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Molecular Docking Study on CD14 Receptor of Human Macrophage Cells by Screening Active Compounds Present in *Clerodendrum Inerme*

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Abstract

CD14 is a key cell surface receptor present on macrophage cells which play a major role in immune functions. CD14 along with TLR 4 activate NF-κB inflammatory pathway, plays a role in innate immunity and host defence, and induce proinflammatory cytokines and much more. CD14 receptor on monocytes has been linked to the activation of monocytes and its implication in the conversion of monocytes into macrophages. The monocyte-macrophage cell lineage is increasingly recognized as a major player in acute and chronic allograft immunopathology. This study aims to identify the various binding capabilities of bioactive compounds present in *Clerodendrum inerme* on the CD14 receptor to identify possible leads to a new immunosuppressive drug lead. The CD14 receptors 3D structure was downloaded from RCSB PDB (PDB ID: 4GLP). Molecular docking studies were performed using iGEMDOCK module and the Absorption Distribution Metabolism Excretion Toxicity (ADMET) properties of the best molecule that fits with CD14 receptor was predicted using admetSAR database. The protein-ligand interaction was visualized using Biovia DSV. The interaction of the extracted compounds showed their Immunosuppressive properties against CD14 Receptor which could be used for further analysis to inhibit immune function of monocyte-macrophage.

Keywords: admetSAR; CD14 Receptor; *Clerodendrum inerme*; Monocytes; Molecular Docking; iGEMDOCK; Biovia DSV; Immunosupression.

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1. Introduction

CD14 is or cluster of differentiation 14 is a surface protein present mainly on macrophages and to a lesser degree on neutrophils and dendritic cells. CD14 is a member of the family of leucine-rich repeat (LRR) proteins with widely disparate functions that also include the TLRs [1]. The CD14 receptor is a pattern recognition molecule in the innate immune response against microorganisms and other exogenous and endogenous stress factors. The most important CD14 signalling co-receptor is toll-like receptor 4 (TLR4), which activates, among others, the nuclear factor-kB (NF-kB) inflammatory pathway [2]. The diversity of activities regulated by CD14 suggests that the coreceptor may be a good target for therapeutic intervention, either for inhibition of hyperactivation of macrophages [3]. The monocyte-macrophage cell lineage is increasingly recognized as a major player in acute and chronic allograft immunopathology.

Monocytes and macrophages contribute to alloimmunity via diverse pathways such as antigen processing and presentation, costimulation, pro-inflammatory cytokine production, and tissue repair [5]. There are many drugs which exhibit inhibition of CD14,

those drugs offer a novel therapeutic strategy for chronic inflammatory and autoimmune diseases characterized by inappropriate monocyte deployment or function [4]. *Clerodendrum inerme* also known as *Volkameria inermis*, is a species of flowering plant in the genus *Volkameria* of the family *Lamiaceae*, found in India and other tropical countries. This plant is commonly used as an ornamental plant for creating hedges and other landscaping purposes. *Clerodendrum inerme* also has found to possess many bioactivities such as hepatoprotective activity [6], antifungal activity [7], antidiabetic activity, antimicrobial, antiparasitic and many other pharmacologically important activities [8]. This study aims to identify the various compounds present in the methanolic extract of *Clerodendrum inerme* which have the capability of binding with CD14 protein receptor and estimating its immune-modulatory activity.

2. Materials and methods

2.1 Gas Chromatography-Mass Spectrophotometer (GC-MS) analysis

GC-MS analysis was carried out according to [12] for the methanolic extract of *Clerodendrum inerme* to identify the extracted phytocompounds. The Gas Chromatogram (Clarus 680) was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were the transfer line temperature of 240 °C, ion source temperature of 240 °C and ionization mode electron impact at 70 eV, scan time 0.2 sec and scan 0.1 sec. The fragments were obtained from 40 to 600 Da. The spectra of the obtained components were compared with the database stored in the GC-MS NIST (2008) library

2.2 Preparation of the Protein Structure

The protein receptor for the docking studies was obtained from the RSCB PDB (Protein Data Bank) at 4.002 Å root mean square deviations (RMSD) resolution which represents a three-dimensional structure of target CD14 receptor (PDB: ID 4GLP) which is a cell surface protein. The ligands and crystallographic water molecules were removed from the protein and the chemistry of the protein was corrected for missing hydrogen. Following the above steps of preparation, the protein was subjected to energy minimization using the Universal Force Field (UFF).

