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In Silico Screening of Phytochemicals of *Styrax Benzoin* Against the Inflammatory Mediators

Swathy B[1,2], Menaka M[2], Kiran G[3], Prabhakar Reddy V[1]*

[1]Department of Pharmaceutics, University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar-509001, Telangana, India; [2]Department of Pharmacy, Annamalai University, Chidambaram, Tamilnadu, India; [3]School of Pharmacy, Anurag Group of Institutions, Hyderabad-500 088, Telangana, India.

ABSTRACT

Introduction: Inflammatory mediators are up-regulated during SARS-CoV-2 infection and also during injury and surgery. The wound is categorized as break down or opening of the skin, which could lead to malfunctioning of skin. Many physiological processes, protein targets, and cellular signalling pathways are involved in wound healing. Many inflammatory mediators are produced during injury and inhibition of the negative inflammatory mediators like 1RAK4 and NLRP3 inflammasome plays a key role in the wound healing process. Therefore, it could essential to find therapeutics for inhibiting the signalling pathways responsible for the release of negative inflammatory mediators. However, the conventional approaches to drug development are time-consuming and expensive.

Objective: In the present study, we have adopted a computational approach to identify lead molecules from *Styrax Benzoin*against the inflammatory mediators.

Method: We have screened ligands from *Styrax Benzoin* library for their ability to bind and inhibit the two potential inflammatory targets such as IRAK4 (Interleukin-1 Receptor Associated Kinase-4) with Protein Data Bank (6F3I) and innate immune signalling receptor NLRP3 (NOD-, LRR-, and pyrin domain-containing-3) inflammasome with Protein Data Bank (6NPY).

Results: We found that p-coumarin cinnamate 6 and coniferyl benzoate 12 could bind at the substrate-binding pocket of inflammatory targets with high binding affinity. Bioavailability properties and Pharmacophore features were also studied.

Conclusion: The results suggest that the Phytoconstituents of *Styrax Benzoin* have the potential to be developed as novel inhibitors of inflammatory mediators. These inflammatory mediators are upregulated during SARS-CoV-2 infections. However, their clinical usage on wound healing is a subject of further investigations and clinical trials.

Key Words: *Styrax Benzoin*, Inflammatory Mediators, SARS-CoV-2, Wound, 1RAK4, NLRP3 inflammasome

INTRODUCTION

Inflammatory mediators are upregulated during SARS-CoV2 infections and also during injury and surgery. Viruses can affect the wound healing process by causing physiological changes. As per WHS (Wound Healing Society), a wound is categorized as break down or opening of the skin, which could lead to malfunctioning of skin.[1] The physiology stages of wound healing are primary and secondary interventions, smaller wounds heal by primary interventions and larger wounds are heal by secondary interventions.[2, 3] Among the different stages in wound healing the first stage is the inflammatory stage and is a very essential phase in wound healing process.[2, 4]. These clots release monocytes and forms macrophages and further produce the cytokines.[5]When the tissue is an injury that going to release inflammatory cytokines from the damaged tissue cells.[6, 7] Neutrophils, also contribute to wound healing by releasing the cytokines and growth factors and also has phagocytosis functions which protect the wound against bacterial infections.[8, 9]. Therefore, it could essential to find therapeutics for inhibiting the signalling pathways responsible for the release of negative inflammatory mediators. Inflammatory mediators like cytokines, chemokines and growth factors play the key role in wound healing by releasing fibroblasts and keratinocytes from cells and replace or restore the skin integrity.[10, 11] NLRP3 (Nod-like receptor protein) inflammasome cellular pathway is involved in wound healing and various inflammatory skin diseases.[12, 13]Yimin Chai group have studied the

Corresponding Author:
Dr. Prabhakar Reddy Veerareddy, University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar-509001, Telangana, India; Mobile: +91-998980499; E-mail: vpreddyindia@gmail.com

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role of NLRP3 expression in diabetic wounds in humans and the results demonstrate the higher expression of caspase1, NLRP3, IL-1β inflammatory mediators.[14] These mediators could be the potential targets for the diabetic wound healing process. IRAK4 (interleukin-1 receptor-associated kinase 4) is an important molecular target for the release of inflammatory substances.[15, 16] IRAK inhibitors could be useful in the prophylaxis treatment of psoriasis, sclerosis, myocardial infarction, lupus erythematous, and arthritis.[17, 18]

