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ORIGINAL ARTICLE

Molecular Docking Insights into the Anticancer Potential of Bioactive Compounds from *Streptomyces coelicolor Kr23* Through Regulation of Apoptotic Proteins

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ABSTRACT
The biological diversity of Streptomyces, with over 500 identified species, highlights its immense potential in producing bioactive compounds with therapeutic applications, including antibacterial, antifungal, antiviral, and anticancer properties. This study focuses on the anticancer potential of Streptomyces coelicolor KR23, particularly its bioactive secondary metabolites, which exhibit cytolytic and antioxidative activities crucial for cancer treatment. Using GC-MS, bioactive compounds were identified and evaluated for their binding affinities against apoptosis-regulating proteins Bcl-2, Bax, and Bak, critical in cancer cell survival and chemotherapy resistance. Molecular docking studies using AutoDock 4 demonstrated significant binding interactions between the identified compounds, including DL-Norleucine, L-Tyrosine butyl ester, Arachidonic acid, and Oleic acid, with target proteins. The findings revealed notable binding affinities: Bcl-2 with Arachidonic acid (-6.4 KJ/mol), Bak with L-Tyrosine butyl ester (-6.1 KJ/mol), and Bax with Arachidonic acid (-5.9 KJ/mol), suggesting a high potential for therapeutic intervention. These interactions disrupt the anti-apoptotic functions of Bcl-2 and promote pro-apoptotic activities of Bax and Bak, triggering apoptosis. Additionally, the study explores the role of these compounds in modulating oxidative stress, mitochondrial membrane permeabilization, and caspase-mediated apoptosis pathways. The findings support the therapeutic versatility of Streptomyces-derived compounds in cancer therapy, addressing the challenges of drug resistance and apoptosis evasion in cancer cells. This research underscores the potential of integrating microbial metabolites with molecular docking insights for developing innovative and targeted cancer treatments.
Keywords: *Molecular Docking, Streptomyces sp. Anticancer, Apoptosis.*

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INTRODUCTION

The biological diversity of Streptomyces, emphasizing its over 500 identified species that produce a wide array of bioactive compounds with therapeutic applications, including antibacterial, antifungal, antiviral, and anticancer properties. It particularly focuses on the significance of antibiotics like streptomycin and tetracycline in treating bacterial infections, especially in light of increasing antibiotic resistance. The immunomodulatory properties of Streptomyces-derived compounds, which enhance the body's ability to fight infections and manage inflammatory conditions. Additionally, it examines the potential of specific compounds, such as Streptozotocin, in cancer therapeutics, particularly for pancreatic cancer, showcasing the versatility of Streptomyces in various medical domains [1]. *Streptomyces coelicolor*, a species within the prolific genus Streptomyces, exhibits significant anticancer properties through its production of bioactive secondary metabolites. These compounds have shown potential in combating various types of cancer, making *Streptomyces coelic*olor a promising candidate for developing novel anticancer therapies. *Streptomyces coelicolor* is known for producing a variety of secondary metabolites, including antibiotics and pigments, which possess anticancer properties. These compounds have demonstrated

cytolytic and antioxidative activities, which are crucial in cancer treatment [2],[3]. While the anticancer properties of *Streptomyces coelicolor* are promising, it is essential to consider the broader implications of using microbial sources for drug development. The versatility of Streptomyces in producing bioactive compounds not only offers potential in cancer therapy but also in addressing antibiotic resistance and other medical challenges. Continued research and development in this area could lead to innovative and eco-friendly therapeutic interventions. The Bcl-2 family includes both anti-apoptotic proteins (e.g., Bcl-2, Bcl-xl) and pro-apoptotic proteins (e.g., Bax, Bak). These proteins regulate the intrinsic pathway of apoptosis by controlling mitochondrial membrane permeability, which is crucial for the release of apoptogenic factors and subsequent cell death [4]. Bcl-2 is primarily known for its role in inhibiting apoptosis by binding to pro-apoptotic proteins, thereby preventing the release of cytochrome c from mitochondria, which is a crucial step in the intrinsic apoptotic pathway[5],[6].Bax and Bak are essential for the permeabilization of the mitochondrial membrane, leading to the release of apoptotic factors. They form oligomers in the mitochondrial membrane, which is a critical step in the intrinsic pathway of apoptosis. Bax and Bak can be inhibited by Bcl-2 through direct binding, which prevents their oligomerization and the subsequent apoptotic cascade [7]. While the Bcl-2 family proteins are central to apoptosis regulation, their roles are complex and context-dependent. The balance between pro- and anti-apoptotic members determines cell survival or death, and this balance is often disrupted in cancer. Understanding these dynamics is crucial for developing effective cancer therapies. However, the redundancy and compensatory mechanisms within the Bcl-2 family pose challenges, necessitating combination therapies and novel approaches to effectively target these pathways in cancer treatment. Present study extracted bioactive compounds from *Streptomyces coelicolor KR23* through GCMS studies and find the binding properties against BCl2, Bax and Bak proteins.

