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Research Article

MOLECULAR DOCKING STUDIES OF OPENED-CHAIN ANALOGUES OF ANTIMYCIN A_3 AS CASPASES INHIBITORS OF APOPTOSIS IN COLORECTAL CANCER

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ABSTRACT

Objective: Studies of open-chain analogues of antimycin A_3 as caspase inhibitors of apoptosis by molecular docking approach through computer-aided drug design. The novelty of this study is finding the potential antimycin A_3 analogues which structurally modified against caspases.

Methods: In finding potential caspase inhibitor of apoptosis in colorectal cancer (CRC) by *in silico* approach has been utilized. Protein structure of caspase has been downloaded from Protein Data Bank (1SHJ). The minimized of caspase was ready for molecular docking analysis. Analogues of antimycin A_3 as lead compounds were designed and assessed using Molsoft drug-likeness. Both protein and lignan derivatives were docked with Autodock 4.2. The best docking score was shown by the lowest binding energy.

Results: Analogues of antimycin A_3 has been done by evaluating their physicochemical properties as lead compounds. From this assessment, it showed that analogue 2 (AMD2), intermediate amide 4 (AMD4) showed good compounds to be drug-likeness by following Lipinski's rule of five (RO5), while intermediate amide 3 (AMD3) and antimycin A_3 (AMY3) showed cannot followed in Lipinski's RO5. From molecular docking result, the most favorable binding of caspase was AMD4 and AMD2 based on its energy that AMD4 (−7.34 kcal/mol) has the best binding interaction compared to AMD2 (−7.33 kcal/mol), AMY3 (−7.26 kcal/mol), and AMD3 (−5.23 kcal/mol), respectively.

Conclusion: This studies demonstrated that the opened-chain analogues of antimycin A_3, AMD2 and AMD4 as a promising candidates of caspase inhibitor of apoptosis in CRC.

Keywords: Open-chain analogue, Antimycin A_3, Caspase, Apoptosis, Anticolorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy in the populations of developed western countries and represents the third leading cause of cancer-associated mortality in the world. The CRC of cancer in males and the second, following breast cancer, in females [1]. Once the disease spreads to distant sites, it is usually incurable using the current systemic treatment options including chemotherapy. Abnormalities in apoptotic function contribute to both the pathogenesis of CRC and its resistance to chemotherapeutic drugs and radiotherapy, both of which act, at least in part, by killing cancer cells [2]. This is largely due to the resistance of cancer cells to apoptosis. Understanding the resistance of cancer cells to apoptosis the identification of novel therapeutic targets and the development of novel treatment options. Many investigators are starting to exploit the recent discoveries about apoptosis to develop new treatments.

The chemotherapeutic agent of anticancer such as antimycin A_3, This agent-induced apoptosis of cancer cells and strong growth inhibitory activity against human CRC COLO201 [3]. The anticancer activity of antimycin A_3 highly depends on the presence of hydroxyl group, amide bond and 3-formamido group. Whereas, the nine-membered dilactone core in antimycin A_3 was less necessary for anticancer activity compared to 3-formamidosalicylyl moiety [4]. These reports suggesting that the nine-membered dilactone core in antimycin A_3 can be modified by another active core to increase its anticancer activity [5].

The non-apoptotic functions of these proteases suggest that they may become activated independently of the apoptotic cascade, regulation of apoptosis is controlled by caspases. The caspase divided into two classes: Initiator/apical caspases (caspase-2, -8, -9 and -10) and effector/executioner caspases (caspase-3, -6 and -7). Effector caspases are constitutively produced in cells as dimers and proteolytic processing by an initiator enzyme is required to trigger their activity. The proteolytic activity of mature caspase-9 and -3 is inhibited by inhibitor of apoptosis proteins [6]. In turn, the inhibitor apoptosis are inactivated and caspase activity restored by proteins such as SMAC/Diablo, which are released from the mitochondria and with SMAC activity predicted it's a potent prognostic in rectal cancer [7]. The aim of this research is study of antimycin A3 analogues as inhibitors of caspases by molecular docking approach through computer-aided drug design. The novelty of this study is finding the potential antimycin A_3 analogues which structurally modified against caspases. The main this work is to identify the most potential drug candidates of caspase inhibitor. We use Lipinski's rule of five (RO5) [8] as complementary analysis in identifying the best lead compounds of antimycin A_3 analogues.

METHODS

Protein preparation

The crystal structure of the target protein caspase was retrieved from Protein Data Bank (1SHJ), and minimization of the protein was generated by Python Molecular Viewer 1.5.6cr3 [9]. The protocol prepares the protein by inserting the add partial charges using Gasteiger method, add polar hydrogens in the protein. And unwanted materials, such as water and ligand molecules, are removed before minimization.

