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Sesame lignans as promising anti-inflammatory agent: Exploring novel therapeutic avenues with *in silico* and computational approach

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Innumerable health-beneficial properties of sesame lignans like sesamol, sesamolin, sesamin and sesaminol make them lucrative agents in the pharmaceutical industry. To specify the mode of action of these phytochemicals, detailed computational physicochemical properties evaluation, and toxicity assessment (using free web servers and databases), as well as binding interactions with physiological inflammatory effectors (such as COX-2, TNF-α, IL-1β, IL-6) by means of rigid ligand-receptor docking (using software), have been thoroughly investigated. Interestingly, sesame lignans are conformed to have drug-likeness, indicating their efficacy and suitability like established therapeutics. These bioactive lignans possess drug-like attributes and effectively act as ligands in the present *in-silico* study. The basic pharmacokinetic profile of these compounds has suggested non-polar solvents or delivery systems for them to enhance their bioavailability in physiological systems. However, all the sesame lignans are toxic to the liver cells with a50 % lethal dose in the range of 500-1500 mg/kg. Toxicity study indicated minimum toxicity of lignans to normal cellular milieu, but noticeable cytotoxic effects against several cancerous cell lines suggesting their anti-carcinogenic properties. Finally, the findings of the molecular docking study have depicted a high affinity of these ligands for target proteins, even better than traditional anti-inflammatory drugs- Indomethacin and Ibuprofen. The molecular interactions have represented sesaminol as the most effective and Sesamol as the least potent ligand for target receptor whereas COX-2 seems to be the most vulnerable target. The docking scores varied widely (-4.7 to -11.0 kcal/mol). The present *in-silico* approach is expected to provide valuable resources for optimizing bioactive molecules as future-generation therapeutics before pre-clinical and clinical studies.

Keywords: Anti-inflammatory, Drug-likeness, Ligand, Molecular docking, Target receptor, Sesame lignans

Inflammation is regarded as the body's natural biological response to maintain physiological homeostasis and cellular integrity following exposure to endogenous or exogenous harmful pathogens, toxins and irritants[1]. Additionally, it contributes to immunological defences against infections and acute phase reactions. Controlled types of inflammation include cytokine storm and chronic inflammation. Numerous pathologies have attracted a lot of research about cytokines and transcription factors[2]. To effectively diminish the detrimental consequences of an injury or infection, the course of inflammation initiates as a complex immunological cascade involving the recruitment of white blood cells, activation of monocytes and macrophages, increased expression of pro-inflammatory as well as suppression of anti-inflammatory cytokines. As a result, synchronization between redundant pro- and anti-inflammatory cytokines has a major impact on regulating the onset and progression of inflammation. Excessive production of pro-inflammatory cytokines brings about the activation of several downstream signalling cascades that ultimately lead to a major shift in haematological parameters, tissue damage, organ failure and the ultimate demise[3]. Interleukin-1 beta (IL-1β), a key mediator of host defence, infection, injury, and pathogen response is generally produced by activated macrophages[4]. In the biological system, it upregulates other pro-nociceptive mediators, enhances immunogenic responses, contributes to the state of pain, and facilitates central sensitization. Besides, it also regulates a variety of cellular activities such as apoptosis, cell proliferation and differentiation, intestinal dysbiosis, carcinogenesis and auto-inflammatory syndromes. Acute phase responses, chronic inflammatory processes, autoimmune disorders, haematopoiesis, cancer, and immunological responses all are stimulated by the temporary production of Interleukin-6 (IL-6) in response to infections and

tissue injuries[5]. It is pleiotropic in nature, aids in host defence as well as play a pivotal role due to its trans-signalling mechanism, which has a wide range of target cells. The transcriptional regulator Tumour Necrosis Factor- α (TNF-α) is a potent pro-inflammatory cytokine that is crucial for the immune system's function during inflammation, cell division, proliferation, and apoptosis[6]. TNF-α activates complex signal pathways that are primarily focused on either apoptosis and cell death or encouragement of cell survival and production of pro-inflammatory genes. Signal strength, production of signalling molecules and regulatory proteins, interaction with other cell signals, as well as final inflammatory pathways all play an essential role in controlling the balance between the two approaches. cyclooxygenase-2 (COX-2) is an inducible protein that is produced in response to certain physiological, inflammatory, and growth factor stimuli. It helps to facilitate the release of prostaglandins mediating pain and promoting the inflammatory response[7]. The majority of traditional NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) act by inhibiting certain receptors to produce an anti-inflammatory and analgesic effect.

Over the past few decades, several types of research have been focused to investigate the anti-inflammatory potential of various phytochemicals and to explore them as therapeutic candidates in acute/chronic inflammatory conditions. Sesame lignans like sesamol, sesamin, sesamolin and sesaminol have been shown to have effective immunomodulatory and anti-inflammatory potential in previous research conducted *in vivo, ex vivo*, and *in vitro*[8]. Most of the time, bioactive lignans work by modifying specific signalling pathways implicated in inflammation. Sesamin specifically inhibits the toll-like receptor 4 (TLR-4) pathway, which reduces the production of pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6 [9]. Previous *in vivo* experiments found that oral supplementation of sesamin for five consecutive months noticeably lowered the production of TNF-α and IL-6 in mice[10]. Sesamin has also been proven to greatly diminish the amount of uncontrolled IL-6 and TNF-α production in myocardial infarction when taken for five days at a dose of 100 mg/kg/day[11]. In a different study, rats given 10 mg/kg/day of sesamin or sesamol for 15 days showed a considerable decrease in the expression of C-reactive protein (CRP), TNF-α, and IL-1 caused by lipopolysaccharide (LPS), by 84 and 83%, respectively[12]. Both sesamin and sesamol are shown to inhibit the NF-κB/mitogen-activated protein kinase (MAPK) downstream cascade, which reduces the inflammation in RAW 264.7 macrophages[13,14]. Additionally, sesamol has been discovered to drastically reduce the upregulation of COX-2 and inducible nitric oxide synthase (iNOS) [15]. Sesamin and sesamolin have been reported to significantly inhibit the p38 MAPK signalling pathway in BV-2 microglial cells and to reduce the production of IL-6, TNF-α, and NO[16]. Moreover, oral administration of sesaminol was also reported to possess anti-inflammatory properties through inhibition of NF-κB (Nuclear Factor Kappa B), TNF-α and IL-6 expression in ethanol-induced colonic inflammation in mice[17]. Despite the various strategies to suppress inflammatory cascade, developing novel therapeutics with these bioactive lignans requires a vivid understanding of drug-likeness and an insight into the molecular docking analysis of these sesame lignans acting as ligands against various inflammatory markers. The present study is aimed to explore these natural bioactive as potent anti-inflammatory agents for minimizing the unintended side effects of the prolonged use of commercial pharmaceutics. In this perspective, our vigorous efforts are engaged to investigate the affinity of sesame lignans to the molecular targets in comparison with the synthetic drugs. The advantage of this *in silico* screening relies on its cost-effectiveness. Perhaps, this is the first venture depicting the detailed mechanism of sesame lignans against inflammation obtained from the comparative molecular docking study. The findings are expected to highlight the probability of these bioactive lignans being used as therapeutic agents and to pave the path for their synthetic modification as per the requirement in future.

