Exploring Cyclooxygenase-2 (COX-2) Inhibitors From Ascidian Derived Ligands For Cancer Treatment: An In-Silico Study.

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ABSTRACT
Cancer remains a significant global health challenge with increasing mortality rates expected in the coming years. Inflammation plays a crucial role in cancer progression, and cyclooxygenase-2 (COX-2) has been identified as a key target for cancer therapy. COX-2 is overexpressed in several cancers and contributes to cancer progression through inflammatory pathways. The therapeutic potential of selective COX-2 inhibitors, such as those derived from non-steroidal anti-inflammatory drugs (NSAIDs), has been widely recognized, although concerns about cardiovascular risks persist. In recent years, marine organisms, including ascidians, have emerged as promising sources of bioactive compounds with anti-inflammatory and anticancer properties. This study focuses on identifying potential COX-2 inhibitors from ascidian-derived molecules using in silico methods to discover the potential drug for the cancer treatment. Through molecular docking and virtual screening, 24 ascidian-derived ligands were evaluated for their interaction with the COX-2 enzyme. The results indicated that stigmasterol exhibited the highest binding affinity, followed by other compounds such as cyclohexanol and pentafluoropropionic acid. Notably, pentafluoropropionic acid, with two hydrogen bond interactions, demonstrated promising stability and could serve as a potential candidate for COX-2 inhibition. This finding highlights the therapeutic potential of marine-derived compounds for cancer management, and further experimental validation is necessary.

INTRODUCTION

Cancer encompasses a complex group of diseases characterized by the uncontrolled and rapid growth of cells, often leading to metastasis.[1,2] It is currently the second leading cause of death worldwide. The World Health Organization (WHO) reported 9.6 million cancer-related deaths in 2018,[3] and projections suggest this number could rise to 21.6 million by 2030.[4] In low- and middle-income countries, cancer represents a major health challenge, contributing to approximately 70% of total mortality rates. Furthermore, the emergence of drug resistance and the adverse effects associated with many current anticancer treatments underscore the pressing need for the development of new, effective, and selectively targeted anticancer agents. This highlights the therapeutic potential of COX-2 inhibitors in cancer management.

A strong link between cancer and inflammation is well-established. A substantial body of work describing this link has generated intense interest in targeting COX enzymes, particularly COX-2, for cancer therapy or chemoprevention. COX-2 is upregulated in 40% of breast cancers, with increases of up to 84% reported in some studies.[5] Clinical studies have noted a reduced risk for breast, lung, prostate, and colon cancers following treatment with non-steroidal anti-inflammatory drugs (NSAIDs), which non-selectively inhibit COX-1 and COX-2, or through selective inhibition of COX-2.[6] This connection involves both intrinsic and extrinsic inflammatory pathways, which contribute to a cancer-supportive microenvironment enriched with inflammatory mediators. These pathways often activate transcription factors directly or indirectly, promoting cancer progression.[7,8]

Inflammation is a vital immune response activated by various triggers, such as chemical agents, physical injuries, immune reactions, and pathogenic infections.[9,10] Non-steroidal anti-inflammatory drugs (NSAIDs) have long been utilized to treat inflammation-related conditions, including rheumatoid arthritis, fever, and everyday pain.[11] The introduction of aspirin in 1898 marked the beginning of NSAIDs in therapeutic applications, followed

by the development of other drugs like celecoxib, ibuprofen, and diclofenac. These medications exert their effects by inhibiting cyclooxygenases (COXs), enzymes that regulate the biosynthesis of prostaglandins (PGs), which are critical mediators of inflammation.[12,13]

COXs exist in two isoforms, COX-1 and COX-2, with distinct roles: COX-1 contributes to physiological functions such as gastrointestinal protection, while COX-2 primarily drives pathological inflammation.[14,15,16] Non-selective NSAIDs inhibit both COX isoforms, leading to effective anti-inflammatory action but also gastrointestinal damage due to the inhibition of protective COX-1-mediated prostaglandin synthesis.[17,18] Selective COX-2 inhibitors, by contrast, specifically target COX-2, thereby alleviating inflammation while preserving the protective effects of COX-1, significantly reducing gastrointestinal side effects.[19]

Recent research has expanded on the anti-inflammatory mechanisms of COX-2 inhibitors, demonstrating their ability to inhibit the NF-κB pathway. COX-2 inhibition reduces the production of reactive oxygen species (ROS), keeping NF-κB in an inactive state bound to its inhibitor, P-IkB. This suppression prevents the production of pro-inflammatory cytokines such as nitric oxide (NO), PGE2, IL-6, and TNF-α.[20,21] Despite their efficacy, COX-2 inhibitors are often associated with cardiovascular risks, including an increased likelihood of heart attack, stroke, and blood clots.[22,23] These limitations underscore the need to develop new selective COX-2 inhibitors that retain therapeutic efficacy while minimizing adverse effects.

