Coumarin exhibits broad-spectrum antibiofilm and antiquorum sensing activity against Gram-negative bacteria: *In vitro* and *in silico* investigation

Faizan Abul Qais [a], Mohammad Shavez Khan [a], Iqbal Ahmad [a*], Fohad Mabood Husain [b], Rais Ahmad Khan [c], Iftekhar Hassan [d], Syed Ali Shahzad [b], Walaa AlHarbi [e]

[a] *Department of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, UP-202002, India.*

[b] *Department of Food Science and Nutrition, King Saud University, Riyadh-11451, KSA.*

[c] *Department of Chemistry, King Saud University, Riyadh-11451, KSA.*

[d] *Department of Zoology, King Saud University, Riyadh-11451, KSA.*

[e] *Department of Chemistry, Faculty of Science, King Khalid University, P.O. Box-9004, Abha 62529, KSA.*

Supplementary Figures

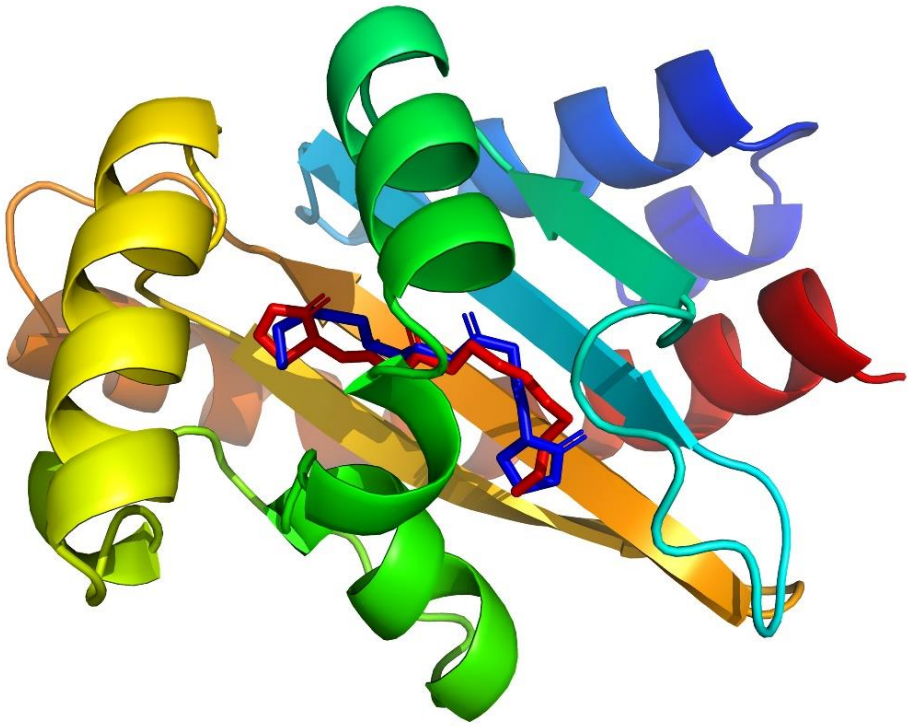

Figure S1. Comparison between crystal structures and docked conformations of N-3-oxo-dodecanoyl-l-homoserine lactone-LasR complex. Stick model of ligand in blue is the crystal structures conformation and stick model of ligand in red is the docked-conformation.

