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INTERACTION OF SELECTED AMINO ACIDS IN ACTIVE SITE OF BAX PROTEIN WITH TWO DERIVATIVES OF SYRINGIC ACID AS AN ANTI-CANCER INITIATING PROCESS: A MOLECULAR SIMULATION STUDY

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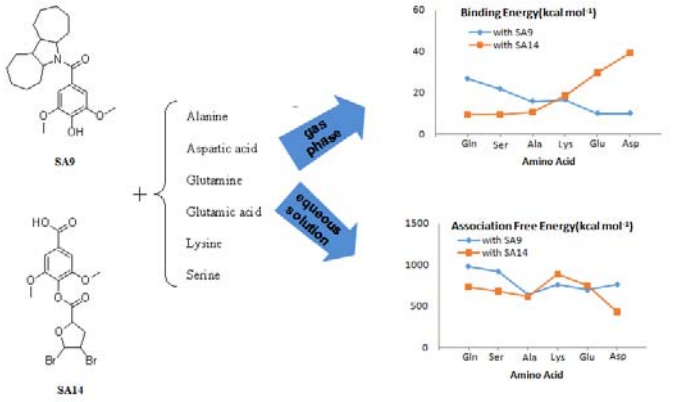
Interaction of drugs with the active site of the BAX protein induces conformational changes in BAX and subsequently starts physiological processes to eliminate damaged cells. Accordingly, the possible interactions of SA9 and SA14, two derivatives of syringic acid, as drugs, with alanine, aspartic acid, glutamine, glutamic acid, lysine and Serine were studied. The binding energies of syringic acid derivative-amino acid complexes were calculated in the gas phase by quantum mechanical method. The calculations showed that lysine, a crucial amino acid involved in BAX activation, was strongly bound to both of the two drugs. Monte Carlo simulations were used to calculate the association free energies in aqueous solution. The results showed that complexes of the syringic acid derivatives with the amino acids were stable in aqueous solution. Moreover, free energy calculations revealed that SA14 bound to Lysine, and SA9 exhibited the highest association free energy with glutamine in aqueous solution.



INTRODUCTION

Apoptosis (programmed cell death) plays a critical role in the normal growth and preservation of tissue homeostasis. It is a biological route that eliminates infected, mutated, or damaged cells in essentially all multi-cellular organisms. The apoptosis mechanism in humans involves a molecular network of a large number of proteins.[1-3] A chain of actions comprised of the signaling, commitment, and execution steps of apoptosis are controlled by these proteins. Abnormal cell growth may be caused by deficiencies in key regulators of the apoptotic machinery. These deficiencies can permit neo plastic cells to live beyond their normal life span, accumulate genetic mutations and continue growth under hypoxia and oxidative stress conditions.[4,5] In one of the main pathways(extrinsic pathway) for transmitting death signals,[6] a pro-apoptotic Bcl-2 protein, such as BAX, interacts with the mitochondria which leads to the release of cytochrome c and activation of apoptosis.[7]

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The three-dimensional structure of BAX consists of two central hydrophobic -helices surrounded by sevenamphipathic -helices of different lengths.[3] Structural analysis of the BAX protein indicated that the active site formed by helices 1 and 6 plays a role in its activation.[8] The conversion of the1-2 loop from closed conformation to open form promotes major conformational[9] changes, bringing about the activation of BAX. Minor changes of the loop residues of BAX due to BIM- BAX interaction have been recognized through NMR analysis.[9-10] One strategy for triggering the pro-apoptotic BAX protein is the use of small molecules that mimic the function of the BIM- BH3 peptide.[11,12] In common cancer cells, BAX could be an effective target for anticancer drugs.[13-15] Several chemotherapeutic agents can prompt BAX-dependent apoptosis in cancer cells.[16] BAX agonists have been developed as a strategy for the treatment of cancer and constitute a class of anticancer drugs.

Earlier studies have revealed that the natural plant-derived Gossypol and its derivatives show antitumor activity by modifying the BAX protein via its BH3 mimetic properties.[17,18]

Xin *et al*.[19] reported that small-molecule BAX agonists, SMBA1, SMBA2 and SMBA3, can induce conformational changes in BAX and facilitate insertion into the mitochondrial membrane.

