PHARMACOPHORE ELUCIDATION AND DOCKING STUDIES ON ANTI-INFLAMMATORY COMPOUNDS OF MEDICINAL PLANTS FOR ULCERATIVE COLITIS

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ABSTRACT

Inflammatory bowel diseases such as Ulcerative Colitis (UC) are becoming common in this aging society throughout the world which includes formation of ulcers or open sores. Since, there is no known medical cure for UC; the therapeutic armamentarium is aimed at reducing the signs and symptoms associated with the disorder. The role of antibiotics in the treatment of severe active UC is controversialbecause the clinically used anti-inflammatory drugs suffer from the disadvantage of side effects and high cost of treatment. In the present study, NF-kB p50/p65 is docked in two different ways, one with the glucocorticoid receptor protein using ZDOCK in Accelrys Discovery Studio 3.5 and the other is screening and docking of400 anti-inflammatory natural compounds derived from plant source which offer a great hope in the identification of lead compounds.These compounds were investigated for their inhibitory activity by molecular docking studies and ADME/T properties of the compounds were analyzed for drug like candidates by using the commercial software's Accelrys Discovery Studio, Lead-IT and GOLD 5.1. Based on the docking results and toxicity analysis using TOPKAT, the best compounds determined are Ginkgetin, Bilobetin and Mesuaxanthone_B. The Pharmacophore studies have also shown that these compounds are having very less side effects and further investigations are requiredto take into clinical trials.

Keywords: Inflammatory bowel disease, NF-kB p50/p65, Glucocorticoid, ZDOCK, TOPKAT

INTRODUCTION

Ulcerative Colitis (UC) is one of the chronic inflammatory disorder of unknown etiology which usually affects the lower colon and rectum causing open sores called ulcers so the disease is generally named as Inflammatory Bowel Disease (IBD). UC may occur in people of all ages irrespective of men and women and appears to run in families. It affects approximately 250,000-500,000 individuals with an incidence of 2-7/100,000 population per year due to their food habbits and bacterial infections. In some people the disease remains for long periods with no symptoms for even years. Inflammation causes the colon to be empty often by triggering bloody diarrhea. Cytokines, which plays a key role in the initiation, augmentation and prepetuation of the disease, since they are directly responsable for mucosal injury. Goals of treatment mainly focuses on maintaining remission by reducing the signs and symptoms of mucosal inflammation in order to provide a better quality of life.

Since, there is no proper medication that can cure the disease and as the clinically used antibiotics suffer from many side effects, it forces the scientific research group to find a new drug as an alterantive. Alternative to these drugs are traditional medicines and natural products, which offer a great hope in the identification of bioactive lead compounds and their development into drugs for treating inflammatory diseases. The present study describes anti-inflammatory natural products derived from plant sources from literatures and articles and to understand the binding patterns of ligands on cristal structure NFkB p50/p65 heterodimer and glucocorticoid receptor protein to obtain more selective and potent drugs for the new potential target. The best lead compound will be brought into light by further trials[1].

MATERIALS AND METHODS

Hardware and software

Docking calculation was carried out on HP Intel® Xeon® processor E3-1200v2 family with 16Gb RAM, 1TB Hard disk, NVIDIA Quadro 2000, windows 7 ultimate 64bit. Accelrys Discovery Studio Client 3.5 was used for docking preparation, Biosolve IT and GOLD 5.1 are docking softwares used for binding energy calculation.

Identification of Target Protein

The crystal structure of the drug target NF-kB p50/p65 heterodimer bound to DNA was retrieved from RCSB Protein Data Bank [PDB ID-1VKX] (http://www.pdb.org).

Approach 1: Protein-Protein docking

Selection of ligand protein

The ligand protein selected is Glucocorticoid Receptor (GR) [PDB ID-1M2Z] which exerts a potent immunosuppressive and anti-inflammatory action through the interruption of cytokine-mediated pathways.Antagonism occurs between the glucocorticoid receptor and NF-kB. After the receptor is bound to glucocorticoid, the activated receptor-glucocorticoid complex may then bind to activated A chain of P65 subunit of NF-kB and prevent it from binding to kB sites on gene that have a role in inflammatory processes. This interaction may occur in cytoplasm or nucleus.

