Design and evaluation of new chemotherapeutics of aloe-emodin (AE) against the deadly cancer disease: an *in silico* study

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Abstract The Bcl-2 family proteins include pro- and antiapoptotic factors acting as critical arbiters of apoptotic cell death decisions in most circumstances. Evasion of apoptosis is one of the hallmarks of cancer, relevant to tumorigenesis as well as resistance to cytotoxic drugs, and deregulation of Bcl-2 proteins was observed in many cancers. Since Bax-mediated induction of apoptosis is a crucial mechanism in cancerous cells, we aimed at conducting *in silico* analysis on Bax in order to predict the possible interactions for anticancer agents. The present report depicts the binding mode of aloe-emodin and its structurally modified derivatives onto Bax. The structural information about the binding site of Bax for docked compounds obtained from this study could aid in screening and designing new anticancer agents or selective inhibitors for chemotherapy against Bax.

Keywords Apoptosis · Bax · Aloe-emodin · Docking · Gromacs

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Introduction

There is an increasing demand for natural compounds that improve human's health. The World Health Organization estimates that approximately 80 % of the world's inhabitants rely on traditional medicine for their primary health care [14]. Plants have long been used in the treatment of cancer [16] and many nutritive and non-nutritive phytochemicals with diversified pharmacological properties have shown promising responses for the prevention and/or intervention of various cancers [51]. Cancer is a major public health burden in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002 [40]. Cancer cells typically possess a number of mutations that have allowed them to ignore normal cellular signals regulating their growth and become more proliferate than normal. In cancer cells, it may have observed that mutations might have occurred that prevent cells from undergoing apoptosis [13]. In these cases, there is no check on the cellular proliferation and consequently the disease licenses to progress to the formation of tumors. In many cases, these tumors can be difficult to kill as many cancer treatments rely on damaging the cells with radiation or chemicals, and mutations in the apoptotic pathway often produce cells that are resistant to this type of attack [39].

The process of apoptosis begins by receiving specific signals that instructs the cells to undergo apoptosis. Upon receiving these specific signals to undergo apoptosis, a number of distinctive changes occur in the cell. They include activation of caspases and Bcl-2 family proteins [1, 9]. The Bcl-2 proteins are a family of proteins involved in the response to apoptosis. Some of these proteins (such as Bcl-2 and Bcl-XL) are antiapoptotic, while others (such as Bad, Bax, or Bid) are pro-apoptotic. The relative ratios of the various Bcl-2

proteins can often determine how much cellular stress is necessary to induce apoptosis [32]. The sensitivity of cells to apoptotic stimuli may depend on the balance of pro- and antiapoptotic Bcl-2 proteins. When there is an excess of pro-apoptotic proteins, the cells are more sensitive to apoptosis; when there is an excess of antiapoptotic proteins, the cells will tend to be more resistant [38, 45]. The pro-apoptotic Bcl-2 proteins are often found in the cytosol where they act as sensors of cellular damage or stress. Following cellular stress, they relocate to the surface of the mitochondria (Fig. 1) where the antiapoptotic proteins are located. This interaction between pro- and antiapoptotic proteins disrupts the normal function of the antiapoptotic Bcl-2 proteins which is likely to lead to the formation of pores in the mitochondria and the release of cytochrome C and other pro-apoptotic molecules from the intermembrane space (Fig. 1) [53]. This in turn leads to the formation of the apoptosome, a multi-protein complex consisting of cytochrome C, Apaf-1, pro-caspase 9, and ATP, and the activation of the caspase cascade (Fig. 1) [10]. This in turn leads to the activation of effector caspase, caspase 3. Caspase 3 plays an important role in the process of the cleavage of chromosomal DNA into nucleosomal units by activating DNases, inhibiting DNA repair enzymes breaking down structural proteins in the nucleus.

