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ORIGINAL RESEARCH

Development of QSAR model for immunomodulatory activity of natural coumarinolignoids

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Abstract: Immunomodulation is the process of alteration in immune response due to foreign intrusion of molecules inside the body. Along with the available drugs, a large number of herbal drugs are promoted in traditional Indian treatments, for their immunomodulating activity. Natural coumarinolignoids isolated from the seeds of *Cleome viscose* have been recognized as having hepatoprotective action and have recently been tested preclinically for their immunomodulatory activity affecting both cell-mediated and humoral immune response. To explore the immunomodulatory compound from derivatives of coumarinolignoids, a quantitative structure activity relationship (QSAR) and molecular docking studies were performed. Theoretical results are in accord with the *in vivo* experimental data studied on Swiss albino mice. Immunostimulatory activity was predicted through QSAR model, developed by forward feed multiple linear regression method with leave-one-out approach. Relationship correlating measure of QSAR model was 99% ($R^2 = 0.99$) and predictive accuracy was 96% ($RCV^2 = 0.96$). QSAR studies indicate that dipole moment, steric energy, amide group count, lambda max (UV-visible), and molar refractivity correlates well with biological activity, while decrease in dipole moment, steric energy, and molar refractivity has negative correlation. Docking studies also showed strong binding affinity to immunomodulatory receptors.

Keywords: coumarinolignoids, immunomodulation, docking, QSAR, regression model

Immunomodulation is the process of alteration in immune response due to foreign intrusion of molecules inside the body. It can be either immunostimulative or immunosuppressive. Along with the available drugs, a large number of herbal drugs are mentioned in Ayurveda (a traditional system of Indian medicine), for their immunomodulating activity.[1–2] In the past, living and attenuated microorganisms' autologous and heterologous proteins and injections of animal organ preparations were used with the aim of restoring an impaired defense mechanism. At present thymus peptides and other biological response modifiers (BRM) (eg, interferon, interleukines), synthetic low molecular weight compounds (eg, Levamisole), chemically modified nucleotides, polysaccharides from fungi (eg, Lentinan), and, especially in Europe and Asia, some plant extracts, are also used for the same purpose.

Many medicinal plant products have been reported to show immunomodulatory effects, such as barberin, boswellic acid, aristolochic acid, cichoric acid, and plumbagin.[2] Natural coumarinolignoids are also among the biologically active compounds which have shown promising immunomodulatory activity affecting both cell mediated and humoral immune response.[3–5] Cleomiscosins are the natural coumarinolignoids extracted from an annual herb *Cleome viscosa* (syn. *C. icosandra*), a common weed of the family Capparidaceae and they have been used in the traditional systems of

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Indian medicine. Considerable phytochemical work on different parts of this plant have been studied well.[3–5] These are newly identified class of natural products in which a lignan group (C_6C_3 unit) is linked with a coumarin moiety through a dioxane bridge.[5] Coumarinolignoids belong to the cycloalkylpropanoic acid class of compounds. Attachment of a phenylpropane unit with a polyphenolic compound through a dioxin bridge was earlier witnessed in the flavono-lignoid, silybin, xanthono-lignoid, and kielcorin.[5] Cleomiscosins are the members of coumarino-lignoids and represent a new class of lignans called coumarinolignans. The isolated compounds showed immunomodulatory effect on Swiss albino mice, weighing 16–21g with LD_{50} value >100 μM/L for racemic mixture of three cleomiscosin molecules *viz.*, A, B, and C.[4]

In the present study, we screen out potential anti-inflammatory and immunomodulatory compound cleomiscosin-B from the isolated racemic mixture of three cleomiscosin isoforms through quantitative structure activity relationship (QSAR) and molecular docking studies. On the basis of binding affinity energy, possible immunomodulatory receptors were identified. For the structural activity relationship, a multiple linear QSAR regression model was developed which successfully establishes the immunomodulatory activity of coumarinolignoids in accord with the *in vivo* experimental data.[4] QSAR modeling also furnishes the activity dependent structural descriptors and predicts the effective dose of other derivatives, thereby suggesting the possible toxicity range. The relationship correlating measure of QSAR model was 99% (R^2 = 0.99) and predictive accuracy was 96% (RCV^2 = 0.96). Druggability of studied compounds was evaluated using Lipinsky's 'Rule of Five' and *in silico* ADME analysis through bioavailability filters. QSAR studies indicate that dipole moment, steric energy, amide group count, lambda max UV-visible, and molar refractivity correlate well with anti-inflammatory and immunomodulatory activity. These results could offer useful references for understanding mechanisms and directing the molecular design of lead compounds with improved immunomodulatory activity.

Materials and methods

Isolation and *in vivo* immunomodulatory activity of coumarinolignoids

The chemical and structural determination of studied coumarinolignoid derivatives from *C. viscosa* have been studied using IR Spectra and nuclear magnetic resonance (NMR) techniques. Isolation and *in vivo* anti-inflammatory and immunomodulatory activity of coumarinolignoids from *C. viscosa* seeds have been carried out in the past by Bawankule et al.[4] Anti-inflammatory and immunomodulatory activity of coumarinolignoids was studied in a lipopolysaccharide- (LPS) induced toxicity model in Swiss albino mice, weighing 16–21 g. Proinflammatory mediators such as cytokines, interleukin-6 (IL-6), or tumor necrosis factor-α (TNF-α) and nitric oxide (NO) were estimated from culture supernatant obtained from peritoneal macrophages stimulated by LPS and anti-inflammatory mediator IL-4 was estimated from culture supernatant obtained from spleenocytes stimulated by concavalin-A (Con-A). For further confirmation, expressions of inflammatory mediators from serum and mortality rate were studied in an LPS-induced toxicity model in mice. Proinflammatory mediator's expression was significantly decreased in the treatment group in a dose-dependent manner, whereas the anti-inflammatory mediator expression was significantly increased at 10 mg/kg treatment. Mortality rate was also significantly reduced in the treatment group in the LPS-induced toxicity model.[4]

