MOLECULAR DOCKING ANALYSIS OF GOSSYPOL ANALOGUES AS HUMAN NEUTROPHIL ELASTASE (HNE), MATRIX METALLOPROTEINASES (MMP 2 AND 9) AND TYROSINASE INHIBITORS

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ABSTRACT

In the present study, five gossypol analogues such as gossypol, gossypol acetic acid, gossypol polyvinyl pyrrolidine, diamino gossypol and ethyl gossypol were assessed on the docking behaviour of Human neutrophil elastase (HNE), Matrix metalloproteinases (MMP 2 & 9) and tyrosinase by utilizing PatchDock method. Furthermore, Molecular physicochemical, Drug-likeness, ADME (Absorption, Distribution, Metabolism and Excretion) analyses were also carried out using molinspiration and Swiss ADME methods respectively. The molecular physicochemical investigation showed that five gossypol derivatives showed two to three violations. Similarly, ADME analysis also predicated to have high gastro-intestinal (GI) absorption effect for the five gossypol analogues. The docking studies showed that diamino gossypol exhibited the highest binding energy for all the targeted enzymes such as HNE, MMP 2 and MMP 9 (except tyrosinase). Thus the present investigation provides new knowledge in understanding gossypol analogues as a possible inhibitor against HNE, MMP 2 & 9 and tyrosinase.

Keywords: Molecular Docking, Gossypol Analogues, Human Neutrophil Elastase, Matrix Metalloproteinase, Tyrosinase.

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INTRODUCTION

Gossypol is a yellowish dye and natural toxin present in the seeds of cotton plants.¹ It is one of examples of “anti-nutritional” factor occurs in plants. Some of the gossypols derivatives are also known for insecticide activity which is commonly present in the *Gossypium* species.² Gossypol was first reported by Longmore (1886).³ Furthermore, Marchlewski (1899) has purified it, by precipitating using acetic acid which results in formation of gossypol acetic acid.⁴ Gossypol is one of versatile molecule from the genus *Gossypium* which has been reported to possess various biological activities especially against cancer, HIV, malaria and psoriasis.⁵ Gossypol and its derivatives have been shown to exhibit various pharmaceutical applications like antifertility or contraceptive^{2,6}, antioxidant^{7,8}, antitumor⁹⁻¹¹, antiparasitic¹²⁻¹⁴, antimicrobial¹⁵⁻¹⁷ and antiviral agent.¹⁸

Cosmetic ingredient review (CIR) committee has recommended cottonseed oil derivatives as cosmetic ingredient, however gossypol content in the cosmetic formulations should not exceeds more than 450 ppm level.¹⁹ Even today cottonseed milk along with coconut milk and jaggery has been consumed by South Indians as traditional health drink. Moreover, Rajam-cottonseed milk mix (Paruthi pal in tamil) is commercial available in the market. The above background prompted us to carry out the present investigation on five selected gossypol analogues which are gossypol, gossypol acetic acid, gossypol polyvinyl pyrrolidine, diamino gossypol and ethyl gossypol. These gossypol analogues were assessed on

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the docking behaviour of Human neutrophil elastase, Matrix metalloproteinases (MMP 2 & 9) and tyrosinase using PatchDock method. Furthermore, Molecular physicochemical, Drug-likeness, ADME (Absorption, Distribution, Metabolism and Excretion) analyses were also carried out in the present study.

EXPERIMENTAL

Ligand Preparation

Chemical structures of ligands, i.e., (i) gossypol (ID 3505), (ii) gossypol acetic acid (ID 227456), (iii) gossypol-vinylpyrrolidone (ID 154932), (iv) diaminogossypol (ID 198041) and (v) ethyl gossypol (ID 374353) were downloaded from ChemSpider and PubMed database. The five selected gossypol ligands were prepared according to the reported procedure²⁰⁻²⁶. These prepared structures were used for further Patch Dock.

