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RESEARCH ARTICLE

Quantum Mechanical Study of Some Biological Important Quorum Sensing Inhibitors in Different Solvent Media

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ABSTRACT

Quorum sensing (QS) is a phenomenon in which microcolonies get converted into a mature biofilm through different processes. Thus, by considering this communication system, it is possible to set up a useful new antimicrobial planning without the risk of growing resistance. The QS inhibition (QSI) process is completely safe for the environment and decreases the use of chemical. Keeping in view of this fact, the hamamelitanin (HAM) is one of the best QSIs, inhibiting the biofilm metabolic activity of all tested bacteria. In this study, molecular docking and *in silico* studies were performed to evaluate the drug likeliness behavior of some ester of gallic acid of D-hamamelose compounds as inhibitors of QS. The study comprised of 34 compounds belonging ester of gallic acid of D-hamamelose along with one standard QSI HAM. The molecular docking of some ester of gallic acid of D-hamamelose with 4g4k protein was performed by the AutoDock 1.5.6 suite. Molecular descriptor properties were evaluated by molinspiration and OSIRIS property explorer. The pharmacophore property has been generated by PharmaGist tools. Out of the 34 derivatives, 10 derivatives have qualified the standard value of the parameters World Drug Index (WDI), modern drug data report (MDDR), drug likeliness, drug score value and attach with the same fragment of protein just like by the natural ligand D-hamamelose or the standard QSI HAM. The binding energies of all the docked complex of compounds have larger negative values than that of HAM. The molecular docking study implied that the qualified compounds may use as a remarkable QSI. The pharmacophore study may be used to design and develop new drugs. This study notably supports a theoretical concept of these compounds as QSIs of *Staphylococcus aureus*.

Keywords: Dipole moment, drug likeness, drug score, highest occupied molecular orbital–lowest unoccupied molecular orbital, molecular docking, quorum sensing inhibitor

INTRODUCTION

Quorum sensing (QS) is a process by which microbes enhance population through communication population.[1] Many species of bacteria use signal molecules through gene expression according to the need of environment.[2,3] Quorum sensing bacteria produce as well as release chemical signal molecules, which are called auto inducers[4] and through this signal the bacteria increase cell density to form colony. QS explores biofilm distribution dispersion which is a colony involving bacterial population through extracellular matrix.[5] In the biofilm, the cells are attached with each other or with the surface. These sticked cells are fixed in a matrix of extracellular polymeric substance.[6] Many fungi, algae, protozoa, debris, and corrosion products explore biofilm formation. It may generally occur on any surface where bacteria and some amount of water are needed. Therefore, several processes have been tested for stopping the QS process and the enzymatic retardation of QS signal molecules (acyl homoserine lactones [AHLs]). This process has been proved to be most effective and applicable one.[7] Several AHL degrading enzymes have been discovered in a number of bacteria.[8] The typical QS system in Gram-negative bacteria consists of three component systems - (a) LuxI synthase homolog receptor, (b) AHL signaling molecules, and (c) LuxR receptor homolog.[9] Gram-positive bacteria use small peptide signaling molecules,[10] while Gram-positive and Gram-negative bacteria exercise autoinducer-2.[11] The biofilm formation

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in *Staphylococcus aureus* is executed by the Agr system, and the QS inhibition (QSI) can inhibit the transformation of mature biofilm from microcolonies.[12] QSIs show no adverse side effect such as the regular bactericidal treatments.[12,13] In this way, it might be possible to found an effective new antimicrobial plan without the threat of developing resistance.[14-16] LuxI/LuxR QS system offers a unique path for developing novel antibiofilm therapies.[12] Unlike the traditional antibiotics that promote the antibiotic-resistant strains of bacteria to limit cell growth of killing the pathogen, a quorum quenching enzymes can do the virulence expression in pathogenic bacteria.[13] The QSI process is completely harmless to the environment and its application minimizes the use of chemicals.[13] The QS process can be diminished by different mechanisms - (i) reducing the activity of AHL cognate receptor protein or AHL synthase and (ii) inhibiting the production of QS signal molecule. There are some special requirements for an effective QSI[17,18] and those are - (a) a small molecule having enough power to minimize the QS-regulated gene expression, (b) be highly specific for a given QS regulator along with no adverse effect on the bacteria or the host, (c) be economically stable and resistant to degradation by various host metabolic systems, and (d) preferably longer than the native AHL. There are various types of Quorum sensing inhibitors (QSIs) comprising Prokaryotic QSIs,[1] animal-based QSIs,[1] marine organism-based QSIs,[1] fungus-based QSIs[1] and plant-based QSIs.[1] QS system in Staphylococcus spp. which consists of the AI RNIII activating protein (RAP) and its target molecule (TRAP). The inhibition of RAP by RNA-III inhibiting peptide (RIP) results in a decrease of virulence. A well-known plant-based QSI hamamelitanin (HAM) which is extracted from the bark of *Hamamelis virginiana* like RIP did not affect the growth of Staphylococcus spp., but it did exhibit the QS regulator RNAIII-prevented biofilm formation and cell attachment in vitro.[19] HAM is a naturally occurring polyphenol extracted from the bark of H. virginiana and it is the ester of D-hamamelose (2-hydroxymethyl-D-ribose) with two molecules of gallic acid [Figure 1]. It prevents graft-associated infections caused by the staphylococci and thereby reduces their virulence.[20] Kiran *et al.* stated that the implantation of grafts into the animal drastically