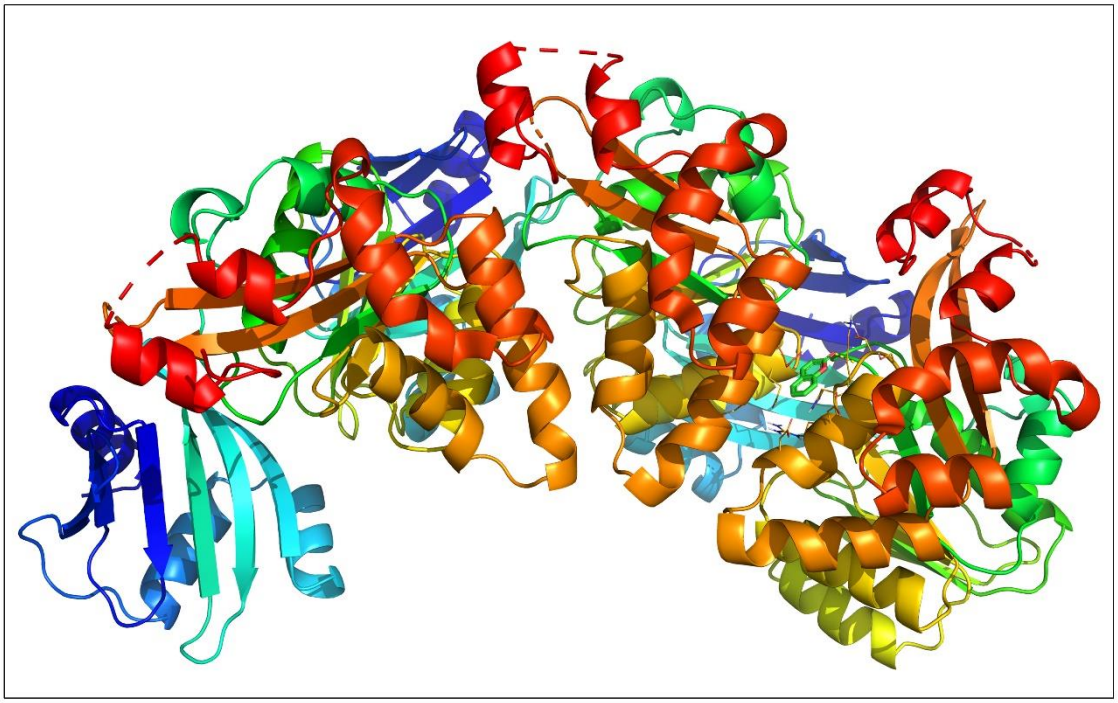

Figure S2. Molecular docked complex of coumarin with PilT (3JVV).

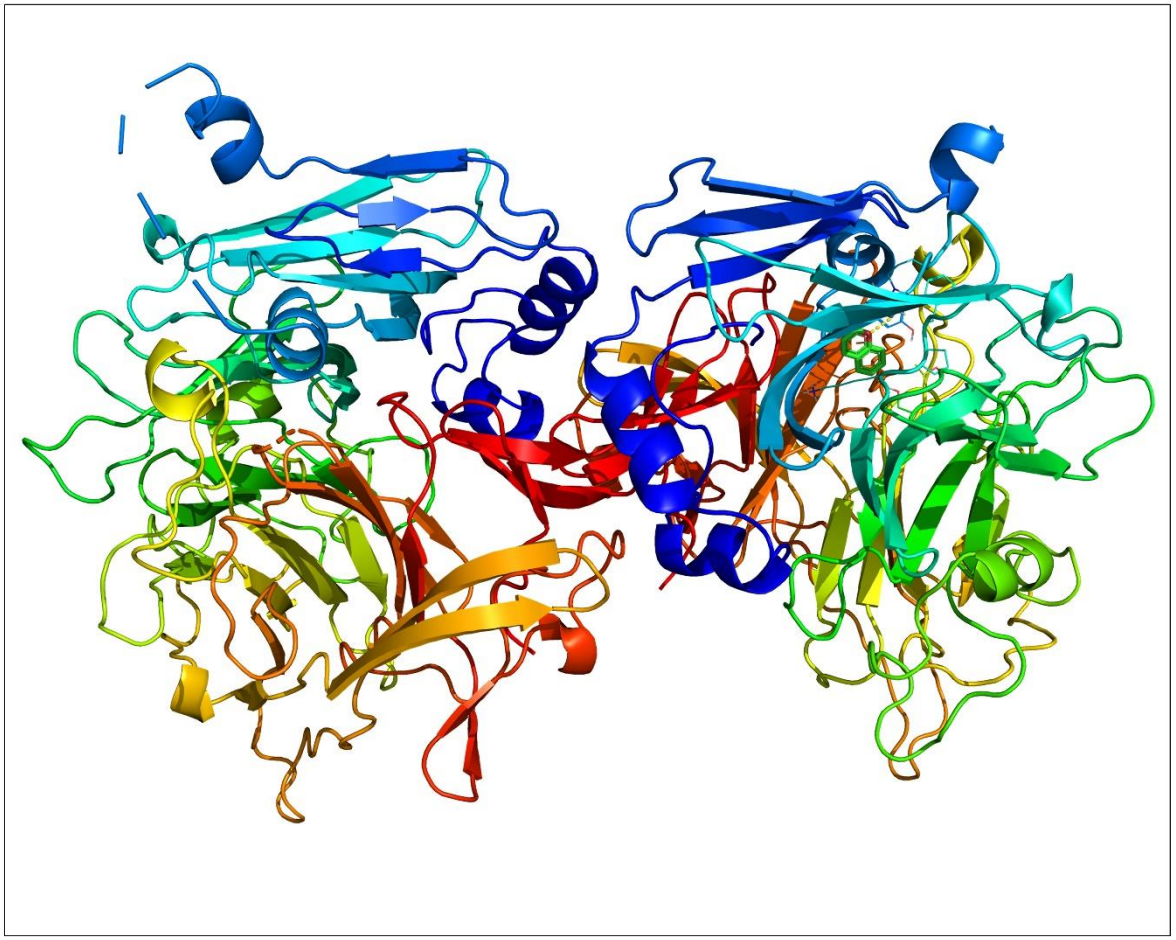

Figure S3. Molecular docked complex of coumarin with PilY1 (3HX6).

