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Design and screening of syringic acid analogues as BAX activators-An *in silico* approach to discover "BH3 mimetics"

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ABSTRACT

Although BAX, which is a molecular hit squad that incentive apoptosis was found to be an attractive emerging target for anticancer agents. The molecular mechanism of small molecules/peptides involved in the BAX activation was remain unknown. The present focus of the study is to identification and development of novel molecules which are precisely activates BAX mediated apoptosis. In this process we identified some syringic acid analogues associated with the BAX hydrophobic groove by a virtual-screen approach. Results from the docking studies revealed that, SA1, SA9, SA10, SA14 and SA21 analogues have shown good interaction with BAX trigger site, of which SA10 and SA14 bound specifically with Lys21 at α1 helix of BAX, a critical residue involved in BAX activation. All docking calculations of SA analogues were compared with clinically tested BH3 mimetics. In this entire *in silico* study, SA analogous have performed an ideal binding interactions with BAX compared to BH3 mimetics. Further, *in silico* point mutation of BAX-Lys21 to Glu21 resulted in structural change in BAX and showed reduced binding energy and hydrogen bond interactions of the selected ligands. Based on these findings, we propose that virtual screening and mutation analysis of BAX is found to be the critical advance method towards the discovery of novel anticancer therapeutics.

1. Introduction

BAX is a pro-apoptotic BCL-2 family member that executes the apoptosis through mitochondrial pathway (Gavathiotis et al., 2010). The relative levels of pro and anti-apoptotic proteins in the cell determine the sensitivity of apoptotic event during a stimuli and the resistance in apoptosis can be due to the excess levels of anti-apoptotic proteins in the cell (Nuessler et al., 1999; Sawada et al., 2000). Structural analysis of BAX/BIM BH3 complex described that; a new binding site is located at N-terminal site formed by the helix1 and 6 of the BAX protein that engage its activation (Gavathiotis et al., 2012). Exposed form of BAX activation requires the major conformational changes prompted by the conversion of α1-α2 loop from closed conformation to open form (Gavathiotis et al., 2008). BIM SAHB-BAX interaction was carried out through NMR analysis, which identifies the minor changes of loop residues located between H1 and H2 of BAX (Gavathiotis et al., 2008; Vogler et al., 2009). Identification of small molecules that mimics the function of BIM BH3 peptide, is one of the strategy for the activation of pro-apoptotic Bcl-2 members (Nguyen et al., 2007; Wang et al., 2006). BAX activity neutralized by their counterparts i.e. anti-apoptotic Bcl-2 proteins; however, in majority of cancer cells, BAX is the most functional protein and it could be an effective drug target for the cancer treatment (Walensky and Gavathiotis, 2011; Lessene et al., 2008; Baell and Huang, 2002).

Several chemotherapeutic agents or knockdown of certain cell cycle proteins can induce the BAX-dependent apoptosis in cancer cells (Zheng et al., 2018). Earlier investigations found that, the natural plant-derived Gossypol and its derivatives showed antitumor activity in *in vitro* and animal models by modulating the BCL-2 members via its BH3 mimetic properties (Azmi et al., 2011; Quinn et al., 2011a, 2011b; Wilson et al., 2010). BTSA1, a pharmacologically optimized BAX activator that potently and specifically binds with the BAX trigger site and induced conformational changes of BAX leading to BAX mediated apoptosis in leukemia cell lines (Reyna et al., 2017). Other findings suggested

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that proapoptotic activity of BAX also can regulate by S184 phosphorylation site located in hydrophobic C-terminal tail of BAX. SMBA1, SMBA2 and SMBA3 are the small-molecule which can induce conformational changes in BAX by blocking S184 phosphorylation (Xin et al., 2014). Syringic acid (SA), a phenolic compound derived from edible plants and fruits (Ramachandran and Raja, 2010) bring its anti-cancer activity through inducing cell-cycle arrest, apoptosis, decreasing invasion, cell migration and prevents the NFkB-DNA binding activity (Abaza et al., 2013). SA exhibits antiangiogenesis activity by down regulation of VEGF expression in Zebra fish embryos (Karthik et al., 2014). Virtually derived SA analogues shown to inhibit proteasome activity in human malignant melanoma cells (Orabi et al., 2013). Some hydrazones derived from SA have shown potent free radicle scavenging activity by inhibition of ROS generated in diseased cell (Belkheiri et al., 2010). Design and screening of small molecules with different chemical moieties capable of targeting Bcl-2 family proteins are of great therapeutic interest (Azmi et al., 2011; Kazi et al., 2011; Delgado-Soler et al., 2012; LaBelle et al., 2012).