The oceans host approximately 90% of the planet's living biomass, making the marine environment an extraordinary source of bioactive natural products. These compounds possess various pharmacological properties, largely attributed to their distinctive chemical and structural characteristics that are absent in terrestrial natural products.[24] Marine organisms have evolved both physiologically and biochemically to survive in their challenging environments. The secondary metabolites they produce, which consist of small molecules, exhibit significant pharmacological effects, such as immunomodulation, anti-inflammatory, antibacterial, and antiviral activities. These molecules often possess chemical properties like a relatively low octanol-water partition coefficient, rotatable bonds, and stereogenic centers, making them attractive candidates for drug discovery.[25] Compounds sourced from a variety of marine organisms, including sponges, mollusks, bryozoans, sea combs, algae, echinoderms, ascidians, and soft corals, are thought to hold potential as candidates for treating inflammatory diseases. Among these, ascidians are a rich source of numerous bioactive molecules that span a wide range of chemical categories. These compounds have potential health applications, including cytotoxic, antimitotic, antiviral, and antimicrobial effects. A series of substituted indole analogs, inspired by the structural motifs of the anti-inflammatory ascidian metabolites herdmanines C and D, were designed and tested for COX-1 and COX-2 inhibition. Among them, compound 5m exhibited balanced COX-1/COX-2 inhibition, suppressed pro-inflammatory mediators, reduced ROS levels, and inhibited NF-κB signalling in LPS-stimulated macrophages.[26]

The present study aims to identify potential inhibitors of the COX-2 gene from ascidian-derived ligand molecules using in silico method. Computational approaches, including molecular docking and virtual screening, were employed to analyze the interactions between the bioactive compounds and the COX-2 enzyme.

MATERIALS AND METHODS

LIGAND PREPARATION

Gas Chromatography-Mass Spectrometry (GC-MS) of crude extract of *Phallusia nigra* (Test), *Phallusia nigra* (Mantle body), *Microcosmus squamiger*, *Didemnum perlucidum* were carried out to identify its bioactive chemical constituents, which were then subsequently considered as ligands for in silico analysis. These identified constituents, known for their pharmacological potential, were subjected to computational docking studies to evaluate their interaction with the COX-2 enzyme. [Table 1-3]

PREDICTION OF LIGANDS ADMET PROPERTY

The ADMET properties (absorption, distribution, metabolism, excretion, and toxicity) of each substance were predicted using the SWISS-ADME prediction tool (http://www.swissadme.ch/).

LIGAND MOLECULE PREPARATION

Twenty four compounds derived from the selected ascidians were shortlisted based on the Lipinski rule of five from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). Some of these compounds lacked three-dimensional structures, so their two-dimensional structures were retrieved and converted into three-dimensional models using OpenBabel. After preparing the ligands, they were uploaded to docking software, which automatically performed necessary steps such as energy minimization and other preparatory processes before docking.

PROTEIN PREPARATION

TARGET PROTEINS

The 3D structure of the COX2 enzyme was retrieved from the Protein Data Bank (https://www.rcsb.org/). Prior to docking, the protein (receptor molecule) was subjected to several preparatory modifications, such as the removal of water molecules, the addition of missing atoms, and energy minimization along with chain B,C, and D using the Biovia Discovery Studio tool. The docking procedure was then carried out following the standard methodology recommended by leading researchers in the field.[27]

MOLECULAR DOCKING

Binding affinities of the target protein and the legand was determined using the Autodock vina with PyRx program. AutoDock Vina is a cutting-edge open-source software tool designed for drug discovery, molecular docking, and virtual screening. It offers enhanced performance through multi-core processing, increased accuracy, and a user-friendly interface. To evaluate the root-mean-square deviation (RMSD) of the ligand during docking, a control ligand was maintained for validation. The software predicts docking results with an RMSD threshold of 2.0 or lower from the experimental positions. Binding affinity results were selected based on the most negative values,[28] which indicate a more stable receptor-ligand complex. A more negative binding affinity relative to the control suggests stronger interactions, with more amino acids involved in hydrogen bonding, thus increasing the reliability of the results.[29-30] The docking results were visualized using Biovia Discovery Studio, which allowed for the clear demonstration of interactions between the receptors and ligands.

Table 1: List of Ligand molecules (Bioactive Compounds) derived from few selected ascidians.

S.No	Ligand Molecule	Molecular Weight	Molecular Formula	Class of Compound	Ascidian Species
1	Stigmasterol	412.69	C29H48O	Tetracyclic Triterpenes	*Microcosmus squamiger*
2	Cyclohexanol, 4-ethenyl-4-methyl-3-(1-methylethenyl)-, (1.alpha.,3.alpha.,4.beta.)-	180.29	C12H20O	Alcoholic compound	*Microcosmus squamiger*
3	Pentafluoropropionic acid, 10-undecenyl ester	316.31	C14H21F5O2	Ester	*Phallusia nigra* (Mantle Body) *Microcosmus squamiger*
4	N-acetylnorephedrine	193.24	C11H15NO2	Carboximidic acid	*Didemnur*
5	8,11,14-eicosatrienoic acid, (z,z,z)	306.48	C20H34O2	Polyunsaturated long chain fatty acids	*Microcosmus squamiger*
6	1,3-benzodioxol-2-one, hexahydro-, cis-	142.15	C7H10O3	Benzene derivative and heterocyclic compound	*Phallusia nigra* (Mantle Body)
7	3,4 - dimethylpentanol	116.2	C7H16O	Primary alcohol	*Phallusia nigra* (Test)
8	1-dodecanol	186.33	C12H26O	Fatty alcohol	*Microcosmus squamiger*
9	Z,z-3,11-octadecadien-1-ol acetate	370.57	C25H38O2	Acetate ester	*Microcosmus squamiger*
10	3-dodecen-1-ol	184.32	C12H24O	Fatty alcohol	*Didemnur*
11	Hexanoic acid	116.16	C6H12O2	Straight chain saturated fatty acid	*Microcosmus squamiger*
12	N-hexadecanoic acid	256.42	C16H32O2	Fatty acid	*Didemnum perlucidum*