Structure cleaning, optimization, and molecular docking

The structures of coumarinolignoid derivatives were constructed using the Scigress Explorer v7.7.0.47 (Fujitsu Ltd., Tokyo, Japan) workspace module. The optimization of the cleaned molecules was done through MO-G computational application that computes and minimizes an energy related to the heat of formation. The MO-G computational application solves the Schrodinger equation for the best molecular orbital and geometry of the ligand molecules. The augmented Molecular Mechanics (MM2/MM3) parameter was used for optimizing the molecules up to its lowest stable energy state. This energy minimization is done until the energy change is less than 0.001 kcal/mol or the molecules are updated almost 300 times. However, the chemical structures of known drugs were retrieved through the PubChem compound database at NCBI (http://www.pubchem.ncbi.nlm.nih.gov). Crystallographic 3D structures of Human's target proteins were retrieved through Brookhaven protein databank (http://www.pdb.org). The valency and hydrogen bonding of the ligands as well as target proteins were subsequently satisfied through the Workspace module. Hydrogen atoms were added to protein targets for correct ionization and tautomeric states of amino acid residues such as His, Asp, Ser, and Glu. Molecular docking of the drugs and the isolated coumarinolignoid derivatives, especially cleomiscosin molecules (A, B, and C), with the immunomodulatory receptors was done using the Fast-Dock-Manager and Fast-Dock-Compute engines available with the Project-leader module of Scigress Explorer (7.7.0.47; Fujitsu

Ltd., Tokyo, Japan). For automated docking of ligands into the active sites we used genetic algorithm with a fast and simplified Potential of Mean Force (PMF) scoring scheme.[6–7] PMF uses atom types which are similar to the empirical force fields used in Mechanics and Dynamics. A minimization is performed by the Fast-Dock engine which uses a Lamarkian genetic algorithm (LGA) so that individuals adapt to the surrounding environment. The best fits are sustained through analyzing the PMF scores of each chromosome and assigning more reproductive opportunities to the chromosomes having lower scores. This process repeats for almost 3,000 generations with 500 individuals and 100,000 energy evaluations. Other parameters were left to their default values. Structure-based screening involves docking of candidate ligands into protein targets, followed by applying a PMF scoring function to estimate the likelihood that the ligand will bind to the protein with high affinity or not.[7–8]

Selection of chemical descriptors for QSAR modeling

For identifying the immunomodulatory activity of the coumarinolignoid derivatives, QSAR study was performed. A total of 52 chemical properties (descriptors) were used for QSAR model development. A total of 61 drugs were involved and lethal dose was considered as the biological activity parameter of the compounds. Forward feed multiple linear regression mathematical expression was then used to predict the biological response of other derivatives. QSAR analysis is a mathematical procedure by which the chemical structures of molecules is quantitatively correlated with a well defined parameter, such as biological activity or chemical reactivity. For example, biological activity can be expressed quantitatively as in the concentration of a substance required to give a certain biological response. Additionally, when physicochemical properties or structures are expressed by numbers, one can form a mathematical relationship, or quantitative structure-activity relationship, between the two. The mathematical expression can then be used to predict the biological response of other chemical structures. QSAR's most general mathematical form is:

$$\text{Activity} = f(\text{physiochemical properties and/or structural properties})$$

A QSAR model attempts to find consistent relationships between the variations in the values of molecular properties and the biological activity for a series of compounds which can then be used to evaluate properties of new chemical entities.[9,23]

Before the novel compounds can be used as potential drugs, the prediction of toxicity/activity ensures the calculation of risk factors associated with the administration of that particular drug. A QSAR model ultimately helps in predicting these important parameters in the form of ED_{50} or LD_{50} values. Some of the important chemical descriptors used in multiple linear regression analysis are: atom count (all atoms), atom count (carbon), atom count (hydrogen), atom count (oxygen), bond count (all bonds), conformation minimum energy (kcal/mole), connectivity index (order 0, standard), connectivity index (order 1, standard), connectivity index (order 2, standard), dipole moment (debye), dipole vector X (debye), dipole vector Y (debye), dipole vector Z (debye), electron affinity (eV), dielectric energy (kcal/mole), steric energy (kcal/mole), total energy (Hartree), group count (amine), group count (carboxyl), group count (ether), group count (hydroxyl), group count (methyl), heat of formation (kcal/mole), HOMO energy (eV), ionization potential (eV), lambda max UV-visible (nm), lambda max far-UV-visible (nm), LogP, LUMO energy (eV), molar refractivity, molecular weight, polarizability, ring count (all rings), size of smallest ring, size of largest ring, and solvent accessibility surface area ($Å_2$).

In silico druggability and ADME

For analyzing druggability, Lipinski's rule of five pharmacokinetics filter was used as a drug likeness test.[9] Briefly, this rule is based on the observation that most orally administered drugs have a molecular weight (MW) of 500 or less, a logP no higher than 5, five or fewer hydrogen bond donor sites, and 10 or fewer hydrogen bond acceptor sites (N and O atoms). In addition, the bioavailability of all derivatives or test compounds was assessed through topological polar surface area analysis. We calculated the polar surface area (PSA) by using termed topological PSA (TPSA), based on the summation of tabulated surface contributions of polar fragments (ChemAxon-Marvinview 5.2.6:PSA plugin).[10] Polar surface area (PSA) is formed by the polar atoms of a molecule. This descriptor was shown to correlate well with passive molecular transport through membranes and therefore allows prediction of transport properties of drugs and has been linked to drug bioavailability. Generally, passively absorbed molecules with a PSA > 140 $Å^2$ are thought to have low oral bioavailability.[11] Calculation of other important absorption, distribution, metabolism, and excretion (ADME) properties of studied compounds was done through QikProp software (version 3.2; Schrödinger, LLC, New York, NY).