With an aim to develop novel anticancer drugs characterized by selective targeting of cancerous cells and low toxicity for normal tissues, we devoted our attention to aloe-emodin (AE), a natural compound that has traditionally been found to have diverse biological activities including anticancer functions [29, 37, 50]. AE (1,8-dihydroxy-3-hydroxymethyl-9,10-

anthracenedione) is a herbal anthracenedione derivative from *Aloe vera* leaves [2, 3, 22]. Recent reports have shown that AE possesses antiproliferation effects on some types of cancer cells, such as lung, squamous, glioma, and neuroectodermal cancer cells [24, 26, 33, 34, 42]. The inhibitory effect of AE on the activity and gene expression of *N*-acetyl transferase, which plays an initial role in the metabolism of aryl amine carcinogens, was found in human malignant melanoma cells [28]. Recently, Lin et al. found that AE-induced apoptosis in T24 human bladder cancer cells was mediated through the activation of p53, p21, Fas/Apo-1, Bax, and caspase 3 [27]. Even if it was found through the work of Lee et al. and Pecere et al. that AE induces apoptosis in many cancer cells by increasing the concentration of Bax and by decreasing the concentrations of Bcl-2 [25, 43]. Inhibitory effect of AE on tumorigenesis-associated cell signaling pathways has made it an interesting molecular entity for antineoplastic studies and formulations. Among all the targets of AE, in the current manuscript that we have chosen Bax as the target of AE because in all the above mentioned investigations, we found that AE by directly or indirectly activating Bax induces the activation of apoptosis in cancer cells. In the present work, we have tried to investigate the binding modes of AE and its structural analogs on Bax because activation of Bax leads to the release of cytochrome C and other pro-apoptotic molecules further which leads to apoptosis. On the basis of these analyses, further suggestions have been given for the better understanding of the mode of action of AE and its derivatives on Bax. This may be helpful in the design of new anticancer compounds by the induction of apoptosis through activating Bax by exploiting natural resources.

Fig. 1 Illustration of the role of Bax in inducing apoptosis in the mitochondria (<http://www.sgul.ac.uk/depts/immunology/%7Edash/apoptosis/mito.html>)

Materials and methods

Protein structures

The starting 3D structure of Bax was obtained from NMR structure in Protein Data Bank [6] (PDB id: 1F16) which consisted of 192 residues [52]. We abbreviated 1F16 as Bax throughout the text. The 3D structure of Bax (Fig. 2) is known to consist of nine α -helices without β -sheets. The overall fold of Bax closely resembles that of Bcl-xL [36] with eight amphipathic α -helices clustered around one central hydrophobic α helix ($\alpha 5$). The α -helices (Fig. 2) are composed of $\alpha 1$ (residues 16–35) (raspberry representation in Fig. 2), $\alpha 2$ (residues 54–71) (green representation in Fig. 2), $\alpha 3$ (residues 74–80) (rendered blue in Fig. 2), $\alpha 4$ (residues 88–99) (represented yellow in Fig. 2), $\alpha 5$ (residues 108–127) (represented magenta pink in Fig. 2), $\alpha 6$ (residues 130–146) (rendered cyan in Fig. 2), $\alpha 7$ (residues 150–154) (rendered orange in Fig. 2), $\alpha 8$ (residues 159–164) (wheat representation in Fig. 2), and $\alpha 9$ (residues 171–188) (rendered red in Fig. 2) [36].

Fig. 2 Initial structure of Bax obtained from PDB represented in cartoon representations

Refinement of the protein structures

Refinement of the 3D structure of Bax was done using 10 ns molecular dynamic (MD) simulations. MD simulations were performed using parallel version of Gromacs 4.0.5 [18] in Gromacs96 43a1 force field implemented on Linux architecture. The geometries of the Bax were fully energy minimized before MD runs. The energy minimized average structure was evaluated for quality determination of protein geometry and structure folding reliability using Procheck [23]. The output data stream was saved in 1,000-ps segments for the easy handling of the output trajectory data. The convergences of energy and root mean square deviation (RMSD) were used to verify the system stability. Subsequently, the dynamic behavior and structural changes of the protein was analyzed by superposing the steady state onto initial structure. The visual analysis of protein structure was carried out using Pymol [12].

Data set

A set of AE derivatives were prepared by inducing structural modifications onto the seed structure of AE using the database of substituents and spacers (linkers) available in Molinspiration server [31]. These substituents are obtained by substructure analysis of a collection of current drugs, development drugs, and other molecules with biological activity containing about 17,000 entries [31]. By using these substituents, a data set of 45 AE derivatives were drawn by ChemDraw ultra 10.0 (Cambridgesoft Inc., USA) and transformed to 3D molecule models by Chem3D ultra 10.0

(Cambridgesoft Inc., USA). All the generated structures were simulated by HyperChem [20]. The properties of the molecules in the dataset including Log *P* (octanol/water partition coefficient), molecular weight, number of rotatable bonds, number of hydrogen bond acceptors, number of hydrogen bond donors, and topological molecular polar surface area were calculated by Mol inspiration server [31]. Among all the 45 designed compounds, 20 compounds were chosen for docking onto Bax based on their ability to follow Lipinski rule of five. The chemical structures and properties of the chosen AE derivatives are given in Table 2.

