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Research Article

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Anti-inflammatory activities of phytochemicals from *Bauhinia variegata* Linn. leaf: An *in silico* approach

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ABSTRACT

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthase (iNOS) plays an important role in inflammation and thus they act as a promising molecular target for the treatment of many inflammatory diseases. Present study was aimed to find out COX-2 and iNOS inhibiting compounds from Bauhinia variegataLinn. 3D structures of compounds reported from GCMS analysis of active fractions of B.variegata were built using Chemsketch software. All the compounds analyzed exhibited antiviral, antibacterial, antineoplastic, antidiabetic and anti-inflammatory properties. Docking studies were performed using Glide (Grid-based Ligand Docking with Energetics) Extra Precision (XP) 5.7 algorithm in Schrodinger Software Suite, 2011 analysis. Drug likeliness (ADME) property using Lipinski RO5 was also predicted. Among the 33 ligands of the active fractions, four ligands were found to have least glide score.Thus, the phytochemicals from the active fractions of B.variegata leaf was found to have appreciable anti-inflammatory activity

Keywords: COX, NOS, docking, inflammation, *Bauhinia variegata*, ligands

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INTRODUCTION

Inflammation is a complex host (systemic/local) response to a wide range of tissue injury and infection, generally marked by increased levels of cytokines, cytokine receptors, adhesion molecules, immuno-regulatory factors and several other mediators[1].Inflammation is a basic way in which the body reacts to infection, irritation or other injury. The key feature being redness, warmth and sensitive nerve endings in our body become irritated. It is mediated by molecules called prostaglandins, a prostanoid.Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain. Currently there are three COXiso enzymes, namely COX1, COX2 &COX3. COX1 is a constitutive enzyme and is found in almost all cell of the body except red cells. The COX 2 enzyme is located specifically in inflammatory areas and it is inducible [2-6]. COX 3 is a splice variant of COX 1 which retains intron one and has a frameshift mutation, thus some prefer the name COX 1b or COX1 variant. Besides, COX has long been recognized to be involved in normal kidney function [7-9], regulating brain function[10-12],maintaining proper glandular architecture of small intestine [13,14], ovulation[15], Uterus contraction [16] and stimulate bone resorption & formation [17,18].

Nitric oxide (NO) is produced from L-arginine in mammalian tissue by Nitric Oxide Synthase (NOS) enzymes. There are three NOS isoenzymes namely nNOS (constitutive in neuronal tissue), eNOS (constitutive in vascular endothelial cells) and iNOS (inducible by cytokine in macrophages and hepatocytes) [19]. Constitutively expresses eNOS and nNOS are responsible for low physiological levels of NO, whereas larger amounts for NO are produced by iNOS. iNOS is induced by microbial products, such as lipopolysaccharide (LPS) and inflammatory cytokines

such as interleukin – 1(IL-1), tumour necrotic factor- α (TNF-α) and interferon –γ (INF- γ) in macrophages and some other cells [20].NO production is increased in response to inflammatory stimuli and mediates the destructive effects [21]. Studies have shown that production of NO by iNOS is implicated in a variety of acute and chronic inflammatory diseases (e.g., sepsis, septic shock, vascular dysfunction in diabetes, asthma, arthritis, multiple sclerosis, and inflammatory diseases of the gut) [22]. Because of the importance of NO derived from iNOS in inflammatory response, there were several research efforts to find a selective iNOS inhibitor. The compounds inhibiting expression or activity of iNOS are proposed to be potential anti-inflammatory agents.

Inflammation is a key etiological factor for several disease conditions such as hypersensitivity, asthma, Inflammatory Bowel Disease (IBD), rheumatoid arthritis and many others. Most of the currently marketed therapeutic drugs are associated with adverse side effects and are not suitable for chronic therapies and so some of them were withdrawn from the markets. For instance, Non-Steroidal Anti-Inflammatory (NSAID's) drugs are reported to have adverse drug interactions and hence are not prescribed along with warfarin, antihypertensives and diuretics. Thus, treatment of these inflammatory disorders still remains a growing health concern and has become a major challenge to the health professionals.

Plants always play an important role in the treatment of many diseases worldwide. The traditional systems of medicine of all the countries have used plants and their products for the treatment of various ailments. *Bauhinia variegata* is a medium sized deciduous tree belonging to the family *Ceasalpinaceae*.Its common names are Mountain ebony, Indian orchid, etc. Various parts of this plant is used for the treatment of piles, dysentery, asthma, menorrhagia, wounds, microbial infections, skin diseases etc.,

The aim of the present study was to identify potential lead compounds of *B.variegata* leaf against various protein targets that are involved in inflammation using molecular docking approaches and subjecting the identified molecules for ADME analysis.

EXPERIMENTAL SECTION

All the computational analysis were carried out using Schrodinger suite version 9 (www.schrodinger.com). Image capturing was carried out using RasMol viewer version 2.7.5.2. (www.rasmol.com).

2.1. Plant material and extraction

*B.variegata*leaves were collected from Chennai and it was authenticated by Dr.P.Jayaraman, Director, Plant Anatomy Research Centre (authentication reference no. PARC/2010/670 dated 22/12/2010).

The leaves were washed with water, shade dried and powdered coarsely. Crude extract was obtained after maceration with 95% ethanol at room temperature for 72 hrs. and repeated till exhaustion of the material. Thereafter, the ethanol crude extract was distilled, evaporated and dried under reduced pressure to yield ethanol extract of *B.variegataleaves*,EBV(yield 8%).

2.2. Column Chromatography and GC-MS Analysis

The ethanol extract of *B.variegata*leaves was separated through silica gel G (60- 120) column chromatography with various solvent of increasing polarity (n-hexane, chloroform, ethyl acetate andmethanol) in gradient step and final elution was performed with 100% methanol. Based on the TLC profiles, the fractions were pooled and finally 11 fractions were obtained. Among 11 fractions, fraction 1 and 10 has shown better cytotoxicity against COLO 320 cells and hence, they were called as Active fraction I and Active fraction II.The active fraction I and II was subjected to GC-MS analysis to identify the bio constituents (unpublished data).

2.3. Ligand preparation

The chemical structures of all these molecules were drawn by using ChemSketch version 11.01 (http://www.acdlabs.com). All these ligands were prepared for docking by using LigPrep. The tautomers for each of these ligands were generated and optimized. Partial atomic charges were computed using the OPLS_2005 force field. The structures of all docked ligands were shown in the figure 1 and 2.