Styrax Benzoin (Latin: Benzoinum) is a balsamic resin and other species are Sumatra Benzoin and Siam Benzoin and belongs to the family Styraceae.[19] It is grown high in tropical rain forests of South-Eastern Asia Countries like Indonesia, Thailand, China and Vietnam. (20) The plants grow up to 14 cm long and flowers are white in colour and bell-shaped. Sumatra benzoin resin contains chemical constituents are balsamic acid which esters of benzoic and cinnamic acids.[21] They also contain Triterpenoid acids like summaresinolic and siaresinolic acids are present.[22] Whereas, Siam Benzoin about 76% of coniferyl benzoate is present as the chief active constituent.[23] The styrax benzoin resin is prepared as a tincture and used as an expectorant, carminative, disinfectant and diuretic.[24-27] It also has biological uses in throat infection and upper respiratory tract infections.[28] this study aims to investigate the *In Silico* computational study of 22 Phytoconstituents of *Styrax Benzoin* against the inflammatory mediators 1RAK4 and NLRP3 inflammasome.

EXPERIMENTAL

Protein Preparation

The present study is aimed to perform the computational studies of phytochemical analogues of *Styrax Benzoin* against COVID-19 negative immune regulators such as IRAK4 (Interleukin-1 Receptor Associated Kinase 4) with PDB ID: 6F3I and innate immune signaling receptor NLRP3 (NOD-, LRR-, and pyrin domain-containing 3) inflammasome with PDB ID: 6NPY.[29,30]The X-ray crystal structures of IRAK4 in complex with pyrrolotriazine inhibitor and NLRP3 bound to NEK7 were retrieved from Protein Data Bank (https://www.rcsb.org/structure/6F3I; https://www.rcsb.org/structure/6NPY). The protein targets were downloaded in PDB format and protein structural preparation in Macromolecule protocol was carried out in Discovery Studio software with default settings. Protein structures were cleaned and missing residues, Hydrogen's were added and 3D protonation was carried out to the target protein and minimized for the selected active residues.

Ligand Preparation

The important phytochemicals of *Styrax Benzoin* were collected from the literature survey and also from the TCIM database. The canonical smiles were saved in .csv format and structures were generated by using Data warrior software and all the **22** Phytoconstituents were saved in SD file. All the ligand structures were energy minimized using CHARMm force field in Small-molecule Protocol and different conformers were generated.

Molecular Docking Studies

Molecular docking was carried out for the 18 Phytoconstituents of *Styrax Benzoin* (**1-22)** to identify the molecular interactions between inflammatory targets 1RAK4 (PDB ID: 6F3I) and NLRP3 inflammasome (PDB ID: 6NPY).[29, 30]All the ligands were docked with by using Accelrys Discovery Studio version 3.5 with Libdocker and CDocker software. The protein structures were retrieved from the protein data bank and the protein preparation and minimization were carried out with the default settings in Discovery Studio. The active site sphere is generated from ligand-binding sites with the current selection of Define and Edit Binding site in receptor-ligand Interaction tools. The binding site sphere specified based on the binding interactions of co-crystal ligand against the target protein, Docking Tolerance as 0.25, Docking Preferences as High Quality. The results were analysed and 3D and 2D interactions were obtained with Discovery Studio Visualizer.

Pharmacophore Modelling

Pharmacophores features are generated between receptor-ligand using Interaction Pharmcophore generations in DS Protocol. Different molecular interactions like HBA (hydrogen bond acceptor), HBD (hydrogen bond donor), HY (hydrophobic centre) and PI (Positive ionisable) were generated for the receptor-ligand complex. The study was done for the best-docked pose of p-coumaryl cinnamate6 and coniferyl benzoate12 for 1RAK4 and NLRP3 complex proteins.

In Silico Absorption Distribution Metabolism Excretion (ADME) and Toxicity Prediction:

All the Phytoconstituents of *Styrax Benzoin***(1-22)** were predicted In silico ADME properties and Toxicity analysis were carried using Discovery Studio, pKCSM web server and Data Warrior Software.[31,32]

RESULTS AND DISCUSSION

Molecular Docking Studies

The molecular docking studies were carried out in Discovery Studio Docking software. The 3-dimensional proteins (PDB: 6F3I and 6NPY) were retrieved from the protein data bank. All the proteins were prepared and their energies were minimized by the protein preparation wizard. The receptor sphere around their co-crystal ligands was generated using the current

selection of co-crystal ligand interactions. All the selected **22** Phytoconstituents of *Styrax Benzoin*were downloaded from Pubmed (**Figure-1)**. The molecular docking using normal mode was carried out and results were analysed.

The initial rationale molecular docking studies of all 22 Phytoconstituents of *Styrax Benzoin* (**1-22)** in the active site of 1RAK4 (PDB ID: 6F3I) and NLRP3 inflammasome (PDB ID: 6NPY)were carried out in order to predict the binding efficiencies.The molecular docking scores are summarized in (**Table:1-3**). Initially the docking studies were carried out for all ligands with Libdock which High Throughput Screen based software and best ligand poses were further docked with CDocker.