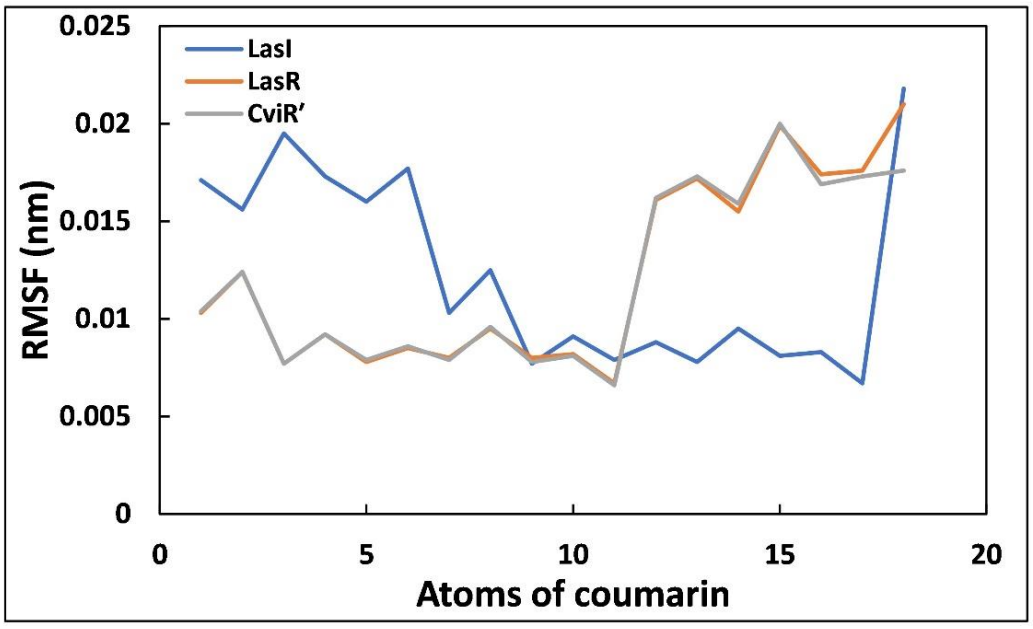

Figure S4. The average RMSF value of each atoms of coumarin during the MD simulation.

Supplementary Tables

Table S1. Details of the chemicals used in this study.

Name	**Catalogue Number**	**Purity**	**Company Name**	**Country**
Coumarin	C4261	≥99%	Sigma-Aldrich	USA
Azocasein	A2765	NA	Sigma-Aldrich	USA
Elastin–Congo red (ECR)	E0502	NA	Sigma-Aldrich	USA
Trichloroacetic acid	RM7570	≥99%	Hi-Media Laboratories	India
2,3,5-triphenyltetrazolium chloride (TTC)	65599	99%	Sisco Research Laboratories	India
Dimethyl Sulfoxide (DMSO)	BP2311	≥99.7%	Thermo Fisher Scientific	India
Chloroform	38376005	≥99%	Thermo Fisher Scientific	India
TRIS-hydrochloride (Tris-HCl)	MB030	99%	Hi-Media Laboratories	India
Orcinol monohydrate	MB242	≥98%	Hi-Media Laboratories	India
Diethyl ether	60-29-7	≥99%	Thermo Fisher Scientific	India
Ethanol	100983	≥99.9%	Merck	India
Crystal Violet	28376	NA	Sisco Research Laboratories	India

NA: Information not available.

Table S2. Details of the grid of proteins/receptors used in this study.

S. No.	Name of protein	Size of grid			Centre of grid		
		x	y	z	x	y	z
1.	LasI	52	48	46	38.371	-12.794	-11.794
2.	EsaI	48	58	52	22.9	-3.461	15.731
3.	LasR	40	44	52	24.865	11.477	77.671
4.	PilY1	88	92	90	21.984	-2.816	21.205
5.	LasA	30	28	30	24.682	2.824	-9.526
6.	PilT	120	68	72	-54.697	25.098	24.212
7.	CviR	48	54	84	31.606	1.806	19.505
8.	CviR′	52	46	48	14.982	18.069	45.838
9.	PqsR	52	50	48	-26.624	63.023	9.982
10.	RhlR	56	80	74	26.079	27.547	-23.006

Table S3. Effect of sub-MICs of coumarin on inhibition of virulence factors in *P. aeruginosa* PAO1.