13	2-amino-3-methyl-1-butanol	103.16	C5H13NO	Amino alcohol	*Microcosmus squamiger*
14	Cyclohexanone, 4-hydroxy	114.14	C6H10O2	Ketone compound	*Microcosmus squamiger*
15	Cyclopropane, pentyl	112.21	C8H16	Cycloalkane	*Phallusia nigra* (Test)
16	Z-10-pentadecen-1-ol	226.4	C15H30O	Straight chain alkane	*Microcosmus squamiger*
17	Carbamimidoylsulfanylacetic acid	134.16	C3H6N2O2S	Carbamimidoyl-acetic acid	*Phallusia nigra* (Test)
18	Z,z-3,11-octadecadien-1-ol acetate	184.23	C10H16O3	Polyunsaturated long-chain fatty acid	*Microcosmus squamiger*
19	2- octadecadecen-1-ol	268.48	C18H36O	Fatty alcohol	*Microcosmus squamiger*
20	Formic acid, hexyl ester	130.18	C7H14O2	Carboxylic acid group	*Phallusia nigra* (Test)
21	Oxirane, (fluoromethyl)	76.07	C3H5FO	Halogenated heterocyclic compound	*Phallusia nigra* (Mantle Body)
22	Cyclopentaneundecanoic acid, methyl ester	268.43	C17H32O2	Fatty acid methyl esters	*Didemnu*
23	Propanenitrile, 3-(hexyloxy)	155.24	C9H17NO	Simple aliphatic nitrile.	*Phallusia nigra* (Mantle Body)
24	Methyl 10,12-pentacosadiynoate	388.63	C26H44O2	Carboxylic acid	*Microcosmus squamiger*

RESULTS AND DISCUSSION:

This study examined 24 ligands derived from secondary metabolites of ascidians through molecular docking, focusing on their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) characteristics and adherence to the Lipinski rule of five, a widely recognized criterion in drug development. The ADMET properties of the ligand molecules were calculated and are summarized in Table 1. The physicochemical properties of the ligand molecules are detailed in Tables 2 and 3.

The binding affinity scores of the ligand molecules were generally satisfactory. Notably, stigmasterol exhibited the highest binding affinity against the COX-2 enzyme, with a score of -8.3 kcal/mol, demonstrating a hydrogen bond interaction with GLU A: 524. This was followed by cyclohexanol, which had a binding affinity score of -5.7 kcal/mol. Pentafluoropropionic acid showed a binding affinity of -5.6 kcal/mol, with two hydrogen bond interactions involving ARG A: 120 and LYS A: 83. Additionally, N-acetyl norephedrine had a binding affinity score of -5.5 kcal/mol, interacting via a hydrogen bond with ARG A: 456. Furthermore, 8,11,14-eicosatrienoic acid (Z,Z,Z) displayed a binding affinity score of -5.0 kcal/mol, interacting with HIS A: 214 through a hydrogen bond. When compared with standard drugs such as celecoxib and rofecoxib, stigmasterol exhibited a similar binding affinity to celecoxib, which formed three hydrogen bonds with residues ASN A: 382, THR A: 212, and ASN A: 222. The only difference in interactions suggests that structural modifications could enhance its potential as a COX-2 inhibitor. Similarly, rofecoxib demonstrated a binding affinity score of -6.5 kcal/mol, with two hydrogen bond interactions involving ARG A: 456 and LYS A: 459, closely aligning with the interactions observed for pentafluoropropionic acid. All ligands demonstrated significant binding affinities, with pentafluoropropionic acid showing strong interactions due to its two hydrogen bonds, indicating stability. This ligand, identified in the GC-MS analysis of selected ascidians, may serve as a promising candidate for COX-2

inhibition. However, further wet lab tests are necessary to validate these interactions and confirm their biological activity through molecular changes.