2.3 Ligand Preparation

The ligand molecules for the docking process were prepared from the methanolic extract of *Clerodendrum inerme* using GC-MS Spectroscopy. The extracted compounds were then analysed and the 3-dimensional structure of the compounds was obtained from the PubChem databases (Table 1). The structure of the compounds was downloaded in (.sdf) format and they were converted into (.mol2) format by using open babel software [13].

2.4 Docking

Docking software iGEM dock was used to dock the human CD14 surface protein with the prepared ligands. iGEMDOCK is an integrated virtual screening (VS) environment from preparations through post-screening analysis with pharmacological interactions [14]. iGEMDOCK provides interactive interfaces to prepare both the binding site of the target protein and the screening compound library. Each compound in the library is then docked into the binding site by using the in-house docking tool iGEMDOCK. Subsequently, iGEMDOCK generates protein-compound interaction profiles of electrostatic (E), hydrogen-bonding (H), and Vander Waal's (V) interactions. Based on these profiles and compound structures, iGEMDOCK infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis. Finally, iGEMDOCK ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring function of iGEMDOCK [15]. The energy penalty was set to 100, RMSD threshold was 2.00 and RMSD calculation by atom ID was set. Docking was conducted between Protein and Inhibitor which results in binding affinities in kcal/mol and docking run time. The compound which gives lowest binding energy is chosen as the best inhibitor.

2.5 Interaction Visualization

The output of the docking procedure will give out the best pose, which is the conformation which best binds with the protein; this best pose can be visualized with the protein. Biovia Discover Studio Visualizer is the visualization software being used. This software allows identifying the various types of bonds being formed between the ligand and the protein, shows 2-Dimentional visualization of compound and bonds, the distance between the bonds and the various amino acids involved in the bonding process. [16]

2.6 ADMET Tools

The adverse properties such as Absorption, Distribution, Metabolism, Excretion and the Toxicity of the compounds are predicted using admetSAR database. They provide the latest and most comprehensive manually curated data for diverse chemicals associated with known ADMET profiles [17].

Table 1: Compounds present in methanolic extract of Clerodendrum inerme

S. No	RT	Name		Molecular Formula	MW g/mol
		Common	IUPAC		
1	9.72	Tricyclo[4.2.0.0(2,4)]octan-5-one	Tricyclo[4.2.0.0(2,4)]octan-5-one, (1.alpha.,2.beta.,4.beta.,6a)-	$C_8H_{10}O$	122.16
2	18.28	oleic acid	Elaidoic Acid	$C_{18}H_{34}O_2$	282.5
3	8.17	2-Methylnorbornane	Bicyclo[2.2.1]heptane, 2-methyl-	C_8H_{14}	110.2
4	10.9	Terpinen-4-ol	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	$C_{10}H_{18}O$	154.25
5	13.3	3-Isopropylbenzoic acid	Benzoic acid, 3-(1-methylethyl)-	$C_{10}H_{12}O_2$	164.2
6	14.08	2,4-Hexadienedioic acid, dimethyl ester, (E, Z)-	2,4-Hexadienedioic acid, dimethyl ester, (E, Z)-	$C_8H_{10}O_4$	170.16
7	14.88	Caryophyllene	beta-caryophyllene	$C_{15}H_{24}$	204.35
8	15.53	Isoflavone	3-phenyl-4H-chromen-4-one	$C_{15}H_{10}O_2$	222.24
9	15.92	6-Methoxyflavone	4H-1-Benzopyran-4-one, 6-methoxy-2-phenyl-	$C_{16}H_{12}O_3$	252.26
10	16.8	Methyl palmitate	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5
11	17.32	1-Eicosene	1-EICOSENE	$C_{20}H_{40}$	280.5
12	18.5	Methyl isostearate	16-Methylheptadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.5
13	19.03	2-(3,4-Dimethoxyphenyl)-7-hydroxy-4-chromanone	2-(3,4-Dimethoxyphenyl)-7-hydroxy-4-chromanone	$C_{17}H_{16}O_5$	300.3
14	20.67	11-Methylenetricosane	1-Tetradecene, 2-decyl-	$C_{24}H_{48}$	336.6
15	22.45	2,4-Tricosanedione	Tricosane-2,4-dione	$C_{23}H_{44}O_2$	352.6
16	25.08	3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione	3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione	$C_{20}H_{18}O_6$	354.4