2.4. Protein structure

X-ray crystal structure and Solution NMR structure of the following proteins were retrieved from Protein Data Bank and the details of their resolution, PDB ID was as follows:

S.No.	Protein Name	PDB ID	Resolution
1	iNOS	4NOS	2.25 Å
2	COX-2	3LN1	2.40 Å

The three dimensional structure of both the proteins are shown in figure 3 and 4.

2.5. Protein Preparation

Protein preparation wizard of Schrodinger software suite version 9 was used. All the water molecules were removed from the original crystal structure before protein preparation process, to analyse the structure and the bond order assigned, hydrogen atoms were added and the geometry of all the hetero groups were corrected. Hydrogen bonds assignment tool was used to optimize the hydrogen bond network. Finally, Impref optimized the position of hydrogen bonds by keeping all the atoms in place. Energy minimization was carried out using default constraint of the 0.3 Å of RMSD and the OPLS_2005 force field.

2.6. Receptor grid preparation

Grids were generated by Receptor Grid Generation panel which defines receptor structure by excluding any other co-crystallized ligand that may be present, settle on the position and size of the active site was represented by receptor grids. Grid generation was performed using OPLS_2005.

2.7. Docking

Virtual screening is the easiest method to identify and rank the potential drug candidates from a database of compounds. Docking studies were performed using Glide (Grid-based Ligand Docking with Energetics) Extra Precision (XP) 5.7 algorithm in Schrodinger Software Suite, 2011 (Schrodinger, Portland, USA). Glide includes ligand-protein interaction energies, hydro-phobic interactions, hydrogen bonds, internal energy,pepstacking interactions and root mean square deviation (RMSD)and desolvation. Finally, the best pose of the particular ligand wasselected based on the Glide score.

2.8. ADME screening

ADME properties were calculated using molsoft(www.molsoft.com).Molsoftpredicts two properties, physically significant and pharmaceutically relevant descriptors. Molsoftprogram will predict the properties of the molecules, with a detailed analysis of principal descriptors and physiochemical properties. It also evaluates the acceptability of the analogues based on Lipinski's rule of 5 (Lipinski *et al*., 1997), which are essential for drug-like pharmacokinetic profile while using rational drug design (Tamilvanan and Hopper, 2013).

RESULTS AND DISCUSSION

3.1. Molecular Docking

The GC-MS analysis of active fraction I and active fraction II identified various bioactive constituents. Active fraction I found to contain 16 compounds and active fraction II contains 17 compounds. The 3D structure of COX-2 and iNOS, were downloaded from the PDB database. All the ligands were docked with these 2 protein targets using Schrödinger software suite version 9 (Glide XP mode).

3.1.1. Docking with COX-2 protein

The docking score (glide score) and docking energy of both active fraction I and II ligands with COX-2 protein has been listed in table 1 and 2. Out of 16 compounds of fraction I, only 15 could dock with COX-2 enzyme. Among 15 compounds of active fraction I, Phenolwas found to have the least docking score. The docking score ranges from – 2.828157to 3.040203. Its docked conformation was shown in figure 5. All the 17 compounds of fraction II could dock with COX-2 protein. Out of 17 compounds in active fraction II, Benzofuranonewas docked with least glide score. Its docked conformation was shown in figure 6.

Binding mode of Phenol (Fraction I Ligand) with COX- 2 Protein

Docking results showed that the ligand Phenol (Fraction I Ligand) with COX- 2 protein occupied the protein binding site with a glide score of – 2.828157 and the glide energy was -14.921756 Kcal/mol. No H bond interaction was observed, but five hydrophobic interactions with Pro 113, Pro 114, Trp 125, Ala 127 and Phe 128 were identified (figure 5). Two polar interactions were observed with amino acid residues Thr 115 and Ser 124.One positive charge interaction was observed with lys 123 residue.

Binding mode of Benzofuranone (Fraction II Ligand) with COX- 2 Protein

Docking results showed that the ligand Benzofuranone (Fraction II Ligand) with COX- 2 protein occupied the protein binding site with a glide score of -3.147224 and the glide energy was -24.739633 Kcal/mol. One hydrogen bond interaction was identified with the backbone amino acid residue Gln 358. Three hydrophobic interactions were observed with amino acid residue Tyr 108, Ileu 110 and Phe 357(figure 6). Three polar interactions with amino acid residues Ser 112, Ser 107 and Gln 356 were observed. One positive charged interactions and one negative charged interaction were observed with amino acid residues Lys 518 and Asp 111 respectively in the ligand binding site.

3.1.2. Docking with iNOS protein

When docked with iNOS, the 16 compounds of active fraction I showed a range of glide score from -9.359683 to -1.949758. Dioctyl phthalate was the lead compound with least glide score of about -9.359683 (table 3). The corresponding glide energy was found to be -48.046527 Kcal/mol. The docked conformation was shown in figure 7. 17 compounds of active fraction II gave a glide score range of about -7.056089 to -0.971996 (table 4). Benzene dicarboxylic acid mono (2-ethyl hexyl) ester got the least glide score and thus might act as the lead compound. A snapshot of docked conformation was documented in figure 8.

Binding mode of Dioctyl Phthalate (Fraction I Ligand) with iNOS Protein

Docking results showed that the ligand Dioctyl Phthalate (Fraction I Ligand) with iNOS protein occupied the protein binding site with a glide score -9.359683 and the glide energy was -48.046527 Kcal/mol. No hydrogen bond interactions were identified.Phe 369 and Trp 194 were involved in the π-π stacking interaction with the ligand. 21 hydrophobic interactions with the amino acid residues Leu 209, Phe 369, Trp 194, Ala 243, Ile 244, Pro 198, Phe 488, Leu 125, Tyr 491, Tyr 450, Ala 197, Val 353, Tyr 489, Met 355, Cys 200, Ile 204, Ala 439, Met 374, Ile 201, Met 434 and Trp 372 were observed. Gln 205, Ser 442, Ser 242 and Asn 370 formed polar interactions and Arg 199 formed the positive charge interactions with the ligand (figure 7).