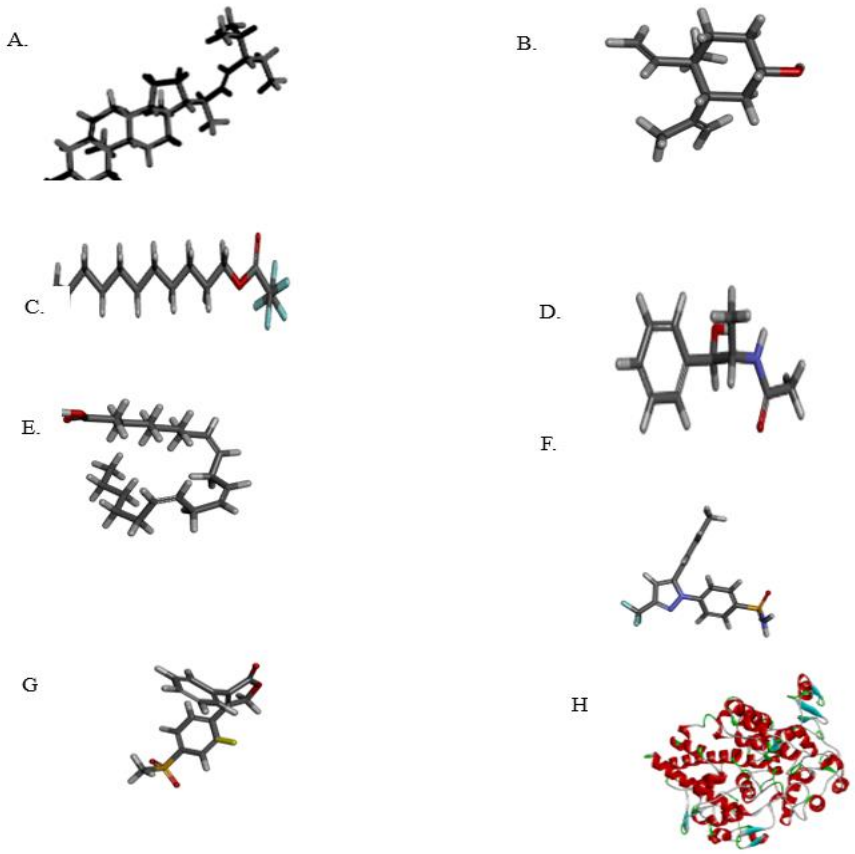
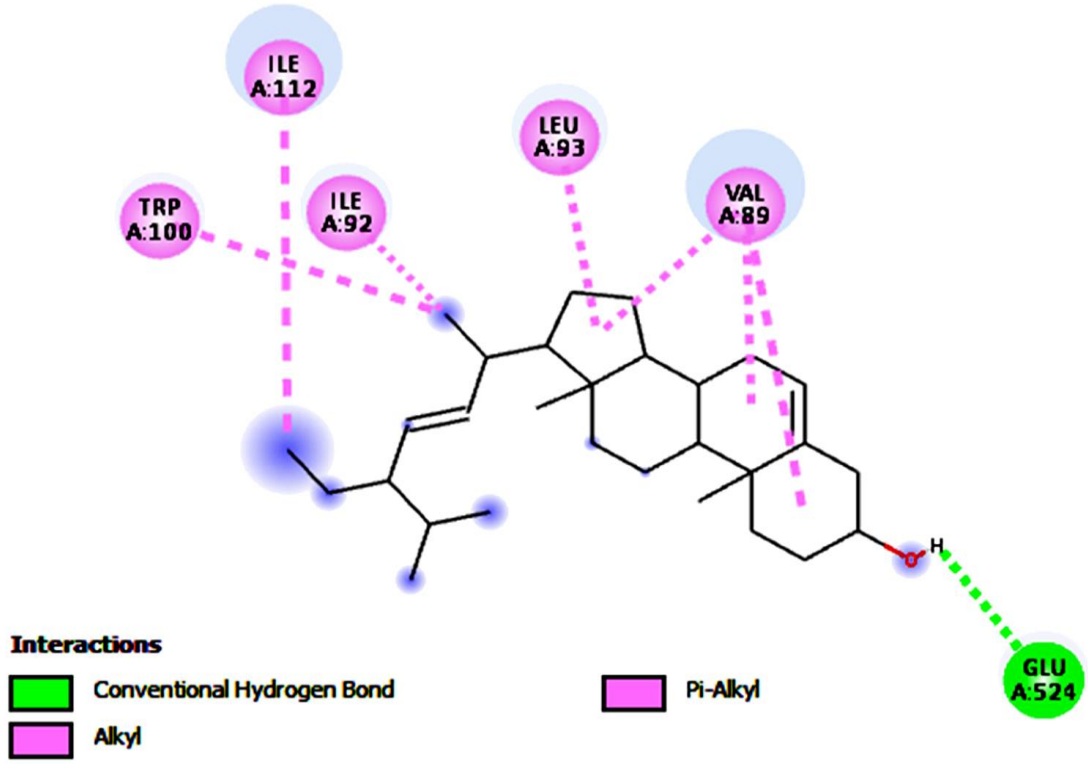
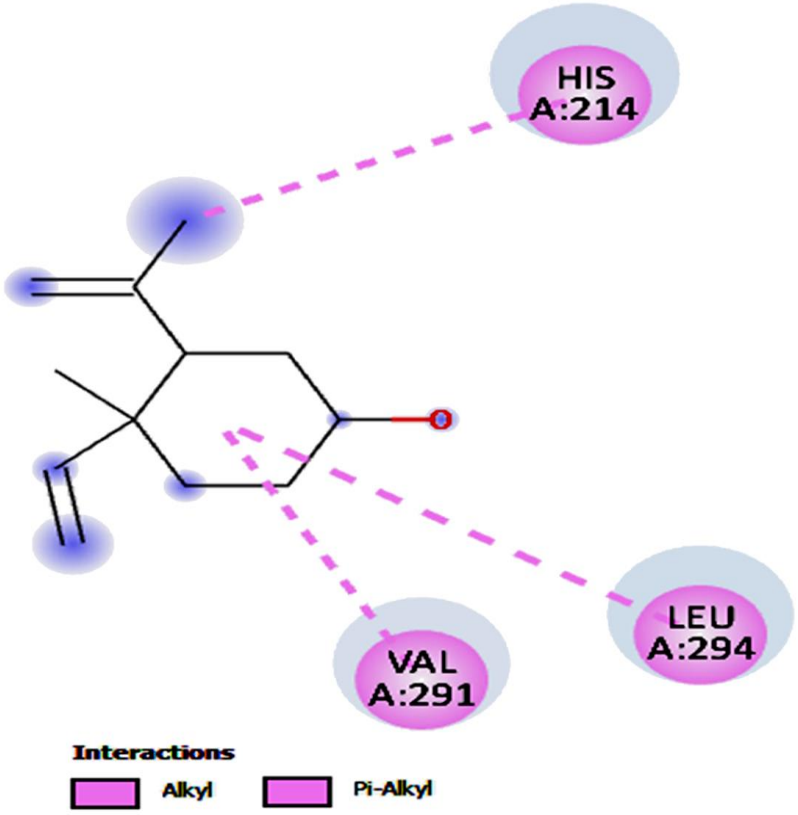


