ORIGINAL ARTICLE

In silico Studies for *Tridax procumbens* Linn Phyto constituents with Anti-inflammatory Receptors

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Abstract

Introduction: A wound is categorized as a breakdown or opening of the skin, which could lead to malfunctioning of the skin. **Materials and Methods:** Interleukin-1 receptor-associated kinases are key components in the signal transduction pathways utilized by interleukin-1 receptor and interleukin-18 receptor. **Results and Discussion:** The critical role of IRAK-4 in inflammatory processes, and modulation of IRAK-4 kinase activity presents an attractive therapeutic approach for the treatment of inflammatory diseases. To identify the potent IRAK-4 inhibitors with new anti-inflammatory therapeutic agents from selected herbal plants and their extract with the help of *in-silico* data. **Conclusion:** In the present study, we have investigated *in silico* computational studies for *Tridax procumbens* phytoconstituents against the molecular targets involved in the anti-inflammatory process. The molecular docking interactions show good binding affinity against the molecular targets of the anti-inflammatory target, namely, ILAK4. Among the ligands docked against ILAK4, Nodakenin (14), and Apigenin 7-O-β-D-glucoside (16) showed excellent free energy binding with dock score values of −9.4 and −10 kcal/mol, respectively. These results suggest that further mechanistic studies are to be done for these phytoconstituents against anti-inflammation.

Key words: Anti-inflammatory, *in silico*, IRAK-4 kinase, *Tridax procumbens*

INTRODUCTION

As per Wound Healing Society (WHS), a wound is categorized as a breakdown or opening of the skin, which could lead to malfunctioning of the skin.[1] The first stage of wound healing is the inflammatory stage and is a very essential phase in the wound healing process.[2] Any wound that penetrates the dermis or below going to cause hemorrhage and cause bleeding.[3] The clots could release monocytes and form macrophages and further produces cytokines[4]

Tridax contains chemical constituents such as flavonoids, sterols, terpenoids, polysaccharides, and fatty acids.[5] *Tridax procumbens* are known to possess much biological significance such as anti-inflammatory,[6] antifungal, anticoagulant, and insect repellant.

Therefore, it is of interest to document the molecular docking analysis of phytoconstituents from *T. procumbens* Linn with IRAK-4 kinase.[7]

MATERIALS AND METHODS

Protein preparation

It aimed to perform the computational studies of phytochemical analogs of *T. procumbens* against 1RAK4 (PDB ID: 6F3I). The X-ray crystal structures of all protein targets in complex with inhibitor were retrieved from Protein Data Bank. The protein targets were downloaded in PDB format and protein structural preparation in macromolecule protocol was carried out in AutoDock software with default settings. Protein structures were cleaned and missing residues, hydrogen was added, and 3D protonation was

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carried out to the target protein and minimized for the selected active residues by Using MGL Tools-1.5.

Ligand preparation

The important phytochemicals of *T. procumbens* were collected from the literature survey and also from the TCIM database. The canonical smiles were saved in csv format and structures were generated using data warrior software and all the 38 phytoconstituents were saved in an SD file. Energy of ligands was minimized by CHARMm force field to small molecules.

All phytoconstituents are given in Table 1.

Molecular docking studies

Molecular docking was carried out for the 38 phytoconstituents of *T. procumbens* (1-38) to identify the molecular interactions between an inflammatory target against inflammatory mediators. The protein and ligand files in pdbqt format were prepared by using MGL Tools-1.5. Receptor grids were generated using 66 × 68 × 66 Grid points in XYZ with a grid spacing of 0.485 Ao. Grid parameter files (.gpf) and docking parameter files (.dpf) were created for docking. Map types were generated using Autogrid 4.2. A grid box was generated by considering active sites. A good dock-scoring molecule was analyzed for its interaction with a target. The protein plus online pose viewer is used to generate 2D interactions.

ADME and toxicity

In silico ADME and toxicity analysis were carried out using Discovery Studio, pKCSM web server, and Data Warrior Software, and all the phytoconstituents of *T. procumbens* (1-38) were predicted safe.