MATERIAL AND METHODS

Ligand Selection

The choice of ligands can significantly influence the efficacy, selectivity, and toxicity of anticancer agents. Various strategies have been explored to identify and optimize ligands that can target cancer cells while minimizing harm to normal cells. Based on GCMS studies of *Streptomyces coelicolor* KR23 extract to find the four different active molecules for this studies. The structure was obtained from NCBI PubChem. After energy minimization, the structures were converted to the PDB format in Autodock 4 in order to molecular docking.

Table 1: Active site for *Streptomyces coelicolor* KR23

S.No	RT	Name of the Compound	Formula	M.W
1	11.808	DL-Norleucine	C6H13NO2	131.17
2	20.225	l-Tyrosine butyl ester	$C_{13}H_{19}NO_3$	237.29
3	21.575	Arachidonic acid	$C_{20}H_{32}O_2$	304.5
4	23.152	9-octadecenoic acid	$C_{18}H_{34}O_2$	282.5

Target Protein Preparation

In this study three proteins, Bax, Bak and Bcl-2 involved in apoptosis were chosen as targets of our study. The crystal structures of these molecules for docking calculation were obtained using the USCF chimera and PyMol programs. All water molecules, ligands and heavy atoms were removed from each protein.

Molecular docking employing AutoDock 4

The AutoDock 4 software was utilized to investigate the binding modes of the compounds to the Bak, Bax, and Bcl-2 proteins. A docking procedure was performed against the crystallographic structure of the anti-apoptotic protein Bcl-2 (PDB ID: 4AQ3), the mitochondrial apoptosis regulator Bak (PDB ID: 2JCN), and the apoptosis regulator Bax (PDB ID: 2K7W) using AutoDock 4.2. The structural configurations of these compounds were refined utilizing the GROMOS force fields. Ultimately, torsional roots and branches were delineated, and Gasteiger-Marsilli atomic charges were allocated for each compound through MGL Tools 1.5.4. Prior to the docking computations, all water molecules and ligands were extricated from each protein. Furthermore, MGL Tools 1.5.4 was employed to amalgamate all non-polar hydrogens and to allocate Gasteiger charges to each atom within the macromolecule. Three-dimensional grids measuring 90 ˚A × 90 ˚A × 90 ˚A with a spacing of 0.402 ˚A, centered on the crystallographic structure of Bak, three-dimensional grids measuring 120 ˚A × 120 ˚A × 100 ˚A with a spacing of 0.375 ˚A, centered on the crystallographic structure of Bax, and three-dimensional grids measuring 66 ˚A × 50 ˚A × 50 ˚A with a

spacing of 0.375 ˚A, centered on the binding site of the crystallographic structure of Bcl-2, were computed for each atom type. Additionally, electrostatic and desolvation maps were generated. Molecular docking computations were executed using AutoDock 4.2, employing the Lamarckian genetic algorithm for ligand conformational exploration. The docking procedure for each molecule encompassed a total of 100 iterations, initiated with a population of 150 individuals, a cap of 25 million energy assessments, a maximum of 270,000 generations, a mutation rate set at 0.02, and a crossover rate of 0.8. The resultant conformations for each molecule were clustered employing a root-mean-square deviation threshold value of less than 3.0 A˚. The molecules exhibiting selective binding to the respective binding sites of each protein were subsequently re-docked employing grids measuring 50 ˚A × 50 ˚A × 50 ˚A, centered in the catalytic site of the protein. It is significant to underscore that all conceivable binding modes were contemplated throughout the entirety of the calculations. To this end, flexibility was permitted for all rotatable bonds of the ligand, while the protein was maintained as a rigid structure.