SMBA1 suppresses lung tumor growth via apoptosis by activating BAX without significant normal tissue toxicity.

Syringic acid (SA) is a phenolic compound derived from edible plants and fruits[20] which also possesses anticancer properties.[21] Cheemanapalli *et al.*[22] have designed several derivatives of SA and screened them for their anticancer potency, toxicity and binding affinity with the BAX protein. Among all studied SA derivatives, SA1, SA9, SA10, SA14 and SA21 displayed the largest docking energy to BAX. The studies involved application of molecular mechanics based calculations to investigate the interactions of drugs and target molecules.

Quantum mechanical calculations can be used to evaluate binding energies more exactly. In polyatomic systems, quantum mechanical calculations are too extensive and time consuming. By considering the interacting site(instead of the whole system)one could reduce the number of degrees of freedom and render quantum mechanical computations more viable.

Since the amino acid residues of the active site groove play a fundamental role in interaction with ligands, the present research investigates the interactions between these amino acids and drugs. In particular, we study the interactions of amino acids of the BAX protein active site with derivatives of Syringic acid (SA9 and SA14) in the gas phase and aqueous solution within quantum mechanical calculation and Monte Carlo simulation. In addition, association free energies are computed to compare the stability of the structures. This paper is organized as follows: section two discusses the details of the model and explains thoroughly the computational methods employed, and section three presents the results and discussion.

COMPUTATIONAL METHOD

1. QM Computation

Cheemanapalli *et al.*[22] found that among SA analogues, SA1, SA9, SA10, SA14 and SA2exhibitedthe maximum docking energy with the BAX protein. Therefore, in the present calculation, we designed the SA9 and SA14 derivatives of SA and predicted their binding affinity with the amino acids of the BAX active site. Cheemanapalli *et al.* have been also analyzed the structural and binding site information of BAX to predict its active site residues. It was indicated that the active site is comprised of Alanine(Ala), aspartic acid(Asp), glutamine(Gln), glutamic acid(Glu), lysine(Lys) and Serine(Ser). In the present research, the binding energies of these amino acids with SA9 and SA14 were calculated in the gas phase. Quantum mechanical calculations were used for the isolated molecules (SA9, SA14, Ala, Asp, Gln, Glu, Lys, Ser) and for the complexes of the amino acids with SA9 and SA14. The most favorable atoms for the binding of the SA derivatives with the amino acids have been indicated to be oxygens of the six member ring[22] (Fig. 1).Therefore, the interactions between the amino acids and the SA derivatives were also considered via these oxygens.

To model a better representation of BAX active site that reacts like real system, cluster approach was also applied.[23] An appropriate part of active site Protein was cut out and was treated with accurate quantum chemical methods.

To investigate interaction of drugs with BAX, a quantum chemical model of BAX active site was designed on basis of crystal structure of the protein (PDB code 1F16).[24]

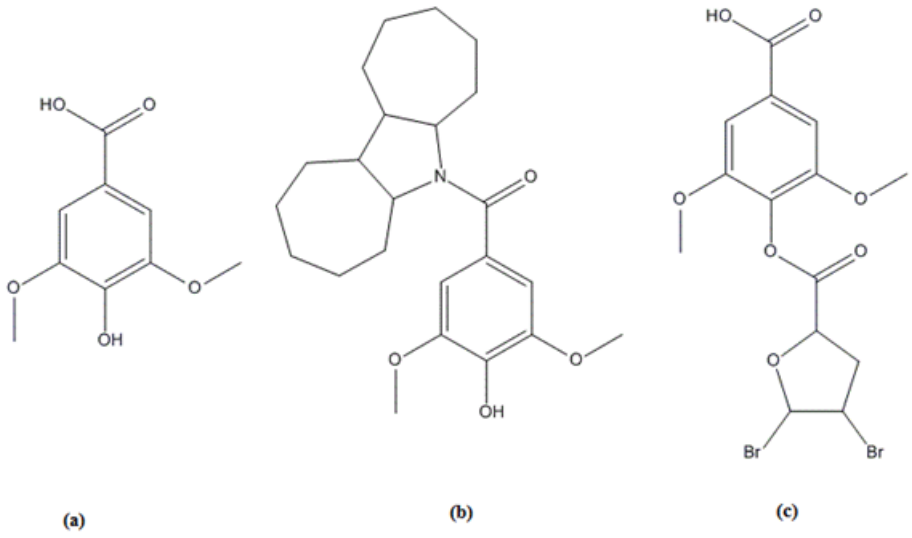


Fig. 1 – Structures of (a)SA, (b)SA9 and (c)SA14.