Binding mode of Benzenedicarboxylic acid mono (2-ethyl hexyl) ester(Fraction II Ligand) with iNOS Protein

Docking results showed that the ligand Benzenedicarboxylic acid mono (2-ethyl hexyl) ester (Fraction II Ligand) with iNOS protein occupied the protein binding site with a glide score of -7.056089 and the glide energy was -41.006085 Kcal/mol. Two Hydrogen bond interactions were identified with the backbone amino acid residue Trp 372 and side chain residue Glu 377(figure 8). 14 hydrophobic interactions were observed with amino acid residues Tyr 373, Cys 200, Ala 243, Leu 209, Ile 244, Tyr 489, Trp 194, Phe 369, Pro 350, Val 352, Met 434, Ala 439, Ile 201 and Met 374. Ser 242, Asn 370 and Gln 205 were involved in polar interactions with the ligand.

3.2. Predicted ADME properties

Physically significant descriptors and pharmaceutically relevant properties of all the lead compounds of both the fractions were analysed using molsoft prediction tool. Molecular weight, log P Octanol/water partition coefficient, H-bond donors, H-bond acceptors, Mol Log S and their positions according to Lipinski's rule of five were presented in table 5 and 6. Almost all the compounds were in the acceptable range of Lipinski's rule of five, indicating their potential for use as drug-like molecules (Lipinski *et al*., 2001)

DISCUSSION

The present *insilico* molecular docking study was done to identify the lead compounds present in both the fractions which could dock with iNOS and COX 2. For the study, two protein targets were selected and their 3D structures were downloaded from PDB database. Docking was done by using Schrodinger software suite version 9.

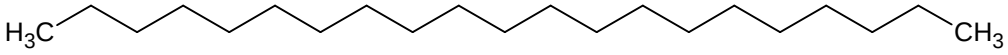
When a ligand binds with a protein, it can either activate or inhibit the protein. In the present study, the docking studies led to the identification of lead molecules which might play an important role in the activation or inhibition of the protein involved.

Anti-inflammatory proteins like COX-2 and iNOS might be inhibited or inactivated upon binding with the lead ligands of both the active fraction I and II. Various authors have reported many inhibitory ligands (from natural origin) for COX-2 protein using molecular docking studies [23, 24]. ArumugamMadeswaran[25] has documented the docking studies of iNOS using quercetin and its derivatives as ligands. In all these previous findings, the proteins COX-2 and iNOS were inhibited by the binding of the ligands. These findings were in agreement with the present study.

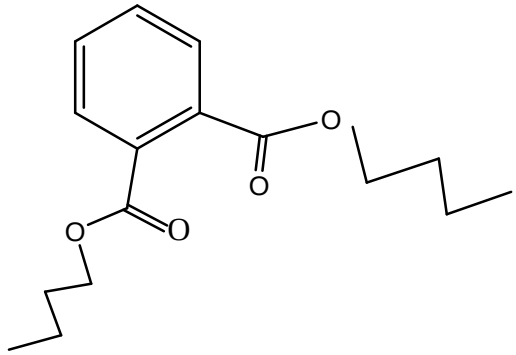
All the 16 ligands of fraction I and 17 ligands of fraction II have been checked for drug likeliness (ADME) property using Lipinski RO5. Almost all the ligands were in the acceptable range of Lipinski's rule of five, indicating their potential for use as drug-like molecules.

Figure1. Chemical Structures of Fraction I ligands

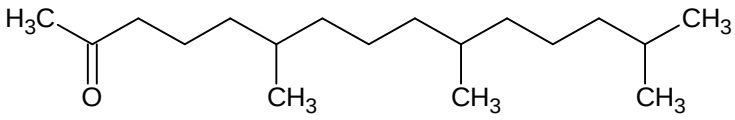
1. Heneicosane



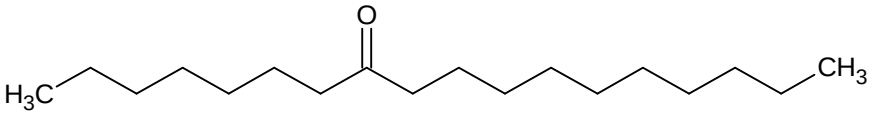
2. 1,2-Benzene Dicarboxylic acid Dibutyl ester



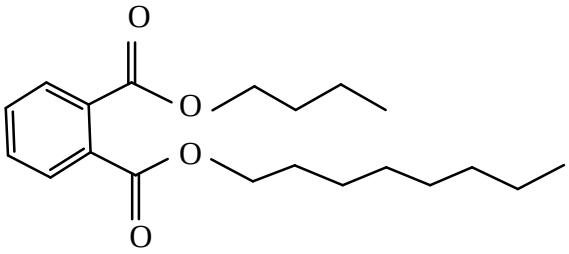
3. 2-Pentadecanone



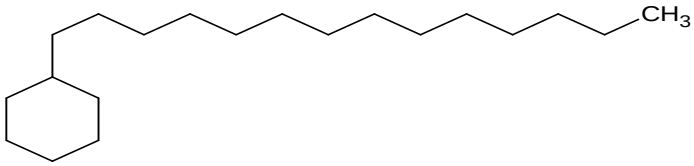
4. 8-Octadecanone



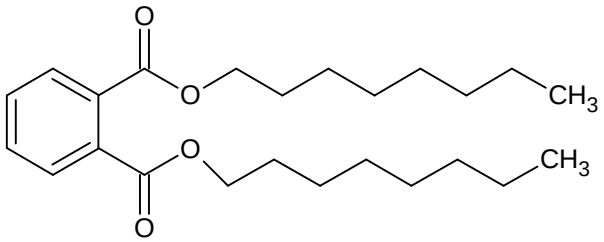
5. Butyloctylphthalate



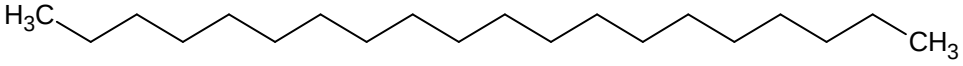
6. Cyclohexane



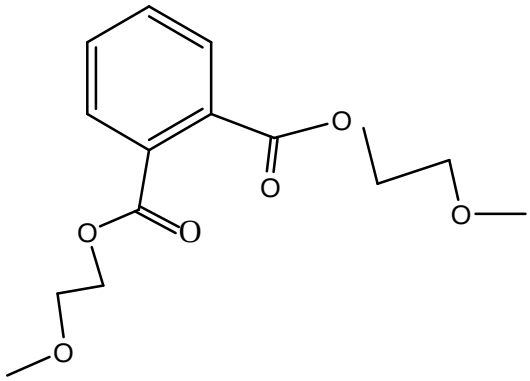
7. Dioctyl Phthalate



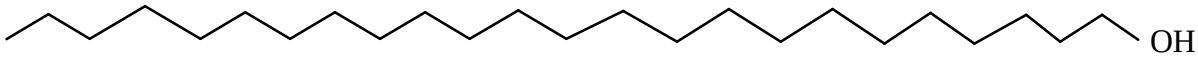
8. Eicosane



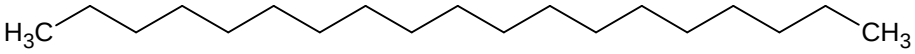
9. Dibutyl phthalate(dimethyl)