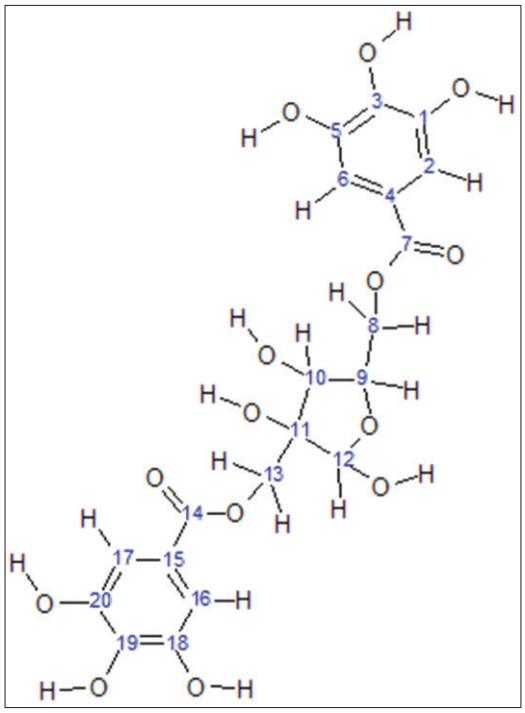

Figure 1: The structure of hamamelitanin

decreased the bacterial load instead of control.[21] A non-peptide analog of RIP (2, 5-di-o-galloyl-d-hamamelose-hamamelitanin) was identified as QSI through virtual screening of available chemicals database,[1] and it HAM decreases *S. aureus* attachment *in vivo* and *in vitro*.[20-22] Earlier it has been suggested that HAM along with vancomycin can quench QS through the trap QS system though the mechanism is still unknown. HAM specifically affects *S. aureus* which is a causative agent of exploring acute and chronic bacterial infections in human and animals through the formation of biofilm by cell wall synthesis and extracellular DNA release of *S. aureus*.[23] HAM increases the susceptibility of *S. aureus* to antibiotic treatment *in vitro* as well *in vivo Caenorhabditis elegans* and mouse mammary gland infection models.[23] It has been compared that the effect of HAM on the susceptibility of *S. aureus* is much superior than so-called antibiotics, for example, cefazolin, cefalonium, cephalexin, cefoxitin, daptomycin, linezolid, tobramycin, and fusidic acid.[23] It has been suggested that the sensitivity toward disruption of biofilm is more effective when HAM is implemented along with VAN, compared to VAN treatment alone.[23] HAM matches the pharmacophore model of RIP, a peptide described to block QS by affecting TRAP.[20] It is widely used for *S. aureus*, Gram-negative and Gram-positive bacteria.[23]

Table 1: Calculated energy values (eV) of hamamelitanin in different solvents using DFT-R B3LYP 6-31(G)

Solvents	E_{HOMO}eV	E_{LUMO}eV	E_{HOMO-1}eV	E_{LUMO+1}eV	$E_{LUMO} - E_{HOMO}$ eV	$E_{LUMO+1} - E_{HOMO-1}$eV	E_{total}eV
Gas	−155.859	−34.207	−161.708	−21.176	121.652	140.532	−49696.25
Water	−159.487	−32.282	−163.263	−25.619	127.205	137.644	−49695.38
DMSO	−159.265	−31.394	−160.746	−23.323	127.871	137.423	−49695.68
CHN	−159.117	−28.876	−160.153	−20.954	130.241	139.199	−49695.85

DMSO: Dimethyl sulfoxide, CHN: Cyclohexyl amine

As the D-hamamelose fragment has the special feature for designing QSIs, so the ester derivatives of gallic acid of D-hamamelose may enable to the development and marketing of a new series of QSIS. A suitable screening of the compounds, using theoretical and computational way, especially pharmacophore study, is the most effective one for discovering the better drug. The physicochemical properties also play an important role for choosing the better drug through *in vitro* and *in vivo* findings. However, it is also found that the intrinsic biological and physicochemical parameters of the molecules depend on many of these properties. However, the complex structure of the whole drug molecule seems difficult to compare with these parameters.[24] Apart from these, the binding affinity parameter may help to design modern drug where the molecular docking study help to predict drug-receptor interaction and it is considered as one of the fundamental approaches for promising drug design.[25]

In the present study, the molecular docking study was done for some D-hamamelose derivatives against 4g4k protein. The various other procedures such as absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction, drug likeness property and drug score analysis are used in the case the docked compounds for characterizing of some D-hamamelose derivatives as QSIs of *S. aureus*.

MATERIALS AND METHODS

The energies of highest occupied molecular orbital (HOMO), lowest unoccupied MO (LUMO), second $HOMO_{-1}$, and second LUMO and the corresponding energy gap in different solvents for standard QSI HAM using the DFT with R-B3LYP 6-31(G) basis set are represented in Tables 1 and 2. The study has been carried out on 34 compounds of the ester of gallic acid of D-hamamelose including standard QSI HAM. In each of the compounds there are different different substituents in the rings, side chains or both [Table 3]. SWISS ADME explorer was used to calculate logp, solubility, drug-likeness, polar surface area, molecular weight, number of atoms, number of rotatable bonds, volume, drug score, and number of violations to Lipinski's rule. The drug-likeness property was assigned by Pre ADMET program and ADMET profile. The SWISS ADME program was used to predict the overall ADMET property of the most active derivatives because some fragments may be generally responsible for the QSI of the studied compounds. The molecular docking was performed using the AutoDock 1.5.6 suite.[26]

Table 2: Computed ionization potential (I), electron affinity (A), chemical potential (μ), global hardness (Ƞ), global softness (S), electrophilicity index (ω) using DFT- R B3LYP 6- 31(G) in different solvents.

Solvent	I eV	A eV	μ eV	ηeV	SeV^{-1}	σ	ω eV
Gas	155.859	34.207	−95.033	60.826	0.0164	95.033	74.239
H_2O	159.487	32.282	−95.885	63.603	0.0157	95.885	72.276
DMSO	159.265	31.394	−95.330	63.936	0.0156	95.330	71.069
CHN	159.117	28.876	−93.997	65.121	0.0153	93.997	67.838

DMSO: Dimethyl sulfoxide, CHN: Cyclohexyl amine

The three-dimensional structure [Figure 2] of the 4g4k protein was collected from the protein data bank (PDB ID: 4G4K).[27] During molecular docking, all the water molecules and ligands were removed from the PDB file. The interface program AutoDock tools were used to prepare all missing hydrogen and side chain atoms of the receptor.[28] The grid box was prepared in such a way that it covered the active site based on the amino acid residues, which are involved in bindings. The grid box size was set at 82, 90, and 98 A^0 (x, y, and z, respectively) using AutoGrid 1.5.6 program integrated in AutoDock 1.5.6. 34 separate molecular docking experiments were carried out using Lamarckian genetic algorithm keeping all other parameters set in default mode. The compounds which have the lowest energy cluster with maximum cluster size were considered for more studies. Interaction has been compared

Table 3: The substituents of the ester of Gallic acid of D-hamamelose and its binding energy