Experimental Section

A subset was chosen from many computational techniques available for predicting the pharmacological characteristics and toxicological effects of chemical compounds on human health. The techniques including user-friendly interfaces and free tutorials (online or open-source) had shown an accuracy of prediction typically higher than 70%.

Ligand structure retrieval

Sesame lignans are taken as ligands in the present *in-silico* analysis to learn the mechanism of bioactive lignans inhibiting inflammatory target proteins. The PubChem database (https://pubchem.ncbi. nlm.nih.gov) was used to determine the structures of four significant sesame lignans such as sesamin, sesaminol, sesamolin and sesamol. The same source also yielded their chemical nomenclature and molecular formula, as well as 2D and 3D molecular structures[18].

Drug-likeness and Basic pharmacokinetic parameters of Ligand

To direct the choice of bioactive lignans to synthesize, test, and promote as well as to identify the favourable ones with the best possibility to become an effective ligand, a vast number of parameters were analyzed. The drug-likeness tool (DruLito 1.0) was used to compute the fundamental pharmacokinetic properties of the target ligand, including the partition coefficient (log P), molecular weight (Mw), total polar surface area (TPSA), H-bond acceptor (HBA), H-bond donor (HBD), octanol-water partition coefficient (AlogP), rotatable bond count (RC), atom molar refractivity (AMR) and number of rotatable bonds (nRB) (NIPER S.A.S., Nagar, India). All the laws were implemented independently of one another. The calculations were completed as per the previously mentioned standard practices. Additionally, Molinspiration software was used to calculate the ligand's volume and surface area[19]. As molecular property filters, the widely used quantitative estimate of drug-likeness (QED), Lipinski's rule, MACCS-II drug data report (MDDR)-like rule, Veber rule, comprehensive medicinal chemistry (CMC)-50-like rule filters, Ghose filter, and blood-brain barrier likeness were successfully utilized here. These filters were executed through the DruLiTo software (http://www.niper.gov).

In silico toxicity prediction

The biological toxicity of the selected ligands was predicted using PASS online web server. The prediction can be acquired from the PASS (Prediction of Activity Spectra for Substances) system, which was created using training sets created using manually curated data from drug labels (http://www.way2drug.com/PASSonline)[20]. Predicting detrimental and adverse pharmacological effects such as cardiac failure, arrhythmia, myocardial infarction, hepatotoxicity and nephrotoxicity was done using the ADVERPred webserver (http://www.way2drug.com/adverpred/)[21].

In silico cell line toxicity prediction

Based on the structural formula, the CLC-Pred (Cell Line Cytotoxicity Predictor) web service was utilized to predict the *in-silico* cytotoxicity of sesame lignans in non-transformed and cancerous cell lines. To evaluate the utility of including a chemical compound in experimental screening, CLC-Pred predicts the cytotoxicity of the compound. Prediction of Activity Spectra for Substances (PASS) technology (http://www.way2drug.com/PASSonline) and a training set constructed employing cytotoxicity data captured from ChEMBLdb are the foundations of this assertion (version 23) (https://www.ebi.ac.uk/chembldb/)[22].

Target structure retrieval and Active site prediction

Discovery Studio Visualizer 3.5 observed the four targets or receptors IL-6 (PDB ID: 1P9M), IL-1β (PDB ID: 2NVH), COX-2 (PDB ID: 4COX), and TNFα (PDB ID: 2AZ5) that were retrieved from the PDB website and used for docking simulation. The RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) was used to locate the crystal structures of the target proteins. The resolution factor for the COX-2 protein was 1.55, R-value of 0.186, while the resolution factor for the other target proteins was 2.30, R-value of 0.237, using the X-ray diffraction method. Once proteins were in PDB format, they underwent processing to remove native ligands and crystalline water before being used in docking research. Using the Computed Atlas for Protein Surface Topography, amino acids important in the creation of active pockets were identified (CASTp). The architecture and active site pockets of proteins were determined using the simple and helpful web application CASTp[23]. Using CASTp and a 1.4 radius probe, the surface volume of the interior cavity of the receptor which binds to the ligands was calculated. Setting the grid box at an active site before docking is crucial.

Molecular docking

The PyRx software was used to assess *in silico* molecular interaction. The macromolecular structures of the ligand and receptor were processed appropriately and set up for docking. The affinity of ligands or macromolecules binding to targets or receptor proteins can be roughly estimated through docking. Docking was used to gather every possible ligand orientation and conformation at the binding site (https://pyrx.sourceforge.io/). Using PyRx software, the macromolecules and ligands were created. Throughout the docking investigation, the macromolecule remained rigid while the ligand molecules were flexible. The intermolecular interactions of protein-ligand binding at an atomic level were assessed and viewed with Discovery studio[24]. The macromolecules and ligands were produced using PyRx software. Initial attempts to repair the protein molecule were executed by adding polar hydrogen and Kollman

charges. Next, to the ligand molecule, Gasteiger charges were added. With the aid of a grid box, PyRxenables were set a specified target site. The X, Y, and Z dimensions, as well as the X, Y, and Z centres were altered and modified by with the active sites of the proteins. The stochastic Lamarckian genetic method was used to examine the output for ligand conformations. The strongest binding and most advantageous conformation for the ligand and protein interaction was indicated by the least negative ΔG. After the docking search was finished, the optimal conformation was selected based on the lowest binding energy and highest binding affinity. Using a graphical user interface and Discovery Studio, the 3D visualisation of docked structures was carried out. In the Discovery studio, there are a few facilities for developing ligands, analysing protein-ligand interactions, and more. The details are given in Table 1.

Results and Discussion

Ligand structure retrieval

The molecular or chemical formula communicates information about the distribution of different atoms that make up the compound, whereas the IUPAC terminology includes compositional or systematic nomenclature of the compound and serves as an international standard for designating compounds. A chemical formula reveals a wealth of information about the chemical substance it represents, including the number, type, and arrangement of atoms constituting the material. The stoichiometry and chemical skeleton of the constituent atoms are represented in 2D and 3D structures, providing insight into their spatial arrangement and the overall shape of a molecule.