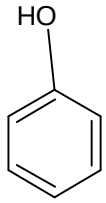
10. 1-Heptacosanol



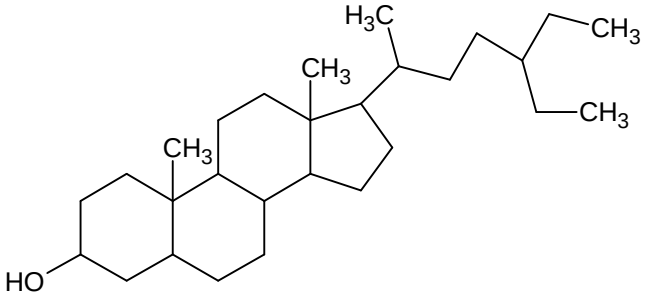
11. Nonadecene



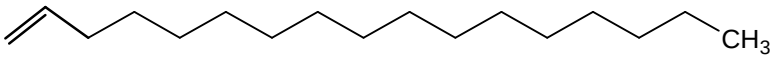
12. Phenol



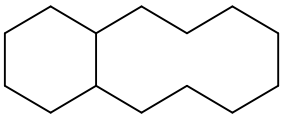
13. Stigmast-5-en-3-ol



14. 1-Heptadecene



15. Tetrahydrobenzo(A)cyclodecene



16. Stigmast-4-en-3-one

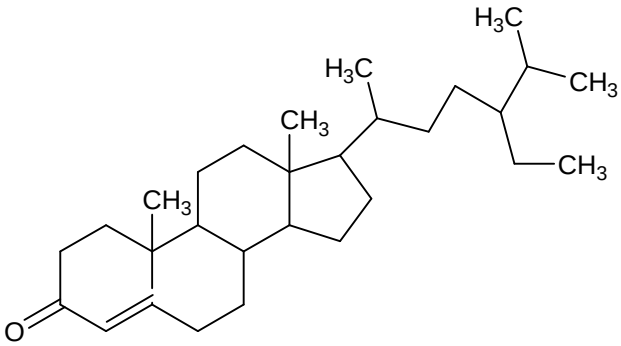
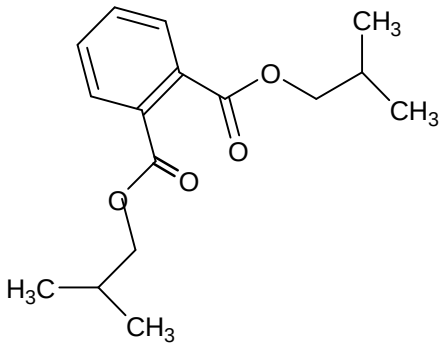
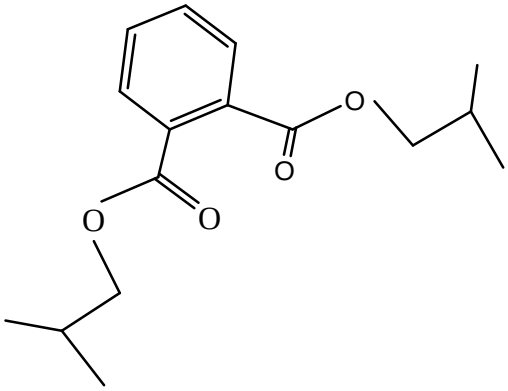


Figure 2.Chemical Structures of Fraction II Ligands

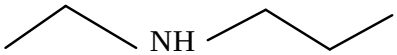
1. Benzenedicarboxylic acid (dimethyl)



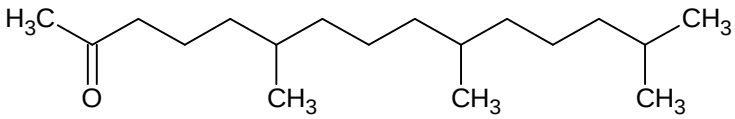
2. Benzenedicarboxylic acid



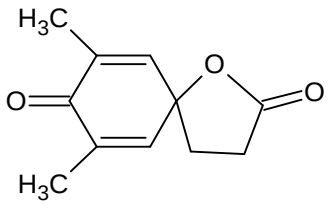
3. 1-Propanamine



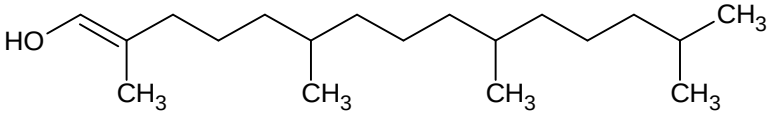
4. 2-Pentadecanone



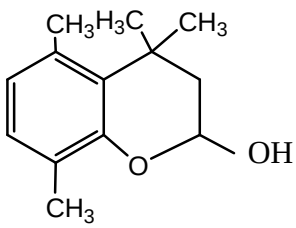
5. 2,8- Dione



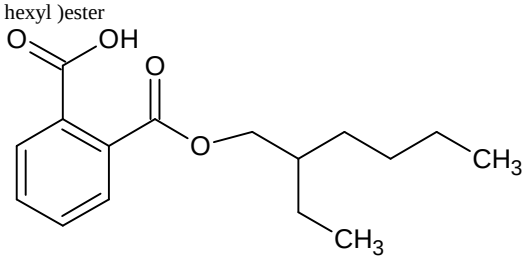
6. 2-Hexadecen-1-ol



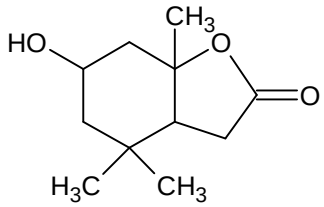
7. 4,4,5,8-Tetramethyl Chroman-2-ol



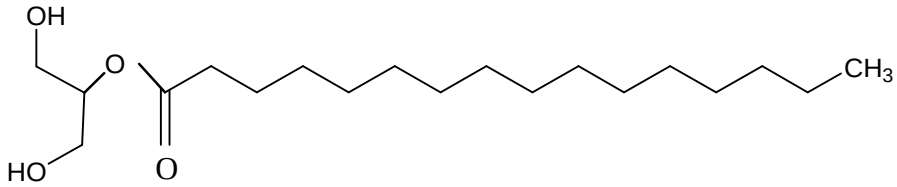
8. Benzene dicarboxylic mono (2-ethyl hexyl)ester