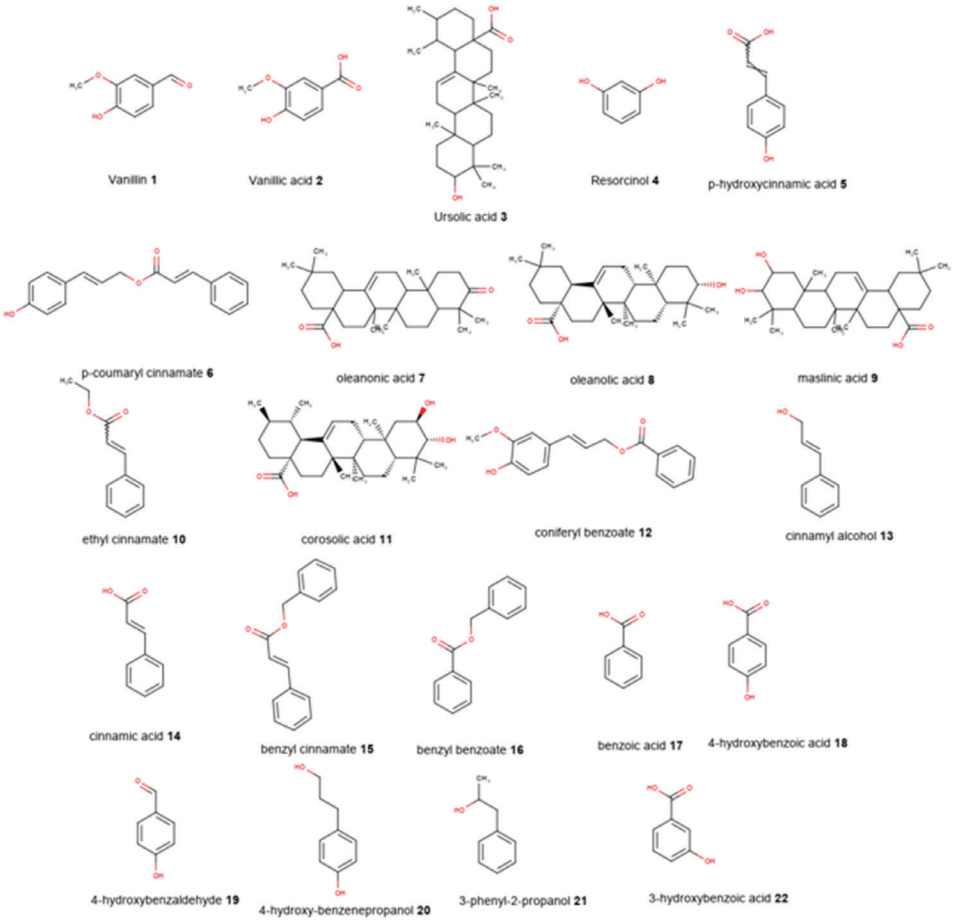

Figure 1: 2D Structures of Phytoconstituents of Styrax Benzoin.

Table 1: Docking studies of 22 Phytoconstituents of Styrax Benzoin against 1RAK4 and NLRP3 inflammasome

Compd ID	Phytochemical	Libdock Score	
		PDB ID: 6F3I	PDB ID: 6NPY
1	vanillin	61.92	58.67
2	vanillic acid	66.13	66.44
3	ursolic acid	98.70	
4	resorcinol	46.36	
5	p-hydroxycinnamic acid	73.01	73.72
6	p-coumaryl cinnamate	105.19	118.76
7	oleanonic acid	102.59	--
8	oleanolic acid	104.27	--
9	maslinic acid	106.11	--

Table 1: ***(Continued)***

Compd ID	Phytochemical	Libdock Score	
		PDB ID: 6F3I	PDB ID: 6NPY
10	ethyl cinnamate	76.38	83.74
11	corosolic acid	98.18	--
12	coniferyl benzoate	99.36	119.99
13	cinnamyl alcohol	63.15	74.23
14	cinnamic acid	68.01	68.64
15	benzyl cinnamate	97.90	104.39
16	benzyl benzoate	82.65	104.26
17	benzoic acid	52.48	58.61
18	4-hydroxybenzoic acid	59.47	58.56
19	4-hydroxybenzaldehyde	55.62	--
20	4-hydroxy-benzenepropanol	66.75	--
21	3-phenyl-2-propanol	62.01	75.71
22	3-hydroxybenzoic acid	58.50	73.47
23	Co-Crystal Ligand	130.28 (CKN)	--