Figure: 1 A. Stigmasterol; B. Cyclohexanol; C. Pentafluoropropionic acid; D. N-Acetyl norephedrine; E. 8,11,14-Eicosatrienoic acid (Z,Z,Z); F. Celecoxib; G. Rofecoxib; H. COX 2 Enzyme.

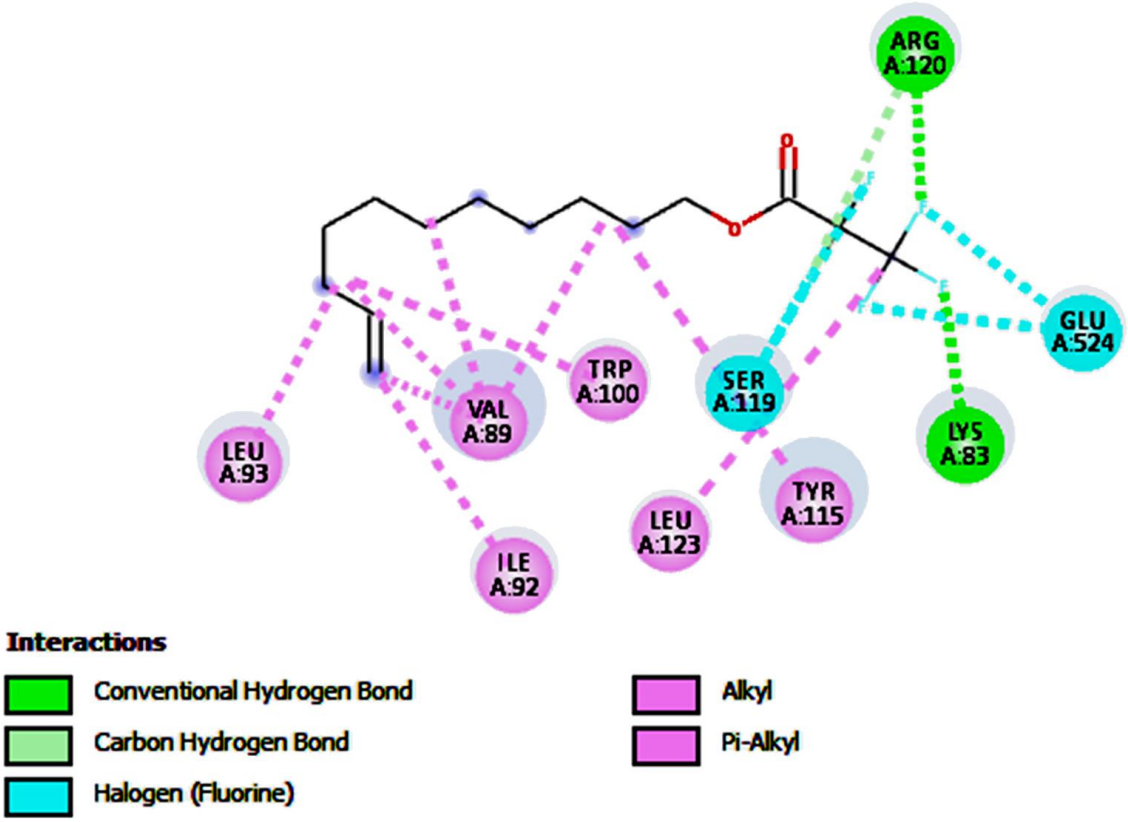
Stigmasterol against COX 2 Enzyme with binding affinity -8.3 kcal/mol



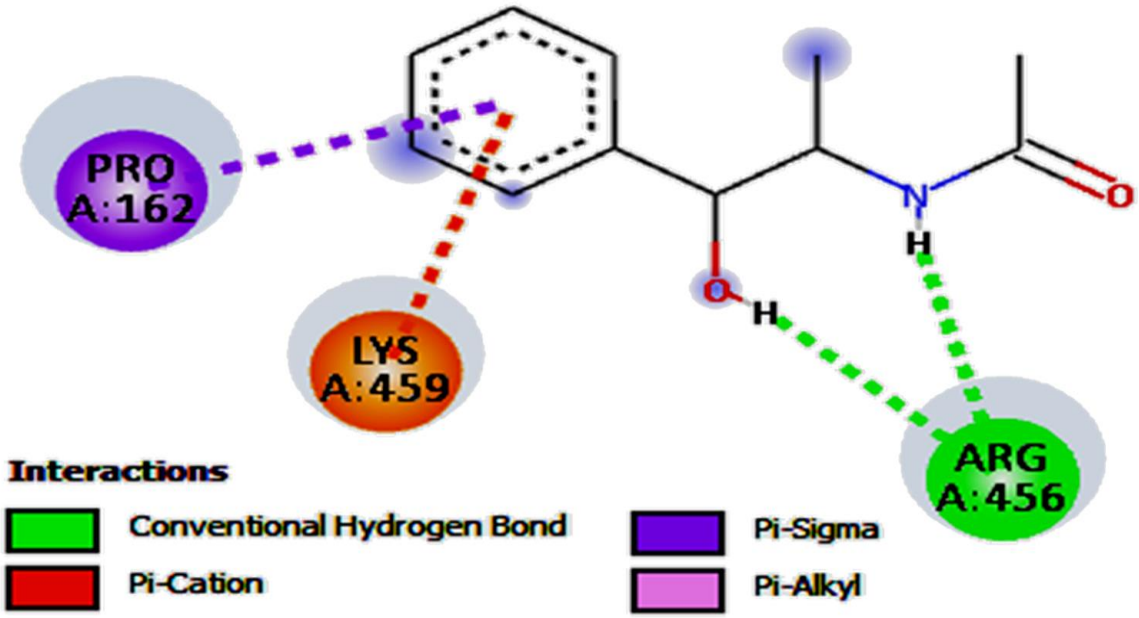
Cyclohexanol against COX 2 Enzyme with binding affinity -5.7 kcal/mol