Antimycin A_3 analogues structures preparation

The structure of antimycin A_3 analogues was generated by Marvin Sketch by Chem Axon [10,11], and their conformational energy was minimized using MMFF94 force field. The analogues were designed by open chain nine-membered dilactone core of antimycin A_3. These molecules are analogue 2 (AMD2), intermediate amide3 (AMD3),

Table 1: Molecular weight, LogP, HBD, HBA, and TPSA of open-chain analogues of antimycin A_3

Compounds	Molecular formula	Berat molekul	LogP	HBD	HBA	TPSA	Lipinski's RO5
AMY3	$C_{26}H_{84}N_2O_9$	568.62	1.61	11	9	137.18	No
AMD2	$C_{16}H_{56}N_2O_8$	404.40	−2.89	5	8	134.33	Yes
AMD3	$C_{23}H_{92}N_2O_8$	524.69	−1.56	6	8	127.00	No
AMD4	$C_{22}H_{89}N_2O_6$	477.67	−0.66	4	6	91.62	Yes

RO5: Rule of five, TPSA: Total polar surface area, HBD: Hydrogen bond donor, HBA: Hydrogen bond acceptors

intermediate amide4 (AMD4), and antimycin A_3 (AMY3). The molecule structures as shown in Fig. 1.

Drug likeliness evaluation

The drug likeliness of the compounds was evaluated with the help of Lipinski drug filter under Molsoft [12]. This rule describes molecular properties important for a drug's pharmacokinetics in the human body and provides the information regarding the utilization of the ligands as a drug [5].

Molecular docking of complexes caspase-antimycin A_3 analogues

The preparative protein and ligand coordinates were saved as pdbqt files. Molecular docking was generated using Autodock 4.0 software (The Scripps Institute) [13]. The ligands are set to have prepared as a rigid structure. The complex of protein and ligand are saved as output in dlg files, the grid box volume was adjusted to 40 × 40 × 40 Å in the x, y and zaxes, respectively, for specific docking of complexes and grid-sizes have space up to 1 Å. The binding energy values were calculated based on the total intermolecular energies (kcal/mol) including hydrogen bond energy, Van Der Waals energy, desolvation energy, and electrostatic energy. The docking program will evaluate this energy to obtain the best binding mode. The root-mean-square deviation which <2.0 Å was scored during running docking program. The results of complexes were generated using Chimera [14].

RESULTS AND DISCUSSIONS

Drug likeliness evaluation

The analysis of the World Drug Index, which leads to Lipinski's "RO5". These rules, which are usually viewed more as guidelines rather than absolute cutoffs, are molecular mass <500 daltons (Da), calculated octanol/water partition coefficient (logP) <5, number of hydrogen bond donors <5, and number of hydrogen bond acceptors <10. Thus, such studies point the most important physicochemical properties and structural characteristic of a good drug in the context of our current knowledge. These properties are then typically used to construct predictive absorption, distribution, metabolism and excretion models and create the basis for what has been property-based design [15,16].

In this study, four compounds have logP values ranging from 1.61 to −2.89 (Table 1). In general, an orally active drug has no more than two violation according to Lipinski's RO5. From this results showed that AMD2 and AMD4 have followed Lipinski's RO5, but not AMY3 and AMD3 due to there were found more than two violations of Lipinski criteria.

Docking results

The refined of caspase inhibitor was done then used for docking interaction analysis. Autodock was used for the docking studies. The docked conformation corresponding to the lowest binding energy was selected as the most favorable binding conformation. The total screened four compounds were docked into the active site of caspase. The docking energies of all the compounds were represented in kcal/mol. The docking scores are tabulated in Table 2. The best conformation of protein-ligand complexes (Fig. 2). The best-docked compounds were shown with the lowest affinity energy. From docking result, it is shown that AMD4 (−7.34 kcal/mol) has the best binding interaction compared to AMD2 (−7.33 Kcal/mol), AMY3 (−7.26 Kcal/mol), and AMD3 (−5.23 Kcal/mol), respectively.