RESULTS AND DISCUSSION

The molecular docking study was carried out for 38 phytoconstituents of *T. procumbens* (1-38) into the active site of 1RAK4 (PDB ID: 6F3I). IRAK family (IRAK1-4) has both

Table 1: Phytoconstituents of *Tridax procumbens* (1-38) used for docking studies

Quercetin (1)	Robinetin (11)	Tannins (21)	Isoquercitin (31)
Luteolin (2)	Puerarin (12)	Catechol (22)	lauric acid (32)
Apigenin (3)	6,8,3-trihydroxy-3,7,4-trimethoxy flavone (13)	α-Amyrin (23)	linolenic acid (33)
Kaempferol (4)	Nodakenin (14)	1-Dotriacontanol (24)	15,16-epoxy-octadeca-9,12-dienoic-acid (34)
Naringenin (5)	β-Stigmasterol (15)	Arachidic acid (25)	Octadecanoate (35)
Daidzein (6)	Apigenin 7-O-β-D-glucoside (16)	β-amyrenone (26)	Esculetin (36)
Genistein (7)	bis (2-ethylhexyl) phthalate (17)	Cynaroside (27)	betulinic acid (37)
Nobiletin (8)	α-sitosterol (18)	delta7-Avenasterol (28)	Oleanic acid (38)
Myricetin (9)	Centaureidin (19)	Docosanoic acid (29)	
Biochanin A (10)	3?-Hydroxy-20 (29)-lupene (20)	Isoquercetin (30)	

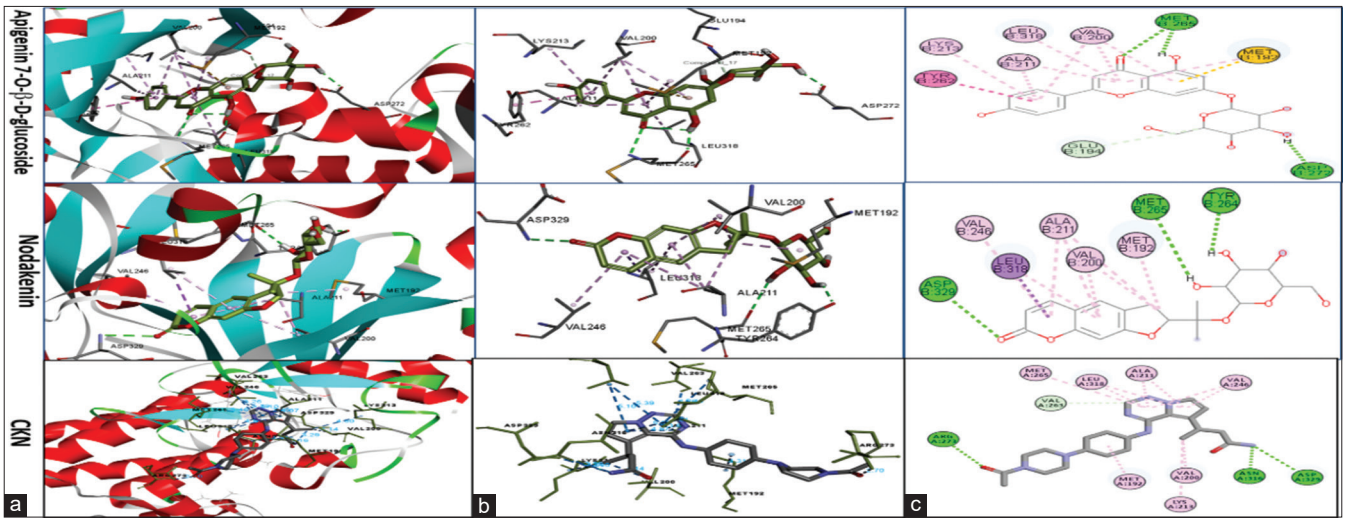

Figure 1: Molecular Docking Interactions of Apigenin-7-O-β-D-glucoside, Nodakenin, and CKN (Co-crystal ligand) against 1RAK4 (PDB ID: 6F3I). (a) Protein-ligand interactions, (b) 3D residual interactions, and (c) 2D interactions