Compounds	substituted group	substituted by	Binding energy kcal/mol
1a	7-C=O, 13-H	7- OH, 13-OH	−6.5
1b	7-C=O, 8-H, 13-H	7- OH, 8-OH, 13-OH	−6.4
1c	7-C=O, 8-H, 12-OH	7- OH, 8-OH, 13-OCH	−6.6
1e	8-H, 12-OH, 14-C = O	8-OH, 12-NH_2, 14-CHOH	−6.4
1f	8-H, 10-OH, 12-OH, 14-C = O	8-OH, 10-NH_2, 12-NH_2, 14-CHOH	−6.7
1g	8-H, 10-OH, 11-OH, 12-OH, 14-C = O	8-OH, 10-NH_2, 11-NH_2, 12-NH_2, 14-CHOH	−6.6
1h	8-H, 10-OH, 12-OH	8-OH, 10-NH_2, 12-NH_2	−6.6
1i	8-H, 10-OH, 12-OH	8-OH, 10-NH_2, 12-$CONH_2$	−6.9
1j	8-H, 10-OH, 12-OH	8-OH, 10-$CONH_2$, 12-$CONH_2$	−7.2
1k	8-H, 10-OH, 11-OH, 12-OH	8-OH, 10-$CONH_2$, 11—$CONH_2$, 12-$CONH_2$	−6.9
1l	8-H, 10-OH, 11-OH, 13-H	8-OH, 10-$CONH_2$, 11-$CONH_2$, 13-OH,	−6.7
1m	8-H, 10-OH, 11-OH, 12-OH, 13-H	8-OH, 10-$CONH_2$, 11-$CONH_2$, 12-$CONH_2$, 13-OH,	−6.5
1n	8-H, 9-H, 10-OH, 12-OH, 13-H	8-OH, 9-OH, 10-$CONH_2$, 12-$CONH_2$, 13-OH	−7.0
1o	10-OH, 12-OH	10-$CONH_2$, 12-$CONH_2$	−7.5
1p	12-OH	12-COOH	−6.6
1q	10-OH, 12-OH	10-COOH, 12-COOH	−7.2
1r	10-OH, 12-OH	10-COOH, 12-$CONH_2$	−7.0
1s	5-OH, 11-OH, 18-OH	5-H, 11-H, 18-H	−6.5
1t	5-OH, 10-OH, 12-OH, 18-OH	5-H, 10-H, 12-H, 18-H	−5.9
1u	5-OH, 11-OH, 12-OH, 18-OH	5-H, 11-H, 12-H, 18-H	−6.3
1v	5-OH, 10-OH, 11-OH, 12-OH, 18-OH	5-H, 10-H, 11-H, 12-H, 18-H	−6.1
1w	1-OH, 5-OH, 11-OH, 18-OH, 20-OH	1-H, 5-H, 11-H, 18-H, 20-H	−6.0
1x	5-OH, 7-CO, 11-OH, 14-CO, 20-OH	5-H, 7-CH_2, 11-H, 14-CH_2, 20-H	−5.8
1y	3-OH, 5-OH, 11-OH, 19-OH, 20-OH	3-CH3, 5-H, 11-H, 19-CH3, 20-H	−6.6
1z	1-OH, 3-OH, 5-OH, 11-OH, 18-OH, 19-OH, 20-OH	1-CH_3, 3-CH_3, 5-H, 11-H, 18-CH_3, 19-CH_3, 20-H	−6.5
1aa	1-OH, 3-OH, 5-OH, 11-OH, 18-OH, 19-OH, 20-OH	1-H, 3-H, 5-H, 11-H, 18-H, 19-H, 20-H	−6.4
1bb	1-OH, 3-OH, 5-OH, 10-OH, 11-OH, 12-OH, 18-OH, 19-OH, 20-OH	1-H, 3-H, 5-H, 10-H, 11-H, 12-H, 18-H, 19-H, 20-H	−5.9
1cc	1-OH, 3-OH, 5-OH, 10-OH, 11-OH, 12-OH, 18-OH, 19-OH, 20-OH	1-CH_3, 3-CH_3, 5-H, 10-H, 11-H, 12-H, 18-CH_3, 19-CH_3, 20-H	−5.4
1dd	3-OH	3-$CONH_2$	−7.3
1ee	3-OH, 19-OH	3-$CONH_2$, 19-$CONH_2$	−7.7
1ff	3-OH, 10-OH	3-$CONH_2$, 10-$CONH_2$	−6.3
1gg	3-OH, 12-OH	3-$CONH_2$, 12-$CONH_2$	−6.5
1hh	3-OH, 10-OH, 12-OH	3-$CONH_2$, 10-$CONH_2$, 12- $CONH_2$	−7.2

based on the amino acid residues of the active site of 4g4k interacting with the natural ligand D-hamamelose and remaining 33 compounds [Figure 2]. The docking result was transformed from.dlg format to.PDB format using Avogadro script. The compounds were structurally arranged to compare ligand-based pharmacophore property using PharmaGist tool.[29]

RESULTS

Among 34 compounds, most of these have successfully qualified MDDR like rule and World Drug Index (WDI) like rule and a few compounds satisfy Lipinski's rule and comprehensive medicinal chemistry like rule [Table 4]. For testing good oral bioavailability [Table 5], some of the compounds have shown excellent permeability, while others have relatively less or poor (in few cases) permeability [Table 6]. The activity of the compounds was also carried out by calculating the physical properties such as ionization potential, electronic energy, and dipole moments. The drug score and drug-likeness of the ligands were also predicted [Table 7]. After careful study of the drug-likeness property of all the tested compounds, it

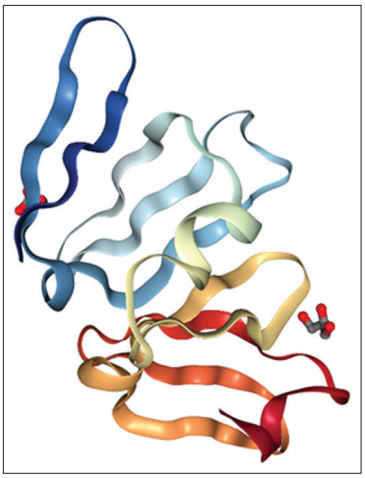

Figure 2: 3-D structure of the *Staphylococcus aureus* AgrA LytTR domain (4g4k)

was found that compounds (1y, 1z, 1aa, 1bb, 1cc, 1dd, and 1ee) have the drug score value in the range of 0.2–0.5 and the rest of the compounds in the range of 0.5–0.77. To assign a good drug, the toxicity property is one of the essential parameters. The drug which has low toxicity or side effect is considered the most effective drug. Taking these concepts, I have performed toxicity prediction using Osiris property explorer and it was shown in color codes. Different colors indicate different properties such as green color representing low toxicity, yellow representing mediocre toxicity, and red representing high toxicity. The results expressed that all the compounds have low toxicity as shown in Table 8.

After performing the molecular docking study, it was expressed that 10 docked complexes of 34 have larger negative binding energy than standard HAM drug as shown in Table 7. The highest binding energy docking model comprising "1ee" ligand with 4g4k is shown in Figure 3 as a sample. The docking study also showed that all the tested compounds under study have occupied the same cavity [Figure 4] like the natural ligand D-hamamelose one.

Table 4: Data representing the qualifications of the substituents for drug likeliness using CMC like rule, MDDR like rule, and WDI like rule along with rule of five as predicted using PreADMET

Compounds	CMC like rule	MDDR like rule	Rule of five	WDI like rule
1	Not qualified	Drug like	Violated	90%
1a	Not qualified	Drug like	Violated	90%
1b	Not qualified	Drug like	Violated	90%
1c	Not qualified	Drug like	Violated	90%
1d	Not qualified	Drug like	Violated	90%
1e	Not qualified	Drug like	Violated	90%
1f	Not qualified	Drug like	violated	90%
1g	Not qualified	Drug like	Violated	90%
1h	Not qualified	Drug like	Violated	90%
1i	Not qualified	Drug like	Violated	90%
1j	Not qualified	Drug like	Violated	90%
1k	Not qualified	Drug like	Violated	90%
1l	Not qualified	Drug like	Violated	90%
1m	Not qualified	Drug like	Violated	90%
1n	Not qualified	Drug like	Violated	90%
1o	Not qualified	Drug like	Violated	90%
1p	Not qualified	Drug like	Violated	90%
1q	Not qualified	Drug like	Violated	90%
1r	Not qualified	Drug like	Violated	90%
1s	Failed	Drug like	Violated	90%
1t	Qualified	Drug like	Suitable	90%
1u	Qualified	Drug like	Suitable	90%
1v	Qualified	Drug like	Suitable	90%
1w	Failed	Drug like	Suitable	Failed
1x	Failed	Drug like	Suitable	Failed
1y	Failed	Drug like	Suitable	Failed
1z	Failed	Drug like	Suitable	Failed
1aa	Failed	Drug like	Suitable	Failed
1bb	Qualified	Drug like	Suitable	90%
1cc	Qualified	Drug like	Suitable	90%
1dd	Not qualified	Drug like	Violated	90%
1ee	Not qualified	Drug like	Violated	90%
1ff	Not qualified	Drug like	Violated	90%
1gg	Not qualified	Drug like	Violated	90%
1hh	Not qualified	Drug like	Violated	90%