Sesame seeds as well as sesame oil include bioactive lignans, which are well-known for their health-promoting activity and therapeutic intervention[25]. Sesame's primary lignans are benzodioxol-substituted furofurans, which are both phytoestrogens and members of the Polyphenol phytochemical class. Coniferyl alcohol dimerization is the first step in the biosynthesis of furofuran lignans, accompanied by the production of dioxoles, oxidation, and glycosylation. These sesame lignans are desirable dietary additives because of their nutraceutical qualities. In sesame, lignans are created by fusing cinnamyl alcohol's oxopropane side chains with a furofuran core (3,7-dioxabicyclo [3.3.0.]octane). Sesamol is a white, crystalline substance that belongs to the benzodioxole family and is a phenol derivative. The compound 3-4-methylenedioxyphenol has two oxygenated positions. Although it is miscible with most oils, it is only weakly soluble in water. Furofuran lignan is the common name for sesamolin. Two phenylpropanoids are combined to create sesamolin by joining them at their central propyl carbon. Sesamolin's numerous biological actions can be attributed to the presence of methylene dioxyphenoxy moieties or its metabolite form, the phenolic hydroxyl group. In terms of bulk, sesamin, and sesamolin makeup only a little portion of sesame oil—on average, 0.14%. Sesamin is an isoflavone or lignan that is composed of 1,3-benzodioxole groups at positions 1 and 4 of tetrahydro-1H,3H-furo[3,4-c]furan. Sesaminol is a furofuran that has a 6-hydroxy-1,3-benzodioxol-5-yl group at position 1S and a 1,3-benzodioxol-5-yl group at position 4S as substitutes. The results of the analysis of the steric and electrostatic fields of sesaminol have shown that the -OH group at the sesaminol R1 position is the active centre of their diverse biological activities. Sesamin, sesaminol, and sesamolin are phenylpropane dimers with two joined tetrahydrofuran rings that are found in nature. The four asymmetric centres in the diagonally substituted tetrahydrofurofuran nucleus allow for three different (+/-) stereoisomer pairs e.g., two aryl groups that are equatorial (eq/eq), one aryl group that is equatorial along with one axial (eq/ax), and two aryl groups that are axial (ax/ax). An additional pair with the two different aryl groups positioning either eq/ax or vice versa is to be anticipated when two separate aryl groups from the equatorial/axial configured compounds are present[26]. The 2D and 3D structure of the ligands chosen for this study along with their IUPAC name have been provided in the supplementary material (Supplementary Information, Fig. S1).

Table 1 — The details of parameters used in molecular docking

Receptor Molecules	Size (X, Y, Z)	Center (X, Y, Z)	Spacing
IL-1 β	46, 40,46	38.501, 13.225, 68.920	0.375
IL-6	124,104,56	57.052, 175.253, 45.160	0.375
TNF-α	72,68,72	-13.803 ,71.851, 27.536	0.375
COX-2	58,80,68	42.052, 33.541, 35.659	0.375

Drug-likeness and basic pharmacokinetic parameters of ligand

Drug-likeness is the similarity between a substance and a drug; molecules more or less similar to drugs are more likely to be administered and show efficacy like drugs in physiological systems. In addition, factors like hydrogen bonding, lipophilicity, molecular volume, and ionizability are also good predictors of bioavailability. Several physicochemical parameters such as solubility, lipophilicity, hydrogen bond donors (HBDs), hydrogen bond acceptors (HBAs), and topological polar surface area (TPSA) influence the pharmacokinetic and pharmacodynamic profile of the drugs[27].

In contrast to the other three lignans, sesamol has a relatively lower molecular weight. The latter three are about three times as heavy as the former. Lipophilicity and aqueous solubility both are crucial characteristics of bioactive substances, and the partition coefficient (log P) value of a molecule typically decides its solubility. Negative values of Log P define the aqueous solubility of a molecule where a positive value indicates lipophilicity. The value is significant because, even in the clinical aspect, aqueous solubility may have an impact on the bioavailability as well as bioactivity in the *in vivo* and *in vitro* conditions. Most *in vitro* assays are conducted utilizing an aqueous medium at the experiment level, particularly when using the cell culture as a model system. To assess the test chemical's pharmacological action, the medium must have all the test compounds completely dissolved in it. Additionally, to perform *in vivo* assays, the drug needs to be optimally dissolved in water, to be disseminated efficiently through the circulation to achieve satisfactory bioavailability and to attain the desired pharmacological effect at the target site. In the present study, all four compounds exhibited a positive log P value much less than 5 indicating their lipophilicity and favourable solubility in octanol (organic solvent) instead of water. Though the aqueous solubility is supposed to be slightly compromised for the substances possessing a positive log P value<5, they are shown to sufficiently absorb through the phospholipid bi-layer of the cell membrane. As per Lipinski's Rule of Five, orally administered drugs with a Log P value between 1.35-1.8 are apt for satisfactory intestinal absorption. Sesamin and sesaminol had fallen in this ideal range of log P value. Furthermore, by Lipinski's rule of five, all sesame lignans have the necessary number of hydrogen bonds, or HBA and HBD, which is the ideal quantity for membrane absorption. The measurement of overall molecule polarity is called molar refractivity. According to our findings, sesamol can be categorized as non-polar in contrast to other molecules because it does not comply with the required polarity of a molecule. The molar refractivity of sesame lignans except sesamol is within the required range and they are all polar in nature.

To optimally bind with most of the target proteins, the bioactive ligand's polar surface area (PSA) is necessary. The bioactive compound's PSA implies how well it is absorbed. A high PSA makes a substance more soluble in water, but a PSA level of more than 140 hinders a substance's capacity to penetrate cells. All four of the sesame lignans used in this investigation have PSA values that fall within the required range; hence they are all regarded as having good permeability. Other crucial metrics for any chemical compound are the number of rotatable bonds (nRB), hydrogen bonds (nHB) and rigid bonds (nRigidB). Sesamolin and sesamin have required nRB and nRigidB but lacked the necessary nHB. In contrast to sesaminol, which adhered to all four parameters, Sesamin conformed to nRB only. All phytochemicals were found to have higher volume and smaller surface area indicating that they take up a lot of room in both the physiological and biological environment. Once more, it may be inferred from their decreased surface area that their S/V ratio is extremely low, and as a result, their chemical reactivity. A compound's ability to be absorbed, transported, and distributed in various solvent systems is determined by its a logP value. Only sesamol has shown an ideal a log P value[28].

Overall, in the present work, all four lignans conformed to Lipinski's guidelines and the CMC-50 regulations, but no one could fully comply with the Ghose rule's criteria as depicted in Table 2. Sesamolin and sesaminol were found to abide by all MDDR regulations. Sesaminol, once more, was the only substance meeting the criteria for crossing the blood-brain barrier; all other substances either lack one or more characteristics that could prevent or restrict their passage through this system. The overall findings indicate that all four phytochemicals are highly potent as natural drug substitutes and can be utilized effectively replacing synthetic pharmaceuticals readily available on the market.