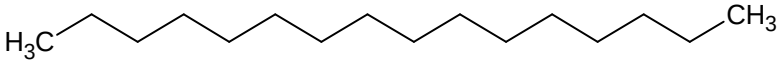
9. Benzofuranone



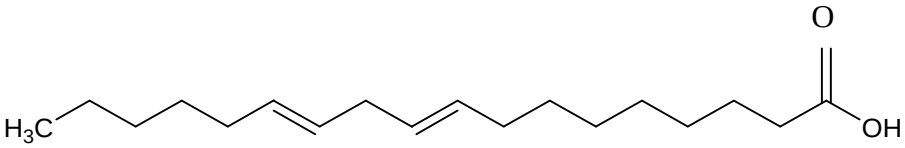
10. Hexadecanoic acid



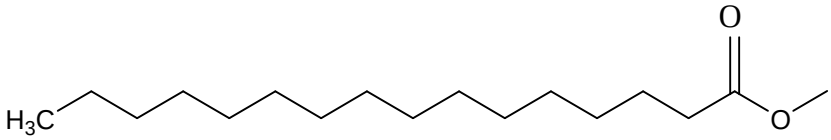
11. Hexadecene



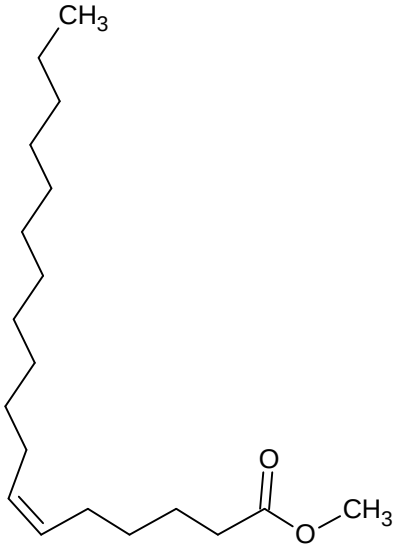
12. Octadecanoic acid, methyl ester



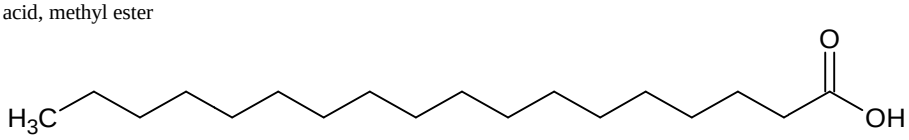
13. Methyl palmitate



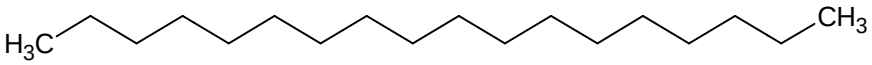
14. Methylpetroselate



15. Octadecanoic acid, methyl ester



16. Octadecene



17. Stigmast-5-en-ol

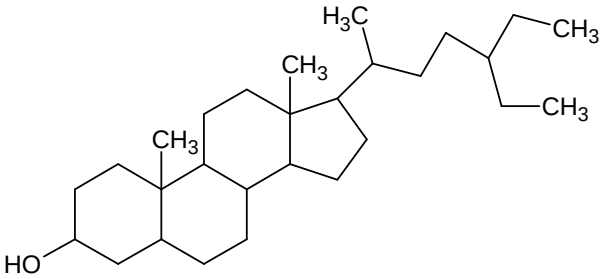


Figure 3. 3D Structure of COX-2 (PDB ID: 3LN1)

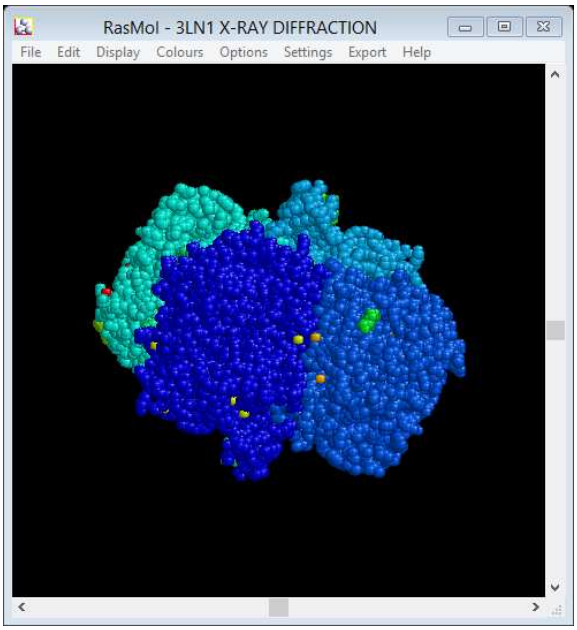


Figure 4. 3D Structure of iNOS (PDB ID: 4NOS)

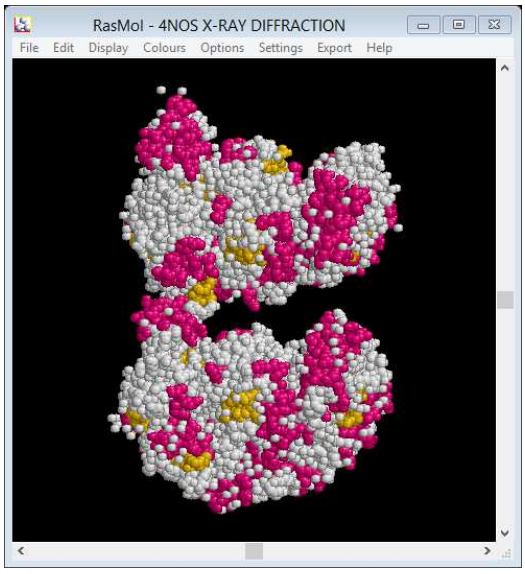


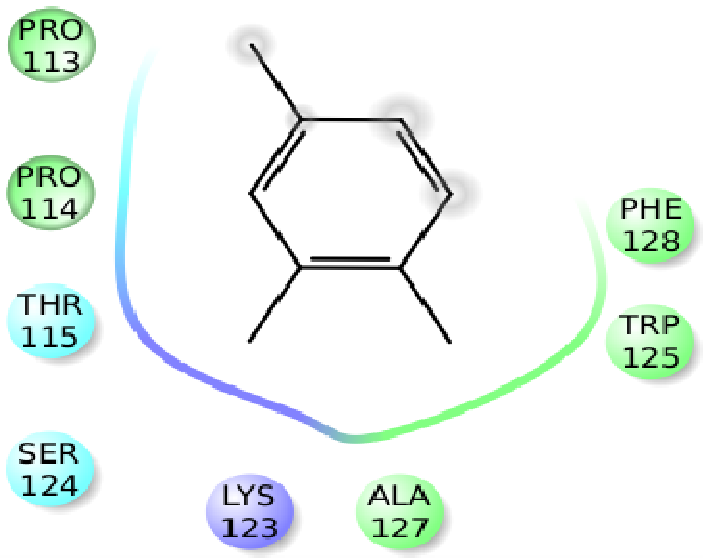
Table 1. GLIDE XP Docking score of Fraction I ligands with COX- 2