Results and discussion

Chemical structure-activity relationship (SAR)

In the present work, 12 derivatives of natural coumarinolignoids were evaluated for their anti-inflammatory and immunomodulatory activity through QSAR, ADME, and docking studies. Later results were compared with experimental *in vivo* activity data, which suggest that only three derivatives of coumarinolignoids (compound 1a, 1f, and 2a) have good anti-inflammatory and immunomodulatory activity. Results of the SAR study suggest in compound 1a (cleomiscosin-A), the phenolic and alcoholic-OH groups in its molecule are responsible for its activity.[3] The presence of a coumarin moiety based on other SAR studies on cleomiscosin-A (1a) has already been well established.[3] Cleomiscosin-C (1f) has an extra -OMe group in the phenylpropanoid unit, and thus it showed less activity. It was found that the resonances for compound-1f were in good agreement with those for 1a, rather than those for 2a (cleomiscosin-B), especially in the chemical shifts for C-7', C-8', and C-9', which were most affected by the structural difference between 1f and 2f. Compound 1f is a racemic compound and therefore has the same structural framework as 1a. Compound 2a (cleomiscosin-B) is the position isomeric compound of 1a and shows striking resemblance with 1a in all its spectral properties, indicating a close structural similarity, thus become most active derivative. The two oxide linkages in compound 2a are at C-7 and C-8 as in 1a (Figures 1–3). Later *in vivo* immunomodulatory biological activity of these compounds was tested on Swiss mice.[4] Since *in vivo* activity was done on the racemic mixture of cleomiscosin A, B and C, compounds (compound 1a, 1f, and 2a), in the present work we tried to explore the most active compound in the mixture based on QSAR modeling, molecular docking, and *in silico* ADME analysis. Results indicate that all compounds produce significant anti-inflammatory and immunomodulatory activity similar to that of the standard drug aristolochic acid. Compound 2a (cleomiscosin-B) exhibits strong anti-inflammatory and immunomodulatory activity, while compound 1a (cleomiscosin-A) exhibits the least activity. *In vivo* dose-dependent experimental data for immunomodulatory effect of studied compounds are summarized in Table 1.

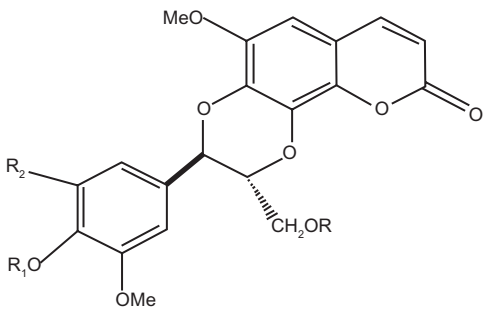
Quantitative structure-activity relationship (QSAR) modeling

Structure activity relationship has been denoted by QSAR model showing significant activity-descriptors relationship accuracy of 99% ($R^2 = 0.99$) and activity prediction accuracy of 96% ($RCV^2 = 0.96$). A total of 61 drugs were used for QSAR modeling against 52 chemical descriptors. Only five descriptors were found to be significant and seem to be responsible for *in vivo* immunomodulatory activity (Table 2). A forward feed multiple linear regression QSAR model was developed using leave-one-out approach for the prediction of biological activity of cleomiscosin molecules. Anti-inflammatory and immunomodulatory drugs fit well into this correlation, which intuitively seems very reasonable. Results indicate that variations in stereochemistry do not markedly affect the binding energy of ligand and receptor. Therefore, we looked for a simpler descriptor for the prediction of biological *in vivo* activity for studied class of compounds. QSAR studies indicate that dipole moment, steric energy, amide group count, lambda max (UV-visible), and molar refractivity correlate well with biological activity (Table 2). The QSAR mathematical model equation derived through multiple linear regression method is given below, showing relationship between *in vivo* experimental activity (LD_{50}) and dependent five chemical descriptors:

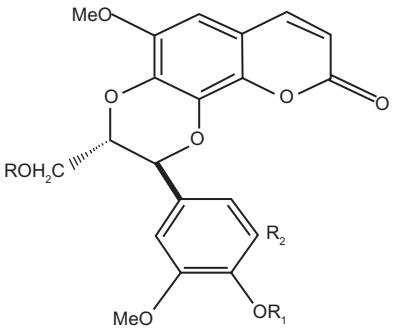
Predicted log LD_{50} (mg/kg) = –0.156436 * dipole moment (debye) –0.00118794 * steric energy (kcal/mole) +0.910351 * group count (amide) +0.0206362 * lambda max UV-visible (nm) –0.00834447 * molar refractivity –1.06753.

[$RCV^2 = 0.96$ (96%) and $R^2 = 0.99$ (99%)]

Since experimental *in vivo* activity was reported for racemic mixture of three cleomiscosin molecules A, B, and C (1a, 2a, and 1f) ie, 100 mg/kg (Table 1), we therefore aimed to predict the activity of each compound separately through QSAR modeling and identify the most active compound. We successfully developed the QSAR model for both anti-inflammatory and immunomodulatory activity. More than 50 known drugs with reported anti-inflammatory as well as immunomodulatory activity were included in the training data set for comparison and evaluation of prediction accuracy of QSAR model. Results showed that predicted activity of cleomiscosin molecules (A, B, and C) were comparable with experimental activity. Results indicate that cleomiscosin-B (2a) had higher immunomodulatory activity than cleomiscosin-C (1f) and cleomiscosin-A (1a). Moreover, based on the results of molecular docking, cleomiscosin-B showed much better binding energy with immunomodulatory receptors, and is therefore considered as the most active compound in the coumarinolignoids mixture. We also checked the compliance of isolated compounds to Lipinski's



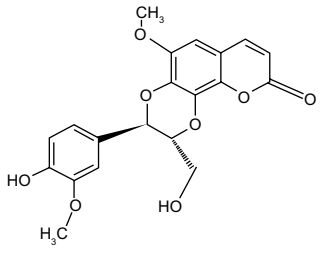
Prototype-1



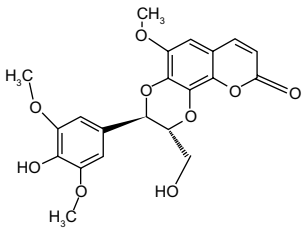
Prototype-2

Derivative	R	R1	R2	Name
1a	H	H	H	Cleomiscosin-A*
1b	H	Me	H	Monomethyl ether
1c	H	Et	H	Monoethyl ether
1d	Ac	Ac	H	Diacetate
1e	Ac	Et	H	Monoacetate
1f	H	H	OMe	Cleomiscosin-C*
2a	H	H	H	Cleomiscosin-B*
2b	H	Me	H	Monomethyl ether
2c	H	Et	H	Monoethyl ether
2d	Ac	Ac	H	Diacetate
2e	H	H	OH	Hydroxy derivative
2f	H	H	OMe	Methoxy derivative

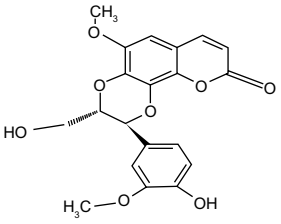
Figure 1 Molecular differences in different coumarinolignoids derivatives. Prototype 1 and 2 are showing fusion of coumarin moiety with the phenylpropanoid unit (C6C3). Bold face indicates active and isolated compounds. Asterisk indicates that compounds were isolated as racemic mixture.