In silico toxicity

Along with bioactivity, therapeutic efficacy, and promising benefits, certain pitfalls of phytochemicals or compounds must be considered. Using ADVERP red

Table 2 — Drug likeness and physicochemical properties of the ligands

Ligand	Drug property rule/classification	Molecular property/Descriptors	Values	Ideal range or value	Inference
Sesamol	*Lipinski's rule*	Molecular weight	138.03	≤ 500	Pass
		Log P	1.142	≤ 5	Pass
		Hydrogen Bond donor	1	≤ 5	Pass
		Hydrogen Bond acceptor	3	≤ 10	Pass
	Ghose filter	AMR	38.69	40 - 130	Fail
		nAtom	16	20 - 70	Fail
		a Log P	-0.356	-0.4 - 5.6	Pass
	CMC-50like rule	TPSA	38.69	≤140	Pass
		nRB	0	≤10	Pass
	MDDR-like rule	RC	2	≥ 3	Fail
		nRigidB	11	≥ 18	Fail
	BBB-likeness	nAcidic group	0	0	Pass
		nHB	6	8-10	Fail
		Total surface area	57.812		
		Volume	115.99		
Sesamolin	*Lipinski's rule*	Molecular weight	370.11	≤ 500	Pass
		LogP	2.27	≤ 5	Pass
		Hydrogen Bond donor	0	≤ 5	Pass
		Hydrogen Bond acceptor	7	≤ 10	Pass
	Ghose filter	AMR	98.74	40 - 130	Pass
		nAtom	45	20 - 70	Pass
		a Log P	-1.198	-0.4 - 5.6	Fail
	CMC-50like rule	TPSA	64.61	≤140	Pass
		nRB	3	≤10	Pass
	MDDR-like rule	RC	6	≥ 3	Pass
		nRigidB	29	≥ 18	Pass
	BBB-likeness	nAcidic group	0	0	Pass
		nHB	7	8-10	Fail
		Total surface area	96.43		
		Volume	309.50		
Sesamin	*Lipinski's rule*	Molecular weight	354.11	≤ 500	Pass
		LogP	1.812	≤ 5	Pass
		Hydrogen Bond donor	0	≤ 5	Pass
		Hydrogen Bond acceptor	6	≤ 10	Pass
	Ghose filter	AMR	96.79	40 - 130	Pass
		nAtom	44	20 - 70	Pass
		a Log P	-0.975	-0.4 - 5.6	Fail
	CMC-50like rule	TPSA	55.38	≤140	Pass
		nRB	2	≤10	Pass
	MDDR-like rule	RC	2	≥ 3	Fail
		nRigidB	29	≥ 18	Pass
	BBB-likeness	nAcidic group	0	0	Pass
		nHB	6	8-10	Fail
		Total surface area	95.76		
		Volume	300.51		
Sesaminol	*Lipinski's rule*	Molecular weight	370.11	≤ 500	Pass
		LogP	1.731	≤ 5	Pass
		Hydrogen Bond donor	1	≤ 5	Pass
		Hydrogen Bond acceptor	7	≤ 10	Pass
	Ghose filter	AMR	98.4	40 - 130	Pass
		nAtom	45	20 - 70	Pass
		a Log P	-1.538	-0.4 - 5.6	Fail
	CMC-50like rule	TPSA	75.61	≤140	Pass
		nRB	2	≤10	Pass
	MDDR-like rule	RC	6	≥ 3	Pass
		nRigidB	30	≥ 18	Pass
	BBB-likeness	nAcidic group	0	0	Pass
		nHB	8	8-10	Pass
		Total Surface area	97.67		
		Volume	308.53		

and ProTox-II web servers, the sesame lignans selected for our study were subjected to toxicity analysis, which offered a complete assessment of the overall toxicity in physiological systems. All four of the substances, according to the PASS server, were found toxic to the hepatic system. Fig. 1 showed the cumulative outcomes. Pa (probability "to be active") calculates the likelihood that the investigated substance belongs to the category of active compounds and Pi (probability "to be inactive") calculates the possibility that the substance under study belongs to the category of inactive substances.

Sesamol was discovered to have the highest level of harmful nature, while sesamin had the lowest level of toxicity. Due to their deleterious effects, these sesame lignans can cause acute or chronic liver injury in physiological systems. Although it can be detrimental, computationally anticipated outcomes need to be validated further by utilizing *in vitro* and *in vivo* systems.

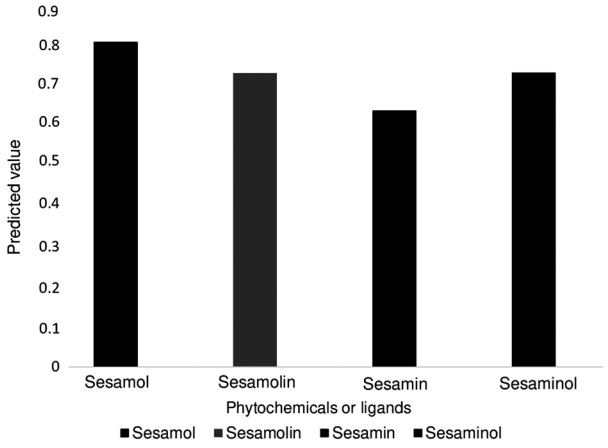


Fig. 1 — Prediction of hepatotoxicity of the ligands

In silico cell line toxicity prediction

Utilizing PASS (Prediction of Activity Spectra of Substances) for *in silico* study, assumptions were made thoroughly. The expected values were shown together with their likelihood of being active (Pa) or inactive (Pi) data. Fig. 2 displayed the Pa values for the stigma sterols' activities against stomach carcinoma, gastric carcinoma cells, lung carcinoma cell etc. From this observation, sesamol was envisaged to be the most active sesame lignan against prostate cancer, normal lung, small and non-small cell carcinoma, melanoma, and brain oligodendroglia. Besides, sesamin was found to possess inhibitory activity against several cancerous cell lines such as melanoma, lung carcinoma, glioblastoma, colon, small as well as non-small cells of the lung, etc. Seaminol was also shown to be similarly effective against multi-cancerous cell lines. Seamolin was found with the least toxic response to various cancers except for lung carcinoma and oligodendroglioma. All the lignans showed toxicity against embryonic lung fibroblast cells.