CMC: Comprehensive medicinal chemistry, WDI: World drug index, MDDR: Modern drug data report

DISCUSSION

HOMO–LUMO energy

The HOMO corresponds to the ability of electron donating and the LUMO for the ability of electron accepting, and the gap between HOMO and LUMO reflects the molecular chemical stability. This energy gap directs the mobility of electron between above said orbitals and it determines the kinetic stability, chemical reactivity, optical polarizability, and chemical hardness-softness of a molecule.[30] The molecule with a large energy gap is known as hard molecule, while a molecule with a small energy gap corresponds to soft molecule. The soft molecule is more polarizable than the hard ones because hard molecule requires high energy to excitation.[31,32] As the gap between HOMO and LUMO is decreased, the possibility of electron transfer is easier which is called the intermolecular charge transfer

Table 5: Molecular descriptor properties of the ligands

Compounds	milogP	TPSA	nON	nOHNH	N violations	N rotb	Volume	N atoms
1	−0.66	243.90	14	9	2	8	382.28	34
1a	−2.19	267.28	15	11	3	8	396.19	35
1b	−2.85	287.51	16	12	3	8	404.23	36
1c	−0.92	236.06	14	9	3	9	405.67	35
1d	−0.30	225.06	14	8	3	10	423.20	36
1e	−2.68	273.08	15	12	3	8	399.46	35
1f	−2.64	278.87	15	13	3	8	402.73	35
1g	−2.60	284.67	15	14	2	8	499.43	35
1h	−1.77	275.71	15	12	2	8	396.87	35
1i	−2.43	292.79	16	12	3	9	415.85	37
1j	−2.19	309.86	17	12	3	10	434.83	39
1k	−2.82	332.72	18	13	3	11	457.09	41
1l	−2.85	330.08	18	13	3	10	442.88	40
1m	−3.48	352.95	19	14	3	11	465.13	42
1n	−3.43	350.31	19	14	3	10	450.57	41
1o	−1.54	289.63	16	11	3	10	426.79	38
1p	−0.77	260.97	15	9	3	9	401.26	36
1q	−0.51	278.04	16	9	3	10	420.25	38
1r	−1.02	283.83	16	10	3	10	423.52	38
1s	0.87	183.21	11	6	2	8	358.55	31
1t	1.87	162.98	10	5	0	8	350.16	30
1u	1.52	162.98	10	5	0	8	350.51	30
1v	2.44	142.76	9	4	0	8	342.46	29
1w	1.85	142.76	9	4	0	8	342.52	29
1x	0.43	149.07	9	6	1	8	354.19	29
1y	3.53	142.76	9	4	0	8	375.64	31
1z	4.46	102.30	7	2	0	8	392.73	31
1aa	2.80	102.30	7	2	0	8	372.37	27
1bb	4.38	61.84	5	0	0	8	310.39	25
1cc	6.03	61.84	5	0	1	8	376.64	29
1dd	0.21	266.76	15	10	3	9	404	36
1ee	0.12	289.63	16	11	3	10	426.79	38
1ff	−1.37	289.63	16	11	3	10	426.79	38
1gg	−1.00	289.63	16	11	3	10	426.79	38
1hh	−1.62	312.49	17	12	3	11	449	40

and the molecules become more active.[33] The molecule with a high HOMO-LUMO energy gap is considered as kinetically stable, i.e. chemically inert and the molecule with small or no HOMO-LUMO energy gap is said to be chemically reactive. Pearson showed that the HOMO-LUMO energy gap intends the chemical hardness of the molecule.[34] The derived parameter electrophilicity index, which originates from flow of electron between donor and acceptor, lowers the energy of ligand.[35]

The complete equations for calculating ionization potential, electron affinity, chemical potential, global hardness, global softness, electronegativity, and electrophilicty index are as follows: Ionization potential(I) = - e_{homo}; electron affinity(a) = -e_{lumo}; chemical potential(μ) = (e_{lumo} + e_{homo})/2; global hardness(η) = (e_{lumo} - e_{homo})/2; global softness(s) = (1/η); electronegativity(σ) = - μ; and eletrophilicity index(ω) = (μ^2/2η).

All of the above calculations were done in four different phases including three different solvents. The structures of all the systems in this work are given in Figure 5. Reactivity of molecules depends on the frontier orbitals HOMO and LUMO. The molecule with a smaller frontier orbital gap is more polarizable, more chemically reactive, and kinetically less stable, and it is assigned as soft molecule. It was found that this gap is decreased with the increase of polarity of the solvent of all the concerned systems. The HOMO and LUMO

Table 6: PreADME prediction of ligands

Compounds	HIA	Caco-2	MDCK	*In vitro* plasma %	*In vitro* blood barrier
1	2.63	8.16	4.78	81.71	0.030
1a	0.4966	9.6563	5.994	76.2	0.028
1b	0.000	10.22	2.175	74.9	0.0282
1c	3.4475	10.55	0.1691	67.69	0.030
1d	6.283	13.03	0.051	60.41	0.0312
1e	0.618	12.62	9.820	63.49	0.028
1f	0.636	13.92	12.41	40.55	0.0280
1g	0.632	14.826	20.67	35.82	0.0278
1h	0.979	14.060	6.732	47.72	0.0282
1i	0.655	14.0096	0.054	57.47	0.0278
1j	0.407	13.592	0.043	72.71	0.0279
1k	0.152	14.168	0.0435	60.35	0.0321
1l	0.066	13.657	0.043	67.669	0.0275
1m	0.000	14.031	0.0434	55.648	0.0293
1n	0.000	11.98	0.0436	60.065	0.0273
1o	1.0547	12.883	0.0443	76.957	0.0285
1p	1.773	10.807	0.0512	78.468	0.0294
1q	1.150	12.648	0.043	78.74	0.032
1r	1.073	12.212	0.043	75.764	0.0348
1s	25.110	14.49	12.01	76.76	0.037
1t	47.07	18.755	0.791	83.43	0.042
1u	47.08	16.50	5.70	81.52	0.042
1v	70.30	19.32	0.617	87.38	0.050
1w	70.64	16.97	13.51	79.08	0.038
1x	49.10	5.62	6.18	81.18	0.045
1y	75.96	17.77	0.366	82.63	0.052
1z	94.97	21.16	0.056	89.22	0.055
1aa	93.51	19.67	18.59	86.18	0.019
1bb	98.34	38.35	4.22	91.60	0.033
1cc	97.82	41.16	0.062	91.49	0.065
1dd	1.75	13.61	1.725	75.86	0.028
1ee	1.07	14.63	8.16	66.31	0.030
1ff	1.06	11.95	0.087	67.5	0.029
1gg	1.058	12.76	2.874	76.99	0.030
1hh	0.586	13.79	0.052	63.66	0.059