Protein docking using ZDOCK in Accelrys Discovery Studio 3.5

ZDOCK is a rigid body protein-protein docking algorithm based on Fast Fourier Transform correlation technique that is used to explore the rotational and translational space of a protein -protein system [2]. Here the crystal structure of NF-kB p50/p65 (1VKX) is used as a receptor protein and glucocorticoid receptor (1M2Z) as a ligand protein were subjected to docking in ZDOCK to calculate the binding energy.

Approach 2: Protein-Ligand docking

Target protein minimization

The crystal structure of the target protein NF-kB p50/p65 was retrieved from PDB and minimization of the protein was done by applying CHARMm forcefield in Accelrys Discovery Studio 3.5. The protocol prepares the protein by inserting the missing atoms and unwanted materials like water and ligand molecules are removed before minimization.

Active Site prediction

From the binding site analysis of 1VKX it was observed that binding pockets are identified and the largest binding pockets are identified and the largest binding pocket (active site 1) was selected for docking studies. After selecting the receptor from PDB 19 active sites were obtained and isolated the B-chain in 1VKX, possible binding residues of receptor was searched and they are [PHE353,ARG354,PHE355,ARG356,TYR357,GLU360,GLY361, PRO362, SER363 , HIS364, GLY365, GLY366, LEU367, PRO368, SER410, LEU411, VAL412, GLY413, LYS414, PHE434 ,ALA435, ASN436, LEU437,GLY438,ILE439,LEU440].

Ligand preparation

400 medicinal plant compounds having anti-inflammatory activity were collected from various articles and literatures. The 2D structure of the compounds and their properties were retrieved from Pubchem [3] and Chemicalize.org [4] databases. The 2D structures of these compounds were converted into 3D in Accelrys Discovery Studio 3.5. The ligands were minimized by applying CHARMm forcefield and saved in MOL2 format for the further use in docking studies.

Drug likeliness evaluation

The drug likeliness of the compounds was evaluated with the help of Lipinski drug filter in Accelrys Discovery Studio 3.5. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligands as a drug [5].

ADME and Toxicity studies

ADMET stands for Absorption, Distribution, Metabolism, Excretion and Toxicity. The prediction of ADMET properties plays an important role in the drug design process because these are responsible for 60% failures of all drugs in the clinical phases. ADME is applied at an early phase of drug development process in order to remove the molecules with poor ADME properties and leads to the significant savings in research and development costs [6].

These studies helped to identify a better anti-inflammatory compound. TOPKAT is used to analyze what body does to the drug.

The Toxicity profile of the compounds are predicted using TOPKAT which uses a range of Quantitative Structure Toxicity Relationship (QSTR) models for assessing special toxicological endpoints. Toxicity profile includes NTP carcinogenicity for male and female rat and mouse, mutagenicity, developmental toxicity and skin irritation test [7].

Molecular Docking

The possible binding modes between the ligands and the target protein (1VKX) were studied using Lead IT and GOLD 5.1 suite. Lead IT is one of the most established protein-ligand docking tools [8]. The active site of the target protein is loaded into the software. A sphere of 40Å radius is selected. Then MOL2 files of screened natural compounds were selected as the docking library. Using the FlexX incremental build up algorithm, the ligands are checked for 200 poses to generate best pose by analyzing the interactions and binding affinities. The docking scores and their poses were saved for further studies.

GOLD is a program that uses Genetic algorithm for protein-ligand docking. It stands for Genetic Optimization for Ligand Docking. It is highly known for its accuracy and reliability although the time taken to predict the result depends on the ligand and protein structures. GOLD achieved a 71% success rate in identifying the experimental binding mode [9]. Wizard is clicked to load the protein structure. Then 8 hydrogen are added in protein setup step. Define the binding site is clicked to set the radius to 40Å. Goldscorep450 is selected in configuration setup. The ligands are loaded which are docked in mol2 format. The fitness function chosen is goldscore. The genetic algorithm search option is set to slow and the gold configuration is finished to run the docking calculation. The docking results with their poses are saved for further reference.