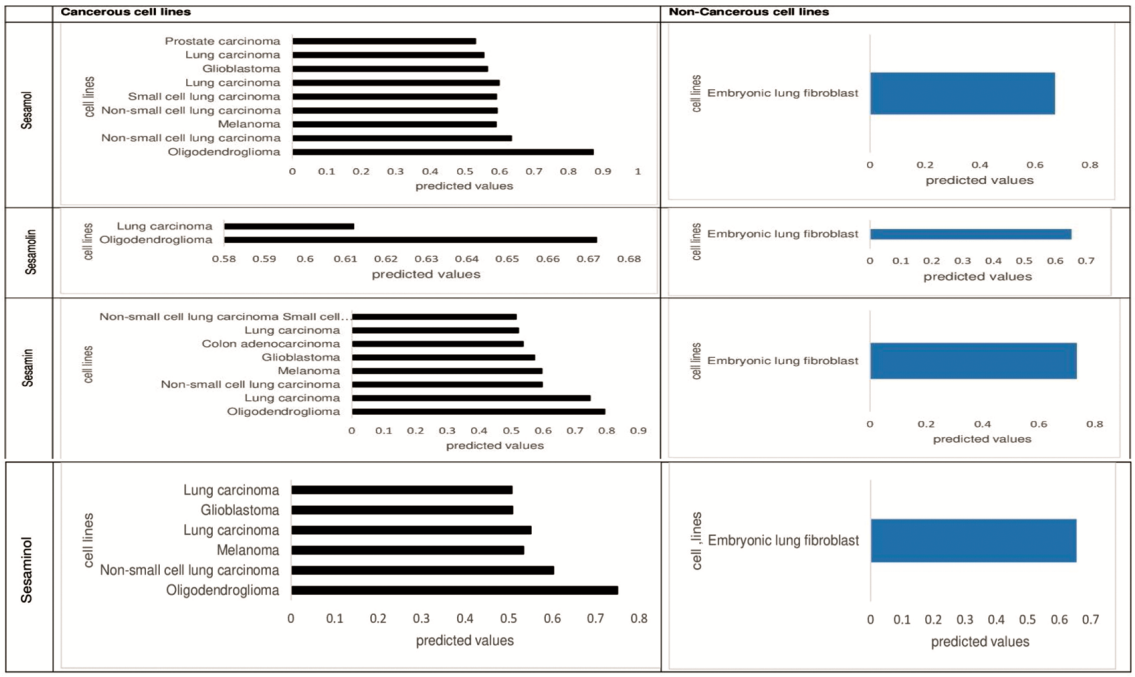


Fig. 2 — Prediction of toxicity of ligands against normal and cancerous cell lines

Target structure retrieval and active site prediction

Four important receptors of inflammatory cascades and signalling pathways were selectively picked up as our study was concerned with the anti-inflammatory effects of sesam lignans. Since the interplay of pro- and anti-inflammatory factors play an important role in inflammatory responses and are promising therapeutic targets so these targets have been chosen for study. These receptors are large molecules and possess large surface areas. The ligands bind to specific cavities or pockets of these molecules commonly known as the active site. The amino acids of these active sites are important and hence need to be identified for ligand specificity. In the present work, the amino acid residues present in the largest active site pocket along with their volume and surface area were computed using a web server. The active sites were calculated irrespective of the ligands in our study and all of them differed in their active site sequences. IL-1β was found with the smallest pocket while IL-6 had an intermediate pocket size. The active site volume of these two proteins was found much higher than the surface area suggesting that this pocket was not near the surface of the receptor and was present as a gorge or cleft in the structure. The area and volume of IL-6 were observed about 15 times more than the other one. As a larger protein, it can bind much larger ligands more effectively. Again, TNF-α was also found to have a large active site pocket with an area twice and volume quadrupled to that of IL-6. The active site of COX-2 was found to be the largest one covering almost the whole area of the protein. The pocket was huge throughout the protein structure as well as the surface, as evident from Fig. 3. The active site pocket shown in the study was the largest and the foremost one only. No other pockets were presented in the receptor.

The amino acid molecules present in the pocket were compared to the docking site residues found by molecular docking. In Fig. 3, the similar residues were marked in black while the non-similar ones were in red. COX-2 amino acids in the active site were all present in the docking approach just opposite IL-6, where none of the residues coincided. The large active site area of the COX-2 receptor may be responsible for this observation. The binding of ligands to IL-6 showed no similar amino acids which inferred that the ligands may bind to other pockets and not to this one. In IL-1β some residues coincided but most of them are not similar. In TNF-α, only one residue of the C chain, LEU 120 coincided with the docking result. These results suggested that most of the ligands did not bind to the major active site or binding pocket of the receptors. They remain bound to other clefts not calculated by the CASTp webservers predominantly.

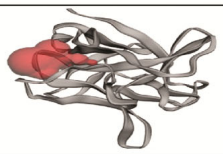
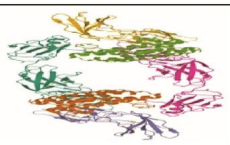
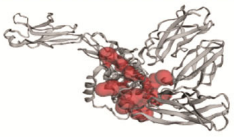
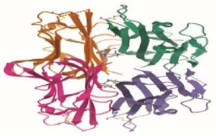
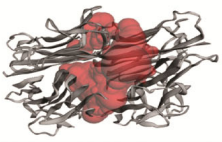
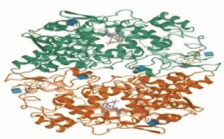
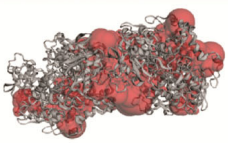
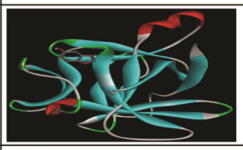
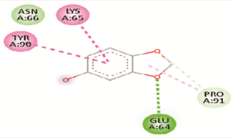
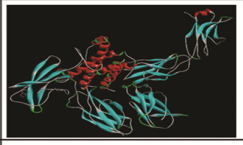
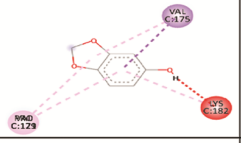
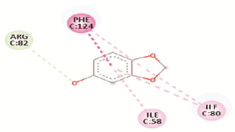
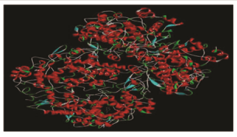
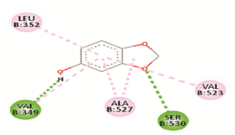
Target protein	PDB ID	Chemical structure	Active site residues	Binding pocket	Area	Volume
Interleukin-1β	2NVH		GLU 64 LYS 65 ASN 66 TYR 90 PRO 91 PRO 2 ARG 4 SER 5 LEU 6 ASN 7 SER 43 TYR 68 PRO 87 VAL 85 LYS 77 LEU 80 PHE 133 LEU A134		96.879	62.159
Interleukin-6	1P9M		PRO C143 MET C155 VAL C175 LYS C182 LYS`A228 THR A258 ARG A259 SER A260 ARG B30 ARG C233 THR C248 TRP C249 MET C250 ARG B30		1562.214	917.876
TNF-α	2AZ5		ILE C58 ILE C80 ARG C82 PHE C124 HIS C73 VAL C74 LEU C76 TRP C114 ILE C118 LEU D63 TYR D115 PRO D117 LEU C75 THR C79 LEU C120 GLN D149 LYS`A228 THR A 258 ARG A259 SER A260 ARG C233 MET C250		2743.256	4514.068
COX-2	4COX		LEU B352 VAL B349 VAL B523 ALA B527 SER B530 CYS C36 CYS C41 GLN C42 ARG C44 GLY C45 CYS C47 PRO C153 ALA C156 LYS C468 ARG C469		19476.376	30534.902

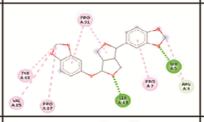
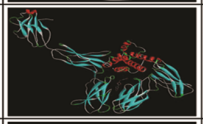
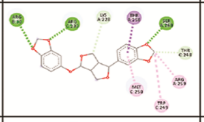
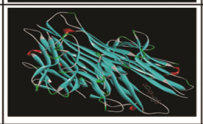
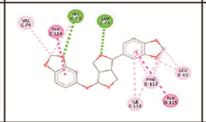
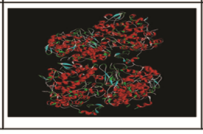
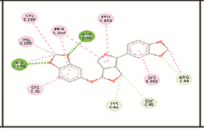
Fig. 3 — Prediction of active sites and structure of the target receptors. (Red indicates non matching and black indicates matching sequences with binding pockets of docking study)