1a Cleomiscosin A

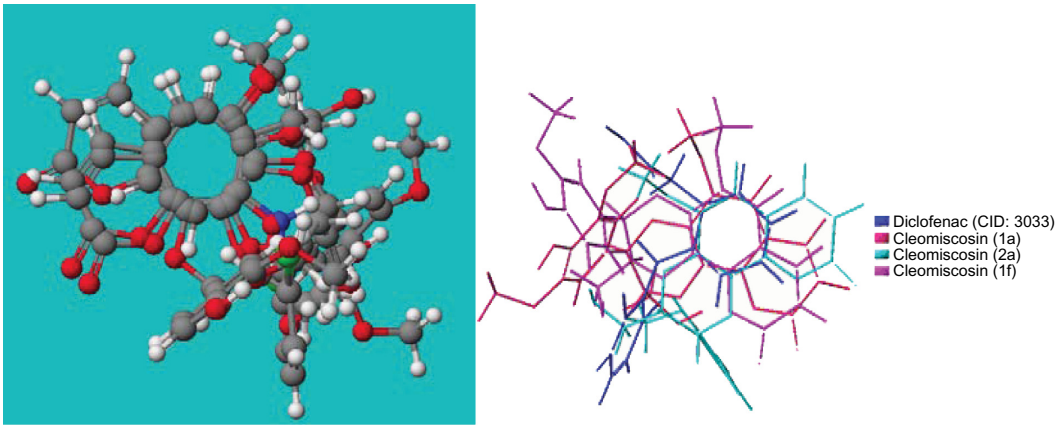


1f Cleomiscosin C



2a Cleomiscosin B

Figure 2 Molecular structure of the purified active natural coumarinolignoids isoforms 1a (Cleomiscosin-A), 1f (Cleomiscosin-C), and 2a (Cleomiscosin-B) isolated from the seeds of *C. viscosa*.



Compd.	Distance RMS (Å)			
	1a	2a	1f	Diclofenac
1a	0	1.612	1.173	0.7942
2a	1.612	0	1.893	1.3895
1f	1.173	1.893	0	1.390
Diclofenac	0.7942	1.306	1.390	0

Figure 3 Superimposition of most favorable conformations of compounds 1a, 2a, 1f, and diclofenac docked into binding site of COX-2 receptor showing common pharmacophore ring structure.

rule-of-five for drug likeness (Table 3). Results indicate that isolated compounds follow most of the ADME properties, thus leading to a good drug candidate for anti-inflammatory and immunomodulatory activity (Table 4). This helped in establishing the pharmacological activity of these isolated novel compounds for their use as potential drugs. Moreover, when we calculated the topological polar surface area (TPSA) as a chemical descriptor for passive molecular transport through membranes, results showed higher TPSA of isolated compounds than standard drugs but within acceptable range (Table 3). TPSA allows for prediction of transport properties of drugs and has been linked to drug bioavailability. Generally, it has been seen that passively absorbed molecules with a TPSA > 140 Å^2 are thought to have low oral bioavailability.[11] On the basis of bioavailability scores, we concluded that isolated compounds have marked immunomodulatory activity but lower bioavailability as compared to standard drugs. Isolated compound cleomiscosin-B (2a) and cleomiscosin-C (1f) showed comparatively low TPSA than cleomiscosin-A (1a).

Table 1 *In vivo* experimental anti-inflammatory and immunomodulatory activity data of isolated mixture of cleomiscosin A, B, and C molecules

Treatment (*in vivo* oral dose)	Response %	Mortality*
Vehicle control	0	0/6
LPS control	100	6/6
Coumarinolignoids (10 mg/kg) + LPS	17	1/6
Coumarinolignoids (30 mg/kg) + LPS	67	4/6
Coumarinolignoids (100 mg/kg) + LPS	50	3/6

Note: * = No. of female Swiss albino mice (n = 6).

Binding affinity of coumarinolignoids for immunomodulatory receptors

The effect of coumarinolignoids when studied in Swiss albino mice for anti-inflammatory and immunomodulatory activity showed a significant decrease in the expression of pro-inflammatory mediators such as IL-6, TNF-α, and nitric oxide in a dose-dependent manner. Also the expression of immunomodulatory mediator IL-4 was found to increase with cleomiscosin A, C, and B (1a, 1f, and 2a)

Table 2 Comparison of experimental and predicted *in vivo* activity data calculated through QSAR modeling based on the five most highly correlated chemical descriptors

Drug/compound	Exp LD_{50} (mg/kg)	Exp log LD_{50}	Pred log LD_{50}	Dipole moment (debye)	Steric energy (kcal/mol)	Group count (amide)	Lambda max UV-visible (nm)	Molar refractivity
Aristolochic acid	81	1.91	1.91	9.48	32.21	0	252.40	85.12
Azimexon	170	2.23	2.22	3.86	509.14	1	194.44	51.20
Bowellic acid	5000	3.70	3.70	2.61	114.53	0	311.39	133.70
Ciamexon	130	2.11	2.15	4.02	251.32	0	223.73	56.96
Cichoric acid	1750	3.24	3.24	4.50	−12.68	0	287.34	112.13
Emetin	32	1.51	1.50	1.91	39.04	0	197.41	139.75
Imemixon	150	2.18	2.16	5.94	134.83	0	220.48	28.47
Isopteropodin	162	2.21	2.22	5.07	40.63	1	196.12	99.27
Levamisol	180	2.26	2.26	4.12	27.81	0	218.54	60.74
Curcumin	2000	3.30	3.71	3.74	5.20	0	302.16	103.42
Celecoxib	2000	3.30	1.39	4.29	52.90	0	191.16	90.98
Calanolide	800	2.90	3.17	4.97	8.49	0	285.83	104.11
Acetylsalicylic	200	2.30	2.47	1.58	5.49	0	201.60	43.95
Cortisol	5120	3.71	2.07	3.42	75.13	0	221.89	97.49
Cyclophosphamide	200	2.30	2.39	4.01	−10.67	0	220.69	58.48
Cleomiscosin-A	100*	2.00	1.50	6.28	5.29	0	211.49	97.37
Cleomiscosin-B			1.44	4.27	5.87	0	193.71	97.37
Cleomiscosin-C			1.49	5.18	5.76	0	205.71	103.84
Diclofenac		2.59	2.30	1.02	51.40	0	204.28	75.46