Table 2: Molecular docking interaction of antimycin A_3 analogues with caspase

Compound	Binding energy (Kcal/mol)	Ki (uM)
AMY3	−7.26	1.47±1.2
AMD2	−7.33	4.21±1.5
AMD3	−5.23	4.58±2.3
AMD4	−7.34	4.17±1.3

Ki is inhibitory activity in μM, expressed in mean value (n=3)±SD. SD: Standard deviation

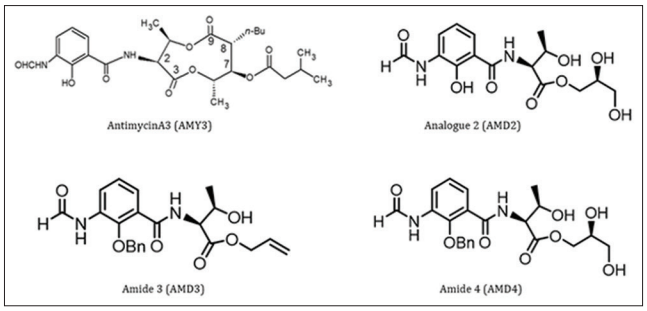

Fig. 1: Structure of antimycin A_3, analogue 2, amide 3, and amide 4

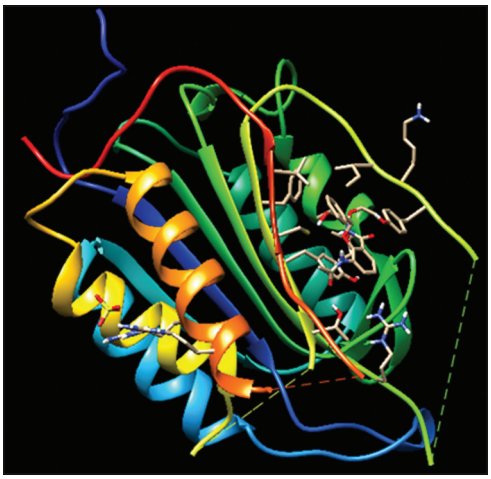

Fig. 2: Complex of caspase-antimycin A_3 analogues through molecular docking analysis

In this study, open chain analogues of antimycin A_3 might be used as leads for developing effective inhibit apoptosis of CRC agent. However, some poor bioavailability and pharmacophore could be structurally modified in nine-membered dilactone core to open chain to improve their bioactivities as lead compounds.

Furthermore, docking simulation analysis shows that AMD2 has a number of hydrogen donors and hydrogen acceptors more than antimycin A_3. This indicates that in comparison to the original compounds antimycin A_3, AMD2 have hydrogen bonding interaction is stronger in the catalytic receptor of caspase targets CRC. All ligand

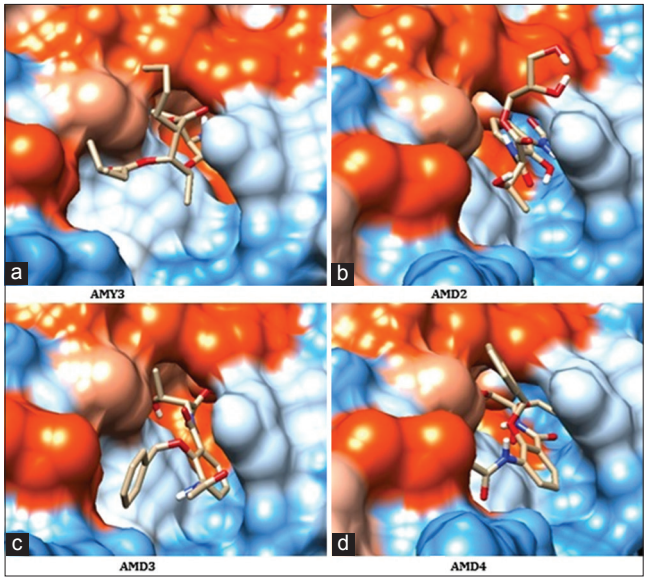

Fig. 3: Conformation of antimycin A_3 analogues into the caspase cavity (surface in gray, blue, and red is pocket structure in inner surface form of caspase, a: AMY3, b: AMD2, c: AMD3, d: AMD4)

conformers were embedded within the active site cavity of protein target. These favored ligand modes were stabilized by hydrogen bonds between the functional group from the ligands with the functional group of side chain residues of caspase protein. From the docking conformation of AMD2 and AMD4, it was observed that nitrogen atom of 3-formamido-2-benzyloxy-benzoic acid enter to the cavity and form hydrogen bonding to site and AMY3 was observed that nine member dilactone of antimycin A_3 cannot enter to cavity (Fig. 3). Beside hydrogen bonding these compounds also showed hydrophobic interactions to various residues in the active site of the enzyme. From the docking conformations of the selected compounds, it was observed that there are some specific functional groups that interact to the important residues and fit well in the binding pocket of caspase protein.

CONCLUSION

In this study, *in silico* approach of antimycin A_3 analogues against caspase. The results of this study demonstrated that AMD2 and AMD4 exhibited the best binding interactions and warrants for the development of potent caspase inhibitors, so the proposed leads need to be presented to the scientific community considered as candidates synthetic drugs superior new and effectiveas caspase inhibitors of apoptosis in CRC.

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