
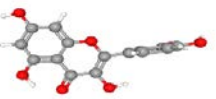
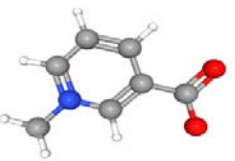
MW	PI	NR	PR	Ext.Co1	Ext.Co2	Instability Index	Aliphatic Index	GRAVY
60393.27	5.71	57	44	112270	111270	56.76	71.76	-0.356

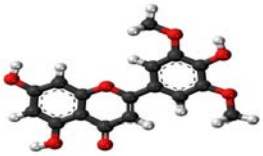
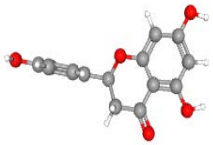
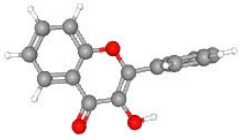
3.1.5. Retrival of Ligands and Energy Minimization

PubChem is the largest repository of freely available databases of chemical information in the world. So, chemical compounds used as ligands were selected from PubChem database [31]. Energy minimization and optimization of ligands were carried out using chem 3D pro ultra software (chem 3D v 12.0.2) [32]. This was a mandatory step in the preparation of ligands for docking because unstable ligands show unreliable vina scores in docking results.

Table 3.6: 3D Structure of Ligands with Molecular Formula and Molecular Weigh**t**

S#	Name	Molecular formula	Molecular weight	3D Structure
1	**Alkaloid**	$C_{18}H_{21}NO_4$	315.4 g/mol	
2	**Flavonoid**	$C_{18}H_{16}O_8$	360.3 g/mol	

S#	Name	Molecular formula	Molecular weight	3D Structure
3	**Phytic acid**	$C_6H_{18}O_{24}P_6$	660.04 g/mol	
4	**4 Hydroxyisoleucine**	$C_6H_{13}NO_3$	147.17 g/mol	
5	**Sapogenin**	$C_{30}H_{50}O_5$	490.7 g/mol	
6	**Quercetin**	$C_{15}H_{10}O_7$	302.23 g/mol	
7	**Trigonelline**	$C_7H_7NO_2$	137.14 g/mol	
8	**Tricin**	$C_{17}H_{14}O_7$	330.29 g/mol	

S#	Name	Molecular formula	Molecular weight	3D Structure
				
9	**Naringenin**	$C_{15}H_{12}O_5$	272.25 g/mol	
10	**Flavonol**	$C_{15}H_{10}O_3$	238.24 g/mol	

3.2 Ligands and their Physiochemical and Pharmacokinetic Properties

The physicochemical and pharmacokinetic properties of a standard drug and the selected ligands are differentiated using certain parameters, including Lipinski's rule of five and ADMET properties [33]. So, in the current research, Lipinski's rule and pharmacokinetic studies were used as primary and secondary filters for the analysis of possible chemical compounds [34]. According to Lipinski's rule, the number of Hydrogen bond donors (HBD) must be less than 5, the maximum number of Hydrogen bond acceptors (HBA) must be 10, the $\log^p$ value must be limited to 5, and molecular weight must be less than 500. All the selected ligands including alkaloid, flavonoid, sapogenin, quercetin, trigonelline, tricin, naringenin, 4-hydroxy isoleucine, and flavonol follow the Lipinski's rule of five. So, they were considered for the current research work except a few ligands including Sennoside A, Teprotide, Rutin, Procyanidin, Pectolinarin, and Phytic acid, which did not follow Lipinski's rule. These ligands were excluded from primary screening. PkCSM is an

online tool that provides an integrated platform to rapidly evaluate the pharmacokinetic and toxicity properties of a drug [35]. This tool is used to determine the toxicity measurements of ligands. The applicability of Lipinski's rule on ligands is shown in Table 3.7 and excluded ligands are mentioned in Table 3.8.