Table 2: Docking score of phytoconstituents of *Tridax procumbens* the active site of 1RAK4 (PDB ID: 6F3I)

S. No.	Phytoconstituent name	Dock score kcal/mol 1RAK4 (PDB ID: 6F3I)
1	Quercetin	−9
2	Luteolin	−9.2
3	Apigenin	−9.2
4	Kaempferol	−9
5	Naringenin	−8.9
6	Daidzein	−9
7	Genistein	−9.4
8	Nobiletin	−8.2
9	Myricetin	−8.8
10	Biochanin A	−9.1
11	Robinetin	−8.7
12	Puerarin	−9.8
13	6,8,3-trihydroxy-3,7,4-trimethoxy flavone	−9
14	Nodakenin	−10.1
15	β-Stigmasterol	−9.3
16	Apigenin 7-O-β-D-glucoside	−10.4
17	bis (2-Ethylhexyl) phthalate	−7.2
18	α-sitosterol	−9.7
19	Centaureidin	−8.9
20	3?-Hydroxy-20 (29)-lupine	−8.6
21	Tannins	−9.7
22	Catechol	−8.4
23	α-Amyrin	−10
24	1-Dotriacontanol	−5.5
25	Arachidic acid	−5.7
26	β-amyrenone	−9.5
27	Cynaroside	−9.9
28	delta7-Avenasterol	−9.7
29	Docosanoic acid	−5.6
30	Isoquercetin	−9.1
31	Isoquercitin	−9.1
32	lauric acid	−5.8
33	linolenic acid	−6.3
34	15,16-epoxy-octadeca-9,12-dienoic-acid	−5.8
35	Octadecanoate	−5.9
36	Esculetin	−7.3
37	betulinic acid	−7.5
38	Oceanic acid	−10.9

positive and negative inflammatory responses by regulating the expression of genes in immune cells. These signals play a key role in the elimination of pathogens as well as in wound healing. The docking score results of all phyto constituents in Table 2.

Table 3: Better docking interactions of phytoconstituents with 1RAK4 (PDB ID: 6F3I)

		Docking interactions 1RAK4 (PDB ID: 6F3I)	
Compound_14	Nodakenin	Asp329 (3.09), Met265 (2.69), Tyr264 (2.15)	Leu318, Ala211, Met192, Val200, Val246, Leu318
Compound_16	Apigenin 7-O-b-D-glucoside	Met265 (1.90), Asp272 (2.10), Glu194 (2.18), Asp272 (2.46), Ala315 (2.90)	Tyr262, Met192, Val200, Ala211, Leu318, Lys213
	CKN	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

Among the ligands docked against IRAK-4, Apigenin 7-O-β-D-glucoside (17) has shown excellent free energy binding with a dock score value of −10.4 kcal/mol. Apigenin 7-O-β-D-glucoside (17) favored H-bond interactions and bond distance in Å with Met265 (1.90), Asp272 (2.10), Glu194 (2.18), Asp272 (2.46), and Ala315 (2.90) amino acid residues and hydrophobic interactions with Tyr262, Met192, Val200, Ala211, Leu318, and Lys213 amino acid residues. The –OCH3 group in the Apigenin has favored the H-bond interactions. Similarly, Nodakenin (15) has exhibited H-bond interactions with Asp329 (3.09), Met265 (2.69), Tyr264 (2.15) amino acid residues and hydrophobic interactions with Leu318, Ala211, Met192, Val200, Val246, and Leu318 amino acid residues with a dock score value of −10.1 kcal/mol. Best docking score compounds data in Figure 3.

In silico drug likeliness and toxicity prediction

All compounds are predicted for toxicity properties using Data Warrior Software. The *in silico* drug likeliness properties of 38 phytoconstituents of *T. procumbens* (1-38) were assessed using drug-likeness quantitative estimation (QED) with RDKit in Galaxy. All the compounds were determined molecular descriptors for Rule 5 (Lipinski rule) which states the oral bioavailability and drug-like properties. The determinants are molecular weight ≤500, no of H-Acceptors ≤10, and no of H-Donor ≤5. Among the calculated chemical descriptors, all the phytoconstituents have passed the Lipinski rule which states phytoconstituents which not violate more than one Ro5. QED (QED Drug-Likeliness) is calculated from eight