energy level of any systems was found to be stabilized in polar solvents, but LUMO level was stabilized little more due to the H-bond and electrostatic interaction. It implies that the decrease in the energy of LUMO was greater than that in the HOMO energy. Consequently, the energy gap between HOMO and LUMO became low[36] and it was supported by the values as shown in Table 1. Thus, the chemical reactivity of the HAM in the various solvents follows the order: Gas > water > dimethyl sulfoxide > cyclohexane. The contours of the occupied and unoccupied molecular orbitals in different solvents using R-B3LYP with 6-31(G) basis set are shown in Figure 5.

Molecular descriptor properties

The selected compounds which are considered as a potential drug are scrutinized by determining the molecular weight, number of rotational bonds, number of hydrogen bonds (nON and nOHNH), and polar surface area (TPSA). In this study, as the molecular weights of all compounds were greater than 500, so first three criteria comprising the number of rotatable bonds (<10), the nON and nOHNH donors and acceptors (<12), and TPSA values (>140) were not solely considered as ideal oral drug index. However, taking into consideration other factors such as logp, drug score, and drug-likeliness, these ligands may be

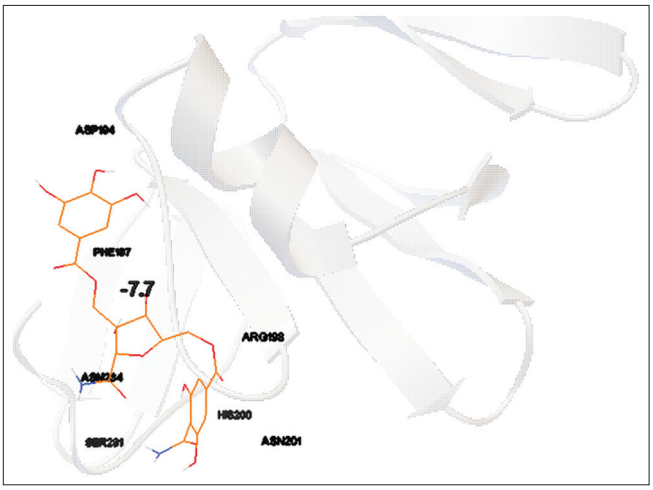

Figure 3: Docking model of the ligand ("1ee") having highest binding affinity with 4g4k protein (protein data bank ID-4g4k)

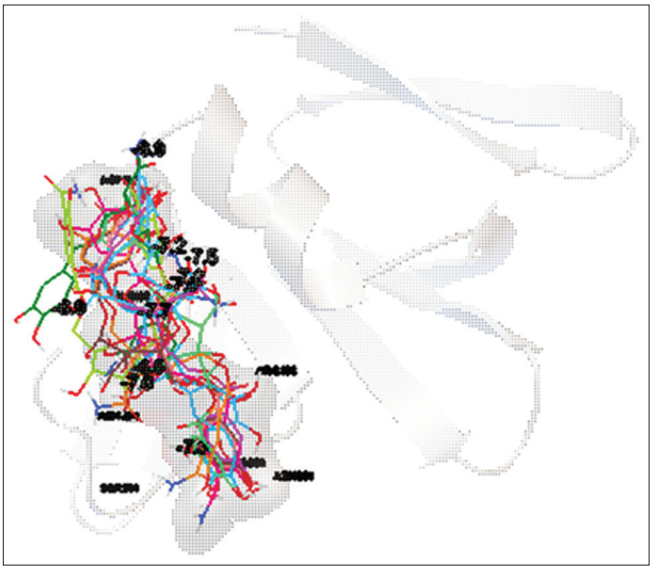

Figure 4: Zoomed view of the active site showing all the docked molecules

considered as a good oral drug.

The active site, responsible for drug-likeness property, is the same for all the systems. The drug-likeliness values of all the compounds are rationally adequate (except 1c, 1d, 1x, 1y, 1z, 1dd, and 1ee) as shown in table 5. The higher drug-likeness values are found in the case of 1j, 1o, 1gg, and 1hh compounds. These results also indicate that these four compounds have the same active fragments and same active functional group just like the existing potential drug HAM.

The drug score values along with logP, solubility, molecular weight, and toxicity were calculated to determine the drug-likeness property.

A better drug must have a better drug score. Our data showed that compound "1t" has the best score (0.77), the compounds 1y, 1z, 1aa, 1bb, 1cc, and 1dd, 1ee were in the range of 0.2–0.5, and the rest of the compounds were in the range of 0.5–0.77. The hydrophobicity of drugs is related with log *P* values [Table 7]. It was found that all the tested compounds have low logp values, i.e. these drugs will be more absorbed and will retain in the body at a measurable time.[37]

ADME prediction

The programmes like PreADMET prediction; MDCK and Caco-2 cell permeability etc. stand for designing modern drug through the computational screening model. The compounds 1z, 1aa, bb, and 1cc under this study have qualified HIA% *in vitro* plasma% (>90% in all cases) and Caco-2 cell permeability (>25 nm/s) considered to be a good drug, while the rest of the compounds satisfies other parameters. A few compounds have shown excellent permeability, while others have relatively less or poor (in some cases) permeability as shown in Table 4.

Molecular docking and pharmacophore study

The binding affinity of gallic acid (−4.5 kcal/mol) [Figure 6] is higher than D-ribose (−4.2 kcal/mol) [Figure 7]. Accordingly, the binding energy of gallic acid-gallic acid derivatives [Figure 8] is higher than gallic acid-ribose derivatives [Figure 9]. Thus, more binding energy was observed when the substitutions were done in gallic acid residue. It was also observed from the fragment-based approach that the incorporation of phosphate group, amine group, halogen, carboxylic group unable to enhance the binding affinity significantly, while amide group shown high binding energy. Moreover, the amide group in the gallic residue shows more binding affinity compared to ribose as shown in Table 3. The molecular alignment of the docked complex revealed that all the tested compounds have occupied the same cavity as occupied by standard drug HAM. The active site of the 4g4k is represented by Ser-190, Arg-207, Asn-234, Asp-194, Arg-198, Ile-210, and Val-235. This phenomenon inferred that the tested compounds may be the competitor to the natural ligand inhibitor. The binding energy was expressed by binding free energy (ΔG) and inhibition constant (Ki) values. Of the 34 tested compounds, 10 models have the best-docked complex, i.e., large negative