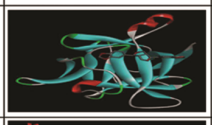
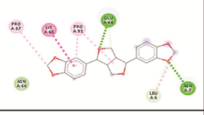
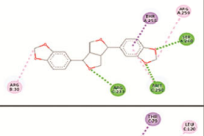
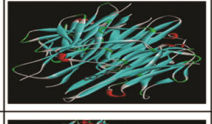
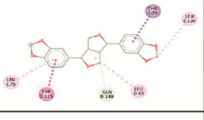
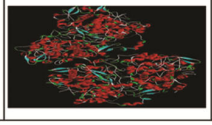
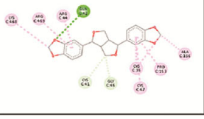
Molecular docking and interaction study

The relative binding affinity of the selected ligands with the target receptor is expressed in terms of ΔG in the molecular docking approach. The docking score is the summation of all the molecular interactions between the ligand and the target depending on their binding affinity or binding energy. Docking scores are not meant to be accurate affinity predictors but in virtual screening, they are supposed to help in categorizing docked ligands and selecting the top scorers for experimental trials. Quantitatively, docking scores having negative binding energy is better than positive energy which confirms that there is a more stable interaction between ligand and target protein. So, the more negative the value was more stable the interactions. However, there is no standard or reference value for this binding energy as some may not show much high negative binding energy. So, the choice of a good binding score is relative to the receptors and ligands chosen, overall binding scores of the chosen ligands as well as drugs in a study, etc. Generally, a score above -7.0 or -8.0 is regarded as a good score and chosen as threshold values for comparison however, there are studies where -10 or -11 is also chosen as a good affinity score. In our study, we also tried to gain insights into the mechanistic approach of sesame lignans as anti-inflammatory mediators.

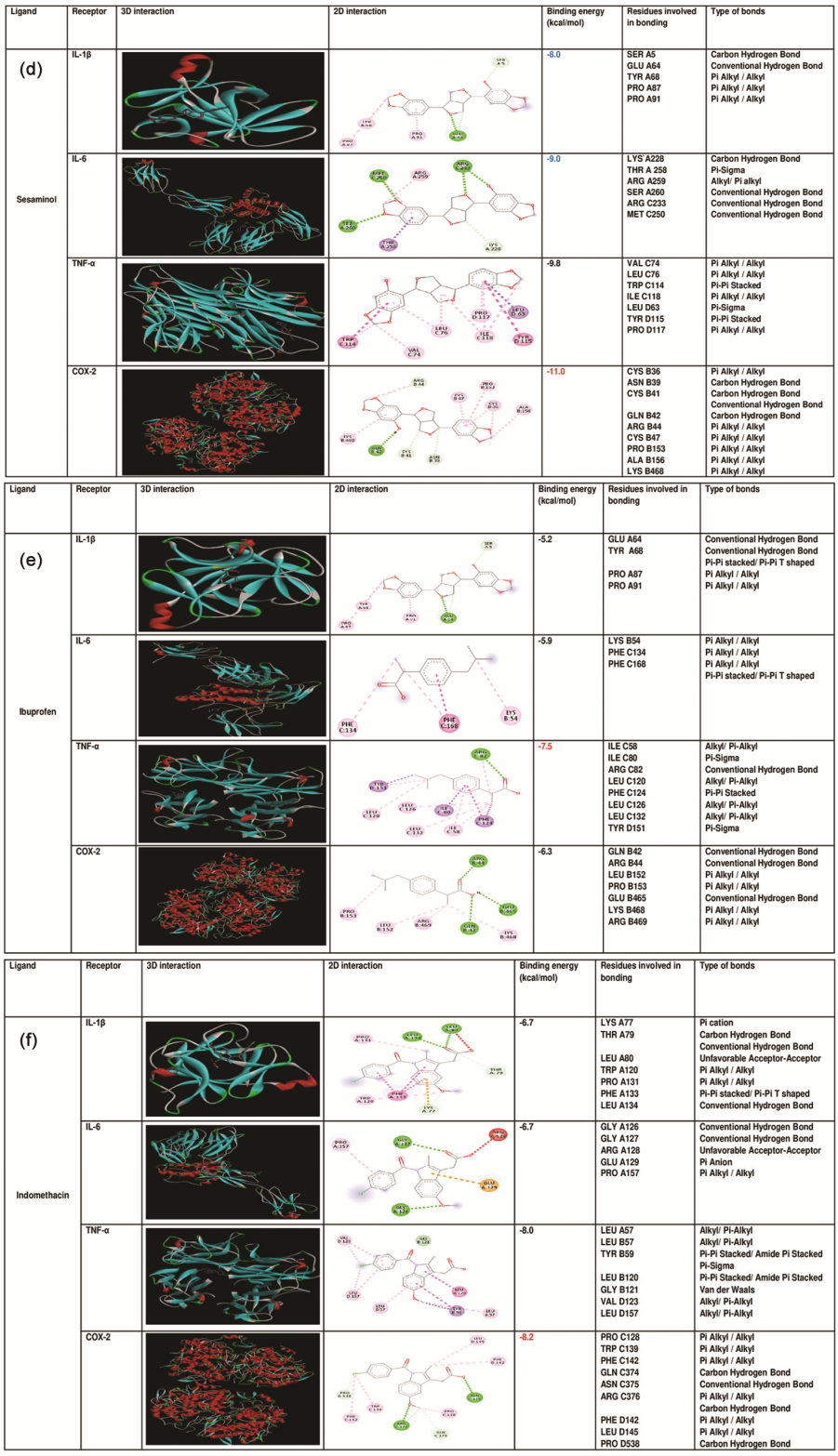
Several *in vivo* and *in vitro* studies have been previously conducted to study the anti-inflammatory effects of sesame lignans but so far *in silico* study has not been carried out in such a detailed manner. This study is an endeavour towards that step paving the way for future computational studies. Several previous bioinformatics studies have been conducted on sesame lignans against several physiological targets such as COVID-19 receptors[29] antioxidant[30], anti-quorum-sensing[31], anticancerous[32] etc. Previous *in silico* study[33]suggested various sesame lignans to be the most efficient ligand for interaction and binding with COX-2 (−9.6 to −10.7 kcal/mol). Potent anti- and pro-inflammatory cytokines and cellular receptors related to inflammation are chosen as targets for the study. In our study, docking scores ranged from -4.7 to -11 kcal/mol. The lignan Sesamol was found to be the least efficient against the chosen receptors of all the sesame lignans (Fig. 4a). All the receptors showed binding affinity below -6.0 kcal/mol. It showed the least affinity for IL-1β and that is almost like IL-6. COX-2 was found to be the most susceptible against this ligand with the highest binding affinity of -5.9 kcal/mol. TNF-α was found to be intermediate affected by having medium similarity (-5.3 kcal/mol). Overall, this ligand did not show a much better affinity for binding, and the docking score just spanned within a range of 1.2 kcal/mol. The amino acids showed different bonds- conventional and alkyl carbon-hydrogen bond, van der Waals interactions, pi-alkyl/alkyl, amide-pi stacked, pi-pi stacked, pi-sigma and carbon-hydrogen bond. An unfavourable donor-donor bond was also found in IL-6. Pi alkyl-alkyl bonds were the major bond interaction shown by the ligand. Sesamolin was found to be the most effective against COX-2 (-10.8 kcal/mol) followed by TNF-α (-9.7 kcal/mol) and IL-6 (-8.5 kcal/mol). IL-1β was the least affected for the chosen ligand. The predominant bonds were carbon-hydrogen bond, pi alkyl/alkyl, pi-sigma, conventional hydrogen bond, pi-pi T shaped etc. This ligand showed no unfavourable bonds. Against this ligand, COX-2 was found to be very efficacious as well as TNF-α. The docking scores spanned for a broad range of 3.4 kcal/mol. Pi alkyl-alkyl bonds and conventional hydrogen bonds are the major interactions prevalent for the ligand (Fig. 4b). Sesamin also showed high binding affinity against COX-2 (-10.6 kcal/mol) followed by TNF-α (-10.2 kcal/mol) and IL-6 (-8.8 kcal/mol) like the previous ligand (Fig. 4c). IL-1β was again the least affected for the chosen ligand. The main interactions are pi alkyl /alkyl, amide-pi stacked, conventional hydrogen bond, pi-sigma, carbon-hydrogen bond, and pi-pi stacked. The final chosen ligand sesaminol showed the highest activity of all the ligands, against COX-2 (-11.0 kcal/mol) (Fig. 4d). The other two receptors showed binding affinity in the similar range- TNF-α (-9.8 kcal/mol) and IL-6 (-9.0 kcal/mol) showing that these can be intermediately affected and chosen as targets in inflammation. IL-1β was again the least affected by this ligand. Conventional hydrogen bonds, carbon-hydrogen bond, pi-sigma, pi alkyl/alkyl, and pi-pi stacked are the main interactions. Overall, among the ligands sesaminol was found to be the most effective among the chosen ligands and sesamol as the least preferred one. COX-2 is the most potent receptor having spanned from about -6.0 to -11.0 kcal. Three of the four ligands showed interaction above -10.0 kcal/mol. TNF-α is the second most prominent receptor against the chosen ligands with two showing above -9.0 and one above -10.0 kcal/mol. IL-6 has intermediate potency against the chosen ligands with three of the four ligands showing docking scores above -8.0 kcal/mol. IL-1β is the least affected receptor spanning between -4.7 to -8.0 kcal/mol.