*Anti-inflammatory and immunomodulatory activity data for racemic mixture of cleomiscosin-A, B, and C.

administration. The expressions of inflammatory mediators from serum and mortality rate were studied in an LPS-induced acute inflammation model.[4] We predicted the orientations and binding affinities of caumarolignoids with proinflammatory proteins and others with the aim of determining which units interact better. We know that the innate immune recognition is mediated by a structurally diverse set of receptors that belong to several distinct protein families. Among them are humoral proteins circulating in the plasma, endocytic receptors expressed on the cell surface, and signaling receptors that can be expressed either on the cell surface or intracellularly.[12] The proinflammatory cytokines such as IL-1, IL-6, or TNF-α have been found to contribute to a variety of inflammatory condition such as ischemic tolerance,[13] rheumatoid arthritis,[14] nephritis,[15] and liver diseases.[16] Nitric oxide generated through inducible NO synthase (iNOS) enzymatic activity has been found to be participating in various

Table 3 Compliance of compounds with computational parameters of drug likeness

S No.	Compd	TPSA (Å^2)	Molecular weight	Log P	H-bond donors (OH group)	H-bond acceptors (O atom)	No. of rule of five violations
1.	Diclofenac	49.33	296.152	3.965	0	2	0
2.	1a	91.29	386.357	1.822	2	8	0
3.	1b	74.22	400.384	1.853	1	8	0
4.	1c	83.45	414.411	2.196	1	8	0
5.	1d	106.59	502.431	2.276	0	12	2
6.	1e	63.22	472.448	2.596	0	10	0
7.	1f	83.45	416.384	1.569	2	9	0
8.	2a	83.45	386.357	1.821	2	8	0
9.	2b	72.45	400.384	1.853	1	8	0
10.	2c	72.45	414.411	2.196	1	8	0
11.	2d	44.76	502.431	2.276	0	12	2
12.	2e	103.68	402.357	1.537	3	9	0
13.	2f	92.68	416.384	1.569	2	9	0
14.	3	101.91	460.48	2.554	0	9	0
15.	4	112.91	448.469	1.886	1	9	0
16.	5	106.91	386.357	1.722	2	8	0

Table 4 Compliance of compounds with computational parameters of ADME

Principal descriptors	Levamisole	Aristolochic acid	Cleomiscosin-A	Cleomiscosin-B	Cleomiscosin-C	(Range 95% of drugs)
Solute molecular weight	204.289	341.276	386.357	386.357	416.384	(130.0/725.0)
Solute dipole moment (D)	5.344	14.344*	8.366	10.631	7.109	(1.0/12.5)
Solute total SASA	427.221	503.667	591.932	598.957	619.895	(300.0/1000.0)
Solute hydrophobic SASA	161.747	156.135	233.265	223.883	282.155	(0.0/750.0)
Solute hydrophilic SASA	26.205	164.026	177.359	163.462	173.297	(7.0/330.0)
Solute carbon pi SASA	191.048	183.506	181.307	211.612	164.443	(0.0/450.0)
Solute weakly polar SASA	48.22	0	0	0	0	(0.0/175.0)
Solute molecular volume (A^3)	698.974	900.195	1084.061	1076.777	1161.989	(500/2000)
Solute vdW Polar SA (PSA)	17.591	110.831	117.094	111.88	125.462	(7.0/200.0)
Solute No. of rotatable bonds	0	3	5	5	6	(0.0/15.0)
Solute as donor – hydrogen bonds	0	1	2	2	2	(0.0/6.0)
Solute as acceptor – hydrogen bonds	2	5.25	7.95	7.95	8.7	(2.0/20.0)
Solute globularity (sphere = 1)	0.892	0.895	0.862	0.848	0.862	(0.75/0.95)
Solute ionization potential (eV)	8.874	9.345	8.951	8.835	8.854	(7.9/10.5)
Solute electron affinity (eV)	0.381	2.484*	1.546	1.393	1.681	(–0.9/1.7)
Polarizability (angstroms^3)	23.643 M	29.698 M	35.751	35.749	38.067	(13.0/70.0)
log P for hexadecane/gas	6.610 M	9.430 M	11.315	11.330	11.880	(4.0/18.0)
log P for octanol/gas	8.972	17.260 M	19.997	20.480	20.872	(8.0/35.0)
log P for water/gas	4.212	9.269 M	13.240	13.369	13.683	(4.0/45.0)
log P for octanol/water	3.108	2.395	1.723	1.798	1.913	(–2.0/6.5)
log S for aqueous solubility	–3.476	–3.281	–3.553	–3.664	–3.613	(–6.5/0.5)
log S – conformation independent	–3.064	–5.011	–4.962	–4.962	–5.262	(–6.5/0.5)
log K hsa serum protein binding	0.112	–0.194	–0.12	–0.15	–0.099	(–1.5/1.5)
log BB for brain/blood	0.462	–0.982	–1.329	–1.252	–1.352	(–3.0/1.2)
No. of primary metabolites	2	2	5	5	6	(1.0/8.0)
Predicted CNS activity	++	–	–	–	–	–2 (inactive), +2 (active)
HERG K^+ channel blockage: log IC50	–4.198	–2.296	–4.627	–4.997	–4.545	(concern below –5)
Apparent Caco-2 permeability (nm/sec)	5589	69	206	279	225	(<25 poor, >500 great)

(*Continued*)

Table 4 (*Continued*)

Principal descriptors	Levamisole	Aristolochic acid	Cleomiscosin-A	Cleomiscosin-B	Cleomiscosin-C	(Range 95% of drugs)
Apparent MDCK permeability (nm/sec)	5839	35	89	124	98	(<25 poor, >500 great)
QP log Kp for skin permeability	–3.392	–3.608	–3.669	–3.307	–3.558	(–8.0 to –1.0, Kp in cm/hr)
Jm, max transdermal transport rate	0.028	0.058	0.023	0.041	0.028	(micrograms/ cm^2-hr)
Lipinski rule of 5 violations	0	0	0	0	0	(maximum is 4)
Jorgensen rule of 3 violations	0	0	0	0	0	(maximum is 3)
% Human oral absorption in GI (±20%)	100	74	78	81	80	(<25% is poor)
Qual. model for human oral absorption	HIGH	HIGH	HIGH	HIGH	HIGH	(>80% is high)

Note: *Indicates a violation of the 95% drug likeness range.

immune and inflammatory reactions. Immunomodulatory cytokines like IL-4, IL-10, and IL-13 are responsible for inhibiting the proinflammatory signaling and hence reduce inflammation. Recent advances in the studies of innate immunity have yielded better understanding of inflammatory mechanisms.