RESULTS AND DISCUSSION

Approach 1: Protein docking results

The binding energy between the protein 1VKX and 1M2Z is calculated using ZDOCK in Accelrys Discovery Studio 3.5. In protein docking with ZDOCK, the docked poses are analyzed and their docking interactions are listed in the table 1 and its poses with Z-Rank score are shown in the figure 1.

Table 1:The target protein (1VKX) - ligand protein (1M2Z) docking interactions in ZDOCK

S. No.	Pose's	Cluster's	Z Rank Score	Protein - Protein interaction				
				1VKX (aa)	1VKX (Atom)	1M2Z (aa)	1M2Z (Atom)	Distance
1	Pose1	Cluster2001_1M2Z	-120.75	B(HIS415)	ND1	A(THR758)	O	2.42609
				B(THR422)	OG1	A(PRO762)	O	2.50652
				B(ARG461)	NE	A(ASN766)	O	1.73048
				B(ARG461)	NH1	A(ASN766)	O	3.01264
				B(ARG461)	NH2	A(ASN766)	O	2.81073

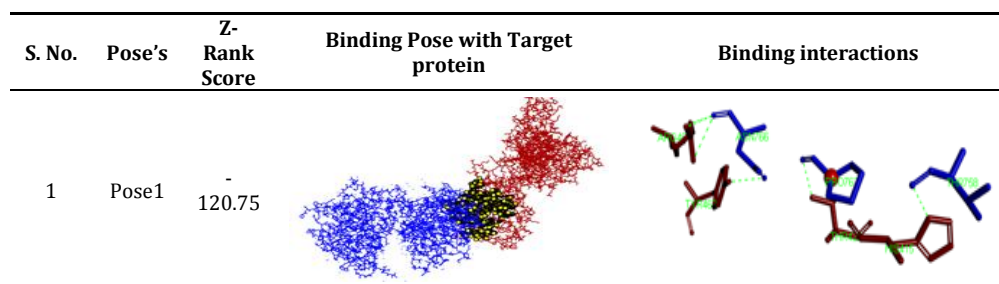| S. No.	Pose's	Z-Rank Score	Binding Pose with Target protein	Binding interactions
1	Pose1	-120.75		

Figure 1: Best binding interactions between target protein (1VKX-BLUE) ligand protein (1M2Z-RED) and binding interface (Black)

The present study was initiated to explore the possibility to develop an inhibitor mimicking the above mentioned interaction. Hence, Structure based drug design approach was implemented to develop the inhibitor. The derived Structure based inhibitor glucocorticoid receptor was docked with NFKB p50/p65 in humans and was found to have very low free energy of binding in ZDOCK with their Z-rank score is -120.75. In protein docking with ZDOCK 2000 different poses were generated and best pose is selected for further studies (Pose1_Cluster2001_1M2Z).This leads to highlyefficient binding

affinity with target protein, leading to the lesser requirement for the inhibition. The inhibitor glucocorticoid receptorform hydrogen bond with P65 unit of NFKB that inhibits the phosphorylation of protein IkB by IkB kinases. Thus, unphosphorylated trimer causes inactivation of target protein NFKB p50/p65 and stop downstream signaling pathway that has a role in inflammatory processes [10]. So, the glucocorticoid can be used in treating Ulcerative Colitis by designing a drug similar to the GR or mimicking the GR in the laboratory and can be used for pre-clinical and clinical trials.

Approach 2: Molecular simulation studies

Energy minimization is performed on structures prior to dynamics to relax the conformation and remove steric overlap that produces bad contacts. CHARMm is a highly flexible molecular mechanics and dynamics program. It performs well over a broad range of calculations and simulations, including calculation of geometries, interaction and conformation energies, local minima, barriers to rotation, time dependent dynamic behavior, and free energy [11]. The results obtained initially and after minimization of protein are shown in the Table **2.**

Table 2: Energy minimization of NFKB p50/p65 (1VKX)

Name -1VKX	Force field -1VKX-CHARMm27	
ENERGY	**INITIAL**	**FINAL**
Potential Energy (kcal/mol)	3144.85064	-35889.24449
Vander Waals Energy (kcal/mol)	16301.56882	-3923.40528
Electrostatic Energy (kcal/mol)	-19814.12500	-37189.39069
RMS Gradient (kcal/(mol x A)	442.73264	1.32367

Minimization Criteria -CONJUG> Minimization exiting with number of steps limit (200) exceeded.