Pentafluoropropionic acid against COX 2 Enzyme with binding affinity -5.6 kcal/mol



N- Acetylnorephedrine against COX 2 Enzyme with binding affinity -5.5 kcal/mol



8, 11, 14- Eicosatrienoic against COX 2 Enzyme with binding affinity -5.5 kcal/mol

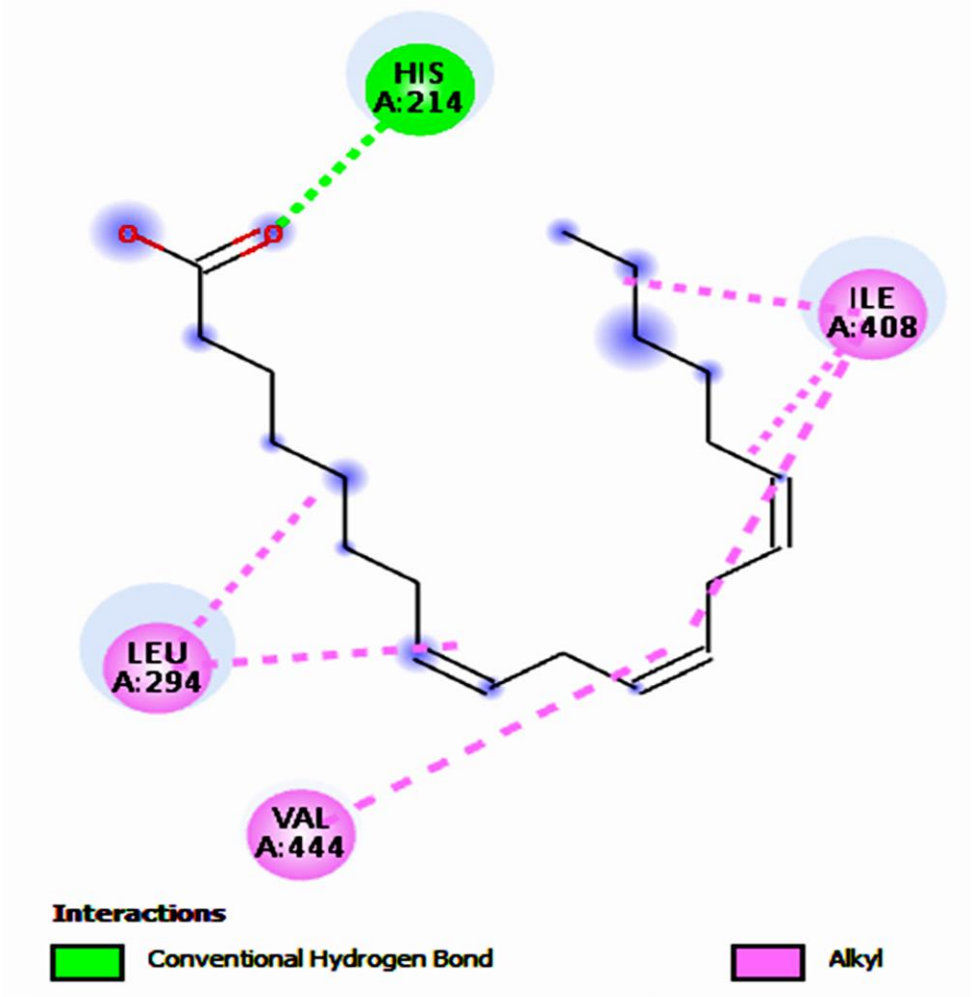
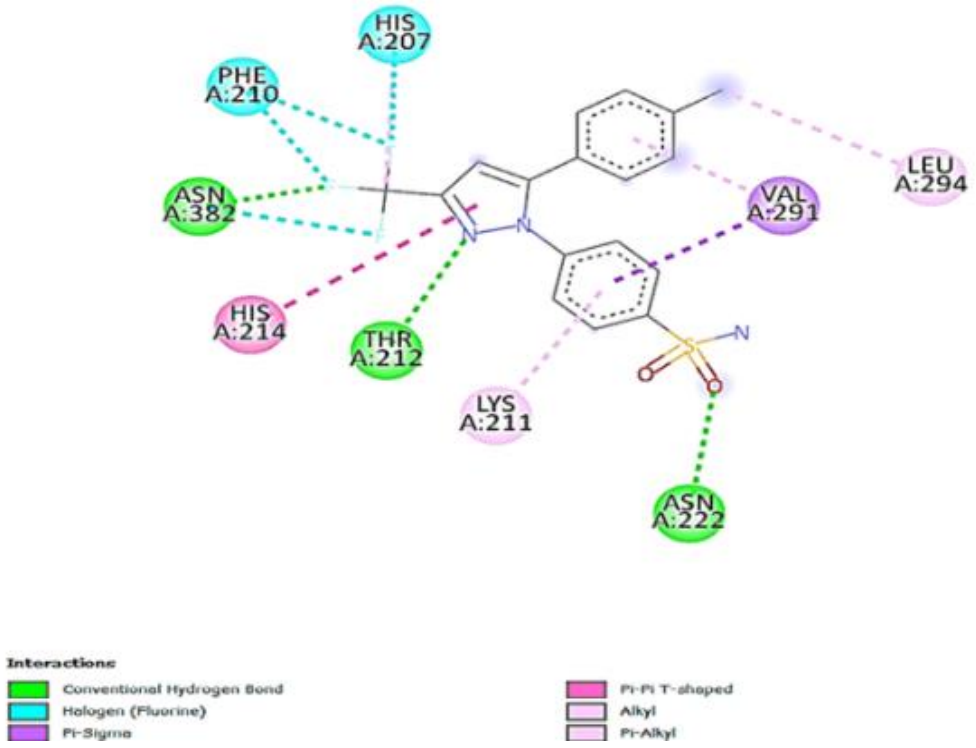