The binding site important shell residues of BAX[22] were included to cluster model. Hydrogen atoms were added manually and the truncated atoms were kept freeze at their crystal positions through geometry optimizations. Thus optimized structures were kept close to the experimental coordinate.

The B3LYP [25,26] hybrid functional was used for the geometry optimization with the 6-31g*,[27,28] basis set for all atoms in SA and the amino acids. The calculations were performed using the GAMESS-US quantum chemistry package.[29] Complexes of SA9 and SA14withthe amino acids were considered in the subsequent Monte Carlo simulation. Computed quantum mechanical atomic charges were applied in the simulation steps.

2. MC Simulation

Transferable intermolecular potential functions[30,31] (TIP3) are used for water molecules, and the standard Lennard-Jones (LJ) potential and Coulombic potential are applied for the short-range and long-range potentials respectively. Each type of atom is characterized by three parameters: the atomic charge, and two LJ parameters ε, and σ, where ε is the potential well depth and σ is related to the interaction range. Appropriate LJ parameters were used for the atoms in SA, the amino acids[32-34] and Br.[35] The LJ parameters between pairs of different atoms are obtained from the Lorentz–Berthelot combination rules.[36]

The free energy difference between two states can be calculated from classical statistical mechanics,[37] via the free energy perturbation (FEP) equation.

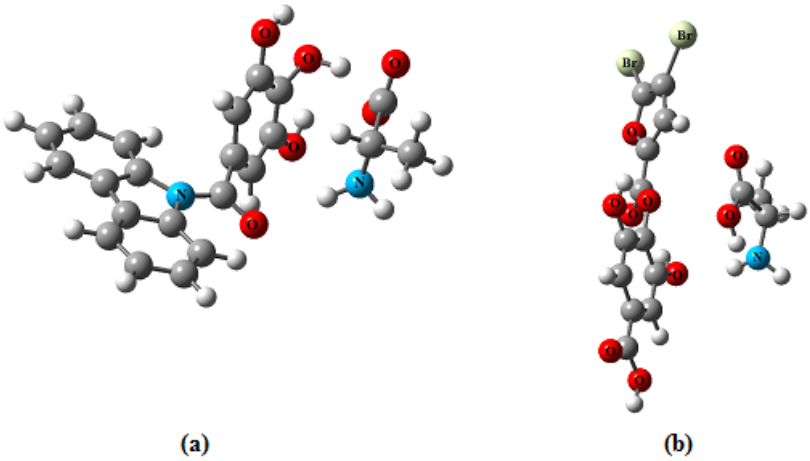
The association free energies (ΔG_{ass}) of the interaction of drugs with amino acids can be calculated using the thermodynamic cycle method,[38,39] which makes use of the free energy for the hypothetical process where the free energy of a species (A) vanishes.

Utilizing the thermodynamic cycle for the drug-amino acid complex formation, one can calculate the free energy of the drug - amino acid interaction in solution, *i.e.* the association free energy, by:

$$\Delta G_{ass} = \Delta G_{sol}(\text{Amino Acid} \rightarrow 0) - \Delta G_{sol}(\text{Amino Acid- Drug} \rightarrow \text{Drug}) \quad (1)$$

Therefore, two simulations must be performed to calculate the free energies of the complex formation of SA9 and SA14 with the amino acids: a simulation corresponding to the vanishing of the amino acid in solution [ΔG_{sol}(Amino Acid→ 0)] and one for the vanishing of the amino acid in the solvated amino acid-drug complex [ΔG_{sol}(Amino Acid- Drug→ Drug)].

