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Piperine derivatives as potential inhibitors of Survivin: An in silico molecular docking

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ABSTRACT

Targeting Survivin, as an inhibitor of apoptosis and a regulator of cell division, has become a worldwide controversial issue. Piperine as a pungent alkaloid has been identified as the most potent adjuvant at enhancing the efficacy of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-based therapies in triple-negative breast cancer (TNBC) cells *in vitro* and *in vivo*, which might be mediated through inhibition of Survivin. In this work, the binding energies, inhibition constants and binding modes of a group of previously synthesized Piperine derivatives at the binding site of Survivin have been studied using molecular docking tools and the best compounds with minimum binding energies are proposed as potential drugs for the inhibition of Survivin. A comprehensive SAR analysis has been done on the results that can be used for designing new Piperine analogs with higher efficacy. Molecular docking computations also show that the studied compounds can bind to BIR domain of Survivin in the same binding site as that of Smac/DIABLO with a suitable binding energy. This binding may result in the segregation of Smac/DIABLO in the cytosol and subsequently free Smac/DIABLO molecules could be available for binding with inhibitors of apoptosis to initiate caspase mediated apoptosis.

1. Introduction

Apoptosis, the process of programmed cell death (PCD), is a natural defense mechanism to remove damaged or abnormal cells that arise in different ways. This process is inhibited by a family of special proteins which are called inhibitors of apoptosis proteins (IAPs) [1]. All of the members of this group have one to three Baculovirus IAP repeat (BIR) domains which is essential to their inhibitory activities. IAPs can bind to caspases which are the essential proteins in apoptosis and subsequently, deactivate their activity [2]. After the apoptotic stimulus, secondary mitochondrial activator of caspase (Smac), which is also referred to as direct IAP binding protein with low pI (DIABLO), is released from the inner mitochondrial membrane into the cytosol; thus, disrupting the inhibitory activity of IAPs on caspases. As a result, Smac/DIABLO acts as a deactivator for apoptosis inhibitors [3]. The identification of Smac as a natural inhibitor of IAPs has motivated scientists to explore Smac mimetics. It has been reported that compounds synthesized based on Smac structure can bind to BIR domains of IAPs, causing caspases to be released from this unit, and thus promoting apoptosis [4]. Survivin, the smallest member of IAPs family is a 16.5 kDa protein with 142 amino acid residues. Its tertiary structure contains a long C-terminal α- helix (amino acid residues: 100–140) and a single N-terminal BIR domain (amino acid residues: 15–89) [5]. The BIR domain consists of a three-stranded antiparallel β-sheet bordered by four small α-helices. Moreover, one zinc which is tetrahedrally coordinated by one histidine and three cysteine residues is responsible for stabilizing the BIR domain. The crystal structure of Survivin shows a homo-dimeric arrangement with the dimer interface shaped by amino acid residues 6–10 and 89–102 [6]. This protein has two outstanding features; besides its significant role in inhibiting apoptosis, it is essential in cell division. Survivin participates in chromosomal passenger complex (CPC). It has been shown that binding of Survivin to the phosphorylated form of the histone H3 N-terminus (H3-T3ph) is essential in CPC formation and thereby functions as a key regulator of chromosomal segregation and cytokinesis [7]. It was shown that Survivin inhibits apoptosis both *in vitro* and *in vivo* [8], perhaps *via* interactions with different regulators of two apoptosis pathways [4]. Survivin is expressed in fetal tissues and different types of cancer cells [9]. Over-expression of Survivin enhances resistance to apoptotic stimuli in many

Abbreviations: TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TNBC, triple-negative breast cancer; IAPs, inhibitors of apoptosis proteins; CPC, chromosomal passenger complex; Smac, secondary mitochondrial activator of caspases; DIABLO, direct IAP binding protein with low pI; RMSD, root mean square deviation

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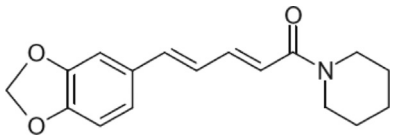

Scheme 1. Chemical structure of Piperine.

malignancies [10]. The most remarkable property of Survivin which has made it unique among other members of IAPs family is its negligible population in differentiated normal tissues [6]. Thus, the inhibition of Survivin as a cancer specific protein that leads to apoptosis in cancer cells, does not affect the normal tissues much. Despite the growing amount of knowledge about Survivin in the last decade, the development of Survivin inhibitors is relatively slow as compared to other therapeutic inhibitors for cancer treatment which is a matter of concern [6]. In other words, one cannot deny the fact that novel inhibitors with higher efficacy must be developed. Alkaloids are diverse plant-origin chemical substances composed of nitrogen atom and a ring structure. They are known as the most significant active ingredients in herbs and rich sources for drug discovery. Alkaloids exhibit antimetastasis and antiproliferation effects on different types of cancers both *in vitro* and *in vivo* [11,12]. Piperine, as a pungent alkaloid with the molecular formula $C_{17}H_{19}O_3N$, isolated from *Piper nigrum* and *Piper longum*, is a compound found in famous spices that have been used for centuries [13] (Scheme1).