Figure: 3 Two dimensional diagram of Predicted interaction among ascidians derived ligand molecules and COX 2enzyme.
Celecoxib (Standard drug) against COX 2 Enzyme with binding affinity -8.3 kcal/mol



Rofecoxib (Standard drug) against COX 2 Enzyme with binding affinity -6.5 kcal/mol

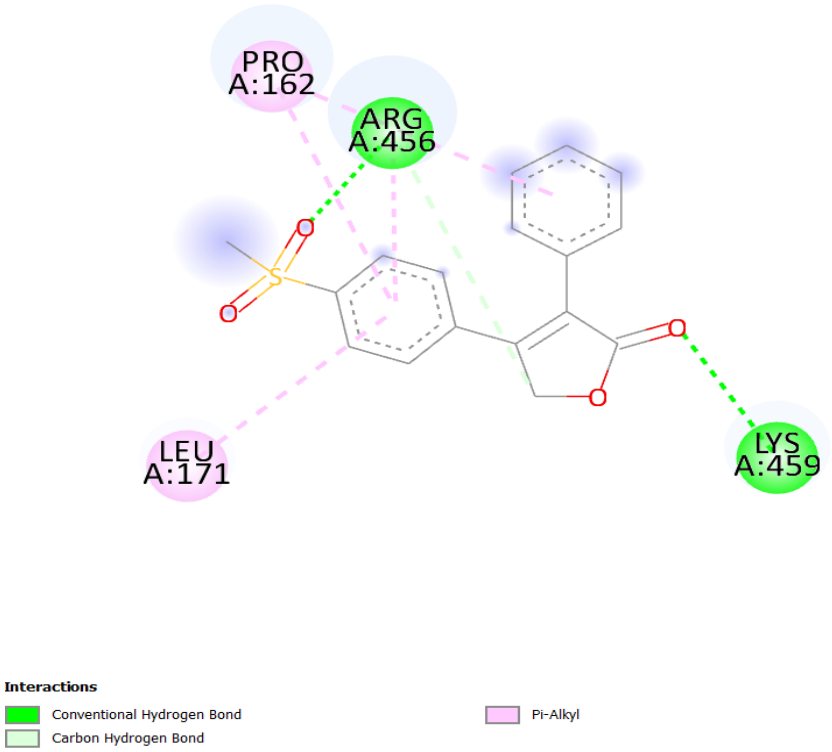


Figure: 2 Two dimensional diagram of Predicted interaction among ascidians derived ligand molecules and COX 2enzyme.

Table 2: Physicochemical Properties, Lipophilicity, Water solubility, Bioavailability & Medicinal chemistry of ligand molecules.