Molecular docking

The optimized structures of the Bax, AE, and its derivatives were used for molecular docking calculations. Molecular docking simulations were carried out with the automated docking program, AutoDock 4.0.1 [35]. AutoDock 4 was launched in a Cygwin interface from AutoDockTools (ADT) [44] in the Linux operating system. All the torsion angles in the small molecules were set free to perform flexible docking. Polar hydrogens were added using the hydrogen module in ADT for Bax. After that, Kollman united atom partial charges were assigned for the receptor Bax. A Lamarckian genetic algorithm (LGA) in combination with grid-based energy evaluation method were used for pre-calculating grid maps according to the interatomic potentials of all atom types present in the receptor Bax and AE derivatives, including the Lennard–Jones potentials for van der Waals interactions and coulomb potentials for electrostatic interactions. A grid map of dimensions 50×50×

50 Å, with a grid spacing of 0.414 Å, was placed to cover the molecule. With the help of ADT [44], the atomic partial charges were calculated by the Gasteiger–Marsili method [15] and other docking parameters were set as default. One hundred LGA runs, each with 300 individuals in the population, and the maximum number of energy evaluations and generations totaling 25,000,000 were performed. The rest of the parameters were set to default values. Docking logs were analyzed in the graphical user interface of ADT according to the instructions in the user guide of AutoDock 4. Results differing by less than 1 Å in a positional RMSD were clustered together. The docked energy is the sum of the intermolecular and the internal energies. For a representative docking instance, the orientation or pose with the lowest estimated free energy (ΔG) of binding (docked energy + torsional free energy – unbound system's free energy) was chosen in each cluster. The final structures were clustered and ranked according to the native Autodock scoring function. In each group, the lowest binding energy configuration with the highest percent frequency was selected as the group representative. From all the docked compounds, the structures with the relative lower binding free energy and the most cluster members were chosen, and these along with AE were subjected to the complex MD simulations.

Molecular dynamic simulations of complexes

For the complex MD simulations, we have chosen five AE derivatives (AE-2, AE-11, AE-15, AE-16, and AE-17) along with AE. The Bax-AE complexes were placed in an octahedron box with the edge of 5.427, 5.260, and 5.324 nm which contains approximately 12,600 water molecules. To neutralize systems, three Na^+ ions are added using the command “Genion” in the Gromacs suite. The Gromacs 4.0.5 package [18] was used to run MD simulations with the Gromacs96 43a1 force field (van Gunsteren et al. 2006) and the SPC water model [5]. The equations of motion were investigated by using a leapfrog algorithm [19] with a time step of 2 fs. The LINCS algorithm [17] was used to constrain the length of all bonds. vdW forces were calculated with a cutoff of 1.4 nm, and particle-mesh Ewald method [11] was employed to treat the long-range electrostatic interactions. The non-bonded interaction pair-list was updated every 10 fs with the cutoff of 1 nm. After minimization by the steepest descent method, position-restrained MD simulations were performed for 2,000 ps to let water molecules move into the active site. Then, the whole system was gradually heated from 0 to 300 K during 500 ps of the MD run. To make sure that the complex is stable, the system was equilibrated for 500 ps at 300 K in the NVT ensemble run with the Berendsen procedure [5] and subsequently in 500 ps NPT ensemble run at pressure of 1 atm. The Parrinello–Rahman pressure coupling [41] has been used. Finally, the system was subjected to 10 ns of full

molecular dynamic simulations with a time step of 0.2 ps. The dynamic trajectories were saved every 2 ps for analysis.