Drug Likeliness Evaluation

The analysis of the World Drug Index (WDI), which leads to Lipinski's 'rule-of-five'. These

rules, which are usually viewed more as guidelines rather than absolute cutoffs, are molecular mass < 500 daltons (Da), calculated octanol/water partition coefficient (CLOGP) < 5, number of hydrogen bond donors <5 and number of hydrogen bond acceptors < 10. Thus, such studies point the most important physicochemical properties and structural characteristic of a good drug in the context of our current knowledge. In the study of 400 compounds 349 have passed the Lipinski rule of 5. These properties are then typically used to construct predictive ADME models and create the basis for what has been called property-based design [12].

ADME studies

Computer aided ADME studies have been done by using the software Accord for Excel in Accelrys Discovery studio 3.5 software. The ADME studies provide insight into the pharmacokinetic property of the compounds. All the parameters calculated are tabulated in the Table 3.

Table 3: ADME values of Top 5 ligands

S.No.	Descriptor	A: Ginkgetin	B: Bilobetin	C:Bowdichione	D:Picen-13-ol	E:Mesuaxanthone_B
1	ADME.PSA.2D	153.583	165.469	90.578	20.815	88.677
2	A LOG P98	4.96	4.735	1.13	5.221	2.238
3	AQ SOl LEV	1	1	3	1	3
4	BBB LEV ADMET	4	4	3	0	3
5	ABSORPTION LEV	3	3	0	0	0
6	PPB LOG	1.12E-08	1.93E-09	2.36E-05	1.97E-05	0.987872
7	CYP2D6	0.0217478	0.000529	5.31E-05	0.0003019	0.169112
8	HEPATOX PROB	0.0015847	0.000159	0.0278931	4.37E-07	0.973142

ADME parameters play a significant role in new drug discovery. Interpretation of the values was done using standards provided by Accelrys Inc. It was observed that A, B and D have slight aqueous solubility and showed good aqueous solubility for C and E compounds. According to CHS Venkataramana et al. [13], the compounds may not be able to penetrate the blood brain barrier. So, the chances of CNS side effects are lower absent for all 5 compounds except D. A, B very poor intestinal absorption, whereas the remaining compounds have good intestinal absorption. Hence the compounds and Bowdichione, Picen-13-ol, Mesuaxanthone_B are expected to possess good human intestinal absorption. The readings of CYP2D6 shows compounds A, B, D and E will not inhibit the CYP450 enzyme during metabolism and PPB scores are reliable so it

shows that the compound binds to carrier proteins in the blood. The compounds have good binding capacity to cross the membrane and bind to plasma protein. The compounds A, B, and C are non-toxic and there is a high probability that these compounds can reach the desired targets.

Virtual Toxicity studies

TOPKAT in Accelrys predicts toxicity endpoints based on chemical structure, including rodent carcinogenicity, Ames mutagenicity, rat oral LD50, rat chronic lowest-observable adverse effect level, developmental toxicity potential and skin sensitization. Various models that can be calculated are tabulated in the Table 4. Models which satisfy all the validation criteria for the query compound are computed and results are recorded.

Table 4: TOPKAT values of top 5 compounds

S. No	Toxicity Models	Compounds				
		Ginkgetin	Bilobetin	Bowdichione	Picen-13-ol	Mesuaxanthone_B
1	NTP Carcinogenicity Call (Female Rat) (v3.2)	0.000	0.000	0.000	1.000	0.000
2	FDA Carcinogenicity Male Rat Non vsCarc (v3.1)	0.000	0.000	1.000	1.000	0.000
3	FDA Carcinogenicity Female Rat Single vsMult (v3.1)	0.000	0.000	0.000	0.025	0.000
4	Ames Mutagenicity (v3.1)	0.000	0.000	0.000	1.000	0.015
5	Rat Oral LD50 (v3.1)	3.331	3.080	2.850	1.817	3.014
6	Rat Maximum Tolerated Dose - Feed/Water (v6.1)	4.455	4.438	3.594	3.035	3.196
7	Skin Irritation (v6.1)	0.000	0.000	0.000	0.000	0.000