Ligand	Receptor	3D interaction	2D interaction	Binding energy (kcal/mol)	Residues involved in bonding	Type of bonds
(a)	IL-1β			-4.7	GLU A64 LYS A65 ASN A66 TYR A90 PRO A91	Conventional Hydrogen Bond Amide-Pi stacked Van der Waals Amide-Pi stacked Alkyl Carbon Hydrogen Bond
Sesamol	IL-6			-4.8	PRO C MET C VAL C175 LYS C182	Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi-Sigma Unfavorable Donor-Donor
	TNF-α			- 5.3	ILE C58 ILE C80 ARG C82 PHE C124	Pi Alkyl / Alkyl Pi Alkyl / Alkyl Carbon Hydrogen Bond Pi-Pi Stacked Pi Alkyl / Alkyl
	COX-2			-5.9	LEU B352 VAL B349 VAL B523 ALA B527 SER B530	Pi Alkyl / Alkyl Conventional Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Conventional Hydrogen Bond

Ligand	Receptor	3D interaction	2D interaction	Binding energy (kcal/mol)	Residues involved in bonding	Type of bonds
(b)	IL-1β			-7.4	PRO A2 ARG A4 SER A5 SER A43 TYR A68 PRO A87 VAL A85 PRO A91	Pi Alkyl / Alkyl Carbon Hydrogen Bond Conventional Hydrogen Bond Conventional Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl
Sesamolin	IL-6			-8.5	LYS A228 THR A258 ARG A259 SER A260 ARG B30 ARG C233 THR C248 TRP C249 MET C250	Carbon Hydrogen Bond Pi-sigma Pi Alkyl / Alkyl Conventional Hydrogen Bond Conventional Hydrogen Bond Conventional Hydrogen Bond Carbon Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl
	TNF-α			-9.7	HIS C73 VAL C74 LEU C76 TRP C114 ILE C118 LEU D63 TYR D115 PRO D117	Conventional Hydrogen Bond Pi Alkyl / Alkyl Conventional Hydrogen Bond Pi-Pi Stacked/ Pi-Pi T shaped Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi-Pi Stacked/ Pi-Pi T shaped Pi Alkyl / Alkyl
	COX-2			-10.8	CYS C36 CYS C41 GLN C42 ARG C44 GLY C45 CYS C47 PRO C153 ALA C156 LYS C468 ARG C469	Pi-Pi Stacked Carbon Hydrogen Bond Conventional Hydrogen Bond Pi-Pi Stacked Carbon Hydrogen Bond Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked

Ligand	Receptor	3D interaction	2D interaction	Binding energy (kcal/mol)	Residues involved in bonding	Type of bonds
(c)	IL-1β			-7.8	LEU A6 ASN A7 GLU A64 LYS A65 PRO A87 PRO A91	Pi Alkyl / Alkyl Conventional Hydrogen Bond Conventional Hydrogen Bond Pi Alkyl / Alkyl Amide-Pi stacked Pi Alkyl / Alkyl
	IL-6			-8.8	THR A258 ARG A259 SER A260 ARG B30 ARG C233 MET C250	Pi-sigma Alkyl/Pi-Alkyl Conventional Hydrogen Bond Alkyl/Pi-Alkyl Conventional Hydrogen Bond Conventional Hydrogen Bond
Sesamin	TNF-α			-10.2	LEU C75 THR C79 LEU C120 LEU D63 TYR D115 GLN D149	Alkyl/Pi-Alkyl Pi-Sigma Alkyl/Pi-Alkyl Alkyl/Pi-Alkyl Pi-Pi Stacked Carbon Hydrogen Bond
	COX-2			-10.6	CYS C36 CYS C41 GLN C42 ARG C44 GLY C45 CYS C47 PRO C153 ALA C156 LYS C468 ARG C469	Pi-Pi Stacked Carbon Hydrogen Bond Conventional Hydrogen Bond Pi-Pi Stacked Carbon Hydrogen Bond Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked Pi-Pi Stacked

(*Contd.*)