S. No	Name of the Molecule	H-bond acceptors	H-bond donors	Molar Refractivity	iLOGP	ESOL Class	Bioavailability Score	Synthetic Accessibility
1	STIGMASTEROL	1	1	132.75	5.01	Poorly soluble	0.55	6.21
2	CYCLOHEXANOL, 4-ETHENYL-4-METHYL-3-(1-METHYLETHENYL)-, (1.ALPHA.,3.ALPHA.,4.BETA.)-	1	1	57.64	2.55	Soluble	0.55	3.33
3	PENTAFLUOROPROPIONIC ACID, 10-UNDECENYL ESTER	7	0	70.55	3.77	Moderately soluble	0.55	2.64
4	N-ACETYLNOREPHEDRINE	2	2	54.8	1.63	Very soluble	0.55	1.95
5	8,11,14-EICOSATRIENOIC ACID, (Z,Z,Z)	2	1	98.6	4.03	Moderately soluble	0.85	3.25
6	1,3-BENZODIOXOL-2-ONE, HEXAHYDRO-, CIS-	3	0	34.29	1.7	Very soluble	0.55	2.92
7	3,4 - DIMETHYLPENTANOL	1	1	36.92	2.09	Very soluble	0.55	1.26
8	1-DODECANOL	1	1	60.96	3.37	Soluble	0.55	1.85
9	Z,Z-3,11-OCTADECADIEN-1-OL ACETATE	2	0	118.27	5.84	Poorly soluble	0.55	3.43
10	3-DODECEN-1-OL	1	1	60.49	3.39	Soluble	0.55	2.85
11	HEXANOIC ACID	2	1	32.73	1.57	Very soluble	0.85	1.17
12	N-HEXADECANOIC ACID	2	1	80.8	3.85	Moderately soluble	0.85	2.31
13	2-AMINO-3-METHYL-1-BUTANOL	2	2	30.02	1.54	Very soluble	0.55	1
14	CYCLOHEXANONE, 4-HYDROXY	2	1	30.2	1.19	Very soluble	0.55	1.37
15	CYCLOPROPANE, PENTYL	0	0	38.46	2.67	Soluble	0.55	1.61
16	Z-10-PENTADECEN-1-OL	1	1	74.91	3.97	Moderately soluble	0.55	2.92
17	CARBAMIMIDOYLSULFANYLACETIC ACID	3	3	32.2	0.17	Very soluble	0.55	2.68
18	Z,Z-3,11-OCTADECADIEN-1-OL ACETATE	3	1	51.68	2.63	Very soluble	0.55	3.81

19	2- OCTADECADECEN-1-OL	1	1	89.33	4.62	Moderately soluble	0.55	3.35
20	FORMIC ACID, HEXYL ESTER	2	0	37.44	2.26	Very soluble	0.55	1.23
21	OXIRANE, (FLUOROMETHYL)	2	0	15.56	1.24	Very soluble	0.55	1.08
22	CYCLOPENTANEUNDECANOIC ACID, METHYL ESTER	2	0	83	4.41	Moderately soluble	0.55	2.54
23	PROPANENITRILE, 3-(HEXYLOXY)	2	0	46.21	2.6	Very soluble	0.55	2.31
24	METHYL 10,12-PENTACOSADIYNOATE	2	0	124.71	6.58	Poorly soluble	0.55	4.47

Table 3: Pharmacokinetics and Drug likeliness of ligand molecules.

S. No	**Molecule**	**GI Absorption**	**BBB Permeant**	**PGP Substrate**	**CYP 1A2 Inhibitor**	**CYP2 C19 Inhibitor**	**CYP 2C9 Inhibitor**	**CYP 2D6 Inhibitor**	**CYP 3A4 Inhibitor**	**LOG KP (CM/S)**	**LIPINSKI #VIOLATIONS**
1	Stigmasterol	Low	No	No	No	No	Yes	No	No	-2.74	1
2	Cyclohexanol, 4-ethenyl-4-methyl-3-(1-methylethenyl)-, (1.alpha.,3.alpha.,4.beta.)-	High	Yes	No	No	No	No	No	No	-4.97	0
3	Pentafluoropropionic acid, 10-undecenyl ester	Low	No	No	No	No	Yes	No	No	-3.77	1
4	N-acetylnorephedrine	High	Yes	No	No	No	No	No	No	-6.9	0
5	8,11,14-eicosatrienoic acid, (z,z,z)	High	No	No	Yes	No	Yes	No	No	-2.95	1
6	1,3-benzodioxol-2-one, hexahydro-, cis-	High	Yes	No	No	No	No	No	No	-6.01	0
7	3,4 -dimethylpentanol	High	Yes	No	No	No	No	No	No	-5.57	0
8	1-dodecanol	High	Yes	No	Yes	No	No	No	No	-3.79	0

9	Z,z-3,11-octadecadien-1-ol acetate	Low	No	No	No	No	No	No	Yes	-2.66	1
10	3-dodecen-1-ol	High	Yes	No	Yes	No	No	No	No	-4.19	0
11	Hexanoic acid	High	Yes	No	No	No	No	No	No	-5.65	0
12	N-hexadecanoic acid	High	Yes	No	Yes	No	Yes	No	No	-2.77	1
13	2-amino-3-methyl-1-butanol	High	No	No	No	No	No	No	No	-6.93	0
14	Cyclohexanone, 4-hydroxy	High	Yes	No	No	No	No	No	No	-7.15	0
15	Cyclopropane, pentyl	Low	Yes	No	No	No	No	No	No	-4.12	0
16	Z-10-pentadecen-1-ol	High	Yes	No	Yes	No	No	No	No	-3.55	0
17	Carbamimidoylsulfanylacetic acid	High	No	No	No	No	No	No	No	-7.28	0
18	Z,z-3,11-octadecadien-1-ol acetate	High	Yes	No	No	No	No	No	No	-6.39	0
19	2-octadecadecen-1-ol	High	No	No	Yes	No	No	No	No	-2.34	1
20	Formic acid, hexyl ester	High	Yes	No	No	No	No	No	No	-5.35	0
21	Oxirane, (fluoromethyl)	Low	No	No	No	No	No	No	No	-6.88	0
22	Cyclopentaneundecanoic acid, methyl ester	High	Yes	No	Yes	No	No	No	No	-2.92	0
23	Propanenitrile, 3-(hexyloxy)	High	Yes	No	No	No	No	No	No	-5.76	0
24	Methyl 10,12-pentacosadiynoate	Low	No	No	Yes	No	No	No	No	-1.3	1

CONFLICT OF INTEREST STATEMENT:
The authors declare that they have no conflict of interest.

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