Toxicity studies include mutagenicity, NTP carcinogenicity and developmental toxicity assays. Mutagenicity predicts the ability of the drug to cause mutation to human cells. Mutagenicity assay is

based on the Ames test. Carcinogenicity assay predicts the ability of the compound to cause cancer to normal human cells. Carcinogenicity test are carried for male and female mouse models.

Toxicity prediction studies serves as a preclinical examination and helps to minimize the time and cost during clinical trials. Skin irritation test provides information on the use of compound for topical applications. TOPKAT features a patented algorithm (US patent 6, 036,349, issued March 14, 2000), which determines whether a query structure lies within the Optimum Prediction Space (OPS) of a respective model. Computed Probability should be used to determine toxicity. If it is between 0 and 0.29 the compound is non-toxic, if it is between 0.3 and .69 the result is indeterminate, and if the score is between 0.7 and 1, the compound is toxic. It has been observed that if the discriminant score is negative then probability of causing cancer is 0 or non-carcinogenic, if discriminant score is positive then probability of causing cancer, mutagenicity and developmental toxicity is 1 or carcinogenic, mutagenic and developmental toxicity exist. For Rat LD50 the model should fall in between the range. From the toxicity analysis Ginkgetin, Bilobetin and Mesuaxanthone_B are having good scores (ie. 0) than others and
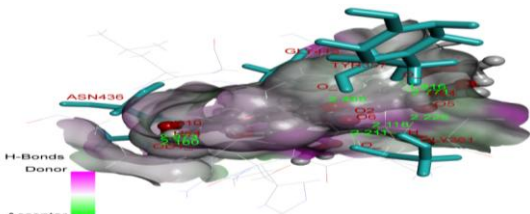
they have proven to be non-toxic in case of NTP Carcinogenicity Call (Female Rat) (v3.2), FDA Carcinogenicity Male Rat Non vs Carc (v3.1), FDA Carcinogenicity Female Rat Single vs Mult (v3.1), non-mutagenic, no skin irriancy effect and Developmental toxicity.

Docking Results

In the present study, Accelrys discovery studio 3.5 was used to find the possible binding site of 1VKX. From the binding site analysis the binding pockets are identified and the largest binding pocket was selected as active site for the docking studies. Then 349 anti-inflammatory natural compounds were docked into NFKB p50/p65 protein using GOLD 5.1 and Lead-IT software all docking solutions were ranked according to the GOLD and Lead-IT fitness function. The docking results showed that all the anti-inflammatory compounds are active NFKB inhibitors. The docking scores are tabulated in the table 5 and table 6 with their Lead-IT and GOLD scores.

Table 5: Ligand-Protein interaction with docking scores in Lead-IT

S.No	Compound Name	Lead-IT score	H-Bond	Lead-IT Amino acid	Amino acid atom	Ligand atom	H-bond length
1	Ginkgetin	-25.3794	8	TYR357	O_	O5	2.77117
				GLY361	O_	O6	2.21135
				GLY361	HN_	O5	2.22518
				GLY361	HN_	O6	2.1178
				GLY413	O_	O2	2.4647
				ASN436	OD1_	O10	2.97339
				TYR357	O_	H54	1.81596
				ASN436	OD1_	H64	2.16008
2	Bilobetin	-24.9645	8	TYR357	O_	O5	2.78219
				GLY361	O_	O6	2.20762
				GLY361	HN_	O5	2.21573
				GLY361	HN_	O6	2.13195
				GLY413	O_	O2	2.44639
				ASN436	OD1_	O10	2.98214
				TYR357	O_	H53	1.82954
				ASN436	OD1_	H61	2.18186
3	Bowdichione	-22.9402	6	ARG354	O_	O6	2.91202
				ARG356	HN_	O6	1.63586
				SER363	HN_	O2	2.15019
				SER363	HN_	O4	1.95922
				GLY366	HN_	O3	2.41513
				ILE439	HN_	O6	2.19
4	Picen-13-ol	-22.7670	4	TYR357	O_	O1	2.86343
				GLY361	O_	O1	2.87803
				GLY361	HN_	O1	1.93669
				TYR357	O_	H37	2.02043
5	Mesuaxanthone_B	-21.9774	9	TYR357	O_	O4	2.89189
				GLY361	O_	O2	1.91037
				GLY361	HN_	O2	1.88541
				GLY361	HN_	O4	2.11811
				VAL412	O_	O3	3.07364
				LEU437	O_	O5	2.83189
				VAL412	O_	H24	2.23658
				TYR357	O_	H25	1.94523
				LEU437	O_	H26	1.89861