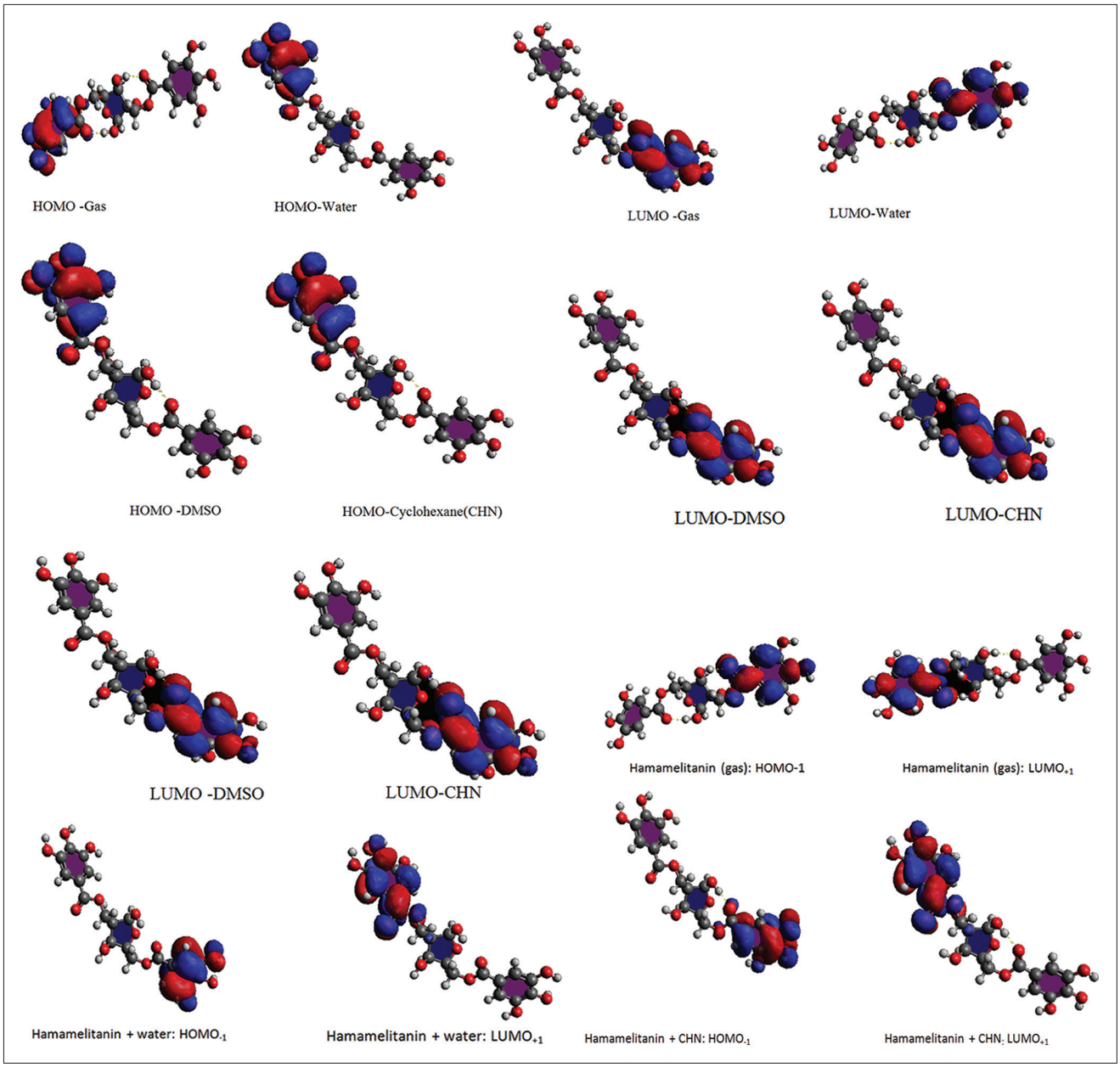

Figure 5: Contours of the occupied and unoccupied molecular orbitals in different solvents using R-B3LYP with basis set 6-31(G)

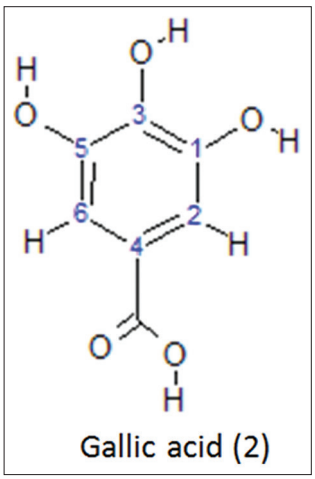

Figure 6: Structure of gallic acid

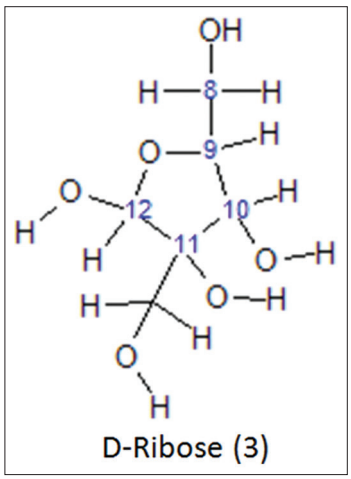

Figure 7: Structure of D-ribose

values of ΔG and low inhibition constant Ki, while remaining 24 compounds have low binding energy and inhibition constant as compared to the standard drug HAM.

Table 7: Fragment-based drug-likeliness of the ligands

Compound	clogP	Solubility	MW	Drug-likeliness	Drug score
1	−0.96	−0.86	484	2.15	0.73
1a	−1.85	−0.23	502	1.73	0.69
1b	−2.56	−0.04	518	1.81	0.67
1c	−0.72	−0.54	500	−0.25	0.53
1d	−0.29	−0.67	514	−0.08	0.53
1e	−2.25	−0.31	501	1.87	0.69
1f	−2.65	−0.38	500	1.87	0.7
1g	−3.05	−0.46	499	1.41	0.67
1h	−2.46	−0.83	498	2.26	0.71
1i	−3.06	−0.87	526	3.45	0.69
1j	−3.22	−0.9	554	3.88	0.66
1k	−3.74	−1.08	581	3.35	0.62
1l	−3.92	−0.72	570	3.64	0.64
1m	−4.44	−0.9	597	3.33	0.6
1n	−4.33	−0.4	586	3.64	0.62
1o	−2.51	−1.09	538	3.88	0.68
1p	−1.56	−0.9	512	1.45	0.53
1q	−1.71	−0.94	540	1.46	0.62
1r	−2.11	−1.01	539	3.59	0.68
1s	0.56	−1.91	436	1.13	0.72
1t	1.35	−2.17	420	1.85	0.77
1u	0.91	−2.09	420	1.24	0.74
1v	1.76	−2.49	404	1.06	0.72
1w	1.25	−2.5	404	1.34	0.75
1x	0.39	−1.65	408	−0.75	0.57
1y	1.94	−3.19	432	−2.18	0.25
1z	3.32	−4.47	428	−2.79	0.34
1aa	1.94	−3.09	372	0.86	0.43
1bb	3.14	−3.67	340	0.71	0.39
1cc	4.52	−5.05	396	2.95	0.28
1dd	−1.56	−1.39	511	−0.9	0.47
1ee	−2.18	−1.77	538	−1.83	0.39
1ff	−2.52	−1.36	538	3.53	0.54
1gg	−2.08	−1.36	538	3.75	0.68
1hh	−3.08	−1.47	565	3.88	0.64