Table 2: Docking studies of Phytoconstituents of Styrax Benzoin against 1RAK4 (PDB ID: 6F3I)

S. No	Compd Id	Phytochemical	Cdocker Energy	Binding Energy (kcal/mol)	H-Bond	Hydrophobic
1	3	ursolic acid	-94.22	-43.44	Ser269(2.33)	Val200, Ala315, Leu318
2	6	p-coumaryl cinnamate	20.90	-36.49	Met265(2.01), Asn316(2.23), Tyr264(2.86)	Val200, Ala211, Leu318, Met192
3	8	oleanolic acid	-61.84	-48.08	Asp272(2.51)	Val200, Ala211, Lys213, Ala315, Leu318, Tyr262
4	9	maslinic acid	-90.53	-58.59	Arg273(2.46), Asp278(1.94)	Met192, Ala315, Val200
5	11	corosolic acid	-93.77	-37.44	Glu194(2.99), Asp272(2.82), Gly193(2.72)	Ala315
6	12	coniferyl benzoate	20.73	-78.73	Glu194(2.43), Met265(1.91), Val263(2.84) Tyr264(2.63)	Val200, Met192, Tyr262, Ala211, Leu318
7	15	benzyl cinnamate	25.53	-48.77	--	Met192, Val200, Ala211
8		CKN	30.24	-82.794	Arg273(1.87), Arg273(2.88), Asn316(2.38), Asp329(2.48), Asp272(2.81), Asp272(2.37),	Met192, Val200, Lys213, Ala211, Val246, Met265, Leu318,

Table 3: Docking studies of Phytoconstituents of Styrax Benzoin against NLRP3 inflammasome (PDB ID: 6NPY)

Compd ID	Phytochemical	Cdocker Energy	Binding Energy	H-Bond	Hydrophobic
1	vanillin	21.95	-32.55	Arg165(2.02), Arg165(2.39), Gly227(2.93), Ile228(2.84)	Leu169, Ile232, Pro410, Ile232,
2	vanillic acid	25.54	-28.63	Arg165(2.20), Arg165(2.10), Arg165(2.08), Gly227(3.00), Ile228(2.70)	Pro410, Leu169, Ile232, Leu411
5	p-hydroxy cinnamic acid	26.17	-43.32	Arg165(1.97), Arg165(1.92), His520(2.24), Arg165(2.12), Gly227(1.89), His520(3.00)	Ile232, Pro410, Leu411
6	p-coumaryl cinnamate	29.03	-67.33	Thr167(1.98), His520(2.17), Thr167(2.02), Gly229(2.41)	Pro410, Ile232, Arg235
10	ethyl cinnamate	24.03	-20.59	Arg165(1.92), His520(2.24),	Ile232, Pro410,
12	coniferyl benzoate	27.54	-63.66	Thr167(2.22), Thr167(3.03), Arg165(2.40), Gly229(2.45), Gly227(2.95),	Phe371, Pro410, Ile232, Leu411, Leu162
13	cinnamyl alcohol	11.55	-22.19	Arg165(1.78)	Leu169, Ile232, Pro410,
14	cinnamic acid	21.16	-38.80	Thr167(2.24), Thr167(1.98)	Ile232, Pro410,
15	benzyl cinnamate	28.74	-36.94	Arg165(2.00), Arg165(2.81)	Trp414, Leu169, Ile232, Pro410
16	benzyl benzoate	26.25	-46.00	Gly229(2.68), Gly229(2.94)	Trp414,Ile232, Leu411
17	benzoic acid	18.05	-20.75	Thr167(1.87), Thr167(1.97)	Phe371, Ile232, Pro410
18	4-hydroxybenzoic acid	21.77	-49.46	Thr167(1.97), Gly227(2.69), Thr167(1.92), Gly229(2.50)	Ile232, Pro410
20	4-hydroxy-benzene-propanol	25.21	-41.72	Arg165(1.94), His520(2.01), Glu150(2.03), Gly227(2.86),	Trp414, Leu162, Ile232
21	3-phenyl-2-propanol	20.96	-26.35	Gly227(2.12), Gly229(2.67)	Trp414, Ile232
23	ADP	40.39	-16.58	Thr167(3.01), Tyr379(2.95), Thr167(2.70), Arg165(2.82), Pro410(2.90), Ile519(3.33), His520(3.77),	Ile232, Trp414

Molecular Docking Studies with 1RAK4 (PDB ID: 6F3I):

The molecular docking study was carried out for 22 phytoconstituents of *Styrax Benzoin* (1-22) into the active site of 1RAK4 (PDB ID: 6F3I). IRAK family (IRAK1-4) plays a central role in positive and negative inflammatory responses by regulating the expression of genes in immune cells.[33] These signals which stimulus the various inflammatory mediators and plays a key role for elimination of pathogens like virus, bacteria and carcinogenic cells, as well as for wound healing.