	Ligand Name	glide gscore	glide energy Kcal/mol
1	Phenol	-2.828157	-14.921756
2	1,2- Benzene Dicarboxylic Acid Dibutyl Ester	-2.634257	-26.262822
3	Stigmast-5-en-3β-ol	-2.439097	-26.632898
4	1-Heptacosanol	-2.09476	-30.067786
5	Butyloctyl Phthalate	-2.029204	-29.648608
6	Stigmast-4-en-3-one	-1.847274	-23.807287
7	2-Pentadecanone	-1.652009	-26.632581
8	1,2-Benzenedicarboxylic Acid,Bis(2-Methoxyethyl)Ester	-1.378248	-25.303502
9	Dioctylphthalate	-1.069724	-33.340573
10	Cyclohexane	-0.741217	-26.579172
11	Nonadecene	-0.334454	-21.950349
12	Heneicosane	0.388586	-24.589758
13	1-Heptadecene	0.837367	-25.532945
14	Eicosane	1.43599	-23.835739
15	8-Octadecanone	3.040203	-24.571029

Table 2. GLIDE XP Docking score of Fraction II ligands with COX- 2

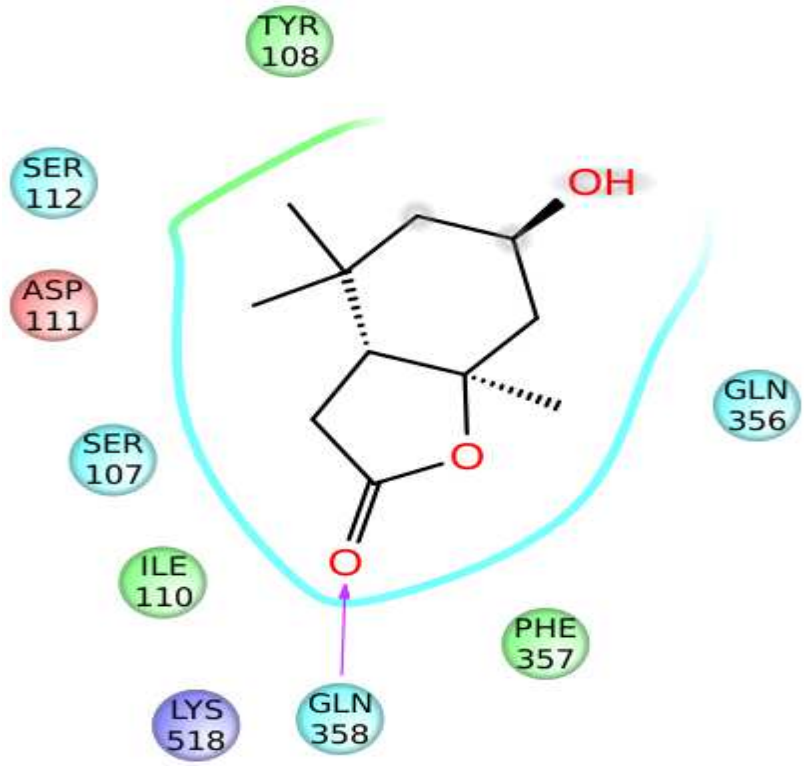
S.No	Ligand Name	glide gscore	glide energy Kcal/mol
1	Benzofuranone	-3.147224	-24.739633
2	2,8, Dione	-2.830344	-23.700961
3	1,2- Benzenedicarboxylicacid,Bis(2-Methylpropyl)Ester	-2.754819	-23.641574
4	Stigmast-5-en-3β-ol	-2.439097	-26.632898
5	Benzenedicarboxylic Acid Mono (2 Ethylhexyl)Ester	-2.067307	-25.749254
6	1,2-Benzenedicarboxylic Acid	-1.741915	-23.807245
7	Methylpetroselate	-1.284036	-28.435498
8	1-Propanamine	-1.051498	-10.81697
9	Methyloctadeca 9,12-Dienoate	0.21421	-21.378733
10	Hexadecanoic acid	0.474492	-27.391194
11	Octadecanoic acid, Methyl Ester	0.535084	-29.019892
12	4,4,5,8-Tetramethyl Chroman-2-ol	0.720601	-15.005137
13	Hexadecene	0.783713	-24.608681
14	Pentadecanone	1.388705	-18.930989
15	2-Hexadecen-1-ol	1.41499	-31.789803
16	Methylpalmitate	1.931463	-22.602188
17	Octadecene	2.353716	-22.256023

Figure 5. Docked conformation of Phenol (Fraction I Ligand) with COX-2 protein



Glide Score	**-2.828157**
Glide Energy (Kcal/mol)	**-14.921756**

Figure 6. Docked conformation of Benzofuranone(Fraction II Ligand) with COX-2 protein