Table 3.7. Applicability of Lipinski's Rule of Five on Ligands

S#	Ligand	log^P Value	Molecular Weight	H-bond Acceptor	H-bond Donor
1	Alkaloid	1.7944	315.4 g/mol	5	1
2	Flavonoid	2.6026	360.3 g/mol	8	3
3	4-Hydroxyisoleucine	-0.5848	147.17 g/mol	3	3
4	Sapogenin	4.6027	490.7 g/mol	5	4
5	Quercetin	1.988	302.23 g/mol	7	5
6	Trigonelline	-1.1254	137.14 g/mol	2	0
7	Tricin	2.594	330.29 g/mol	7	3
8	Naringenin	2.5099	272.25 g/mol	5	3
9	Flavonol	3.1656	238.24 g/mol	3	1

Table 3. 8. Applicability of Lipinski's Rule on Excluded Ligands

S.No	Ligand	log^P Value	Molecular Weight	H-bond Acceptor	H-bond Donor
1	Phytic acid	-3.1326	660.04 g/mol	12	12
2	Sennoside A	-1.09	862 g/mol	18	12
3	Teprotide	-1.658	1101.3 g/mol	12	10
4	Rutin	-1.6871	610.521 g/mol	16	10
5	Procyanidin	2.7327	594.525 g/mol	13	10
6	Pectolinarin	-0.7867	622.576 g/mol	15	07

3.3 Molecular Docking of Target Proteins and Ligands

Molecular docking has become a powerful tool for lead discovery and optimization. A large number of docking programs have been developed during the last three decades, based on different search algorithms and scoring functions. Docking was performed using Cox-2, mPGES-2, HNE, and tyrosinase proteins and ligands (alkaloids, flavonoid, sapogenins, quercetin, trigonelline, tricin, naringenin, flavonol, and 4-hydroxy isoleucine). To automatically predict binding modes without information about binding sites, we used a user friendly blind docking webserver called CB Dock [36]. It predicts and estimates the binding site for a given protein, calculates centres and sizes with a novel rotation cavity detection method, and performs docking with the popular docking program named AutoDock Vina [36]. CB Dock gives the five best interacting confirmations for each ligand molecule. These confirmations are arranged based on their binding affinity. Then, the finest confirmation selection is carried out based on the highest affinity score of protein-ligand interaction.

AMDock (Assisted Molecular Docking) is a user friendly graphical tool employed to assist in the docking of protein-ligand complexes using AutoDock Vina and AutoDock4. AMDock integrates several external programs (Open Babel, PDB2PQR, AutoLigand, ADT scripts) to accurately prepare the input structure files [37]. AMDock relies on PyMOL for visualization at two different stages: 1) setting up the grid box location and dimensions (the search space), and 2) analysis of the docking results [38]. Ligands with the best binding score values via CB Dock and AMDock with Cox-2, m PGES-2, HNE, and tyrosinase are shown in Table 3.9 and Table 3.10, respectively**.**

Table 3.9. Ligands' Binding Score Values with Cox-2, m PGES-2, HNE, and Tyrosinase via CB Dock

S.#	Name of Compound	Binding Score with COX-2	Binding Score with mPGES-2	Binding Score with HNE	Binding Score with Tyrosinase
1	Alkaloid	-6.7	-5.9	-5.0	-6.7
2	Flavonoid	-9.0	-8.1	-9.0	-8.1
3	4-Hydroxyisoleucine	-5.5	-4.9	-4.6	-5.3
4	Sapogenin	-9.6	-9.1	-9.4	-7.6
5	Quercetin	-9.6	-8.5	-7.5	-9.3
6	Trigonelline	-5.9	-5.8	-4.9	-5.9
7	Tricin	-9.3	-8.0	-7.7	-7.7
8	Naringenin	-8.8	-8.8	-7.5	-7.7
9	Flavonol	-9.4	-8.4	-7.4	-9.4

Table 3.10. Ligands Binding Score Values with Cox-2, m PGES-2, HNE, and Tyrosinase via AMDock