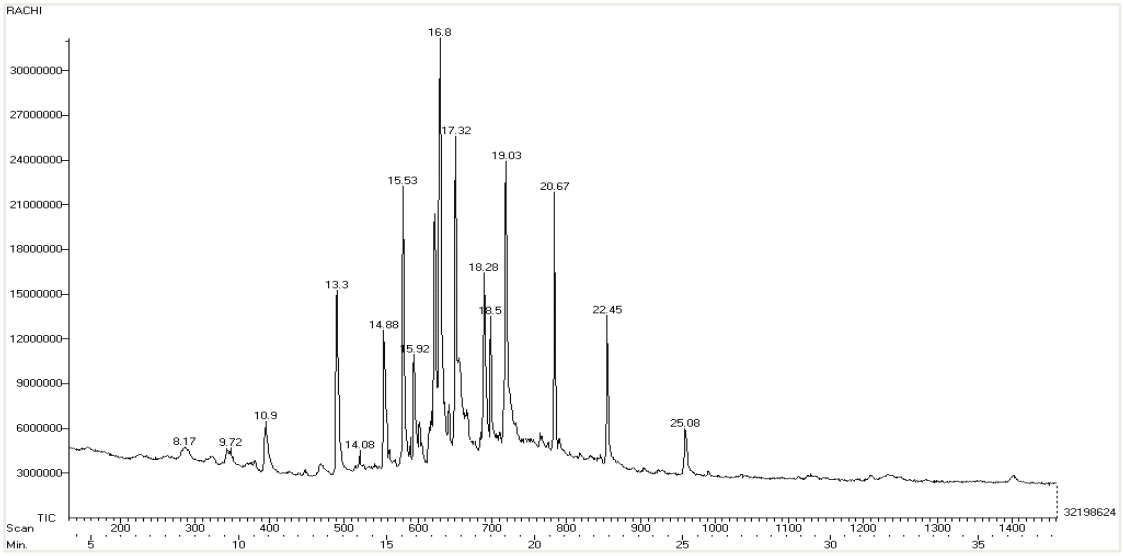

Figure 1: Gas Chromatogram for the methanolic extract of *Clerodendrum inerme*

Table 2: Interaction Profile of protein-ligand interaction

#Ligand	Total Energy	VDW	H Bond	Elec	Aver Con Pair
CD14-11-Methylenetricosane 2d-1.pdb	-122.29	-122.29	0	0	31.375
CD14-methyl isoesterate 2d-1.pdb	-113.964	-113.964	0	0	35.5238
CD14-1-Eicosene-1.pdb	-113.143	-113.143	0	0	35.55
CD14-2,4-Tricosanedione-1.pdb	-112.448	-103.734	-8.71384	0	28.12
CD14-3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-1.pdb	-106.712	-95.533	-11.1791	0	26.7692
CD14-6-Methoxyflavone-0.pdb	-93.5116	-88.9964	-4.51522	0	35.9474
CD14-Methyl palmitate-1.pdb	-89.3329	-80.8329	-8.5	0	31.3158
CD14-2-(3,4-Dimethoxyphenyl)-1.pdb	-86.7987	-74.6333	-12.1654	0	23.5455
CD14-Isoflavone-1.pdb	-83.4618	-83.4618	0	0	29.3529
CD14-oleic acid-0.pdb	-74.9905	-62.0737	-12.9403	0.0235	21.9
CD14-2,4-Hexadienedioic acid-0.pdb	-71.8858	-51.8665	-20.0193	0	34.9167
CD14-Terpinen-4-ol-1.pdb	-66.2045	-62.7045	-3.5	0	34.6364
CD14-Caryophyllene-1.pdb	-61.2445	-61.2445	0	0	26.4667
CD14-3-Isopropylbenzoic acid-0.pdb	-61.234	-56.614	-4.61996	0	34.9167
CD14-Tricyclo[4.2.0.0(2,4)]octan-5-one-1.pdb	-57.5538	-51.7983	-5.75557	0	36.4444
CD14-2-Methylnorbornane-1.pdb	-52.1902	-52.1902	0	0	38.625

3. Results and Discussion

3.1 Gas Chromatography-Mass Spectrophotometer (GC-MS) analysis

The GC-MS analysis of a methanolic extract of *Clerodendrum inerme* yielded 16 different compounds; the gas chromatogram for the fraction is shown in Figure: 1.