MM-PBSA method

The binding free energy is defined by the following equation;

$$\Delta G_{\text{bind}} = G_{\text{complex}} - G_{\text{free-protein}} - G_{\text{free-ligand}} \quad (1)$$

In the MM-PBSA approach, one has:

$$\begin{aligned} G &= E_{\text{gas}} + G_{\text{solvation}} - TS \\ G_{\text{solvation}} &= G_{\text{PB}} + G_{\text{sur}} \\ G_{\text{sur}} &= \gamma A + b \end{aligned} \quad (2)$$

The energy in the gas phase E_{gas} is as follows:

$$E_{\text{gas}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{tors}} + E_{\text{vdw}} + E_{\text{elec}} \quad (3)$$

Local terms E_{bond} , E_{angle} , and E_{tors} come from covalent bonds, bending, and torsion interactions. E_{vdw} and E_{elec} are the van der Waals and the electrostatic interaction energies, respectively. Total molecular mechanical energies E_{gas} are calculated by using Gromacs utility with same force field used in the MD simulations, but no cutoff is used for the evaluation of non-bonded interactions. Within the MM-PBSA approach [21], the free energy is additive (Eqs. 1–3) containing contributions from different types of interactions. Such decomposition is useful for understanding the nature of complex systems and is also possible in the more rigorous free energy perturbation approach [7, 8]. $G_{\text{solvation}}$ represents the free energy of salvation and TS is the solute entropic contribution. $G_{\text{solvation}}$ consists of two parts: the polar salvation (G_{PB}) and nonpolar salvation energy (G_{sur}). G_{PB} arises from the electrostatic potential between solute and solvents and it is determined by using the continuum solvent approximation [47]. The APBS software package [4] was employed for finding the numerical solution of the corresponding linear Poisson–Boltzmann equation. The Gromos radii and charges were used to generate the PQR files. The continuum medium is assumed to have the dielectric constant of water with no salt $\epsilon=80$ and the solute dielectric constant $\epsilon=2$. The surface or nonpolar solvation term G_{sur} is defined by the solvent accessible surface area A and two empirical parameters $\gamma=0.0072$ kcal/(mol Å²) and $b=0.92$ kcal/mol [49]. Here, A was estimated using the Shrake–Rupley numerical approximation [48] implemented in the APBS package.

After equilibration, snapshots were taken every 10 ps for estimating the enthalpy. In the MM-PBSA approximation, snapshots collected from the MD run for the protein–ligand complex are used for estimating $G_{\text{free-protein}}$ and $G_{\text{free-ligand}}$. Solute entropy contributions were estimated for the last snapshots taken from MD runs. The structures were minimized with no cutoff for non-bonded interactions by using conjugate gradient and low-memory Broyden–Fletcher–

Fig. 3 The trajectories of the 10-ns molecular dynamic simulations. Snapshot taken every 1,000 ps were superposed. **a** Represents the snapshots taken in first 5,000 ps. They were superposed onto the initial

structure. **b** Represents the snapshots taken in last 5,000 ps. They were further superposed onto the initial structure

Goldfard–Shanno method [46] until the maximum force was smaller than 10^6 KJ/(mol nm). Normal mode analysis was performed by calculating and diagonalizing the mass-weighted Hessian matrix.

Results and discussion

Molecular dynamic simulation study

In order to gain insight into the stability and dynamic properties of the 3D structure of the Bax, Bax alone was subjected to explicit solvent MD simulation for 10 ns. It can be seen that the starting structure of the Bax at the N-terminal region from residues 1–14 and at the C-terminal tail was highly distorted (Fig. 2). In addition, the N-terminal loop was partially unrolled. Figure 3 illustrates the motion along the dynamic simulations and it was observed that during the dynamic simulation the protein had undergone folding and distortions are removed at both the ends. The geometries of energy minimized structures were calculated using Procheck (Table 1). The calculated Ramachandran plot before energy minimization suggests that 76.5, 17.3, 4.9, and 1.2 % of the residues were in the most favored, additional allowed, generously allowed, and disallowed regions, respectively. The final refined structure has shown that 84.6 % residues were in most favored regions, 12.8 % residues in additionally allowed regions, 2.0 % residues in generously allowed regions, and 0.6 % residues in disallowed regions. Altogether, 99.4 % of the residues in the refined model of Bax were placed into the combined favored and allowed categories. Thus, Procheck validated the folding integrity of the structure

after energy minimization. The total energy of the whole system and RMSD from the starting structure was relatively stable during overall MD simulations of 10 ns. These RMSD values and total energy of values indicate that the employed simulation time has been adequate to obtain equilibrium structure of the Bax and the further molecular docking work can be carried out with the structure that has been obtained after 10 ns simulations.