Among the ligands docked against IRAK-4, coniferyl benzoate (**12**) has shown excellent free energy binding with a Lib dock score of 99.36 and with Cdock score of -20.73 and with a binding energy value of -78.73 kcal/mol. Coniferyl benzoate(**12**) exerted H-bond interactions and bond distance in Å with Glu194(2.43), Met265(1.91), Val263(2.84) Tyr264(2.63) amino acid residues and hydrophobic interactions with Val200, Met192, Tyr262, Ala211, Leu318 residues. Similarly, p-coumaryl cinnamate has exhibited H-bond interactions with Met265(2.01), Asn316(2.23), Tyr264(2.86) residues and hydrophobic interactions with Val200, Ala211, Leu318, Met192 amino acid residues with Libdock Score 105.19and Cdock score -20.90and binding energy value of -36.49 kcal/mol. (**Table-1 & 2**)

Whereas, Crystal ligand CKN (Pyrrolotriazine) has involved key interactions with residues forming H-bond with Arg273(1.87), Arg273(2.88), Asn316(2.38), Asp329(2.48), Asp272(2.81), Asp272(2.37), and hydrophobic interactions with Met192, Val200, Lys213, Ala211, Val246, Met265, Leu318, residues with Libdock Score 130.28 and Cdock score 30.24 and binding energy value of -82.79 kcal/mol. It signifies that Coniferyl benzoate(**12**) is occupying the same residues of the active site of the crystal ligand. (**Figure: 2 & 3**).

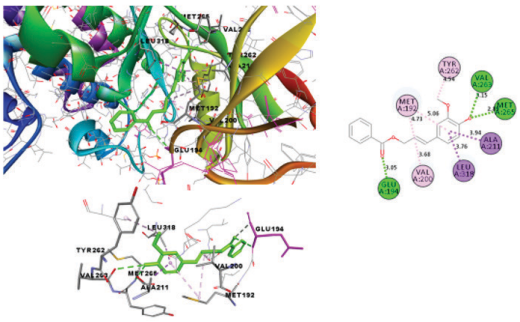

Figure 2: 3D and 2D Interactions of Coniferyl Benzoate 12 with 1RAK4 (PDB ID: 6F3I).

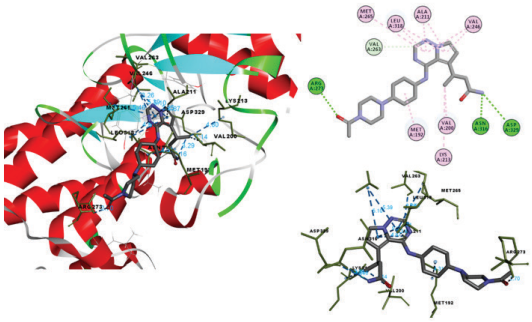

Figure 3: 3D and 2D Interactions of CKN with 1RAK4 (PDB ID: 6F3I).

Molecular Docking Studies with NLRP3 inflammasome (PDB ID: 6NPY):

The molecular docking study was carried out for 22 phytoconstituents of *Styrax Benzoin* (**1-22**) into the active site of NLRP3 inflammasome (PDB ID: 6NPY). NLRP3 inflammatory signalling pathway plays a key role in the release of inflammatory mediators, on activation of NLRP3 abrupt the cytochrome storms viz Interleukins (IL-1β, IL-6) and TNF-αand causes inflammatory in the lower respiratory tract.[34] On the inhibition of NLRP3 will stop the release of negative inflammatory mediators.

Among the ligands docked against IRAK-4, coniferyl benzoate (**12)** has shown excellent free energy binding with Lib dock score 119.99and with Cdock score -27.54 and with a binding energy value of -63.66 kcal/mol. Coniferyl benzoate(**12)** exerted H-bond interactions and bond distance in Å with Thr167(2.22), Thr167(3.03), Arg165(2.40), Gly229(2.45), Gly227(2.95), amino acid residues and hydrophobic interactions with Phe371, Pro410, Ile232, Leu411, and Leu162 residues. Similarly, p-coumaryl cinnamate**(6)** has exhibited H-bond interactions with Thr167(1.98), His520(2.17), Thr167(2.02), Gly229(2.41) residues and hydrophobic interactions with Pro410, Ile232, Arg235 amino acid residues. (**Table 1 & 3**)

Whereas, Crystal ligand CKN has involved key interactions with residues forming H-bond with His163 and Glu166 with a bond distance of 2.06 A° and 2.18 A°, respectively and hydrophobic interactions with His41, Met49 and Met165 residues. It signifies that p-coumaryl cinnamate**(6)** and Coniferyl benzoate(**12)** is occupying the same residues of the active site of crystal ligand. (**Figure: 4-6**).