Coumarin concentration	Virulence factors production					
	Pyocyanin[α]	Pyoverdin[β]	Total protease[γ]	Elastase activity (lasB)[δ]	Rhamnolipid[Ω]	Swimming motility[ε]
Control	6.58±0.49[a]	761.51±26.12[a]	0.61±0.02[a]	0.42±0.01[a]	0.88±0.02[a]	88.66±1.15[a]
DMSO (0.5%)	6.69±0.35[a]	752.59±17.39[a]	0.60±0.02[a]	0.42±0.01[a]	0.86±0.04[a]	89.33±1.15[a]
31.25 µg/ml	4.50±0.68[b] (31.70)	660.58±27.23[b] (13.25)	0.53±0.02[a] (12.76)	0.39±0.01[ab] (07.07)	0.74±0.04[b] (15.48)	48.33±3.05[b] (45.48)
62.5 µg/ml	3.76±0.37[b] (42.79)	501.57±22.98[c] (34.13)	0.42±0.02[b] (31.73)	0.36±0.01[b] (13.83)	0.65±0.01[c] (26.69)	33.00±2.64[c] (62.78)
125 µg/ml	2.30±0.51[c] (64.97)	332.06±18.46[d] (56.39)	0.36±0.03[b] (41.27)	0.28±0.02[c] (31.91)	0.54±0.02[d] (39.09)	21.66±2.51[d] (75.56)
250 µg/ml	0.83±0.14[d] (87.25)	228.07±43.90[e] (70.05)	0.14±0.03[c] (76.07)	0.17±0.03[d] (58.64)	0.45±0.03[d] (48.94)	16.66±4.04[d] (81.20)

The data is presented as average±SD of three replicates. For analysis of statistical significance, one-way ANOVA was performed using Tukey's test at significance level of 0.05. Different letters above SD represent different significant groups at p-value=0.05 and are in descending order of values starting from letter "a". Values in parenthesis are the percent inhibition.

[α]Pyocyanin concentrations were expressed as µg/ml of culture supernatant.

[β]Pyoverdin results are expressed in fluorescence intensity (arbitrary unit).

[γ]Total protease activity is expressed as the absorbance at 400 nm.

[δ]Elastase activity is expressed as the absorbance at 495 nm.

[Ω]Rhamnolipid is expressed as the absorbance at 421 nm.

[ε]Swimming motility is expressed as swarm diameter in mm.

Table S4. Binding energies of top five poses for the interaction of coumarin with different proteins obtained using AutoDock Vina.

S. No.	PDB ID	Protein name	Binding energy (kcal mol^{-1})				
			Pose I	**Pose II**	**Pose III**	**Pose IV**	**Pose V**
1.	1RO5	LasI	-5.7	-5.6	-5.5	-5.5	-5.4
2.	1KZF	EsaI	-6.6	-6.3	-6.3	-6.2	-5.9
3.	2UV0	LasR	-8.1	-7.6	-7.5	-7.4	-7.4
4.	3IT7	LasA	-7.5	-6.6	-6.5	-6.0	-5.9
5.	3QP5	CviR	-7.7	-7.6	-6.9	-6.6	-6.5
6.	3QP1	CviR′	-7.7	-7.3	-7.1	-7.1	-6.9
7.	4JVI	PqsR	-6.5	-6.4	-5.8	-5.7	-5.7
8.	3JVV	PilT	-6.3	-6.1	-6.1	-6.1	-5.9
9.	3HX6	PilY1	-6.3	-6.2	-6.2	-6.0	-5.9
10.		RhlR	-6.4	-6.2	-6.2	-6.0	-6.0

Table S5. RMSD, R_g, and SASA of protein only, coumarin-protein complex, and coumarin only calculated in MD simulation.

Name of Proteins	Proteins/complexes	Proteins		
		RMSD (nm)	**R_g (nm)**	**SASA (nm^2)**
LasI	LasI only	0.263±0.039	1.609±0.010	104.746±2.377
	LasI -coumarin complex	0.272±0.030*	1.580±0.011*	102.032±2.307*
	Coumarin only	0.012±0.002$^{*\#}$	0.227±0.001$^{*\#}$	003.153±0.168$^{*\#}$
LasR	LasR only	0.137±0.024	1.494±0.007	90.682±1.623
	LasR-coumarin complex	0.137±0.016*	1.483±0.007*	89.498±1.598*
	Coumarin only	0.012±0.002$^{*\#}$	0.226±0.001$^{*\#}$	03.138±0.161$^{*\#}$
CviR′	CviR′ only	0.167±0.034	1.572±0.012	98.630±1.908
	CviR′-coumarin complex	0.208±0.031*	1.565±0.011*	97.660±2.002*
	Coumarin only	0.014±0.003$^{*\#}$	0.227±0.001$^{*\#}$	03.155±0.168$^{*\#}$

RMSD: Root-mean square deviation, R_g: Radius of gyration, SASA: Solvent accessible surface area. * indicates significantly different from protein only at $p < 0.05$. $^{\#}$ indicates significantly different from protein-coumarin complex at p # 0.05.