S#	Name of Compound	Binding Score with COX-2	Binding Score with mPGES-2	Binding Score with HNE	Binding Score with Tyrosinase
1	Alkaloid	-5.2	-4.3	-4.0	-3.7
2	Flavonoid	-9.5	-6.0	-7.0	-5.4
3	4-Hydroxyisoleucine	-4.9	-2.5	-3.3	-3.2
4	Sapogenin	-9.6	-6.6	-6.7	-6.5
5	Quercetin	-8.6	-5.5	-6.1	-5.9
6	Trigonelline	-4.1	-2.7	-3.6	-2.7
7	Tricin	-9.0	-5.3	-5.7	-4.0
8	Naringenin	-8.1	-5.6	-5.8	-5.3
9	Flavonol	-7.8	-5.3	-3.5	-4.7

Table 3.11. Ligands with Cavity Size, Grid Map Values, Maximum Energy, and Minimum Energy Values via CB Dock

S#	Ligands	Cavity Size	Grid Map Via CB Dock	Max-energy Kcl/mol	Min-energy Kcl/mol
1	Alkaloid	1232	14	8.2145	0.2962
2	Flavonoid	1232	14	34.7004	6.5377
3	4-Hydroxyisoleucine	3795	73	1.3770	-3.3147
4	Sapogenin	3377	58	88.1844	0.9317
5	Quercetin	4717	36	8.6926	3.3080
6	Trigonelline	4247	20	36.0689	0.0000
7	Tricin	3377	58	22.9008	2.0687
8	Naringenin	1129	38	5.4830	-1.1725
9	Flavonol	4247	20	21.2189	3.8292

Table 3.12. Ligands with Cavity Size, Grid Map Values, Maximum Energy, and Minimum Energy Values via AMDock

S#	Ligands	Ligand Efficiency	Grid Map Via AM Dock	Max-energy Kcl/mol	Min-energy Kcl/mol
1	Alkaloid	-0.47	15	8.2145	0.2962
2	Flavonoid	-0.37	20	34.7004	6.5377
3	4-Hydroxyisoleucine	-0.49	11	1.3770	-3.3147
4	Sapogenin	-0.27	22	88.1844	0.9317
5	Quercetin	-0.28	18	8.6926	3.3080
6	Trigonelline	-0.36	10	36.0689	0.0000
7	Tricin	-0.38	58	22.9008	2.0687
8	Naringenin	-0.40	19	5.4830	-1.1725
9	Flavonol	-0.29	15	21.2189	3.8292

Table 3.8 shows CB Dock docking results of the targated proteins (Cox-2, m PGES-2, HNE, and tyrosinase) with selected ligands (alkaloid, flavonoid, 4-Hydroxyisoleucine, sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol) [39]. Table 3.9 shows AMDock docking results of targated proteins (Cox-2, m PGES-2, HNE, and tyrosinase) with selected ligands (alkaloid, flavonoid, 4-Hydroxyisoleucine, sapogenin, quercetin, trigonelline, tricin, naringenin, and flavonol).

If both types of docking scores are compared, there is a clear difference between them. These scores prove that CB Dock results are much better then AMDock results. Grid map facilitates incredibly quick docking calculations. AutoGrid was used to draw these maps. Grid point spacing typically ranges from 0.2 Å to 1.0 [40]. The potential energy of a 'probe' atom or functional group (due to all the atoms in the macromolecule) is stored at each position on the grid map [41]. If the grid map values of CB Dock and AMDock are compared, the former is much better then the latter. Overall, it indicates that CB Dock shows better results then AMDock. Tables 3.11 and 3.12 show ligands with cavity size, grid map values, maximum, and minimum energy values.