The minimum binding energy (maximum stability) was observed in "1ee" tested compound having binding energy −7.7 kcal/mol. The incorporation of $–CONH_2$ in the two benzene ring residues of the compound "1ee" enhances electrostatic interaction, Van der Waals interaction, H-bond interactions, solvation interaction, and torsional interaction with the amino acids lys-187, ser-190, arg-207, asn-234, arg-198, asn-201, ser-231, arg-233, his-200, and phe-196 implying the highest binding energy.[38] In this tested compound, it was interesting to notice that the amino acids which are involved in abovementioned interactions with the compound are present in the active site of 4g4k. The ligand "1ee" formed three hydrogen bonds, of which the HZ1 atom of Lys-187 forms hydrogen bond with the ligand at a distance 2.188 A°, the HH12 of Arg-207 at 2.095 A°, and HD21 of Asn-234 at 2.219 A°. It was reported that the key amino acids Arg-207 and Asn-234 are more effective for binding and biological activity.[25] The maximum binding energy or minimum stability was found in 1cc (−5.4 kcal/mol) which forms only one hydrogen bond with the Arg-207 residue of the receptor. It was also observed from the binding energy values that incorporation of amide group at –3C and −19C shows pronounced interaction with the enzyme in comparison to compounds with hydroxyl group at the respective positions.

Table 8: Toxicity prediction as per output of Osiris program

Compounds	Mutagenic	Tumorigenic	Irritant	Reproductive effect
1	Green	Green	Green	Green
1a	Green	Green	Green	Green
1b	Green	Green	Green	Green
1c	Green	Green	Green	Green
1d	Green	Green	Green	Green
1e	Green	Green	Green	Green
1f	Green	Green	Green	Green
1g	Green	Green	Green	Green
1h	Green	Green	Green	Green
1i	Green	Green	Green	Green
1j	Green	Green	Green	Green
1k	Green	Green	Green	Green
1l	Green	Green	Green	Green
1m	Green	Green	Green	Green
1n	Green	Green	Green	Green
1o	Green	Green	Green	Green
1p	Green	Green	Green	Yellow
1q	Green	Green	Green	Green
1r	Green	Green	Green	Green
1s	Green	Green	Green	Green
1t	Green	Green	Green	Green
1u	Green	Green	Green	Green
1v	Green	Green	Green	Green
1w	Green	Green	Green	Green
1x	Green	Green	Green	Green
1y	Green	Red	Green	Green
1z	Green	Green	Green	Green
1aa	green	Green	Red	Green
1bb	Green	Green	Red	Green
1cc	Green	Green	Green	Green
1dd	Green	Green	Green	Green
1ee	Green	Green	Green	Green
1ff	Green	Green	Green	yellow
1gg	Green	Green	Green	Green
1hh	Green	Green	Green	Green

The introduction of –OH group in place of –H at −7C and −13C, CH2 in place of CO within the ligand "1o", decreases the binding energy, indicating a reduction of non-covalent interaction with the 4g4k protein. The HG1 of Ser-190 forms hydrogen bond with carbonyl group (−13C) of the ligand at the distance 1.957 A°, HH12 of Arg-207 with –OH group (−19C) at 2.102 A°, and HD22 of Asn-234 with –OH group (−12C) at 2.011 A°. The incorporation of OH in place of –CO in the side chain (8th position) , -OCH3 and -NH2 in place of –OH (10th and 12th position respectively) in the ribose ring residue, reduces the dipolar repulsion causing lower binding energy of all the compounds '1a' to "1h" [Figure 1]. The substitution of –OH group by –CONH2 in the ribose ring decreases hydrogen bonding distance, enhances dipolar and hydrogen bonding interaction causing high binding energy of the compound "1i." The incorporation of the second amide group increases the binding energy of the compound "1" for the same reason. However, the introduction of third amide group in the same, enhances high steric crowding and decreases the binding energy of the compound "1k." In the compound "1l" at −13C, the substitution of –H by –OH decreases hydrophobic interaction reflecting less binding energy. Due to the steric hindrance phenomenon caused by the incorporation of three amide groups in the ribose ring of the ligand, "1m" further

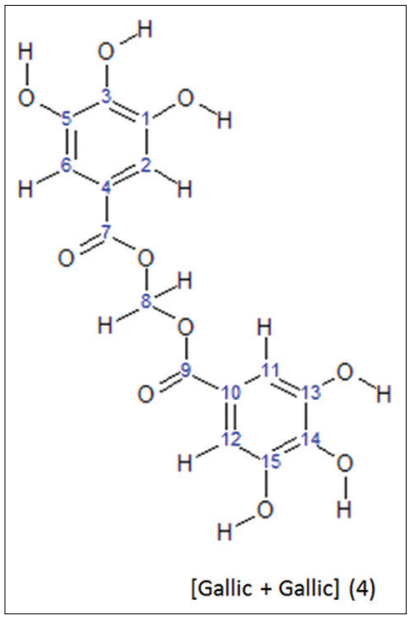

Figure 8: Structure of gallic acid-gallic acid

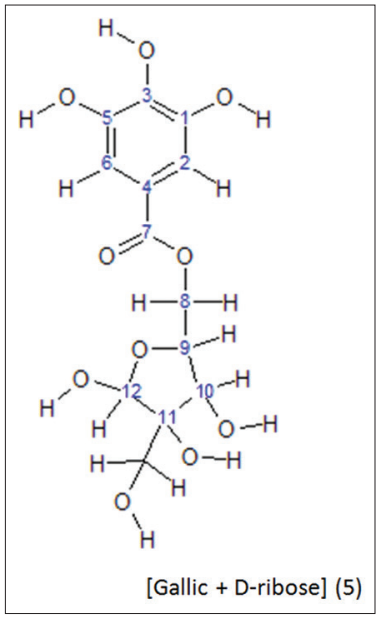

Figure 9: Structure of gallic acid-ribose

reduces the binding energy. In the compound "1n," the number of amide group is decreased, steric crowding decreases, and binding energy increases. When the –OH group is replaced by –H atom at −8C and −13C of the compound, the "1n" compound was converted into "1o" increases the hydrophobic interactions well as binding affinity. The incorporation of –COOH gr in the place of –OH at the −12C decreases the polarity and thereby decreases binding energy of the compound "1p." However, the introduction of second –COOH gr at −10C increases the polarity as the number of hydrogen bond increases, and hence increases binding energy of the compound "1q." In the compound "1r" the high polar group –COOH and –CONH2 induces high binding energy for the same reason. All the compounds from "1s" to "1x" where the polar gr. -OH is substituted by less polar –H group decreases the interaction and follow less binding energy. In the compound "1y" and "1z" the presence of less polar group –OCH3 imply moderate hydrophobic interaction so binding energy lie in the range −6.5–−6.6 kcal/mol. When all the polar –OH group are replaced by –H atom in the two gallic acid residues of the compound "1aa," the interaction decreases and binding energy lies at the value −6.4 kcal/mol. Furthermore, the replace of –OH group in the ribose ring of the compound '1bb' decreases the binding energy due to lack of hydrogen bonding. The introduction more hydrophobic –CH3 group in the place of –OH group within the Gallic acid residue in the compound "1cc" shown lowest binding energy due to least interactions.