S.No	Compounds	Lead-IT Scores	Binding pose with target protein
1	Ginkgetin	-25.3794	

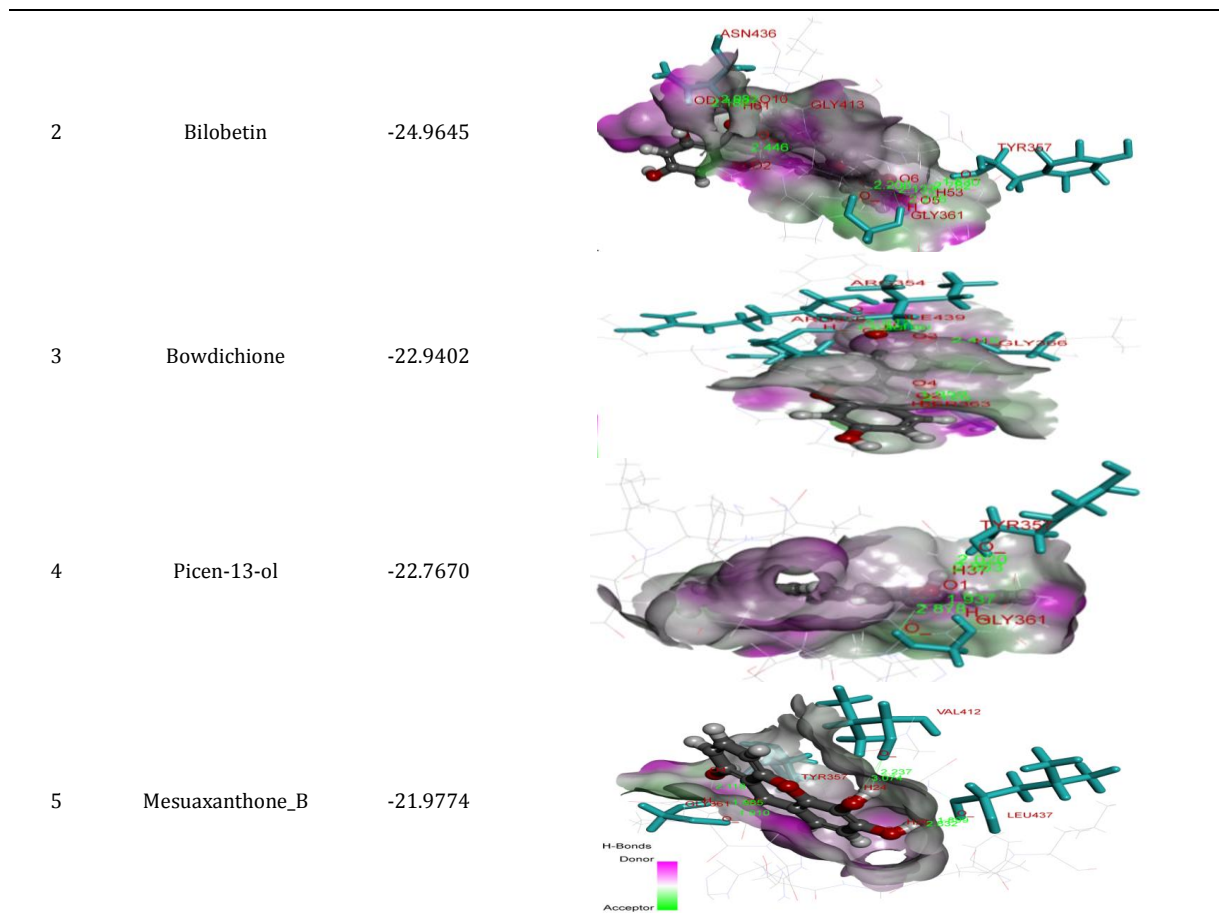|			
2	Bilobetin	-24.9645	
3	Bowdichione	-22.9402	
4	Picen-13-ol	-22.7670	
5	Mesuaxanthone_B	-21.9774	