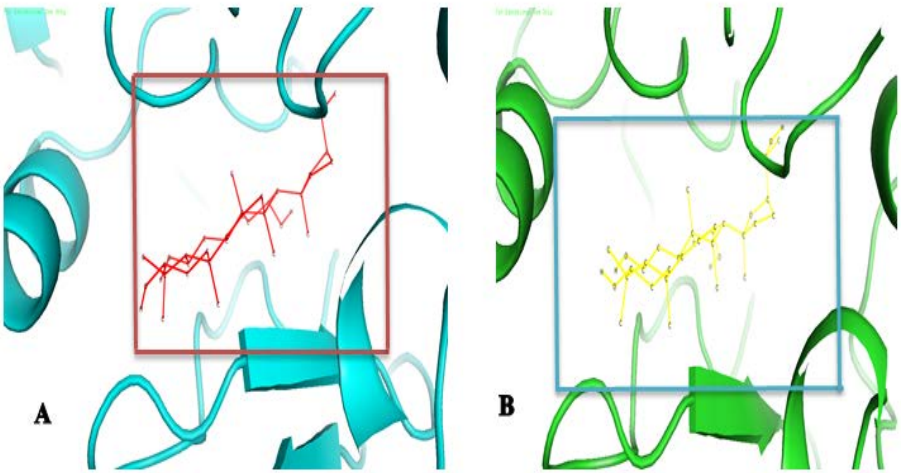


Figure 3.5. A shows that ligand bind with Cox-2 after performing docking via CB Dock and the ligand bind at the same position involving in biological process. B shows that ligand bind with Cox-2 after performing

docking via AMDock and the ligand bind at the same active site involving in biological process.

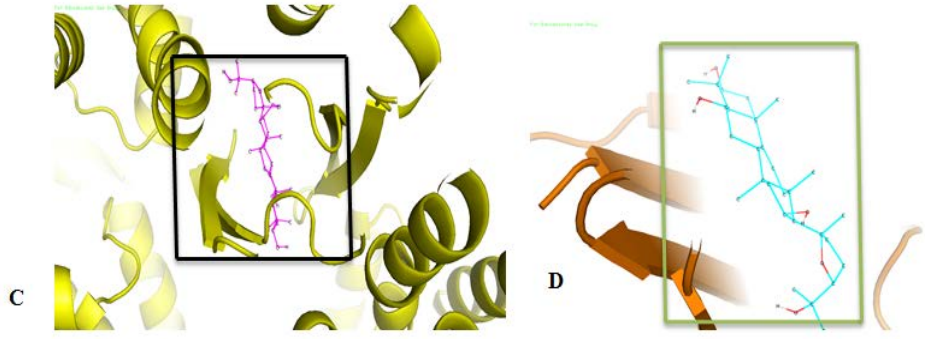


Figure 3.6. C shows that ligand bind with m PGES-2 after performing docking via CB Dock and the ligand bind at the same active site. D shows that ligand bind with m PGES-2 after performing docking via AM Dock and the ligand bind with the same position.

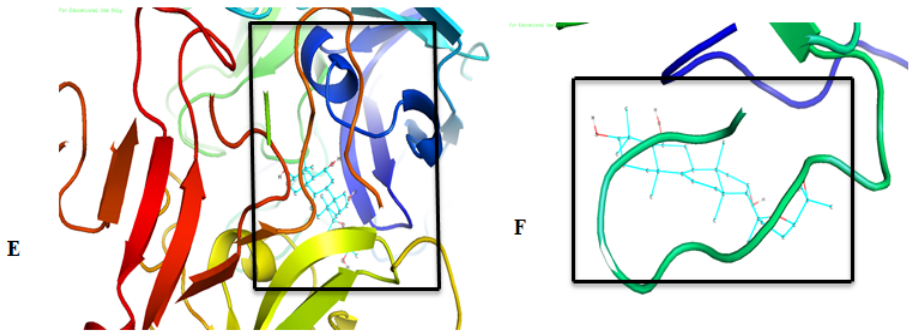


Figure 3.7. E shows that ligand bind with HNE after performing docking via CB Dock and the ligand bind at same active site position involving in biological process. F shows that ligand bind with HNE after performing docking via AMDock and the ligand bind at different active site which already shown in Figure 3.7.