3.2 Docking

Molecules selected from the plant source were docked using iGEMDOCK software and docked scores of those molecules were represented in (Table 2), with their binding energy, Vanderwaal energy, electrostatic and hydrogen bond profiles. Binding energies of the protein-ligand plays a crucial role in understanding how the drug binds to the target protein. The compounds ,*11-Methylenetricosane*(-122.29), *Methyl isostearate*(-113.96), *1-Eicosene*(-113.14), *2,4-Tricosanedione*(-112.44) and *3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione*(-106.71) were found to dock into the binding pockets CD14 protein receptor. From the above results were can quantitatively say that *11-Methylenetricosane* has a significant binding capability towards CD14 receptor thus effective proving their immunomodulatory activity on monocytes, neutrophils and dendritic cells. The docked poses of the compound 11- Methylenetricosane with the CD14 receptor are shown in Figure 1.

3.3 Interaction Visualization

The best pose was extracted from the docking software and visualized in Biovia DSV along with the protein. The protein and ligand were assigned as CD14 receptor and docked pose of 11-Methylenetricosane respectively. The ligand interaction option allows the user to visualize the various interactions such as type of bonds, bond distance, and the amino acids partaking in bond formation. The 3D interaction is shown in Figure 2 and the 2D interaction is shown in Figure 4. The active site can also be evaluated by the software by either defining the active site, from protein surface cavities or PDB site records. The active site was identified and the interaction of the ligand with the active site is shown in Figure 2. From the images, we can see the interactions such as pi-pi bonds, pi- pi-alkyl bonds and van der Waals interaction being formed with the respective various amino acids which are ILE 177, LEU 149, VAL 152, TRP 154, LYS 127, ALA 180, HIS 181, TRP 160, SER 153, GLY 157, GLN 179, ASN 151 and ALA 178.

Table 3: ADMET Predicted models – Classification for 11- Methylenetricosane

Model	Result	Probability	Model	Result	Probability
Human Intestinal Absorption	+	0.9206	CYP inhibitory promiscuity	-	0.5959
Caco-2	+	0.7367	UGT catelyzed	-	0
Blood-Brain Barrier	+	0.9966	Carcinogenicity (binary)	+	0.6571
Human oral bioavailability	-	0.6714	Carcinogenicity (trinary)	Non-required	0.4727
Subcellular localzation	Lysosomes	0.4502	Eye corrosion	+	0.8457
OATP2B1 inhibitior	-	0.8496	Eye irritation	+	0.9866
OATP1B1 inhibitor	+	0.9333	Ames mutagenesis	-	0.94
OATP1B3 inhibitor	+	0.9377	Human either-a-go-go inhibition	-	0.7152
MATE1 inhibitior	-	0.98	micronuclear	-	0.99
OCT2 inhibitior	-	0.75	Hepatotoxicity	-	0.725
BSEP inhibitior	-	0.6775	Acute Oral Toxicity (c)	III	0.8543
P-glycoprotein inhibitior	-	0.7605	Estrogen receptor binding	-	0.8611
P-glycoprotein substrate	-	0.9307	Androgen receptor binding	-	0.8383
CYP3A4 substrate	-	0.7207	Thyroid receptor binding	-	0.7306
CYP2C9 substrate	-	0.8315	Glucocorticoid receptor binding	-	0.8351
CYP2D6 substrate	-	0.7358	Aromatase binding	-	0.7414
CYP3A4 inhibition	-	0.9595	PPAR gamma	-	0.7909
CYP2C9 inhibition	-	0.9179	Honey bee toxicity	+	0.8788
CYP2C19 inhibition	-	0.9117	Biodegradation	+	0.8
CYP2D6 inhibition	-	0.9301	crustacea aquatic toxicity	+	0.8318
CYP1A2 inhibition	-	0.5761	Fish aquatic toxicity	+	0.9971

Table 4: ADMET Predicted models – Regression for 11- Methylenetricosane

ADMET predicted profile --- Regressions	Value	Unit
Water solubility	-4.755	logS
Plasma protein binding	0.792	100%
Acute Oral Toxicity	1.712	kg/mol
Tetrahymena pyriformis	2.011	