Molecular docking

Table 2 summarizes the chemical properties of compounds used in docking experiments. The log *P* value of all compounds was found to be less than 5 which defines their lipophilic nature and qualify these compounds for proper absorption and permeation in host body [30]. Among all the designed compounds, AE-15 showed the lowest log *P* value (less than AE) demonstrating its potential as a drug in the field application. However, the compounds AE-2, AE-11, AE-16, and AE-17 also exhibited the comparable log *P* values to that of AE-15, proposing that these compounds could be as effective as AE-15 in absorption. The large solvent accessible

Table 1 Ramachandran plot analysis of Bax

Bax	Most favored regions (%)	Additionally allowed regions (%)	Generously allowed regions (%)	Disallowed regions (%)
Bax before energy minimization	76.5	17.3	4.9	1.2
Bax after energy minimization	84.6	12.8	2.0	0.6

Table 2 Properties of compounds used in docking studies onto bax

Compound	Structure	Molecular weight	Hydrogen bond acceptors	Hydrogen bond donors	Topological surface area	Log <i>P</i>	Violations
AE		286.2630	5	2	111.47	0.6541	0
AE-1		354.3580	6	2	100.900	2.6743	0
AE-2		360.3650	4	2	83.83	1.4020	0
AE-3		375.3800	5	3	95.860	3.7603	0
AE-4		362.337	5	2	93.060	3.6741	0
AE-5		375.380	4	3	95.860	4.0156	0
AE-6		376.364	5	3	104.060	3.9879	0
AE-7		371.348	4	3	99.620	4.1468	0
AE-8		388.419	4	2	83.830	4.3420	0
AE-9		376.364	6	3	104.066	3.8284	0
AE-10		388.41	5	2	86.890	4.1411	0

Table 2 (continued)

AE-11		272.2360	5	2	110.470	0.6830	0
AE-12		388.419	4	2	89.630	3.6504	0
AE-13		298.250	5	2	100.600	1.7985	0
AE-14		299.2820	6	2	87.670	1.7286	0
AE-15		328.2560	5	3	135.990	0.1560	0
AE-16		325.3860	5	3	138.690	0.5916	0
AE-17		327.2920	6	3	112.9300	1.3562	0
AE-18		314.2930	6	3	104.0600	1.6485	0
AE-19		269.2198	6	3	93.060	2.2601	0
AE-20		213.8123	5	3	94.830	1.6195	0

Table 3 Various energies of docked compounds calculated by AutoDock

Compounds	Total clusters	Binding energy (kcal/mol)	Docked energy (kcal/mol)	Intermolecular energy (kcal/mol)	Torsional energy (kcal/mol)	Internal energy (kcal/mol)	RMSD (Å)
AE	58	-15.72	-17.05	-16.89	+1.17	-0.165	0.10
AE-1	36	-6.38	-8.01	-7.59	+1.21	-0.42	0.30
AE-2	67	-15.66	-16.95	-16.28	+0.62	-0.67	0.37
AE-3	38	-4.84	-6.00	-5.68	+0.84	-0.32	0.39
AE-4	45	-6.31	-7.95	-7.54	+1.23	-0.41	0.33
AE-5	48	-6.85	-8.30	-7.91	+1.06	-0.39	0.45
AE-6	48	-5.80	-7.39	-6.51	+0.71	-0.88	0.68
AE-7	36	-7.90	-9.37	-8.79	+0.58	-0.89	0.38
AE-8	53	-8.64	-10.32	-9.63	+0.99	-0.69	0.59
AE-9	48	-9.86	-11.24	-10.93	+1.07	-0.31	0.71
AE-10	63	-12.97	-14.16	-13.89	+0.92	-0.57	0.34
AE-11	69	-17.51	-18.51	-18.16	+0.65	-0.35	0.25
AE-12	52	-12.56	-14.19	-13.61	+1.05	-0.58	0.56
AE-13	50	-13.56	-15.05	-14.58	+1.02	-0.47	0.30
AE-14	40	-11.87	-13.28	-12.76	+0.89	-0.52	0.47
AE-15	74	-18.26	-19.38	-18.89	+0.63	-0.49	0.28
AE-16	58	-16.31	-17.70	-17.32	+1.01	-0.38	0.22
AE-17	49	-15.37	-16.45	-15.88	+0.51	-0.57	0.49
AE-18	38	-14.89	-16.84	-16.12	+1.23	-0.72	0.77
AE-19	47	-13.40	-15.30	-14.32	+0.92	-0.68	0.39
AE-20	42	-14.47	-16.01	-15.36	+0.89	-0.65	0.44

surface (van der Waals) of compounds AE-15 and AE-16 suggests that because of their bigger structure these compounds can show high interactions with the proteins.