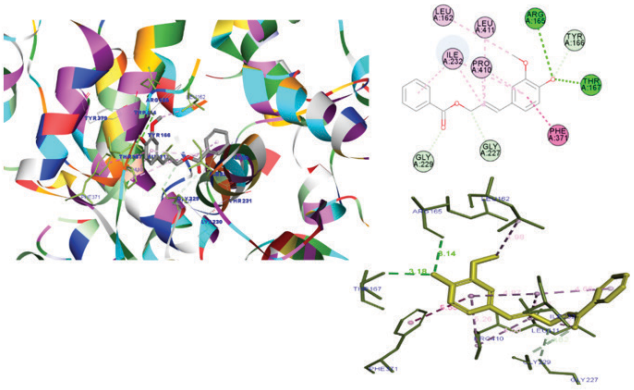

Figure 4: 3D and 2D Interactions of Coniferyl Benzoate with NLRP3 (PDB ID: 6NPY).

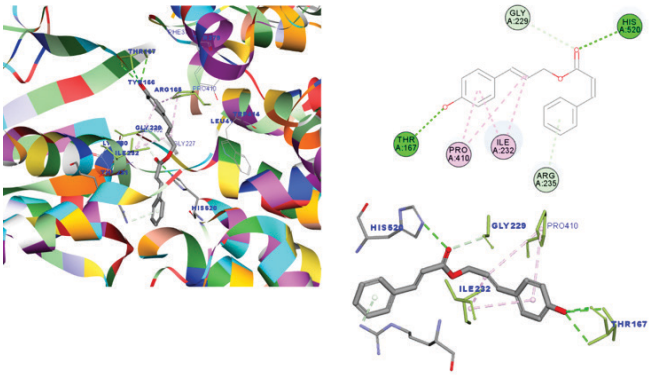

Figure 5: 3D and 2D Interactions of p-coumaryl cinnamate with NLRP3 (PDB ID : 6NPY).

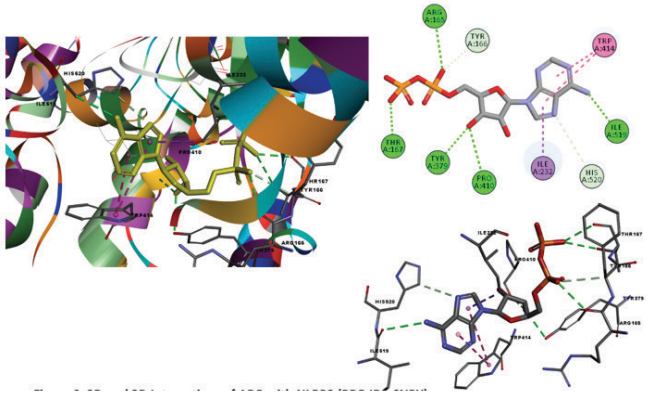

Figure 6: 3D and 2D Interactions of ADP with NLRP3 (PDB ID: 6NPY).

Structure-Based Pharmacophore: The best protein-ligand pose of docking were further analysed for Pharmacophore features using Interaction Pharmacophore Generation Protocol. **Table-4** give the details about Common Pharmacophore Feature for ligand p-coumaryl cinnamate **6** has HHDA pharmacophore feature and fit value 3.99 and rank 9.13 and whereas, coniferyl benzoate **12** has HHHDA pharmacophore features with fit value 4.99 and rank 11.50 which shows better features. The interaction pharmacophore features for receptor-ligand complex among the result coniferyl benzoate-6F3I complex has 10 pharmacophore models with H-bond interactions Glu194, Val263, Tyr264, Met265 and with hydrophobic interactions Met192, Val200, Ala211, Tyr262, Leu318. Similarly, with coniferyl benzoate-6NPY complex has 10 pharmacophore models with H-bond interactions Arg165, Thr167, Gly227 and hydrophobic interactions Leu162, Ile232, Phe371, Pro410, Leu411. The interaction pharmacophore features are shown in **Figure-7**.