The substitution of one –OH group by highly polar $–CONH_2$ at −3C in the active site Gallic ring in the compound "1dd" results in high interaction and high binding energy of the value −7.3 kcal/mol. The incorporation of second $–CONH_2$ group in the second gallic ring at −19C in the compound "1ee" maximum interaction takes place and shows highest binding energy of the value −7.7 kcal/mol. However, one amide group in the gallic ring and other at less active moiety ribose interaction not take so much amount in the compound "1ff" and "1gg" and the binding value decreases and the introduction of three amide groups, of which one in ribose and other two in gallic acid moiety, increases the binding affinity of the compound "1hh", well supported by the binding energy value −7.2 kcal/mol.

The compounds "1i," "1j," "1k," "1n", "1o," "1q," "1r," "1dd," "1ee," and "1ff" found binding energies in the range of −6.9–−7.7 kcal/mol. All these ten tested compounds were used to develop a ligand-based pharmacophore study [Table 6] using SWISS ADME tool, which could be used further for the development of new, improved, and optimized drug and could be acted as inhibitors to QS.

The pharmacophore study of all these ten tested compounds envisaged that there are eight rotational bonds, fourteen hydrogen bond acceptors, and nine hydrogen bond donors which enables in making several non-covalent interactions, hydrogen bonding, electrostatic interactions, etc.

These pharmacophores successfully qualified MDDR rule, WDI like rule, drug likeliness, logp, drug score values and could be considered as a better drug. In this way one can able to modify the better drug molecule by analyzing the therapeutic index and by enhancing the kind of interactions between the drug and the target protein.

CONCLUSION

The molecular docking study signified that the compounds can act as a useful inhibitor to QS compared to standard drug HAM. The ten compounds have also successfully qualified the WDI like rule and MDDR like rule and drug likeliness drug core value. All these ten compounds have low logp values, good drug-likeness property, and high drug score values, i.e., better pharmacological properties. Moreover, these concerned compounds have low toxicity value and the compounds were predicted to be safe (non-mutagenic as well as non-carcinogenic as shown in Table 8). This study has enlarged the scope of producing more specific and effective drugs for QS.

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SUPPORTING INFORMATION

The binding energy values of various derived compounds, were shown in the supplement Table1

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SUPPLEMENT

Supplement Table 1: Substituents of the ester of gallic acid of D-hamamelose and its binding energy

Compounds	Substituted group	Substitute by	Binding energy kcal/mol
4a	8-CH_2	8-CH_2CH_2	−6.1
4b	8-CH_2	8-$CH_2CH_2CH_2$	−6.2
4c	8-CH_2	8-$CH_2CH_2CH_2CH_2CH_2CH_2$	−5.6
5a	3-OH	3-CH_3	−5.8
5b	3-OH	3-COOH	−5.9
5c	3-OH	3-$CONH_2$	−6.1
5d	1-OH, 3-OH	1-$CONH_2$, 3-$CONH_2$	−6.6
5e	3-OH, 5-OH	3-$CONH_2$, 5- NH_2	−6.1
5f	3-OH, 5-OH	3-$CONH_2$, 5- NMe_2	−5.7
5g	3-OH, 5-CH_2OH	3-CONH2, 5-$CH_2O_4PH_2$	−6.2
5h	3-OH, 12-OH	3-$CONH_2$, 12-OPO_3H_2	−6.4
5i	3-OH, 12-OH	3-$CONH_2$, 12-$O_7P_2H_3$	−6.5
5j	3-OH, 8-CH_2	3-$CONH_2$, 8-CH_2HPO_4	−5.9
5k	1-OH, 3-OH, 5-OH	1-NH_2, 3- NH_2, 5- NH_2	−5.7
5l	1-OH, 3-OH, 5-OH, 12-OH	1-NH_2, 3-NH_2, 5-NH_2, 12-OPO_3H_2	−5.9
5m	1-OH, 3-OH, 5-OH,	1-NH_2, 3-$CONH_2$, 5-NH_2	−5.8
5n	1-OH, 3-OH, 5-OH, 12-OH	1-NH_2, 3-$CONH_2$, 5-NH_2, 12- OPO_3H_2	−6.1
5o	1-OH, 3-OH, 5-OH, 11-OH	1-NH_2, 3-$CONH_2$, 5-NH_2, 11- OPO_3H_2	−6.2
5p	8-H	8-OH	−6.1
5q	3-OH, 8-H	3-$CONH_2$, 8-OH	−6.7
5r	1-OH, 3-OH, 5-OH, 8-H	1-NH_2, 3-$CONH_2$, 5-NH_2, 8- OH	−6.2
5s	3-OH, 11-CH_2OH	3-$CONH_2$, 11-CH_3	−6.3
5t	3-OH, 10-OH	3-$CONH_2$, 10-CH_3	−6.1
5u	3-OH, 11-CH_2OH	3-$CONH_2$, 11-CH_3	−6.0
5v	3-OH, 10-OH, 11-CH_2OH	3-$CONH_2$, 10-CH_3, 11-CH_3	−6.1
5w	3-OH, 8-CH_2	3-$CONH_2$, 8-CH_2CH_2	−6.0
5x	3-OH, 8-CH_2	3-$CONH_2$, 8-$CH_2CH_2CH_2$	−5.7
5y	3-OH, 5-OH	3-$CONH_2$, 5-Cl	−6.2
5z	3-OH, 5-OH, 8-CH_2	3-$CONH_2$, 5-Cl, 8-CH_2CH_2	−6.2
5aa	3-OH, 5-OH, 8-CH_2	3-$CONH_2$, 5-Cl, 8-$CH_2CH_2CH_2$	−6.0
5bb	1-OH, 3-OH, 5-OH	1-Cl, 3-$CONH_2$, 5-Cl	−6.1
5cc	1-OH, 3-OH, 5-OH, 8-CH_2	1-Cl, 3-$CONH_2$, 5-Cl, 8- CH_2CH_2	−5.9
5dd	1-OH, 3-OH, 5-OH, 8-CH_2	1-Cl, 3-$CONH_2$, 5-Cl, 8- $CH_2CH_2CH_2$	−6.0