Ligand	Receptor	3D interaction	2D interaction	Binding energy (kcal/mol)	Residues involved in bonding	Type of bonds
(d) Sesaminol	IL-1β			-8.0	SER A5 GLU A64 TYR A68 PRO A87 PRO A91	Carbon Hydrogen Bond Conventional Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl
	IL-6			-9.0	LYS A228 THR A 258 ARG A259 SER A260 ARG C233 MET C250	Carbon Hydrogen Bond Pi-Sigma Alkyl/ Pi alkyl Conventional Hydrogen Bond Conventional Hydrogen Bond Conventional Hydrogen Bond
	TNF-α			-9.8	VAL C74 LEU C76 TRP C114 ILE C118 LEU D63 TYR D115 PRO D117	Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi-Pi Stacked Pi Alkyl / Alkyl Pi-Sigma Pi-Pi Stacked Pi Alkyl / Alkyl
	COX-2			-11.0	CYS B36 ASN B39 CYS B41 GLN B42 ARG B44 CYS B47 PRO B153 ALA B156 LYS B468	Pi Alkyl / Alkyl Carbon Hydrogen Bond Carbon Hydrogen Bond Conventional Hydrogen Bond Carbon Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl
(e) Ibuprofen	IL-1β			-5.2	GLU A64 TYR A68 PRO A87 PRO A91	Conventional Hydrogen Bond Conventional Hydrogen Bond Pi-Pi stacked/ Pi-Pi T shaped Pi Alkyl / Alkyl Pi Alkyl / Alkyl
	IL-6			-5.9	LYS B54 PHE C134 PHE C168	Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi-Pi stacked/ Pi-Pi T shaped
	TNF-α			-7.5	ILE C58 ILE C80 ARG C82 LEU C120 PHE C124 LEU C126 LEU C132 TYR D151	Alkyl/ Pi-Alkyl Pi-Sigma Conventional Hydrogen Bond Alkyl/ Pi-Alkyl Pi-Pi Stacked Alkyl/ Pi-Alkyl Alkyl/ Pi-Alkyl Pi-Sigma
	COX-2			-6.3	GLN B42 ARG B44 LEU B152 PRO B153 GLU B465 LYS B468 ARG B469	Conventional Hydrogen Bond Conventional Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Conventional Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl
(f) Indomethacin	IL-1β			-6.7	LYS A77 THR A79 LEU A80 TRP A120 PRO A131 PHE A133 LEU A134	Pi cation Carbon Hydrogen Bond Conventional Hydrogen Bond Unfavorable Acceptor-Acceptor Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi-Pi stacked/ Pi-Pi T shaped Conventional Hydrogen Bond
	IL-6			-6.7	GLY A126 GLY A127 ARG A128 GLU A129 PRO A157	Conventional Hydrogen Bond Conventional Hydrogen Bond Unfavorable Acceptor-Acceptor Pi Anion Pi Alkyl / Alkyl
	TNF-α			-8.0	LEU A57 LEU B57 TYR B59 LEU B120 GLY B121 VAL D123 LEU D157	Alkyl/ Pi-Alkyl Alkyl/ Pi-Alkyl Pi-Pi Stacked/ Amide Pi Stacked Pi-Sigma Pi-Pi Stacked/ Amide Pi Stacked Van der Waals Alkyl/ Pi-Alkyl Alkyl/ Pi-Alkyl
	COX-2			-8.2	PRO C128 TRP C139 PHE C142 GLN C374 ASN C375 ARG C376 PHE D142 LEU D145 PRO D538	Pi Alkyl / Alkyl Pi Alkyl / Alkyl Pi Alkyl / Alkyl Carbon Hydrogen Bond Conventional Hydrogen Bond Pi Alkyl / Alkyl Carbon Hydrogen Bond Pi Alkyl / Alkyl Pi Alkyl / Alkyl Carbon Hydrogen Bond

Fig. 4 — Molecular interaction and docking analysis of (a) sesamol, (b) sesamolin, (c) sesamin, (d) sesaminol, (e) ibuprofen, and (f) indomethacin against receptors. (The red values indicate the best binding score against a particular ligand or drug. The blue score indicates the maximum value of a particular receptor amongst all the interactions)

We have also compared the activities of two anti-inflammatory drugs-Ibuprofen and Indomethacin against our ligands. Ibuprofen, a commercial NSAID is globally known for its huge anti-inflammatory and analgesic efficacy. Non-selective, reversible inhibition of two COX isoforms is suggested to be the underlying mechanism of ibuprofen. Compared to R-ibuprofen, S-ibuprofen is found to be a more potent enantiomer showing more intense inhibitory activity against COX-1 rather than COX-2. In addition, ibuprofen shows a prominent free radical scavenging activity *in vitro*. In the present study, ibuprofen was found to be the least effective of all the chosen ligands (Fig. 4e). This drug showed the highest affinity against TNF-α (-7.5 kcal/mol) unlike other chosen ligands which showed against COX-2. The other receptors showed very less binding affinity to this drug of around -6.0 kcal/mol. COX-2 showed the second most potency among all and the other two showed docking scores above -5.0 kcal/mol. In parallel, indomethacin, a traditional nonsteroidal indole derivative is widely used for its promising anti-inflammatory and anti-carcinogenic properties. In this study, the drug showed highly positive effects against COX-2 (-8.2 kcal/mol) and TNF-α (-8.0 kcal/mol), and was found to be more effective as compared to sesamol but relatively less than the other three ligands (Fig. 4f). The drug showed a medium binding affinity for the other two receptors and binding energy in a similar range of around -6.5 kcal/mol. From the docking result, it can be inferred that the sesame lignans are more effective and promising as anti-inflammatory agents and even better than the two conventional drugs, Indomethacin and Ibuprofen commonly used for such therapeutic purposes. In such computational biology studies such drugs are chosen for comparative efficacy since these drugs possess well known mechanism of action, structures, and extensive studies and literature based on which we can comment on any novel compounds.

Conclusion

We attempted a preliminary *in silico* study to gain insights into the anti-inflammatory activity of sesame lignans utilizing free web servers and software. This study depicted that sesame lignans are highly efficacious molecules that can be successfully utilized as either prophylactic or therapeutic agents in various inflammatory disorders. The bioactive lignans conform to drug-like molecules possessing optimum physicochemical properties, solubility as well as minimal toxicity. The phytochemicals also have shown selective toxicity against cancerous cell lines showing their anti-carcinogenic efficacy. The comparison of the active site of the chosen receptors has exhibited similarity with the binding residues in docking postures. Finally, through docking and binding interaction analysis, highly negative binding energies of the compounds in the range of -7 to -11 kcal/mol proved that they can be widely used as pharmaceutical agents with minimal side effects in physiological disorders. The drugs ibuprofen and indomethacin are also found to be less effective in comparison to these compounds. Thus, the endeavour offers novel directions for future clinical and pre-clinical trials to establish the bioactive lignans as unique pharmaceutical agents against chronic/acute inflammation.

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Supplementary Information

Supplementary information is available on the website http://nopr.niscpr.res.in/handle/123456789.

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