Glide Score	**-3.147224**
Glide Energy Kcal/mol)	**-24.739633**

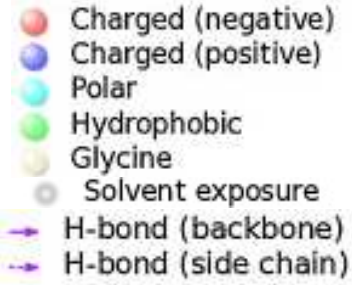
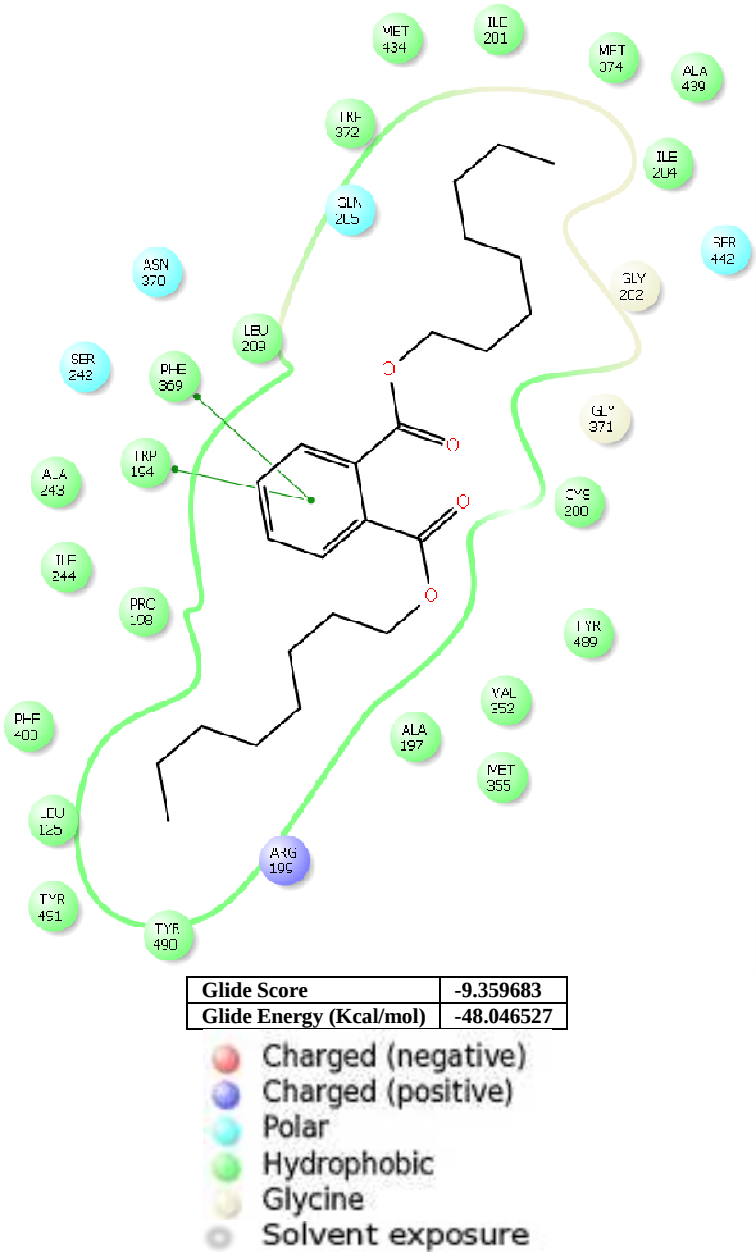


Table 3. GLIDE XP Docking score of Fraction I ligands with iNOS protein

S.No.	Ligand Name	glide gscore	glide energy Kcal/mol
1	Dioctylphthalate	-9.359683	-48.046527
2	Butyloctyl phthalate	-7.686333	-41.420846
3	1,2-Benzenedicarboxylic acid,bis(2-Methoxyethyl)Ester	-6.144439	-34.861536
4	Stigmast-4-en-3-one	-5.923689	-34.178628
5	1,2- Benzene dicarboxylicacid dibutylester	-5.316044	-33.795957
6	Heneicosane	-4.952599	-32.830178
7	2-Pentadecanone	-4.875764	-26.674431
8	Stigmast-5-en-3β-ol	-4.839684	-29.856107
9	8-Octadecanone	-4.36555	-33.690744
10	Cyclohexane	-4.329966	-29.893488
11	1-Heptadecene	-4.267861	-29.752862
12	Eicosane	-4.092293	-29.562972
13	Nonadecene	-3.92592	-29.435161
14	Phenol	-3.763636	-20.103475
15	1-Heptacosanol	-3.651125	-38.98107
16	Tetradecahydrobenzo(A) Cyclodecene	-1.949758	-20.335788

Figure 7. Docked conformation of Dioctylphthalate (Fraction I Ligand) with iNOS



Glide Score	-9.359683
Glide Energy (Kcal/mol)	-48.046527

Charged (negative)
Charged (positive)
Polar
Hydrophobic
Glycine
Solvent exposure

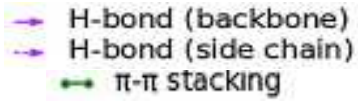
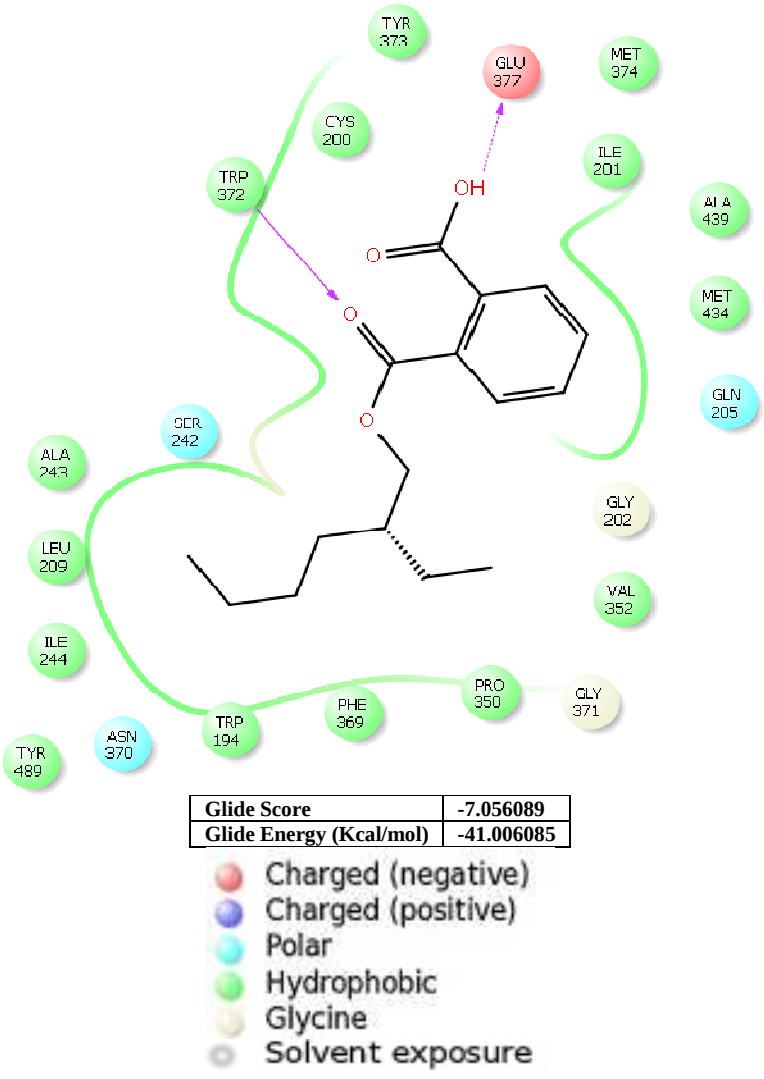