It shows several pharmaceutical activities and can also inhibit breast stem cell self-renewal without affecting the differentiated cells [14,15]. The inhibition of solid tumor growth and antimetastatic properties against lung metastasis in mice have also been reported for this compound [16,17]. Moreover, Sherif et al. screened 55 compounds from natural productsin combination with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in triple-negative breast cancer (TNBC) cells. They identified Piperine as the most potent adjuvant at enhancing the efficacy of TRAIL-based therapies in TNBC cells *in vitro* and *in vivo*, which might be mediated through inhibition of Survivin [18]. This hypothesis has been also examined on Withanone as another herbal ligand and its binding properties to Survivin have been comprehensively investigated using molecular docking tools. After analyzing the results, it has been suggested that Withanone could be a potent anticancer drug candidate due to its potential to inhibit Survivin activity through interfering with the inhibitory action of Survivin against caspases [2]. Moreover, Muthukumar et al. investigated molecular interaction of Survivin and Piperine through molecular docking analyses for neuroblastoma targeting. Binding mode as well as interactions are explained in this study and their results represent the possible binding of Piperine to the active site of Survivin with a good energy level (the estimating binding free energy (ΔG) was -14.04 kJ/mol) [19].

Considering the significant role of Survivin in cancer progression and the ability of Piperine in inhibiting Survivin and regarding the fact that there is not any computational study on the interactions of Piperine analogs and Survivin in the literature, it would be of interest to do computational studies on the binding of Piperine and its analogs with Survivin to gain knowledge about their inhibition properties with a certain level of accuracy. For this purpose, in the present study the interactions of this natural compound and some of its previously synthesized analogs with Survivin have been investigated using molecular docking tools. The selected Piperine analogs have been previously synthesized and showed inhibitory activity on monoamine oxidase [20]. The results of this study would help us to explore structure–activity relationship of these analogs that consequentely would lead us to design new compounds with higher efficacy. Furthermore, their binding modes, binding energies, the number of hydrogen bonds and the essential functional groups participated in the interactions of Piperine analogs and Survivin have been reported. All the docking results were performed with AutoDock 4.2. Finally, the interactions of H3-T3ph with Survivin in its binding site were also explored using molecular docking calculations. Afterwards, the binding site of this compound and that of the Piperine derivatives were compared in order to find out whether these compounds can interfere with the formation of Survivin–H3-T3ph complex and therefore disrupt the function of Survivin in cell division.

2. Computational methods

2.1. Receptor and ligands

The initial coordinates of Survivin–Smac/DIABLO complex were obtained from Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB IDs: 3UIH).This file of protein was chosen because it has no missing atom, has a reasonable resolution and contains Smac/DIABLO. The chemical structures of ligands were drawn in GaussView. In order to get the most stable geometry, these structures were optimized through employing the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid density functional theory at the 6–31 G^{**} basis set using the quantum chemistry software Gaussian03. The output files were saved in PDB format.

2.2. Molecular docking setup

2.2.1. AutoDock 4.2

Molecular docking calculations were performed byAutoDock4.2 program package using the AutoDock empirical free energy function and the Lamarckian genetic algorithm with local search. First of all, water molecules were removed from the initial structure of protein as well as Smac molecule and then missing hydrogens and Gasteiger charges were added to the system during the preparation of the protein input file. AutoDockTools was used for the preparation of coordinate files of ligands and protein (PDBQT). Afterwards, pre-calculation of grid maps was done using AutoGrid in order to save a lot of time during the docking. After docking Smac/DIABLO as the reference ligand back into Survivin, the RMSD (root mean square deviation) value between the docked Smac/DIABLO and the reference Smac/DIABLO was 1.45 Å. The superimposition of the obtained binding modes of Smac/DIABLO from docking calculation and experimental data represents high similarity between the two. This high similarity and the calculated RMSD value confirm the validity of AutoDock 4.2 for the docking of ligands to Survivin. Fig. 1a and b shows the binding mode and detailed interactions of Smac/DIABLO in the binding site of Survivin, respectively. With respect to this figure, Smac interacts with Leu54, Leu64 and Trp67 as hydrophobic and Glu51, Glu63, Glu65, Glu76, Lys62, Asp71 and His80 as hydrophilic residues. The aforementioned interactions along with the hydrogen bond between Val2 residue of Smac and Glu65 of protein, stabilize the binding mode of Smac.

The 3D structure of Survivin–Smac/DIABLO complex shows that Smac binding site is located in BIR domain of this protein. Therefore, the docking calculations were performed by locating a grid map with $60 \times 60 \times 60$ Å^3 points and a grid-point spacing of 0.375 Å which was centered on the Smac binding site in a way that all the amino acid residues in this site were incorporated in the grid. The number of independent docking runs performed for each docking simulation was set to 200 with 25,000,000 energy evaluations for each run. The default values of program were used for other docking parameters.