Toll-like receptors (TLRs) have been found to recognize and respond to the moieties related to tissue injury and microbial infections.[17] TLRs are mediators of various cell mediated and humoral immune response caused by different agents or TLR specific ligands. Different TLRs have been found to respond to variety of pathogen-associated molecular pattern (PAMP) such as microbial agents, viral proteins, RNA, CpG DNA, bacterial lipopolysaccharides (LPS), and peptidoglycan. Signaling through TLRs results in inflammatory reactions mediated by various cytokines such as TNF-α, IL-6, IL-8, and IL-1ß. The inhibitors of TLR mediated signaling of inflammatory reactions are the decoy receptors, signaling inhibitors, and immunomodulatory cytokines (IL-4, IL-10, and IL-13).[13–18] Additionally, cluster of differentiation (CD) plays a very important role in the various immunological cascades of reactions and acts as a costimulatory signaling molecule for the activation of several lymphocytes. This activity is responsible for producing numerous immune responses such as production of T-helper cells, T-cytotoxic cells, macrophage activation, and antibody production.[19–22] Results of molecular docking were comparable with the experimental results,[4] which suggest that proinflammatory mediator expression was significantly decreased in the coumarinolignoids treatment group in dose dependent manner. This suggests that oral administration of coumarinolignoids inhibits the proinflammatory mediators and enhances the production of the immunomodulatory mediator (Table 5; Figures 4–6).

Conclusion

Molecular modeling calculations accompanied by *in vivo* experimental data on Swiss albino mice were used to predict potential immunomodulatory compounds among natural coumarinolignoids namely, cleomiscosin-A (1a), cleomiscosin-C (1f), and cleomiscosin-B (2a) isolated from *C. viscosa* seeds. The obtained results indicate that all studied compounds possess significant anti-inflammatory and immunomodulatory activity after oral administration and that cleomiscosin-B possess higher immunomodulatory activity comparable to standard drugs eg, Levamisole and cyclophosphamide. The QSAR analysis established the immunostimulatory activity of the cleomiscosin molecules in a dose dependent manner, which is in accord with the *in vivo* data. Results of molecular docking combined with *in vivo* data for inhibition of the human proinflammatory mediators suggest that compound cleomiscosin-B is preferentially more active than others with strong binding affinity to most of the immuno-modulatory receptors.

Table 5 Molecular docking based identification of potential immunomodulatory targets of cleomiscosin molecules

Coumarinolignoids	Potential target
Cleomiscosin A (1a)	TLR-4
Cleomiscosin B (2a)	iNOS, COX-2, CD14, IKK ß
Cleomiscosin C (1f)	CD86, COX-1

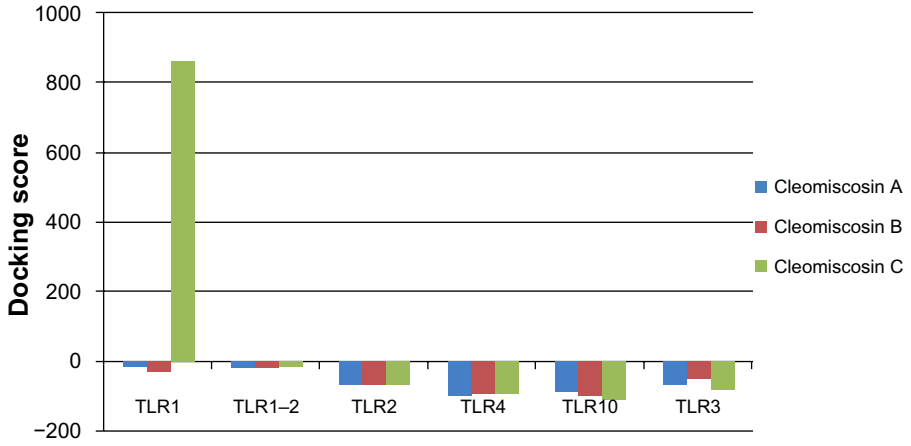


Figure 4 Binding affinity of cleomiscosin A, B, and C against toll-like receptors (TLRs). Docking scores (kcal/mol) in negative are acceptable.

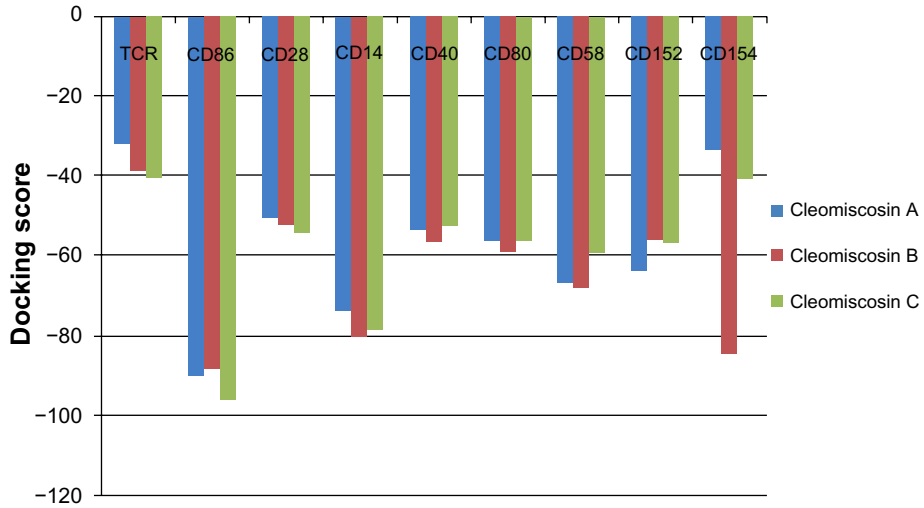


Figure 5 Binding affinity of cleomiscosin A, B, and C with various cluster of differentiation molecules (CD molecules) and T-cell receptor proteins. Negative docking scores (kcal/mol) are acceptable.