To study the binding modes of these designed compounds and to reveal the most essential amino acid residues involved in the designed ligand recognition, onto the receptor Bax that

Fig. 4 The binding mode of AE and its derivatives in the catalytic site of Bax (yellow). Bax is represented in surface representations; AE is shown in ball stick model. And derivatives of AE are represented in lines

Fig. 5 Docking conformations of AE and its derivatives bound to Bax. The Bax is represented in electrostatic potential surfaces. AE and its derivatives are represented in ball and stick representations

was obtained after 10 ns of molecular dynamic simulations, we performed molecular docking with AutoDock 4.0. AutoDock uses binding free energy evaluation to find the best binding mode. Energy terms calculated by AutoDock are characterized by intermolecular energy (consists of van der Waals energy, hydrogen bonding energy, desolvation energy, and electrostatic energy), torsional energy, and internal energy of ligand. During all these interactions, the electrostatic interaction between ligands and receptor is most important because in most cases it can decide the binding strength and location of ligand. The energy information is listed in Table 3, and the interaction modes of the all the docked compounds are depicted in Fig. 4. Among all the compounds used for docking, compound AE-15 owns the highest docking score, i.e., -19.38 kcal/mol and it is higher than that of AE. Additionally AE-11 and AE-17 had also shown higher docking scores than that of AE. Except for AE-11, AE-15, and AE-17, the rest of the compounds showed less docked energies than that of AE. Compounds AE-2 and AE-16 had shown almost similar docked energy to that of AE. Remaining compounds even though formed hydrogen bonding interactions with that of receptor Bax but they

failed to show higher docking energies than AE. Residues located within $5 \text{ \AA}$ distance from ligand were considered as binding pocket. Few residues such as Lys58, Cys62, and Leu162 were common residues in the binding pocket of Bax that have shown interactions with almost all the docked compounds.

AE binding site was (Fig. 5a) formed of Leu26, Lys58, Cys62, Leu162, and Gly166. All these residues showed hydrogen bonding interactions with the 3-hydroxymethyl group, 8-hydroxyl, 9, 10-oxygen atoms of AE. The obtained results of the molecular docking of the compound AE-1, AE-6, and AE-7 were almost similar. Two hydrophilic residues, Lys58 and Cys62, formed hydrogen bonding with the 3-amino and 8-hydroxy side chains of AE-1, 3-acetyl and 8-hydroxy side chain of AE-6, and 3-hydroxy ethyl side chain and 9-oxygen atom of AE-7. The 1-hydroxyl side chain and 10-oxygen atoms of these compounds were found to be located inside the pocket formed by Leu59 and Leu162. The introduction of hydroxyl group onto ethyl side chain of compound AE-7 led to significant improvement in potency (docked energy -9.37 kcal/mol) over the remaining

two compounds AE-1 and AE-6. The possible reason may be that the compound AE-7 is more rigid than AE-1 and AE-6 and has lost less torsional free energy compared with the other two compounds AE-1 and AE-6 during docking. The molecular docking of compounds AE-2, AE-3, AE-4, AE-9, and AE-10 showed that two hydrophilic residues Lys58 and Cys62 formed hydrogen bonding interactions with the 8-amino and 3-hydroxy methyl side chains of AE-2, 8-acetyl side chain and 10-oxygen atoms of AE-3, 8-imino side chain and 9-oxygen atoms of AE-4, 8-methoxy and 3-hydroxy methyl side chains of AE-9, and 8-aldehyde side chain and 9-oxygen atoms of AE-10. The proposed binding mode of compound AE-2 in Fig. 5b suggests that it is surrounded by binding pocket of six hydrophobic residues formed of Leu26, Ala54, Leu59, Leu162, and Phe165. The data obtained for compound AE-5 and AE-8 had shown the similar binding mode as that of AE-3, AE-4, and AE-10 by forming hydrogen bonding interactions with that of Lys58 and Cys62. In addition, these two compounds developed hydrogen bonding interactions with Ser163 also.