All of the MC simulations were performed using a standard Metropolis sampling technique[40] in the canonical (T, V, N) ensemble. Each setup included two sections: a solute fragment and water molecules. All calculations were performed in a cubic box at the experimental density of water. The optimum box size was 30 Å, which corresponds to almost 900 H_2O molecules of pure solvent. An acceptance rate of 50% for new configurations was achieved by using ranges of ±0.15 Å for the translations and ±10° for the rotation about a randomly chosen axis. The results showed that the best length of the Markovian chains included configurations.



(a) (b)

Fig. 2 – Optimized structure of a)SA9-ala b) SA14-ala.

Table 1

QM results for drugs, amino acids and their complexes in the gas phase

Structure	**Dipole moment (Debye)** **(Debye)**	**Energy (kcal mol^{-1})**	**Binding energy (kcal mol^{-1})**
SA14	4.638	-3846798.017	
SA9	4.222	-682234.641	
Ala	6.261	-203085.279	
Asp	5.576	-321029.223	
Gln	2.637	-333576.500	
Glu	4.396	-345694.216	
Lys	5.259	-312045.608	
Ser	5.841	-250263.582	
SA9-Ala	3.476	-885335.647	-15.728
SA9-Asp	21.964	-1003273.783	-9.919
SA9-Gln	3.961	-1015837.675	-26.534
SA9-Glu	19.769	-1027938.711	-9.854
SA9-Lys	17.368	-994296.733	-16.484
SA9-Ser	5.087	-932519.870	-21.647
SA14-Ala	4.992	-4049893.801	-10.505
SA14-Asp	10.920	-4167866.188	-38.948
SA14-Gln	4.294	-4180383.772	-9.255
SA14-Glu	3.338	-4192521.701	-29.468
SA14-Lys	17.667	-4158861.907	-18.282
SA14-Ser	5.328	-4097070.737	-9.138

RESULTS AND DISCUSSION

1. QM

The optimized structures of the SA9- and SA14 –alanine complexes are displayed in Fig. 2

The binding energies of the interaction of drug with amino acid were calculated according to the expression

$$E_b = E(\text{amino acid\_drug}) - [E(\text{drug}) + E(\text{amino acid})] \quad (2)$$

where E(amino acid_ drug), E(drug) and E(amino acid)]denote the total energy of the optimized structure of the complexes of SA9- and SA14- amino acid,SA9 and SA14, and amino acid respectively. The results are presented in the Table 1.

All calculated binding energies are in the range 9–40 kcal mol^{-1}which is in the hydrogen bond range. As it is seen in Table 1, the binding energies of SA9 and SA14 with the amino acids are in the following order:

Binding with SA9: Gln > Ser > Lys > Ala > (Asp & Glu)

Binding with SA14: Asp > Glu > Lys > Ala > (Gln & Ser)

The partial charges on the atoms of SA9 and SA14 cause these drugs have a lower affinity towards alanine witch is an amino acid with non polar side chains. Hence, the binding energy of alanine with the drugs is lower than the other amino acids.

Comparing the binding properties of SA derivatives indicates that Asp and Glu have the largest binding energies with SA14 and the lowest with SA9. Conversely, Gln and Ser have the lowest binding energies with SA14 and the largest with SA9. The different affinity toward amino acids can be attributed to the structural differences between SA14 and SA9. Moreover, the properties of amino acid residue side chain also affect their interactions with drugs. Addition of brominated furan to the hydroxyl group of SA affects its affinity towards hydrogen bonding.

The distance between the hydrogen bond interactions of all the amino acid-drug compounds were found to be in the range of 1.5–5 Å. It has been reported that in BAX-drug interaction, the donor atoms in the hydrogen bonding are from BAX residues and a very few are from SA derivatives.[22] Br atoms are electron withdrawing groups and increase the hydrogen bond strength. Therefore, the binding energy ofSA14with amino acids without charged side chains like Asp and Glu is increased. Conversely, SA9 has the largest interaction energy with polar side chain amino acids. Among all amino acids, Lys shows strong interactions with both two SA derivatives and seems to play a critical role in the interactions of BAX with SA9 and SA14.