Table 4. GLIDE XP Docking score of Fraction II ligands with iNOS

S.No.	Ligand Name	glide gscore	glide energy Kcal/mol
1	Benzenedicarboxylic Acid Mono (2 Ethylhexyl)Ester	-7.056089	-41.006085
2	1,2-Benzenedicarboxylic Acid	-6.808813	-25.92854
3	2-Hexadecen-1-ol	-5.798607	-33.81167
4	4,4,5,8-Tetramethyl Chroman-2-ol	-5.738466	-23.214541
5	2,8, Dione	-5.401741	-27.809172
6	Methyloctadeca9,12-Dienoate	-5.348522	-35.065114
7	Octadecanoic Acid, Methyl Ester	-5.267897	-36.738881
8	Methylpetroselate	-5.247409	-33.420309
9	1,2- Benzenedicarboxylicacid,Bis(2-Methylpropyl)Ester	-4.943035	-32.766823
10	Hexadecanoic acid	-4.856299	-31.236112
11	Stigmast-5-en-3β-ol	-4.839684	-29.856107
12	Methylpalmitate	-4.737175	-32.661108
13	Octadecene	-4.619959	-28.264826
14	Pentadecanone	-4.416638	-28.339029
15	Hexadecene	-3.467976	-27.54903
16	Benzofuranone	-2.590511	-24.008735
17	1-Propanamine	-0.971996	-9.983476

Figure 8. Docked conformation of Benzenedicarboxylic acid mono (2-ethyl hexyl) ester (Fraction II Ligand) with iNOS



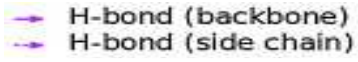


Table 5. Principal Descriptors calculated by Lipinski's rule of five

Lead Molecules	Molecular Weight[a]	Number of HBA[b]	Number of HBD[c]	Mol Log P[d]	Mol Log S[e]
1,2- BENZENE DICARBOXYLIC ACID DIBUTYL ESTER	310.21	4	3	4.49	-3.43
1,2-BENZENEDICARBOXYLIC ACID,BIS(2-METHOXYETHYL)ESTER	314.17	6	3	2.95	-2.24
1-HEPTACOSANOL	396.43	1	1	12.2	-10.57
1-HEPTADECENE	270.33	0	0	8.76	-7.89
2-PENTADECANONE	268.28	1	0	7.3	-4.93
8-OCTADECANONE	268.28	1	0	7.6	-6.28
BUTYLOCTYL PHTHALATE	350.25	4	1	7.14	-6.28
CYCLOHEXANE	280.31	0	0	9.26	-7.86
DIOCTYLPHTHALATE	390.28	4	0	8.59	-7.55
EICOSANE	282.33	0	0	10.47	-9.16
HENEICOSANE	296.34	0	0	10.47	-9.16
NONADECENE	268.31	0	0	9.5	-8.31
PHENOL	198.2	1	2	3.39	-3.7
STIGMAST-4-EN-3-ONE	444.43	1	0	9.51	-8.19
STIGMAST-5-EN-3β-OL	402.39	1	1	9.27	-7.18
TETRADECAHYDROBENZO(A) CYCLODECENE	194.2	1	1	4.99	-4.24

a. Molecular weight of the molecule (160 to 500)
b. Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (not more than 10).
c. Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (not more than 5).
d. Log P for octanol/water (−2.0 – 6.5).
e. Predicted aqueous solubility, log S. S in moldm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (−6.5 – 0.5).

Table 6. Principal Descriptors calculated by Lipinski's rule of five

Lead Molecules	Molecular Weight[a]	Number of HBA[b]	Number of HBD[c]	Mol Log P[d]	Mol Log S[e]
1,2- BENZENEDI CARBOXYLIC ACID, BIS(2-METHYLPROPYL)ESTER	294.18	4	1	3.29	-2.94
1,2-BENZENEDICARBOXYLIC ACID	294.18	4	1	3.29	-2.94
1-PROPANAMINE	103.14	1	2	1.34	-1.82
2,8, DIONE	252.17	3	0	1.15	-2.48
2-HEXADECEN-1-OL	282.29	1	1	8.54	-5.58
4,4,5,8-TETRAMETHYL CHROMAN-2-OL	254.22	2	4	5.64	-5.71
BENZENEDICARBOXYLIC ACID MONO (2 ETHYLHEXYL)ESTER	278.15	4	1	4.9	-3.9
BENZOFURANONE	198.13	3	1	0.99	-2.62
HEXADECANOIC ACID	378.37	4	6	8.24	-6.28
HEXADECENE	226.27	0	0	8.06	-7.05
METHYLOCTADECA9,12-DIENOATE	296.27	2	3	7.93	-5.92
METHYLPALMITATE	286.29	2	2	8.2	-6.43
METHYLPETROSELATE	296.27	2	0	7.5	-6.25
OCTADECANOIC ACID, METHYL EXTER	284.27	2	1	7.62	-6.51
OCTADECENE	254.3	0	0	9.02	-7.89
PENTADECANONE	242.26	1	2	7.74	-5.54
STIGMAST-5-EN-3β-OL	402.39	1	1	9.27	-7.18

a. Molecular weight of the molecule (160 to 500)
b. Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (not more than 10).
c. Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (not more than 5).
d. Log P for octanol/water (−2.0 – 6.5).
e. Predicted aqueous solubility, log S. S in moldm–3 is the concentration of the solute in a saturated solution that is in equilibrium with the crystalline solid (−6.5 – 0.5).

CONCLUSION

This study extends the understanding on the molecular mechanism underlying the diverse biological activities of the various phytochemicals that are present in active fractions (I and II) of EBV. Thus the study reveals the appreciable anti-inflammatory activity of *B.variegata*. Further separation and purification of individual ligands of the active fractions followed by various analysis might pave way for the identification of new anti-inflammatory drugs.

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