According to the models obtained for compounds AE-12, AE13, AE-14, AE-18, AE-19, and AE-20, the additional aromatic ring that was added to these ligands showed more hydrogen bonding interactions with that of receptor Bax. All these compounds were found to establish hydrogen bonding interactions with Lys58, Cys62, Ser163, and Gly166. In addition, these compounds developed hydrophobic interactions with Leu59 and Leu162. The molecular docking performed for compound 11 (Fig. 5c) suggests that the hydrogen atoms of its 8-toluene group and 3-hydroxyl group are hydrogen bonded to the amino group Lys58, sulfur group Cys62, and hydroxyl group of Ser163. More importantly, 3-hydroxy methyl group forms hydrogen bonding interactions with the Phe165. Additionally, the oxygen atom present in the 1-hydroxy group

is hydrogen bonded to the Leu59 and Ala54. Another hydrogen bond has established to the 9-oxygen atom of AE-15 with that of Leu162 and Gly166. Oxygen atom present at the 10th position of AE-11 is hydrogen bonded to Leu26. The binding mode of compound AE-15 with Bax is distinct to that of other compounds (Fig. 5d). The Lys58 is hydrogen bonded to 10-oxygen atom of the AE-15, while the three hydroxyl groups of the 1-oxy toluene side chain of the ligand formed hydrogen bonding interactions with the backbone of Leu26, Leu59, Cys62, and Phe165. The 9-oxygen atom was observed to interact with the Gly166 through hydrogen bonding interactions. In particular, the hydrogen bonding interaction between the 8-hydroxyl of the ligand and the Leu162 make this compound to bind with the receptor more tightly. The docking results obtained for compounds AE-16 and AE-17 shown in Fig. 5e, f suggested that the side chains that were added to these compounds were found to develop hydrogen bonding interactions with three hydrophilic residues Lys58, Cys62, and Ser163. The Leu26, Ala54, Leu162, Phe165, and Gly166 formed a suitable pocket for these ligands to interact with the receptor Bax.

The obtained results of the molecular docking of the AE derivatives allowed us to propose a general binding mode of these ligands and to determine residues involved in the ligand recognition. The obtained models demonstrate that Lys58, Cys62, and Ser163 anchor the hydroxyl moieties of the docked compounds in the pocket. The Leu162, Phe165, and Gly166 are mainly interacting with the side chains that are added onto AE. The oxygen atoms present on the ligands are essential for interaction with the hydrophobic residues such as Leu26, Ala54, and Leu59. Moreover, Lys58 interacted with the lowest binding energy with AE-15 as shown in Table 4 while other residues exhibit comparable interaction with the docked compounds and involved in proper anchoring of ligands.

Table 4 Binding free energies of AE and its derivatives to Bax obtained by the MM-PBSA method

Energy term	AE	AE-2	AE-11	AE-15	AE-16	AE-17
ΔE_{ELE}	-52.14	-52.96	-58.34	-60.59	-50.04	-50.02
ΔE_{VDW}	-60.39	-62.38	-52.56	-65.64	-61.32	-63.39
ΔE_{INT}	0.00	0.00	0.00	0.00	0.00	0.00
ΔE_{GAS}	-112.53	-115.34	-111.34	-126.18	-111.36	-113.41
ΔG_{PBSUR}	-8.49	-8.40	-8.58	-8.61	-8.64	-8.56
ΔG_{PBCAL}	38.32	42.10	41.30	40.12	46.12	46.84
ΔG_{PBSOL}	29.83	33.70	32.72	31.51	37.48	38.28
ΔG_{PBTOT}	-82.70	-81.64	-78.62	-94.67	-73.88	-75.13
$T\Delta S$	-20.20	-21.12	-22.80	-23.59	-15.84	-16.11
ΔG_{bind}	-14.12	-11.04	-14.84	-19.31	-14.02	-14.73

The energies are given in kilocalorie per mole

ELE electrostatic energy, *VDW* van der Waals contribution, *INT* internal energy from bond, angle, and dihedral terms in the MM force field, *GAS* ELE+VDW+INT, *PBSUR* and *PBCAL* nonpolar and polar contributions to the solvation free energy calculated by PB calculations, *PBSOL* PBSUR+PBCAL, *PBTOT* PBSOL=GAS, ΔS entropic contribution

Fig. 6 Time-dependence RMSDs of AE and its selected derivatives in 10 ns if MD simulations