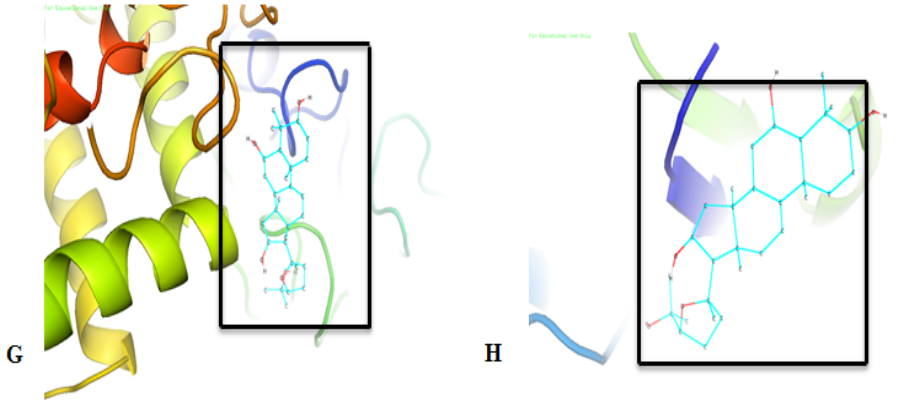


Figure 3.8. G shows that ligands bind with tyrosinase at the same active site after performing docking via CB Dock. H shows that ligand bind with tyrosinase at the same active site after performing docking via AMDock.

The comparison of docking results shows that ligands bind with targated proteins at the same active site Figure 3.7 (F), except Human Neutrophil Elastase protein (HNE) which does not bind with ligands at the same position. It indicates that its active site is not involved in biological processes akin to other poses [42].

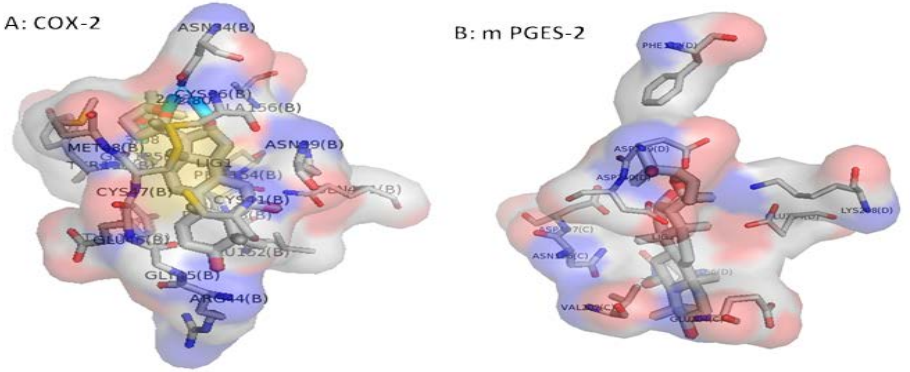


Figure 3.9. A shows the COX-2 active site involved in biological processes. B shows the microsomal PGES-2 active site**.**