RESULT AND DISCUSSION

To understand the interaction between DL-Norleucine, l-Tyrosine butyl ester, Arachidonic acid, 9-octadecenoic acid with apoptosis regulatory proteins, Bak, Bax and Bcl-2, AutoDock 4 was used. Crystal structures of Bax, Bak and Bcl-2 were performed using the USCF chimera and PyMol programs. Processing of the proteins included the deletion of water molecules, ligands and heavy atoms from each protein (**Fig. 1**). Molecular docking with AutoDock 4 exhibited the interaction of DL-Norleucine, l-Tyrosine butyl ester, Arachidonic acid, 9-octadecenoic acid with the binding site of target proteins Bax, Bak and Bcl-2 (**Fig. 2**). The higher negative score indicates a greater binding affinity of the ligand with receptor. The affinity of Bcl2 with arachidonic acid (-6.4 KJ/mol), Bak with Tyrosine butyl ester (-6.1 KJ/mol) and Bax with arachidonic acid (-5.9 KJ/mol). The Bcl2 protein was higher than towards Bak and Bax targets. The conformations of the lead compounds binding to the target proteins were analyzed as well Table 1). DL-Norleucine's potential role in anticancer therapy may be linked to its interaction with the Bcl-2 family of proteins, which are crucial regulators of apoptosis. The Bcl-2 family includes both anti-apoptotic proteins like Bcl-2 and Bcl-xL, and pro-apoptotic proteins such as Bax and Bak. These proteins are central to the survival of cancer cells and their resistance to chemotherapy. Targeting these proteins, particularly by inhibiting the anti-apoptotic members, can restore the apoptotic process in cancer cells, making them more susceptible to treatment. Bcl-2 and Bcl-xL are often overexpressed in cancer cells, contributing to tumor growth and resistance to apoptosis [8]. These proteins inhibit apoptosis by interacting with pro-apoptotic proteins Bax and Bak, preventing their activation[9]. Bax and Bak are crucial for mitochondrial membrane permeabilization, a key step in apoptosis. Bax-derived peptides can directly induce apoptosis by targeting mitochondria and releasing apoptogenic factors [10]. L-Tyrosine butyl ester docking apoptosis involves exploring the potential of L-Tyrosine derivatives in inducing apoptosis through molecular docking studies. The research papers provided offer insights into the mechanisms by which L-Tyrosine and its derivatives can influence apoptosis, particularly in cancer cells. Ester-based compounds, including those derived from L-Tyrosine, have demonstrated anticancer activity by inducing caspase-mediated apoptosis, which is a crucial pathway for programmed cell death[11],[12]. Molecular docking studies have been employed to predict the binding affinity and interaction of L-Tyrosine esters with target proteins, such as caspase-3, which plays a significant role in the apoptosis pathway. Docking studies of L-Tyrosine esters have shown that the presence of a phenolic moiety enhances their biological activity, including anticancer properties, by altering their interaction with proteins. L-Tyrosine esters have been found to possess antioxidant and anticancer activities, with their efficacy being influenced by the chain length and presence of functional groups[13]. Arachidonic acid (AA) and its role in cancer apoptosis involve complex interactions with the BAX and BCL-2 proteins, which are crucial regulators of the apoptotic pathway. BAX, a pro-apoptotic protein, and BCL-2, an anti-apoptotic protein, are part of the BCL-2 family that governs mitochondrial outer membrane permeabilization, a key step in apoptosis. The balance between these proteins determines cell fate, with BAX promoting apoptosis and BCL-2 inhibiting it. Arachidonic acid can influence this balance, particularly through oxidative stress mechanisms. BAX is a pro-apoptotic protein that plays a significant role in initiating apoptosis across various cancer types. It is highly expressed in many cancers and is associated with poor prognosis in several types, indicating its potential as a biomarker for cancer prognosis and therapy efficacy[14]. Therapeutic strategies targeting BAX involve small molecules that promote its insertion into mitochondrial membranes, leading to apoptosis. These compounds can induce BAX-dependent apoptosis, offering potential as cancer therapeutics[15]. BCL-2 is an anti-apoptotic protein that prevents apoptosis by sequestering pro-apoptotic proteins like BAX. Its overexpression is linked to poor prognosis and resistance to chemotherapy in various cancers[16]. Oleic acid, also known as 9-