Molecular dynamic simulation of AE and its derivatives with Bax

To study the stability and dynamic properties of AE and its derivatives interacting with Bax, based on docking results, five derivatives of AE with the best docking scores, AE-2, AE-11, AE-15, AE-16, and AE-17 bound to Bax, were selected to perform complex MD simulations, and MM-PBSA method was used to calculate the binding free energy. The RMSDs of AE and its derivatives and Bax versus time are given in Fig. 5. It shows that the conformations of AE and its derivatives are stable in all simulations. The RMSD of AE in Fig. 6 (shown in black) achieved equilibrium at 0.8 ns and afterwards was found to be stabilized at 1.8 Å. The RMSD of AE-15 in Fig. 6a (shown in red) displays that it achieves equilibrium at 0.4 ns and afterwards stabilizes at 1.2 Å, indicating that the structure of AE-15 keeps stable during the simulations and was found to more stable than AE by

achieving stability at lesser RMSD in less time than AE. However, the RMSD of AE-11 in Fig. 6b is very smooth and keeps at 2.0 Å after 0.5 ns, indicating that AE-11 is more stable than during dynamic simulations. In Fig. 6c, the RMSD of AE-2 is basically maintained at 3.0 Å but shows a great fluctuation. We examine the dynamics trajectory and find that the structure of AE-16 is changed during the simulations. From the RMSD of AE-16 in Fig. 6d, we find that AE-16 is stable in binding pocket. The average structure of AE-16 in MD simulations reveals that AE-16 has shown fluctuations at 3.5 Å during simulations. The RMSD of AE-17 in Fig. 6e reveals that AE-17 has achieved equilibrium at 0.5 ns and was found to get stabilized at 3.5 Å during dynamic simulations. In addition, we performed hydrogen bond analysis from the dynamics trajectories. The corresponding atomic labels are shown in Fig. 5a–e, respectively. It can be seen that the hydrogen bonds of these complexes are mainly distributed between tricyclic core segment and Lys58 as well as sulfonyl group

from Cys62 hydroxyl group of Ser163 and hydrophobic side chains Leu26, Leu59, and Leu162. In particular, the hydrogen bonds between Lys58, Cys62, and Ser163 and tricyclic core segment are more stable. Energy terms contributing to the binding free energy by MM-PBSA method [21] are listed in Table 4. We can see that the major contributions to the binding free energy come from the van der Waals and electrostatic interactions for these six systems. However, the polar solvation free energies are unfavorable to the binding because of positive ΔG_{PBAL} values (about ~ 46 kcal/mol), while the nonpolar solvation free energies (~ -8.5 kcal/mol) provide similar favorable contribution to the binding. For almost all conformations of AEs derivatives, the major discrepancy comes from the electrostatic interaction energies and the polar solvation free energies, respectively.

From the dynamics simulations, we can see that the complex of AE-15 with Bax is most stable and the binding free energy is the lowest; therefore, introduction of three hydroxyl groups into the 1-oxy toluene of AE is more favorable to the binding. The segment is very important for interacting with Bax at its binding pocket. On one hand, it can make strong hydrogen bonds with Lys58, Cys62, and Ser163 to maintain the stability of the complex; on the other hand, it can contact with the hydrophobic surface between Leu26, Ala54, Leu59, and Leu162, and simultaneously, it forms van der Waals interactions with the aromatic ring of Phe165. These interactions enhance the binding between AE-15 and Bax.

Conclusion

Bax plays a crucial role in the control of cancerous cell growth by activating apoptosis, and the design of new inhibitors providing a higher activity and selectivity for this protein represent one of the most ambitious challenges in the treatment of cancer. AE, a hydroxyanthraquinone present in *A. vera* leaves, inhibits tumor cell growth by activating apoptosis. The present study was aimed at suggesting novel anticancer drugs characterized by selective targeting and low toxicity for dividing normal host tissues. In this study, we devoted our attention to a natural compound AE that has been used to treat a variety of diseases including cancer. In this work, the crystallographic structure of the Bax (1F16) was retrieved from PDB. The obtained structure of Bax was refined by 10 ns MD simulation, and energy minimizations improved the general structure and stable RMSD, and total energy throughout the simulation obtained indicated that the structure has considerably good geometry. The molecular docking of 20 designed compounds of AE revealed the main differences of binding modes of these compounds and aid in the recognition of critical amino acids that are involved in the interactions of docked compounds. The observed difference in the binding modes of docked compounds with Bax was firmly

associated with their varied chemical structure. Further, the stability of the complexes was studied by complex molecular dynamic simulations. The combination of binding free energy and hydrogen bonding interaction data suggests that Lys58, Cys62, and Leu162 are the most favorable common sites for binding to Bax. Molecular docking and dynamics studies identified that the compound AE-15 possess high binding affinity towards the Bax. However, further studies are desired to know the real efficacy of these compounds and for use of this as an anticancer drug in future.

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