Therapeutic strategies to selectively activate apoptosis in cancer have focused on inhibiting anti-apoptotic BCL-2 proteins. In the present study, we designed several analogues of SA and screened for their druggability, toxicity and binding affinity with BAX protein using *in silico* method. Only few selected compounds were further docked with BAX trigger site for the investigation of BH3 mimetics by observing the binding mode of standard BAX activator (BAM-7). The *in silico* point mutation of BAX was developed using pyMOL mutagenesis wizard, which impaired the binding strategy and geometry of active site for the ligands examined in the present study. These are clearly explained by changes in ProSA web Z values and electrostatic potential energy of native and mutant models. Based on these *in silico* analysis, first time we have reported some SA analogues that selectively binds to BAX trigger site, as evident from the *in silico* mutation and docking studies. This study provides insights for the development of BH3 mimetics/direct BAX activators which can induce the BAX mediated apoptosis in cancer cells.

2. Materials and methods

2.1. Data set preparation

A series of SA analogues were prepared by inducing structural modifications onto the source structure using the database of substituents and linkers available in Molinspiration server (Lipinski et al., 2001). These were designed by maintaining the synthetic procedure. A data set of 563 SA analogues were drawn by Chem Draw ultra 8.0 (Cambridge Soft, Cambridge, MA, USA) and all the generated structures were simulated by Hyper Chem as described elsewhere (http://www.hyper.com, 2003).

2.2. Virtual Screening and Drug-Likeness PredictionVirtual screening and drug-likeness prediction

Virtual screening of chemical database is a supportive approach to identify novel and potential leads which are suitable for further optimization. In this study, the *in silico* physicochemical properties of all designed SA analogues were predicted according to the Lipinski's Rule of Five (Lipinski et al., 1997) using the molinspiration software. This data predicted the desired ranges of physiochemical properties for newer molecules and they were used as filters for drug development.

2.3. ADMET prediction

The filtered hits were further screened by ADMET properties which refer to the adsorption, distribution, metabolism, excretion and toxicity of a molecule within an organism. ADMET properties of the novel compounds were calculated using the pre-ADMET server (http://preadmet.bmdrc.org/) (Irvine et al., 1999; Zhao et al., 2001).The ADMET properties of *in vitro* plasma protein binding, *in vitro* MDCK cell permeability, human intestinal absorption, *in vivo* BBB penetration and *in vitro* Caco-2 cell permeability were predicted using this server. Finally, the compounds, which passed the above various filters, were subjected to molecular docking studies and visual inspection. Among all designed analogues, 29 compounds include SA were chosen for docking on to BAX trigger site based on their ability to follow Lipinski rule of five and ADMET properties.

2.4. Molecular docking

The SA based structural analogues were used for virtual screening against the selected active site of the BAX protein to understand their molecular interaction and binding mode by docking analysis. The docking studies were carried out with an automated docking program Auto Dock4.2 (Morris et al., 2009). Before docking all the components such as BAX, SA, and its analogues were optimized. BAM-7 and other previously characterized BH3 mimetics are used as reference compounds for comparative docking analysis.

2.4.1. Protein and ligand preparation

The crystal structure of BAX (PDB: 1F16) was obtained from NMR structure in Protein Data Bank (Berman et al., 2002). Prior to initiating the docking simulations, all non-protein molecules were removed, for any alternative atoms locations only the required location was retained. The 3D structures of the SA and its designed analogues were generated by PRODRG server (Schuettelkopf and Van Aalten, 2004) and pre-optimized using the MMFF94x force field. 3D structures of reference compounds (BH3 mimetics) were retrieved from Pubchem database. All ligand molecules were docked against energy minimized BAX using Auto Dock4.2 docking program.

2.4.2. Prediction of BAX active site residues

The structural and binding site information of BAX was analyzed through PDBSum (http://www.ebi.ac.uk/pdbsum) and is linked to Computed Atlas of Surface Topography of Proteins (CASTp) program (http://cast.engr.uic.edu). The confluence of α-helices 1 and 6 formed the BH3 trigger site on BAX and is structurally defined by a hydrophobic groove comprising the amino acids Met20, Ala24, Leu27, Ile31, Ile133, Met137 and Leu141 and a perimeter of charged and hydrophilic residues, including Lys21, Gln28, Gln32, Glu131 and Arg134.

2.4.3. Docking method

Protein-ligand docking method employed to understand the binding interaction of SA analogues onto BAX protein. The complete docking protocol was followed according to method described by Cheemanapalli et al., 2016. The hierarchies of ranking positions for docked ligands are assigning based on lowest binding energies (Kcal/mol). The higher negative value of docking score indicates a greater binding affinity of the ligand with receptor. After docking, the ligand-receptor complexes were analyzed by PyMOL visualizing program (Delano, 2006).

2.5. In silico *point mutation of BAX*

For understanding the significance of binding efficiency of BAX with selected ligands, we performed the single amino acid substitution in the α1 helix of its trigger site. The BH3-induced BAX activation and binding of BIM SAHB to BAX can be weakened