3.4 *ADMET results for 11-Methylenetricosane*

ADMET profile was evaluated using the admetSAR database for. admetSAR predicted classification and regression values for*11-Methylenetricosane*for different types of models such as Caco2 permeability, blood-brain barrier, human intestinal absorption. The compound does not show any shows binding with androgen, oestrogen, androgen, oestrogen, glucocorticoid and thyroid receptor binding. The compound is predicted to possess low oral bioavailability. The compound also shows the low probability of binary carcinogenicity, crustacea aquatic toxicity, fish and honeybee toxicity. In the case of metabolism, various Cytochrome P450 (CYP) substrate and inhibitor models were calculated and the results show that they are Nonsubstrate and Non-inhibitor. The compound does not show hepatoxicity but shows a low probability for eye irritation and corrosion

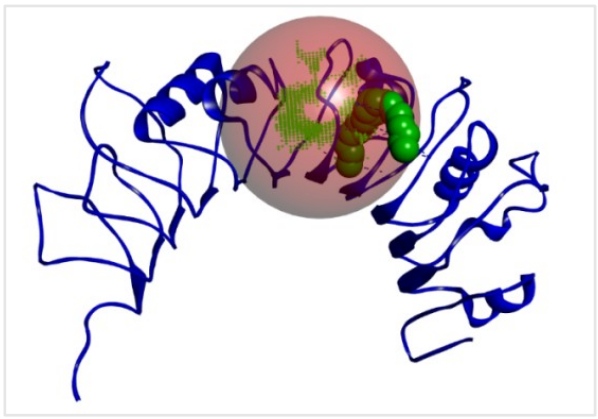

Figure 2: 11-Methylenetricosan binding with the CD14 protein receptor.

The green coloured compound represents the ligand and the blue ribbons represent the protein.
The red colour region represents the active site of the protein.
From the figure, it is clear that the compound binds at the active site of the protein.

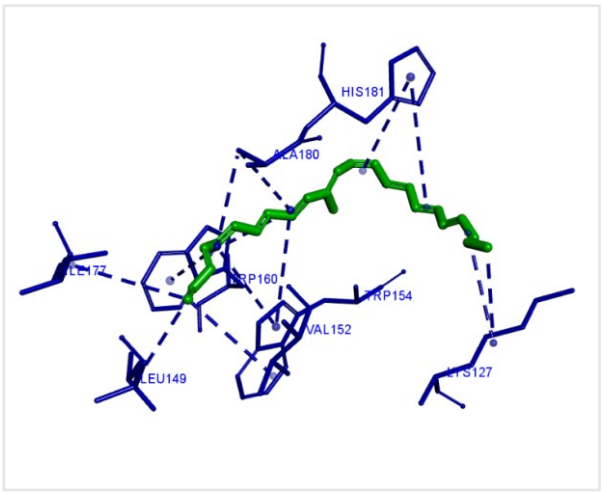

Figure 3: Bonds formed between 11-Methylenetricosane with specific amino acids of CD14 receptor

The green compound represents the ligand and the blue sticks represent the amino acids

4. Conclusion

Understanding the protein-ligand interaction is a key factor when considering new leads for the various drug. From performing a docking stimulation certain compounds from *Clerodendrum inerme* were successfully identified which possess binding capabilities with CD14 receptor. The compound 11- Methylenetricosane showed the highest binding affinity to the CD14 receptor (PDB ID: 4EO6). The compound seems to fit directly into the active site of the receptor. The major finding for admetSAR is that this compound is not 100% safe and needs to be optimized further to improve upon its toxicity and carcinogenicity characteristics. Nevertheless, compound *11-Methylenetricosane* from *Clerodendrum inerme* may one day become a new drug lead for the immunosuppressive drug.

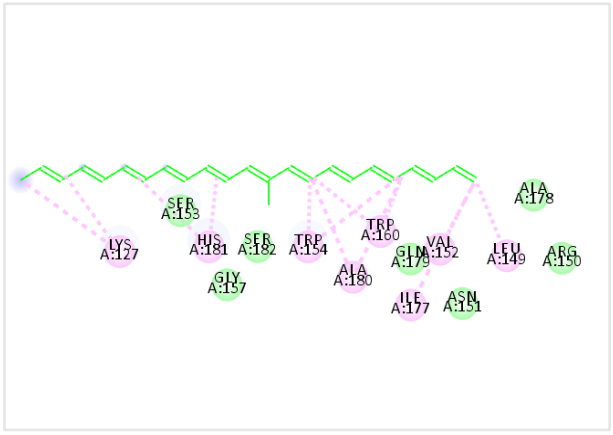

Figure 4: 2D visualization of Protein-Ligand interaction

The various amino acids and the respective bonds being formed

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Conflict of Interest

No conflict of interest to be declared by any of the authors. 4

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