Target Protein Identification and Preparation

The three dimensional (3D) structures of the HNE (PDB ID: 1H1B with resolution of 2.00 Å) and MMP 2 (PDB ID: 1QIB with resolution of 2.80 Å), MMP 9 (PDB ID: 4H1Q with resolution of 1.59 Å) and Tyrosinase (PDB ID: 2Y9W with resolution of 2.30 Å) were obtained from Protein Data Bank (PDB). A chain of these proteins was processed individually by removing another chain (B, C and D) as well as ligands by utilizing UCSF Chimera software.²¹

ADME Analysis

ADME analysis was performed using Swiss ADME analysis, according to reported procedure.²⁰

Docking Studies

Docking investigations were done by using PatchDock online server. Furthermore, the binding site analysis was done by PyMOL software.²³⁻²⁶

RESULTS AND DISCUSSION

-1 represents the two-dimensional (2D) structures of all five selected gossypol analogues. It is essential to identify the physiochemical and drug-likeness activities of these 5 ligands before performing the docking investigations. Moreover, Lipinski's rule of five (or) thumb rule of five was applied to identify the above said activities and furthermore helps to determine bioavailability of lead molecule²⁷. However, in the present study, gossypol, gossypol-vinylpyrrolidone and diaminogossypol (three ligands) have shown three violations, as shown the Table-2.

Table-1: The Two-dimensional (2D) Structure of Selected Gossypol Analogues

S. No.	Ligand Name	Structure
1	Gossypol
2	Gossypol acetic acid
3	Gossypol-vinylpyrrolidone

4	Diaminogossypol
5	Ethyl Gossypol

Table-2: Molecular Physicochemical Analysis of Gossypol Analogues by Utilizing Molinspiration Online Software

S. No.	Gossypol Analogues	Log A ¹	TPSA ²	Natoms ³	MW ⁴	No N ⁵	nOH NH ⁶	Nviolat ions ⁷	Nrotb ⁸	Volume ⁹
1	Gossypol	6.57	155.51	38	518.56	8	6	3	5	462.53
2	Gossypol acetic acid	7.24	135.28	38	516.59	7	52	2	5	471.07
3	Gossypol-vinylpyrrolidone	6.57	155.51	38	518.56	8	6	3	5	462.53
4	Diaminogossypol	5.39	207.56	40	548.59	10	10	3	5	485.11
5	Ethyl Gossypol	6.62	138.44	36	490.55	7	6	2	4	443.55

¹ Octanol-Water partition coefficient, ² Polar surface area, ³ Number of non-hydrogen atoms, ⁴ Molecular weight, ⁵ Number of hydrogen bond acceptors [O and N atoms], ⁶ Number of hydrogen bond donors [OH and NH groups], ⁷ Number of Rule of 5 violations, ⁸ Number of rotatable bonds, ⁹ Molecular volume.

With regard to Drug-likeness score, if the score is greater than '0' is active, between -5.0 to -0.0 is moderate active and less than "-5.0" is inactive.²⁸ All the gossypol analogues displayed active bioactivity score towards enzyme inhibitor descriptor as shown in Table-3.

Table-3: Drug-likeness Property Analysis of Gossypol Analogues Using Molinspiration Online Software

S.No.	Gossypol analogues	GPCR ¹ ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	Gossypol	-0.02	-0.13	-0.07	0.08	-0.17	0.11
2	Gossypol acetic acid	-0.02	-0.14	-0.12	0.11	-0.15	0.13
3	Gossypol-vinylpyrrolidone	-0.02	-0.13	-0.07	0.08	-0.17	0.11
4	Diaminogossypol	-0.06	-0.19	-0.16	-0.14	-0.23	0.09
5	Ethyl Gossypol	0.01	-0.05	-0.02	0.14	-0.18	0.16

¹GPCR- G Protein coupled receptors

Similarly, ADME prediction is also essential to perform before the docking investigation and which is recommended in the early stage of Drug discovery, Drug selection and Drug design, due to its unique property.²⁹⁻³⁰ Table-4 displayed the ADME profile of gossypol analogues; all the ligands are predicted to have low gastrointestinal (GI) absorption effect.