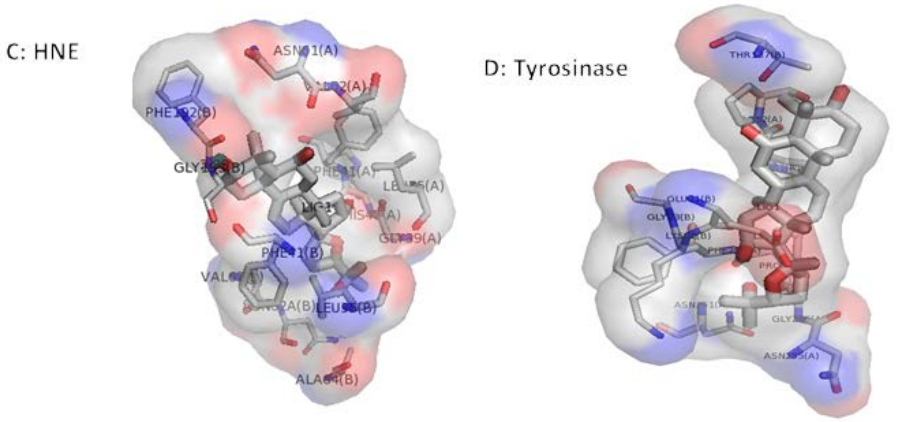


Figure 3.10. C shows the HNE active site. D shows the tyrosinase active site involved in biological processes.

Asn34, Val256, Gly135 residues in COX-2, Val523, Asn34, His244, Val283, and Asn196 in microsomal PGES2, His49, His263, Phe264 in tyrosinase and His40 and Gly193 in HNE, that are involved in biological process [43].

3.4. Comparision of Celebrex and Anti-Inflammatory Agents

A comparison between celebrex and sapogenin would help to identify the better treatment for chronic inflammatory diseases based on various parameters, such as ADMET properties and physiochemical properties of the standard drug celebrex and the lead compound sapogenin. If the physiochemical properties of the anti-inflammatory agent (sapogenin) with the standard drug (celebrex) are compared, it shows that the $\log^p$ value, hydrogen bond acceptor, hydrogen bond donor, and molecular weight of sapogenin are greater than celebrex [44].

For docking purpose, CB Dock and AMDock tools were used. Sapogenin and celebrex were used as ligands, while COX-2, mPGES-2, HNE, and tyrosinase were used as receptors. The docking process yielded five (5) best confirmation results and the finest were selected. The binding and rotatable bonds value of celebrex was found to be higher. However, the $\log^p$ value, hydrogen bond acceptor, hydrogen bond donor, and molecular weight of sapogenin are greater as compared to celebrex.

The comparison between physiochemical properties and docking results of celebrex and sapogenin is depicted in Table 3.13 and Table 3.14 respectively, while hydrogen and hydrophobic interactions of the standard drug and lead compound are shown **in Table 3.15. The** interactions of the active pockets of ligand and protein were calculated for the interpretation of docking results.

Two types of interactions were studied, namely hydrogen bonding and hydrophobic bonding interaction. PyMOL (version 2.5) and Ligplot^{+} (version v.1.4.5) softwares were used for visualization and protein ligand interactions. The interaction of standard drugs and the identified lead compound with COX-2 protein are represented in Fig 3.11 and Fig 3.12, respectively.

Table 3.13. Comparison between Physiochemical Properties of Celebrex and Sapogenin

S#	Drug	LogP Value	H-bond Acceptor	H-bond Donor	Molecular Formula	Molecular Weight
1	Celebrex	3.51392	4	1	$C_{17}H_{14}F_3N_3O_2S$	381.379 g/mol
2	Sapogenins	4.6027	5	4	$C_{30}H_{50}O_5$	490.725 g/mol

Table 3.14. Comparison between the Docking Results of Celebrex and Sapogenin

S#	Properties	Celebrex	Sapogenin
1	Binding Score	-10.4	-9.6
2	HBD	1	4
3	HBA	4	5
4	logP	3.51392	4.6027
5	Molecular Weight g/mol	381.379	490.725
6	Rotatable Bonds	3	2
7	Grid Map	58	58
8	Min-energy Kcl/mol	9.3513	0.9317
9	Max-energy Kcl/mol	217.4415	88.1844
10	Cavity Size	3377	3377