Figure 2: Binding interactions between selected 5 ligands with target

Pharmacophore-based drug design

Pharmacophore are the lead compound against a desired target. A pharmacophore is a 3D arrangement of functional groups within a molecule and these are necessary to bind to a macromolecule or active site. Identification of the pharmacophore is an important step in understanding the interaction between receptor and ligand. Pharmacophore mapping was accomplished by the Ligand scout software according to Wolber G et al. [14]. The pharmacophore models produced were evaluated qualitatively through visual inspection and according to their ability to generate the target pahrmacophores. The pharmacophore expresses constraints on the 3D structure of the molecule by specifying relative atom positions that should be maintained to increase the likelihood that the molecule will bind with the receptor site [15, 16]. For all 3 ligand pharmacophore was generated. Figure 3 shows pharmacophore model generated for Ginkgetin, Bilobetin and Mesuaxanthone_B which were found to be better in their interactions and have very good anti-inflammatory effect could be used as a skeleton for designing a new class of drugs and the probable compounds identified from docking and pharmacophore study are listed in the Table 6 with their Lead-IT, GOLD and TOPKAT scores.
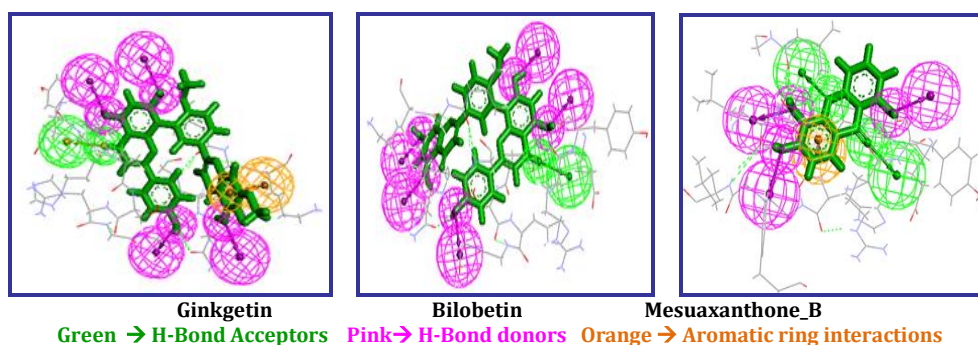

Ginkgetin   **Bilobetin**   **Mesuaxanthone_B**
Green → H-Bond Acceptors   Pink→ H-Bond donors   Orange → Aromatic ring interactions

Figure 3:Pharmacophore based study

Table 6: Best compounds obtained after Pharmacophore analysis and docking Study

S.No.	Compound Name	Natural compound		
		Lead-IT (score)	Gold (score)	TOPKAT
1	Ginkgetin	-5.3794	76.5696	7/13
2	Bilobetin	-4.9645	75.0929	7/13
3	Mesuaxanthone_B	-1.9774	51.1584	7/13

CONCLUSION

In this study, two alternative types of drugs have been studied for treating Ulcerative Colitis. One mimicking the glucocoricoid activity with the target protein NF-kB p50/p65 (1VKX). Other is the *insilico* approach of natural compounds against NFkB p50/p65. The results of present study clearly demonstrated that Ginkgetin, Bilobetin and Mesuaxanthone_B exhibited the best binding interactions among 400 anti-inflammatory compounds and warrants for the development of potent NFkB inhibitors so the proposed leads need to be presented to the scientific community for further investigational confirmation for the treatment of the disease.

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