Table-4: ADME Analysis of Gossypol Analogues Using Swiss ADME Method

S. No.	Gossypol Analogues	GI ¹	BB B ²	P-gp ³	CYP 1A2 ⁴	CYP219 ⁵	CYP2C9 ⁶	CYP2D6 ⁷	CYP3A ⁴ ⁸	Log Kp ⁹
1	Gossypol	Low	No	No	No	Yes	Yes	No	No	-4.54
2	Gossypol acetic acid	Low	No	No	No	Yes	No	No	No	-4.93
3	Gossypol-vinylpyrrolidone	Low	No	No	No	Yes	No	No	No	-5.03

4	Diaminogossypol	Low	No	No	No	No	Yes	No	No	-5.70
5	Ethyl Gossypol	Low	No	No	No	Yes	Yes	No	No	-4.39

¹-Gastrointestinal, ²-Blood-brain barrier permeant, ³-P-gp-P-glycoprotein substrate, ⁴⁻⁸-Cytochrome P450 Inhibitors, ⁹-Skin Permeation (cm/s).

Gossypol has been reported to inhibit lactate dehydrogenase (LDH) activity³¹ and similarly it has been reported to inhibit a number of oxidoreductases activities.³² Gossypol has been reported to inhibit both arachidonate 5 and 12 Lipoxygenases (5, 12- LOX) activities.³³ In the present study human neutrophil elastase (HNE) is one of the target protein/enzyme, which its docking investigation showed diaminogossypol has the highest atomic contact energy (-373.10 kcal/mol). Interestingly, diaminogossypol (Fig.-1) and ethyl gossypol have shown interaction with Asn 61 amino acid residue of HNE as given in the Table-5.

Table-5: Binding Energy Analysis of Gossypol Analogues with Human Neutrophil Elastase (HNE) using PatchDock Method

S. No.	Gossypol Analogues	-ACE** (-kcal/mol)	Interaction of Amino Acid Residue	Bond Distance (Å)
1	Gossypol	277.10	Val 216	2.9
2	Gossypol acetic acid	79.47	Asn 101 Arg 129	3.1 3.0
3	Gossypol-vinylpyrrolidone	260.50	Glu 156 Arg 147	2.5 3.5
4	Diaminogossypol	373.10	Asn 61	3.1
5	Ethyl Gossypol	288.40	Asn 61	3.1

** Atomic contact energy

Above outcome was in good agreement with previous publication, where gossypol (from *Thespesia populnea*) shown to inhibit elastase activity.³⁴

MMPs are a group of metallo-enzymes (especially contains zinc metal) that to particularly MMP 2 and MMP 9 have been elevated in the pathological diseases like ageing, cancer, inflammation and wound healing.²¹ In the present study, MMP 2 and MMP 9 are another two target proteins/enzymes where the docking investigation showed diaminogossypol has the highest atomic contact energy with of MMP 2 (-568.66 kcal/mol) and MMP 9 (-583.33 kcal/mol) respectively. Interestingly, gossypol, gossypol acetic acid and diaminogossypol have shown interaction with Thr 227 amino acid residue of MMP 2 as tabulated in Table-6.