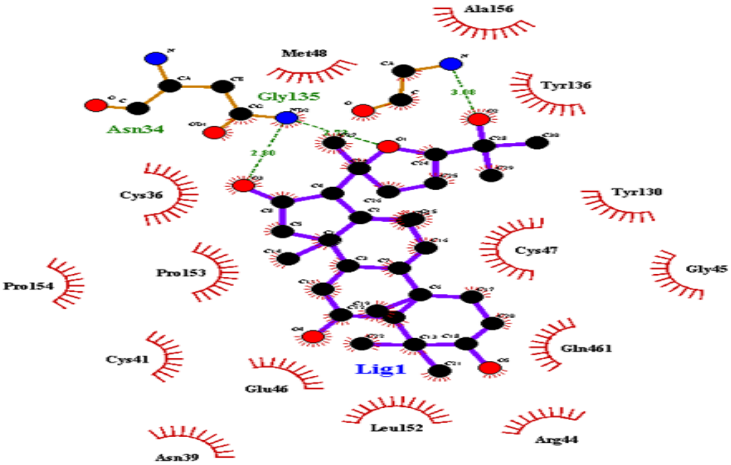


Figure 3.11. Interaction of Lead Compound Sapogenin with Cox-2

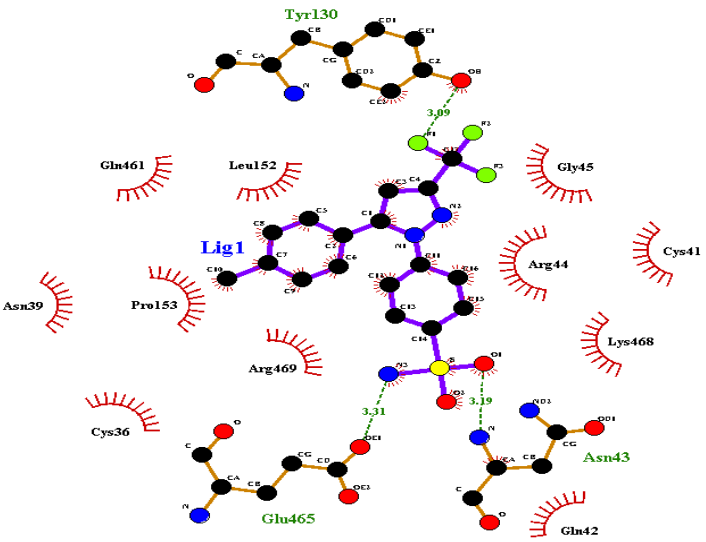


Figure 3.12. Interaction of Standard Drug Celebrex with Cox-2

Table 3.15. Celebrex and Sapogenin Showing Hydrogen and Hydrophobic Interactions

S#	Ligand Name	No. of HBs	Hydrogen Bonding		Hydrophobic Interactions
			Amino Acids	Distance	
1	Celebrex	3	ND:TYR: O N: GLU: OE N: ASN: O	3.09 3.31 3.19	ARG469 LYS468 GLN461 PRO153 LEU152 GLY45 ARG44 GLN42 CYS41 ASN39 CYS36 ALA156
2	Sapogenin	2	ND-ASN: O ND: GLY: O	2.80 2.72	ASN39 ARG44 CYS36 CYS41 CYS47 GLY45 GLU46 GLN461 LEU152 MET48 PRO153 PRO154 TYR130 TYR136

3.5 Comparison of ADMET Properties

ADMET properties include the values regarding drug absorption, distribution, metabolism, excretion, and toxicity. These values help us to determine drug activity and efficiency [45]. ADMET properties of ligands were extracted using pkCSM online tool. This tool was also used to find the toxicity and ADMET properties of the targeted drug. ADMET properties of the identified lead compound and the standard drug are presented in tables 3.16, 3.17, 3.18, 3.19, and 3.20, respectively. The intestine absorption and Caco2 permeability of sapogenin was found to be greater as compared to celebrex. Moreover, P-glycoprotein I inhibitor was found to be present in both celebrex and sapogenin but P-glycoprotein II inhibitor was only present in celebrex. So, it was determined that sapogenin is easily absorbed in the body due to the absence of P-glycoprotein II inhibitor [46]. Similarly, the CYP3A4 substrate was found to be present in both celebrex and sapogenin but CYP1A2 inhibitor was present only in sapogenin. It helps in the metabolism of the drug. Volume distribution value of celebrex was also lesser, while the volume distribution value of sapogenin was relatively higher. It can help in the distribution of the drug in the body. The total clearance value of sapogenin in the body was found to be greater. It helps in the excretion of drug from the body. The most important parameter of pharmacokinetics is toxicity. The maximum tolerated dose for celebrex is 0.178 and for sapogenin it is -0.888. Moreover, the oral acute toxicity rate of sapogenin is greater, while the chronic toxicity rate of celebrex is higher. It indicates that sapogenin is safer as compared to celebrex (the standard drug). Due to chronic toxicity, hepatoxicity and *t.pyriformis* toxicity [47], the standard drug celebrex is unsafe as compared to the identified lead compound sapogenin.