Table 4: Common Pharmacophore Features of Phytochemicals

Compd Id	Phytochemical Name	Pharmacophore features	Fit value	Rank	Max Fit
6	p-coumaryl cinnamate	HHDA	3.99	9.13	4
12	Coniferyl Benzoate	HHHDA	4.99	11.50	5

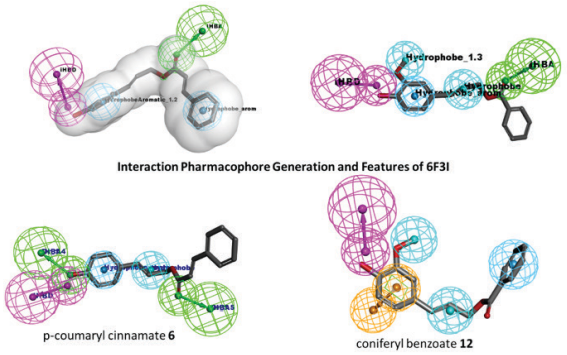

Figure 7: Interaction Pharmacophore Generation and Features of 6NPY

In Silico ADMET Prediction:

In Silico Prediction of ADME and Toxicity parameters were analysed for the Phytoconstituents of *Stryax Benzoin* by using Discovery Studio, pKCSM webserver and Data Warrior Software. All the phytochemicals have obeyed Lipinski's rule of 5 in which the Mol. Wt is below 500, logP <5, No of Hydrogen Bond Donors is <5 and Acceptors < 10 and Molar refractivity (2) between 40-130. Almost all the phytochemicals have not deviated from the rule of 5 and hence they have the potential for oral absorption and less toxicity. **(Figure-8)**.

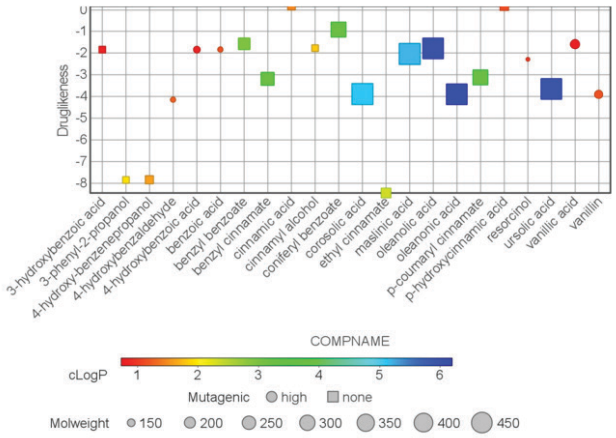

Figure 8: ADMET Properties of Phytochemicals of Styrax Benzoin.

Some of the potential Phytochemicals (**1-7, 9, 11-16, 20**) which have shown good binding affinity with the docking studies were analysed for ADMET using pKCSM webserver(**Table-5**). The absorption properties of the compounds are studied by Caco-2 permeability, human intestinal absorption, skin permeability, and p-gp substrate or inhibitor. The predicted value >0.90 indicates high Caco-2 permeability. Vanillin **1,** vanillic acid **2**, ursolic acid **3**, resorcinol **4,** p-OHcinnamic acid**5**, p-coumaryl cinnamate **6,** oleanonic acid **7,** masilinic acid**9**, corosolic acid **11**, coniferyl benzoate**12**, cinnamyl alcohol**13**, cinnamic acid**14,** benzyl cinnamate**15,** benzyl benzoate**16,** 4-hydroxy-benzenepropanol**20** were predicted to have high cacao-2 permeability. All phytochemicals have good intestinal absorption. In terms of skin permeability if the compounds have log Kp > -2.5 consider being low skin permeability. Among analysed compounds, cinnamyl alcohol**13** was considering to have low skin permeability with log Kp = -1.70.

None of the phytochemicals is the substrate for p-glycoprotein which is an efflux transporter that excretes chemicals or drugs from the cells. All phytochemicals have the apparent volume of distribution the limits are VDss is low if log VDss <-0.15 and high if logVDss >0.45. For Blood-Brain Barrier permeability, compounds with log BB > 0.3 consider crossing the BBB and log BB < -1 are impermeable to the brain. Phytoconstituents 12-16 & 20 will cross the BBB as predicted values are logBB>0.3. Regarding CNS permeability, phytochemicals **6, 7, 9, 11, 13, 14, 15, 16 & 20** have high CNS permeability (log PS > -2).