As shown in Table 1, the binding energy of SA14-Lys is larger than SA9-Lys. These results are compatible with the findings of Cheemanapalli *et al.* which have concluded that SA14 binds particularly with Lys at the helix of BAX.[22] Although, the calculated binding energies indicated that both SA14 and SA9 can interact with all the studied amino acids in the gas phase.

To understand the role of surroundings on interaction of drug and amino acids, we also investigated interactions of SA9 and SA14 with residues of BAX active site in a cluster model. A substantial part of BAX protein was selected and included in model and the binding energies were determined. The optimized structures of the SA9-active site cluster complexes are displayed in Fig. 3.

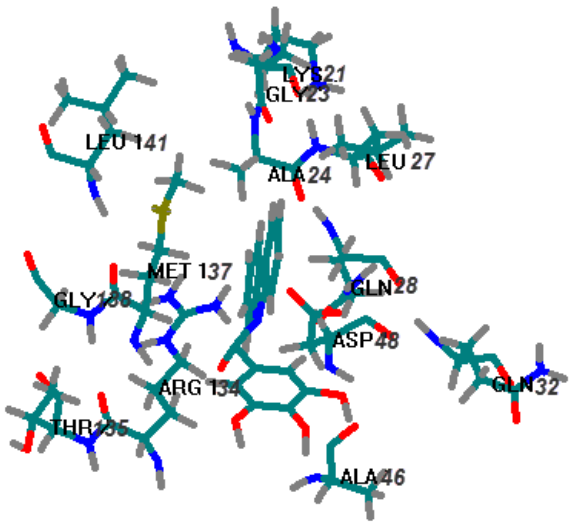


Fig. 3 – Structure of SA9- BAX active site complex.

The results are presented in Table 2. Calculated binding energies indicated that both drugs interact with BAX strongly. In fact results of interactions of all residues in active site and drugs were induced powerful binding. By comparing of binding energies, binding interactions of SA14-BAX active site is stronger than SA9- BAX active site. Hence, Quantum mechanical calculations of cluster model are compatible with calculations of individual active site amino acids.

Table 2

QM results for active site cluster and complexes with two drugs

Species	E (k cal/mole	Eb (kcal/mole)	Dipole moment (Debye)
Active site cluster	-3448905.202		65.681
SA9-BAX Active site	-4136195.925	-5056.203	48.370
SA14-BAX Active site	-7301302.931	-5599.531	51.861

As it is seen in table, dipole moment of SA9-BAX and SA14-BAX are 48.370 and 51.861 respectively. Hence, dipole moment of active site is reduced after interactions with drugs. In fact interactions with drugs induced conformational changes in BAX and therefore, its dipole moment is altered. BAX active site is confluence of α-helices 1 and 6 that included a hydrophobic groove involving the residues Ala24, Met137, Leu141 and perimeter of hydrophilic amino acids such as Lys21, Gln28, Gln32, Glu131, Asp48 and Arg134. Due to partial charges on atoms of drugs, hydrophobic polar residues have a higher affinity towards these drugs. After interaction of SA9 and SA14 with perimeter residues, conformational changes in hydrophobic groove are encouraged and therefore dipole moment is decreased. As it is mentioned before, conformational changes, will be bringing about activation of BAX.[9]

2. MC

The total interaction energies of the compounds in aqueous solution have been calculated by Monte Carlo simulation and tabulated in Table 3. This table also includes the number of solvent molecules (N_{H2O}) in the cubic box, and the electrostatic and van der Waals contributions to the solute-water interaction energy.

The results show that electrostatic interactions constitute the main contribution to the total energy for all the aqueous complexes. It can be noted that the largest total interaction energies correspond to the complexes of lysine with SA9 and SA14 in aqueous solution, -622.772 and -516.129 kcal mol^{-1} respectively. This can be attributed to the strong intermolecular interaction in the lysine complexes. The Lysine-amino acid complexes also exhibit the largest electrostatic contribution. The electrostatic terms of intermolecular energies can be affected by the dipole moment and partial atomic charges of solute. As it is seen in table 1, the dipole moments of the complexes of lysine with SA9 and SA14 are 17.368 and 17.667 Debye respectively. The SA14-Lys complex exhibits the largest dipole moment among SA14 complexes. However, SA9-Glu has a larger dipole moment than SA9-Lys, but the total interaction energy of SA9-Lys in aqueous solution is larger than that of SA9-Glu. This is due to the fact that besides the dipole moment, the partial charges of atoms in a molecule may also have an impact on the electrostatic contribution of solute-solvent interactions. The electrostatic contributions in solute-solvent interaction energies of SA9-Glu and SA9-Lys are -435.546 and -634.078 kcal mol^{-1} respectively. Consequently, the total interaction energy of SA9-Lys in water is greater than other SA complexes.