Table-6: Binding Energy Analysis of Gossypol Analogues with Matrix Metalloproteinase (MMP 2) Using PatchDock Method

S. No.	Gossypol Analogues	-ACE** (-kcal/mol)	Interaction of Amino Acid Residue	Bond Distance (Å)
1	Gossypol	526.81	Gly 216 and Thr 227	2.6 & 2.9
2	Gossypol acetic acid	549.08	Thr 227	3.4
3	Gossypol-vinylpyrrolidone	556.92	Ala 165 Leu 164 Ala 165	2.3 2.9 3.3
4	Diaminogossypol	568.66	Pro 215 Tyr 223 Gly 216 Gly 162 Thr 227	1.6 2.1 2.1 2.5 3.1
5	Ethyl Gossypol	524.02	Pro 215 and Ala 165	1.8 & 2.7

** Atomic contact energy

Similarly gossypol, gossypol acetic acid, gossypol polyvinyl pyrrolidine and diamino gossypol showed interaction with Arg 249 amino acid residue of MMP 9 as given in Table-7. The present result was in good align with previous literature, where gossypol (from *Abelmoscus esculentus*) has shown to dock with MMP 9 using molecular docking method.³⁵

Table-7: Binding Energy Analysis of Gossypol Analogues with Matrix Metalloproteinase (MMP-9) Using PatchDock Method

S. No.	Gossypol Analogues	-ACE** (-kcal/mol)	Interaction of Amino Acid Residue	Bond Distance (Å)
1	Gossypol	530.55	Leu 188 Leu 188 Arg 249	3.4 3.5 2.5
2	Gossypol acetic acid	553.43	Arg 249 Pro 240	3.2 3.5
3	Gossypol-vinylpyrrolidone	568.84	Arg 249	3.3
4	Diaminogossypol	583.33	Arg 249 Pro 240 Leu 188 Ala 189 Ala 189	2.8 3.5 3.3 2.0 3.4
5	Ethyl Gossypol	552.38	Pro 240 Glu 241 Leu 188 Ala 189	3.1 2.8 3.3 2.0

** Atomic contact energy

Tyrosinase is the main regulatory enzyme in melanogenesis pathway especially in the initially two stages such as (i) tyrosine hydroxylation to 3, 4-dihydroxyphenylalanine (DOPA) and (ii) the oxidation of DOPA to dopoquinone³⁶. In this present investigation tyrosinase is the another target enzyme where the docking result showed gossypol-vinylpyrrolidone has the highest atomic contact energy (-137.04 kcal/mol). Similarly, gossypol acetic acid showed very atomic contact energy (+19.22 kcal/mol) with tyrosinase, which might due to unfavorable interactions phenomenon as reported by Castro and co-workers.³⁷ Interestingly, gossypol and gossypol-vinylpyrrolidone have shown interaction with Gln74 amino acid residue of tyrosinase as tabulated in Table-8.

Table-8: Binding Energy Analysis of Gossypol Analogues with Tyrosinase Using PatchDock Method

S. No.	Gossypol Analogues	-ACE** (-kcal/mol)	Interaction of Amino Acid Residue	Bond Distance (Å)
1	Gossypol	44.94	Glu67 Lys70 Gln74 Gln74	2.3 3.2 2.3 3.3
2	Gossypol acetic acid	+ 19.22	Thr308 Lys372 Lys373	3.2 2.4 2.6
3	Gossypol-vinylpyrrolidone	137.04	Gln74 Gln74	2.3 3.2
4	Diaminogossypol	77.24	Gln72 Tyr343 Tyr343	3.1 2.2 2.8
5	Ethyl Gossypol	59.68	Asp3 Lys70	1.7 2.5

** Atomic contact energy

Fig.-1: Represents the Diaminogossypol has shown to interaction with all four target enzymes, especially with the Amino Acid Residues (Top row- Left (Human neutrophil elastase-HNE) and Right (Tyrosinase); Bottom row: Left (Matrix metalloproteinase 2) and Right (Matrix metalloproteinase 9).

CONCLUSION

In conclusion, gossypol derivatives have shown potential to dock with all the four selected proteins (or) enzymes (namely HNE, MMP 2 & MMP 9 and tyrosinase). The present docking investigation has shown diamino gossypol as exhibited the maximum atomic contact energy for three selected enzymes, except for tyrosinase. Hence, the present study strongly suggested that potential of these five gossypol derivatives as HNE, MMP 2 & MMP 9 and tyrosinase inhibitory agents.

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