Cytochrome P450 is an important enzyme for the biotransformation of drugs in the liver and inhibitors of CYP450 will affect the pharmacokinetic properties of the drug. All phytochemicals are not substrate for CYP2D6, Compound **12, 13,**

Table 5: *In Silico* ADMET Predicted properties of Phytoconstitutents of Styrax Benzoin

Properities	1	2	3	4	5	6	7	9	11	12	13	14	15	16	20
Absorption															
Water solubility (log mol/L)	-1.31	-1.84	-3.07	-0.76	-2.38	-4.45	-3.09	-3.04	-3.04	-4.03	-1.85	-2.61	-4.07	-3.58	-1.22
Caco2 permeability (log Papp in 10-6 cm/s)	1.22	0.33	1.17	1.68	1.21	1.37	1.28	0.63	0.64	1.33	1.61	1.72	1.60	1.43	1.69
Intestinal absorption (%Absorbed)	84.98	78.15	100	86.86	93.49	91.23	100	100	100	90.88	92.67	94.83	95.79	95.84	81.82
Skin Permeability (log Kp)	-2.83	-2.73	-2.74	-2.62	-2.72	-2.41	-2.74	-2.74	-2.74	-2.71	-1.70	-2.70	-2.13	-2.10	-2.63
P-glycoprotein substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Distribution															
VDss (human) (log L/kg)	-0.15	-1.74	-1.09	-0.02	-1.15	-0.06	-1.12	-1.28	-1.28	-0.21	0.30	-1.05	0.04	-0.05	0.03
Fraction unbound (human)	0.43	0.52	0.00	0.62	0.43	0.00	0.00	0.03	0.04	0.00	0.34	0.38	0.00	0.08	0.40
BBB permeability (log BB)	-0.24	-0.38	-0.14	-0.32	-0.23	0.28	-0.08	-0.49	-0.47	0.15	0.48	0.45	0.31	0.28	-0.16
CNS permeability	-2.24	-2.63	-1.19	-2.08	-2.42	-1.52	-1.07	-1.48	-1.51	-2.21	-1.76	-1.83	-1.32	-1.38	-2.06
Metabolism															No
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
CYP1A2 inhibitior	No	No	No	No	No	Yes	No	No	No	Yes	Yes	No	Yes	Yes	No
CYP2C19 inhibitior	No	No	No	No	No	Yes	No	No	No	Yes	No	No	Yes	Yes	No
CYP2C9 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitior	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitior	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	
Excretion															
Total Clearance (log ml/min/kg)	0.60	0.63	0.08	0.24	0.66	0.66	-0.13	-0.07	0.09	0.56	0.25	0.78	0.76	0.73	0.29
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity															
AMES toxicity	No	No	No	No	No	Yes	No	No	No	Yes	No	No	No	No	No
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Oral Rat Acute Toxicity (LD50)	1.94	2.45	2.05	2.14	2.16	1.95	2.26	2.52	2.51	1.86	1.92	2.09	1.83	1.73	1.98
Hepatotoxicity	No	No	Yes	No	No	No	No	Yes	Yes	No	No	No	No	No	Yes
Skin Sensitisation	No	No	No	Yes	No	No	No	No	No	No	Yes	No	Yes	Yes	Yes
T.Pyriformis toxicity	-0.01	0.27	0.29	0.11	0.32	1.19	0.29	0.29	0.29	1.02	-0.13	0.25	1.66	1.36	-0.08
Minnow toxicity	1.90	1.93	-0.79	2.19	1.61	-0.62	-1.22	0.24	0.28	-0.15	1.87	1.72	-0.54	0.83	1.90

16 & 20 are substrates for CYP3A4. Compounds **6, 12 & 13** are inhibitors for CYP1A2 inhibitors.

Regarding toxicity compound **6** has Ames toxicity, and compounds **3, 9, 11** have hepatoxicity, **4, 13, & 20** has skin sensitisation problems. All phytochemicals have hERG toxicity and are free from cardiotoxicity. From these observations it could be revealed that all phytochemicals of *Styrax Benzoin* have shown good drug-like properties viz., no toxicity, good oral absorption, metabolism and excretion and no interaction with cytochrome P450 enzymes and free from cardiotoxicity.

CONCLUSION

In Silico computational studies like molecular docking, pharmacophore model, ADMET prediction could provide helpful information for the rapid design of drugs. Wound infection causes cytokine storms and releases negative inflammatory mediators such as 1RAK4 and NRLP3. Therefore, it could essential to find therapeutics for inhibiting the signalling pathways responsible for the release of negative inflammatory mediators. To find potent inhibitors for inflammatory targets 1RAK4 and NRLP3 inflammasome we performed molecular

docking studies for 22 Phytoconstituents of *Styrax Benzoin* using Libdocker and C docker. The results have shown p-coumaryl benzoate **6** and coniferyl benzoate **12** have a high binding affinity against the protein targets. Detailed pharmacophore features were also generated against these docked complexes. ADMET prediction studies indicate that all the Phytoconstituents are having good oral absorption and less toxicity. The results suggest that the Phytoconstituents of *Styrax Benzoin* have the potential to be developed as novel inhibitors of inflammatory mediators. These inflammatory mediators are upregulated during SARS-CoV-2 infections. However, their clinical usage against inflammatory mediators is a subject of further investigations and clinical trials.

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