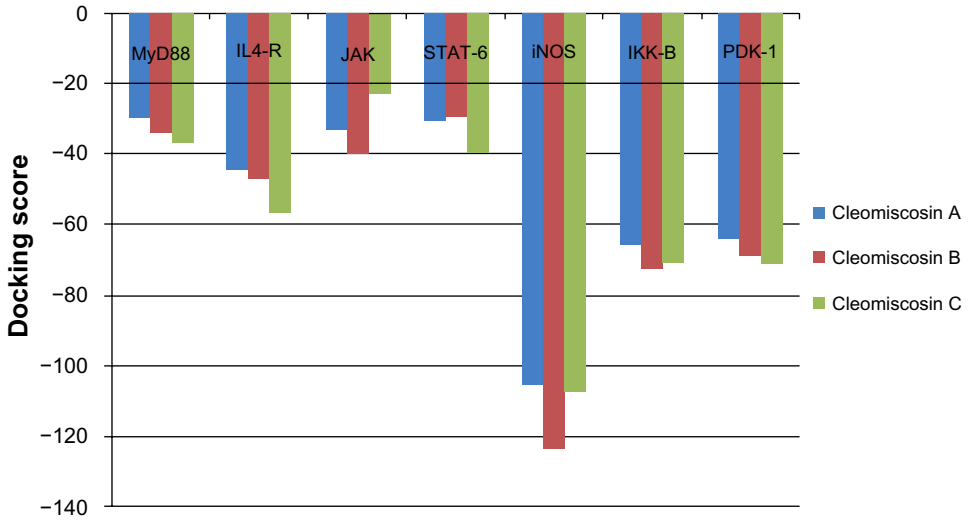


Figure 6 Binding affinity of cleomiscosin A, B, and C with various immune reaction cascade proteins and inducible nitric oxide synthase (iNOS) protein. Negative docking scores (kcal/mol) are acceptable.

Disclosure

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Appendix

Appendix 1 List of training data set of drugs/compounds used in QSAR modeling. Anti-inflammatory and immuno-stimulatory drugs/compounds. Asterisk mark indicates that compounds retrieved from PubChem database, NCBI, USA (www.pubchem.ncbi.nlm.nih.gov)

S No.	Compound	Reference* (PubChem ID)
1.	Aristolochic acid	CID: 2236
2.	Cichoric acid	CID: 5281764
3.	Indometacin	CID: 3715
4.	Plumbagin	CID: 10205
5.	Berberine	CID: 2353
6.	Emetin	CID: 10219
7.	Isopteropodin	CID: 122813
8.	Bowellic acid	CID: 168928
9.	Gelsemin	CID: 6713959
10.	Azimexon	CID: 47294
11.	Ciamexon	CID: 71759
12.	Imemixon	CID: 68791
13.	Methyl inosin monophasphate	CID: 454158
14.	Diethyl dithiocarbamate	CID: 28343
15.	Levamisol	CID: 26879
16.	Urushiol	CID: 5478166
17.	Ubiquinone	CID: 4462
18.	Saikosaponin	CID: 11968912
19.	Tabernanthine	CID: 442136
20.	Helenalin	CID: 23205

Appendix 2 List of training data set of drugs/compounds used in QSAR modeling. Immuno-suppressive drugs/compounds

S No.	Drug/compound	Reference
1.	5-Fluorouracil	Drug bank database
2.	Cytarabine hydrochloride	Drug bank database
3.	Busulfan	Drug bank database
4.	Thalidomide	Drug bank database
5.	Saikosaponin	http://articles.directorym.com/chai_hu_-a853270.html
6.	Methyl cellosolve	Drug bank database
7.	Butenolide	Moniliformin and butenolide: effect on mice of high-level, long-term oral intake pdf
8.	Methoxyacetic acid	Drug bank database
9.	Carboxylic acid	http://www.maximpowercorp.com/_pdf/deerland%20project/alberta%20environment/EPEAdeerlandapplication_appendix%20G_16oct07.pdf
10.	Azodicarbonamide	http://www.sciencelab.com/xMSDS-azodicarbonamide_F_C_C-9922989
11.	Cyclophosphamide monohydrate	http://www.sciencelab.com/xMSDS-cyclophosphamide_monohydrate-9923635
12.	Gemcitabine/gemzar	http://www.flexyx.com/g/gemcitabine%20hcl.html
13.	Rapamycin (sirolimus)	www.fermentek.co.il/MSDS/rapamycin-MSDS.htm
14.	Tacrolimus fujimycin	Drug bank database
15.	Melphalan	Drug bank database
16.	Dimethyl sulphate	www.chembargains.com/attachfile/msds-100234.doc
17.	Thalidomide	http://msds.chem.ox.ac.uk/TH/thalidomide.html
18.	Radanil/benznidazol	http://wisda.pharmazie.uni-marburg.de/index.html?http&&&wisda.pharmazie.uni-marburg.de/dossiere.php?s_inn=benznidazol
19.	Mizoribine	http://www.msdshazcom.com/web_docs/emd/doc/wcd00008/wcd008a9.pdf
20.	Nitrosodimethyl urea	http://hazard.com/msds/tox/f/q140/q167.html
21.	Azaserine	http://www.msdshazcom.com/WEB_DOCS/EMD/docs/wcd0000b/wcd00b3d.pdf
22.	Thiotepa	http://wfldelearn.pssd.com/binderview_PSS/vault/001/001100.pdf. In the search for new anticancer drugs/Sosnovsky G, Li SW
23.	Cytarabine hydrochloride	http://msds.chem.ox.ac.uk/CY/cytarabine_hydrochloride.html
24.	Cytarabine/cylocide	http://www.labseeker.com/chemicalbiotech/chem-moreinfo. asp? catalog_ no =21384
25.	Cytoxan	http://www.pfeist.net/ALL/cytoxan.html
26.	Coformycin (deoxy-coformycin)	Purine metabolism in adenosine deaminase deficiency chapter authors: H Anne Simmonds, A Sahota, CF Potter, D Perrett, K Hugh-Jones, JG Watson
27.	Thioinosine	http://www.coleparmer.in/catalog/msds/26568.htm
28.	Leflunomide	Drug bank database
29.	Dibutyltin dichloride	http://www.aladdin-reagent.com/msds/18969.htm
30.	Azathioprine/imuran	Drug bank
31.	Mycophenolate mofetil	Drug bank
32.	Triamcinolone acetonide	http://msds.chem.ox.ac.uk/TR/triamcinolone_acetonide.html
33.	Lantadin/deflazacort	http://www.labseeker.com/cn/chemicalbiotech/chemmoreinfo.asp?catalog_no = 23945