Based on the docking parameters, physiochemical properties and ADMET properties, it was determined that sapogenin fares better as compared to celebrex because celebrex induces hepatotoxicity and increases the risk of heart attack and stroke. On the other hand, sapogenin proved to be more effective for human health.

Table 3.16. Absorption Properties of Celebrex and Sapogenin

S#	Ligand	Celebrex	Sapogenin
1	Water solubility	-4.45	-5.433
2	Caco2 permeability	0.839	1.055
3	Intestinal absorption (human)	92.995	95.08
4	Skin Permeability	-2.692	-3.702
5	P-glycoprotein substrate	Yes	Yes
6	P-glycoprotein I inhibitor	Yes	Yes
7	P-glycoprotein II inhibitor	Yes	No

Table 3.17. Distribution Properties of Celebrex and Sapogenin

S#	Ligand	Celebrex	Sapogenin
1	VDss (human)	-0.273	0.258
2	Fraction unbound (human)	0.133	0.151
3	BBB permeability	-0.931	-0.514
4	CNS permeability	-2.052	-2.995

Table 3.18. Metabolic Properties of Celebrex and Sapogenin

S#	Ligand	Celebrex	Sapogenin
1	CYP2D6 substrate	No	No
2	CYP3A4 substrate	Yes	Yes
3	CYP1A2 inhibitor	No	Yes
4	CYP2C19 inhibitor	Yes	No
5	CYP2C9 inhibitor	Yes	No
6	CYP2D6 inhibitor	No	No
7	CYP3A4 inhibitor	Yes	No

Table 3.19. Excretion Properties of Celebrex and Sapogenin

S#	Ligand	Celebrex	Sapogenin
1	Total Clearance	0.435	1.218
2	Renal OCT2 substrate	No	No

Table 3.20. Toxicity Values of Celebrex and Sapogenin via pkCSM Tool

S#	Model Name	Predicted values	
		Celebrex	**Sapogenin**
1	Max.tolerated dose (human)	0.178	-0.888
2	hERG I inhibitor	No	No
3	hERG II inhibitor	No	No
4	Oral rate acute toxicity	1.975	2.395
5	Oral rate chronic toxicity	1.526	1.462
6	Hepatoxicity	Yes	No
7	Skin sensitization	No	No
8	t.pyriformis toxicity	0.43	0.304
9	Minnow toxicity	0.86	1.486

4. Conclusion

The aim of this research was to identify novel compounds that could be used in near future in an efficient drug to treat chronic inflammatory diseases using computational methods.Data mining was performed on the literature database and , ten ligands were selected for the current research work. The proteins used for virtual screening were COX-2, Human Neutrophil Elastase (HNE), microsomal PGES-2, and tyrosinase. CB Dock automated version of AutoDock vina and AMDock was used for docking studies. Protein ligand interactions of the selected ligands were analyzed using Ligplot^{+} (version v.1.4.5). After the detailed analysis of their binding score, physiochemical properties, and ADMET properties, sapogenin was identified as a potent inhibitor for inflammation. From the above mentioned physiochemical and ADMET values, it was concluded that sapogenin fares better in comparison to celebrex for human health.

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