Table 4: *In silico* ADME and toxicity prediction of phytoconstituents of *Tridax procumbens* (1-38)

Compd No	MW	ALOGP	HBA	HBD	ROTB	LRo5	QED	Mutagenic	Tumorigenic	Irritant
1	302.24	1.98	7	5	1	0	0.44	high	High	none
2	286.24	2.28	6	4	1	0	0.51	none	None	none
3	270.24	2.57	5	3	1	0	0.65	high	None	none
4	286.24	2.28	6	4	1	0	0.56	high	None	none
5	272.26	2.51	5	3	1	0	0.72	none	None	none
6	254.24	2.87	4	2	1	0	0.72	none	None	none
7	270.24	2.57	5	3	1	0	0.65	high	High	none
8	402.4	3.51	8	0	7	0	0.61	high	High	none
9	318.24	1.69	8	6	1	1	0.37	high	None	none
10	284.27	2.88	5	2	2	0	0.78	none	None	none
11	302.24	1.98	7	5	1	0	0.44	high	None	none
12	416.38	0.38	9	6	3	1	0.38	none	None	none
13	360.32	2.60	8	3	4	0	0.68	none	None	none
14	408.4	–0.38	9	4	4	0	0.52	none	None	none
15	414.72	8.02	1	1	6	1	0.48	none	None	none
16	432.38	0.05	10	6	4	1	0.36	none	None	none
17	390.56	6.43	4	0	14	1	0.3	high	High	low
18	414.72	8.02	1	1	6	1	0.44	none	None	none
19	360.32	2.60	8	3	4	0	0.68	high	High	none
20	426.73	8.02	1	1	1	1	0.47	none	None	none
21	636.47	–0.27	18	11	7	3	0.05	none	None	none
22	290.27	1.54	6	5	1	0	0.48	none	None	none
23	426.73	8.16	1	1	0	1	0.47	none	None	none
24	466.88	11.70	1	1	30	1	0.10	none	None	high
25	312.54	7.11	2	1	18	1	0.30	none	None	none
26	424.71	8.37	1	0	0	1	0.38	none	None	none
27	448.38	–0.24	11	7	4	2	0.28	none	None	none
28	412.7	7.94	1	1	5	1	0.50	none	Low	high
29	340.59	7.89	2	1	20	1	0.24	none	None	none
30	464.38	–0.53	12	8	4	2	0.25	none	None	none
31	464.38	–0.53	12	8	4	2	0.25	none	None	none
32	200.32	3.99	2	1	10	0	0.52	high	High	high
33	278.44	5.66	2	1	13	1	0.32	none	None	none
34	280.45	5.88	2	1	14	1	0.30	none	None	none
35	283.48	4.99	2	0	16	0	0.36	none	None	none
36	178.14	1.20	4	2	0	0	0.4	none	High	none
37	456.71	7.0	3	2	2	1	0.48	none	None	none
38	456.71	7.23	3	2	1	1	0.45	none	None	none

MW: Molecular weight, ALOGP: Octanol-water partition coefficient, HBD's: Number of hydrogen bond donors, HBAs: Number of hydrogen bond acceptors, PSA: Molecular polar surface area, ROTBs: Number of rotatable bonds

properties such as M.W, ALOGP, HBA, HBD, PSA, ROTBs, AROMs, and ALERTS. The value ranges from 0 to 1.

All the phytoconstituents have free from toxicity [Table 3]. Table 4 gives the data of in toxicity and ADME of all constituents.

CONCLUSION

In the present study, we have investigated *in silico* computational studies for *T. procumbens* phytoconstituents against the molecular targets involved in the anti-inflammatory process. The molecular docking interactions

show good binding affinity against the molecular targets of the anti-inflammatory target, namely, ILAK4. Among the ligands docked against ILAK4, Nodakenin (14), and Apigenin 7-O-β-D-glucoside (16) showed excellent free energy binding with dock score values of –9.4 and –10 kcal/mol, respectively. These results suggest that further mechanistic studies are to be done for these phytoconstituents against anti-inflammation.

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