To further characterize the possible interactions of SA9 and SA14 with the amino acids in aqueous solution, the association free energies were also computed. As stated above, to calculate the association free energies ((ΔG_{ass})) using eq.1, requiresthe free energies of the reactions: amino acid→0 and amino acid-drug→drug. The results are presented in Table 4.

It is noteworthy that the association free energies of all amino acid-drug interactions are negative, indicating that both SA9 and SA14 can bind to the amino acids in the active site of the BAX protein in aqueous solution. Between the two drugs, SA9 has relatively higher absolute association free energies with the amino acids. The earlier docking studies[22] of SA based structural analogues to the selected active site of the BAX protein have also revealed that among all the SA analogues, SA14 and SA9havethe highest binding energies, with first and second docking scores among all docked molecules. However their binding energy differences were reported to be about 4%.

Table 3

MC simulation results (energies are in kcal mol^{-1})

Structure	**N_{H2O}**	**Electrostatic Contribution in solute-solvent interaction energy**	**van der Waals contribution in solute-solvent interaction energy**	**E_{total}**
SA9-Ala	907	-38.834	1.266	-36.697
SA9-Asp	908	-201.685	6.241	-192.414
SA9-Gln	904	-47.523	0.477	-41.766
SA9-Glu	908	-435.546	14.735	416.01
SA9-Lys	898	-634.078	13.475	-622.772
SA9-Ser	906	-40.539	0.328	-37.489

Table 3 (continued)

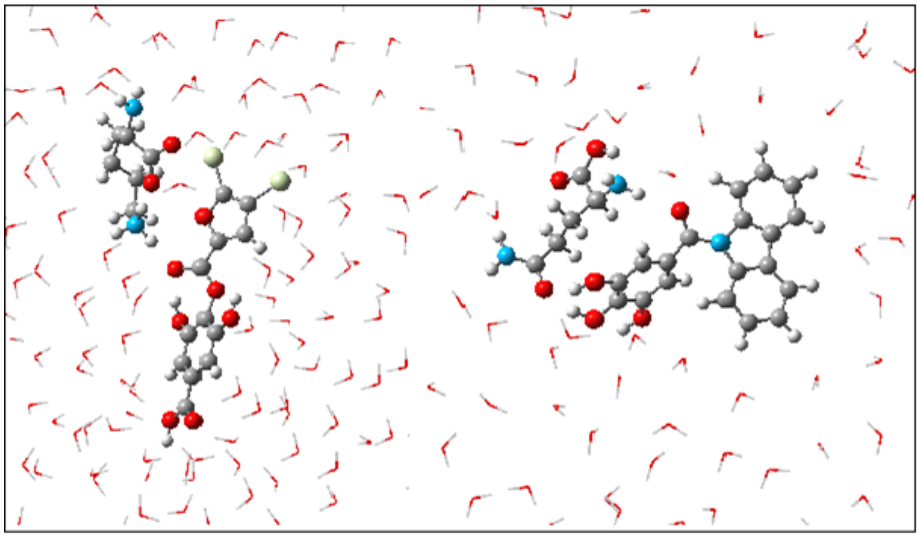
SA14-Ala	912	-357.086	0.260	-352.897
SA14-Asp	918	-245.357	0.633	-246.090
SA14-Gln	905	-310.464	0.292	-305.209
SA14-Glu	910	-365.412	10.920	-351.470
SA14-Lys	907	-526.268	11.304	-516.129
SA14-Ser	912	-300.481	0.161	-298.687