Appendix 2 (*Continued*)

S No.	Drug/compound	Reference
34.	D-amethopterin hydrate/ D-methotrexate	http://www.aladdinreagent.com/msds/18362.htm
35.	Glimepiride	http://wisda.pharmazie.uni-arburg.de/ http://www.labseeker.com/chemical biotech/chem-moreinfo.asp?catalog_no = 26445
36.	Gusperimus	themerckindex.cambridgesoft.com/themercklndex/themercklndex/reversed/M0004582.txt
37.	Ledertrexate	http://www.flexyx.com/L/Ledertrexate.html
38.	Hexabutylditin oxide	https://fscimage.fishersci.com/msds/08596.htm
39.	Tetrandrine	Pharmacology and applications of Chinese materia medica books. Google.co.in/books?Isbn 9810236921
40.	Pheanthine	http://www.chemdrug.com/databases/13_0_vwdsmntgaekxfsfg.html http://www.chemcas.com/msds/cas/msds87/1263–79–2_v2.asp
41.	Methotrexate	Drug bank database

List of chemical descriptors used in QSAR modeling

1. Atom count (all atoms)
2. Bond count (all bonds)
3. Conformation minimum energy (kcal/mole)
4. Connectivity index (order 0, standard)
5. Connectivity index (order 1, standard)
6. Connectivity index (order 2, standard)
7. Dipole moment (debye)
8. Dipole vector x (debye)
9. Dipole vector y (debye)
10. Dipole vector z (debye)
11. Electron affinity (ev)
12. Dielectric energy (kcal/mole)
13. Steric energy (kcal/mole)
14. Total energy (Hartree)
15. Group count (aldehyde)
16. Group count (amide)
17. Group count (amine)
18. Group count (sec-amine)
19. Group count (carbonyl)
20. Group count (carboxyl)
21. Group count (carboxylate)
22. Group count (cyano)
23. Group count (ether)
24. Group count (hydroxyl)
25. Group count (methyl)
26. Group count (methylene)
27. Group count (nitro)
28. Group count (nitroso)
29. Group count (sulfide)
30. Group count (sulfone)
31. Group count (sulfoxide)
32. Group count (thiol)
33. Heat of formation (kcal/mole)
34. HOMO energy (eV)
35. Ionization potential (eV)
36. Lambda max visible (nm)
37. Lambda max UV-visible (nm)
38. Lambda max far-UV-visible (nm)
39. Lambda max far-UV-visible (nm)
40. Log P
41. LUMO energy (eV)
42. Molar refractivity
43. Molecular weight
44. Polarizability
45. Ring count (all rings)
46. Size of smallest Ring
47. Size of largest Ring
48. Shape index (basic kappa, order 1)
49. Shape index (basic kappa, order 2)
50. Shape index (basic kappa, order 3)
51. Solvent accessibility surface area (angstromsquare)
52. Formal charge

Details of some important descriptors

1. **Molecular formula (MF):** The molecular formula of the molecule.
2. **Molecular weight (MW):** The molecular weight of the molecule.
3. **Log P:** The octanol-water partition coefficient.
4. **Solvent accessible surf area (SASA):** The molecular surface area accessible to a solvent molecule.
5. **Polarizability (P):** The molecule's average alpha polarizability.
6. **Shape index order 3 (SI3):** A topological index quantifying the shape of a chemical sample. The shape index of order 3 (Kappa 3) quantifies the degree of branching toward the center of the chemical sample.

7. **Shape index order 2 (SI2):** A topological index quantifying the shape of a chemical sample. The shape index of order 2 (Kappa 2) quantifies the degree of linearity or star-likeness of the chemical sample.
8. **Shape index order 1 (SI1):** A topological index quantifying the shape of a chemical sample. The shape index of order 1 (Kappa 1) quantifies the number of cycles in the chemical sample.
9. **Largest ring size (LRS):** The number of atoms forming the largest ring in the chemical sample, or 0 if the chemical sample contains no ring of size 12 or less.
10. **Smallest ring size (SRS):** Information about rings present in the compound. Rings with more than 12 atoms are ignored. The number of atoms forming the smallest ring in the compound, or 0 if the compound contains no ring of size 12 or less.
11. **Ring count (RC):** The number of rings present in the compound. Rings with more than 12 atoms are ignored. The number of rings with 12 or fewer atoms (All = all aromatic, small, 5-membered, 5-membered aromatic, 6-membered, 6-membered aromatic, large, large aromatic).
12. **Molar refractivity (MR):** The molar refractivity of the compound.
13. **LUMO energy:** The energy gained when an electron is added to the lowest unoccupied molecular orbital (LUMO).
14. **Lambda max far-UV-visible (LMFUV):** The maximum absorption line in the far UV-visible spectrum (150–1000 nm).
15. **Lambda max-UV-visible (LMUV):** The maximum absorption line in the UV-visible spectrum (190–1000 nm).
16. **Ionization potential (IP):** The energy required to remove an electron from a molecule in its ground state.
17. **HOMO energy:** The energy required to remove an electron from the highest occupied molecular orbital (HOMO).
18. **Heat of formation (HF):** The energy released or used when a molecule is formed from elements in their standard states.
19. **Conformation minimum energy (CME):** Energy calculated for an optimized conformation of the compound.
20. **Formal charge (FC):** The net positive or negative charge on the molecule. **Atom count (AC):** The number of atoms.
21. **Bond count (BC):** The number of bonds. Weak and ionic bonds are ignored.