Table 4

Free energy calculations results

STRUCTURE	**ΔGsol(L→0)**	**ΔGsol(LR→R)**	**ΔG(ass)**
SA9-ALA		-25.960	-629.565
SA9-ASP		-181.519	-756.817
SA9-GLN		-29.800	-977.739
SA9-GLU		-403.915	-687.652
SA9-LYS		-610.198	-752.111
SA9-SER		-27.073	-916.716
SA14-ALA		-329.269	-609.067
SA14-ASP		-225.766	-429.759
SA14-GLN		-279.097	-728.442
SA14-GLU		-349.025	-742.542
SA14-LYS		-482.128	-880.181
SA14-SER		-266.781	-677.008
Ala	-655.525		
Asp	-938.336		
Gln	-1007.539		
Glu	-1091.567		
Lys	-1362.309		
Ser	-943.789		

*L=aminoacid, R=drug (SA9 & SA14), Free energies are in kcal mol^{-1}



(a) **(b)**

Fig. 4 – Snapshot of the simulation ofSA14-Lys andSA9-Glu.

By comparing the association free energies, it is noted that glutamine has the largest ΔG_{ass} with SA9(-977.739kcal mol^{-1}) and lysine has the largest ΔG_{ass} with SA14(-880.181kcal mol^{-1}). Fig. 4 shows one snapshot of the equilibrium state in the MC simulations of SA14-Lys andSA9-Glu in water.

Docking studies have shown similar results.[22] It has been revealed that SA14 bindswith the active site residues of Lys and Thr, and that SA9possessesthe highest docking score with Gln of BAX.

CONCLUSIONS

The BAX protein has a critical role in initiating the cytochrome c-mediated apoptosis, a physiological process to eliminate damaged or undesirable cells. As discussed above, the interactions of drugs with the residues of BAX will bring about conformational changes in BAX and promote its activation. In this research the interactions of syringic acid analogous, particularly SA9 and SA14, with selected amino acids in the active site of the BAX protein were investigated. The study included modeling SA9 and SA14 and their amino acids complexes by quantum mechanical calculations, as well as Monte Carlo simulation to calculate complexation free energies for the related structures in aqueous solution.

The development of theoretical approaches influences results quality strongly. The most accurate theoretical methods are based on quantum mechanics which are very CPU-intensive. Therefore, we used individual amino acids interactions and also cluster approach to minimize degree of freedom of the system. Moreover, for the real system with the solvent molecules, it is inevitable to apply MC simulations based on molecular mechanics force fields.

Binding affinity is the strength of interactions between these two drugs and BAX which is defined by binding energy (E_b) or association free energy (ΔG_{ass}). Experimental researchers tend to determine binding affinity using binding constant, while in computational approaches, binding free energy is calculated to define binding strength. By concerning direct correlation between dissociation constant and binding free energy, both computational methods (such as QM and MC) and experimental techniques mention to the binding affinity and can be compared with each other.

However, applying an unsuitable concentration of drug and or protein is common mistake and time consuming in experimental techniques. Actually experimental planning can be achieved according to computational information about binding affinity. Essentially computational methods are strongly scrambled with the experimental approaches. The use of estimated affinity by computational method can help to select drugs properly. However, accuracy of computational methods depended on the level of applied routine. By comparing relative binding affinities, instead of absolute ones, accuracy and capability of computational data have been improved. Affinity estimation methods in drug design process finally introduce drug candidates for preclinical trials from initial compounds.

To enhance the potency of SA as an agonist drug, binding affinity of interaction of SA9 and SA14 with amino acids in active site of BAX were determined computationally by QM and MC methods. The results indicated that both SA9 and SA14 have high binding affinity to BAX active site. However, computed binding energy of SA14 is higher than SA9. When both drugs compete for interaction with BAX active site, SA14 binds powerful in distinct concentration.

The QM results showed opposite trends for the binding energies of SA9 and SA14 with the amino acids. However, Lys exhibited relatively high binding scores with both of the two drugs. Monte Carlo simulation results indicated that lysine complexes have the highest total interaction energy in water for both drugs.

It was found that SA9- Gln and SA14-Lys possessed the largest association free energies among the amino acid complexes, signifying that Gln and Lys are critical amino acids in the interactions with SA9 and SA14 respectively.

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