octadecenoic acid, has been identified as a potential complementary therapy in cancer treatment due to its anti-cancer properties. It is known to inhibit cancer cell proliferation, induce apoptosis, and act as an antitumor agent. The mechanisms through which oleic acid exerts these effects include modulation of oncogene expression and intracellular signaling pathways. Additionally, the Bcl-2 family of proteins, which are involved in cancer cell survival, present another target for cancer therapy, with strategies aimed at inhibiting their activity showing promise. Oleic acid has been shown to inhibit the proliferation of various cancer cell lines, including human lung carcinoma and breast cancer cells [17],[18]. It induces apoptosis in carcinoma cells, potentially through increased intracellular ROS production or caspase 3 activity. Present study promising to supporting report of invitro studies against the cancer cell line.

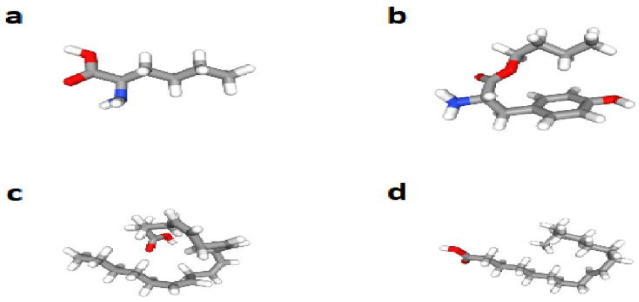

Fig 1: 3D conformers of the ligand compounds retrieved from Pubchem database A) DL-norleucine B) 1-tyrosine butyl ester C) Arachidonic acid D) octadecenoic acid

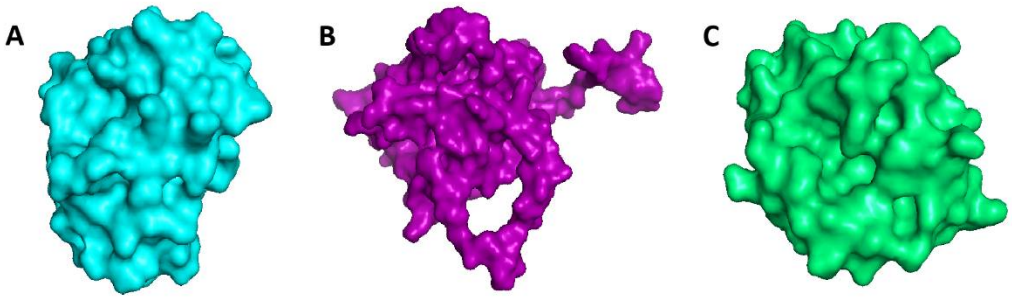

Fig 2: Optimized crystal structures of the target proteins Bak, Bax and Bcl2 from Protein Data Bank database

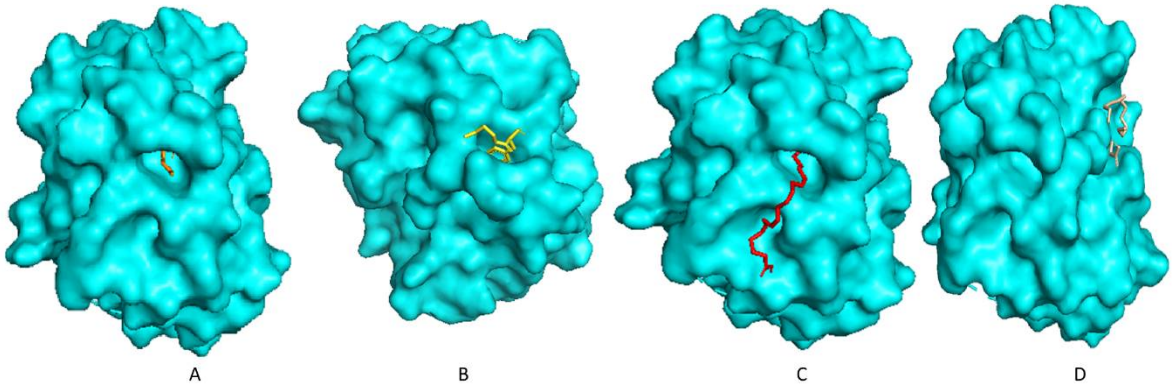

Fig 3: Bak protein with A) DL-norleucine B) 1-tyrosine butyl ester C) Arachidonic acid D) octadecenoic acid

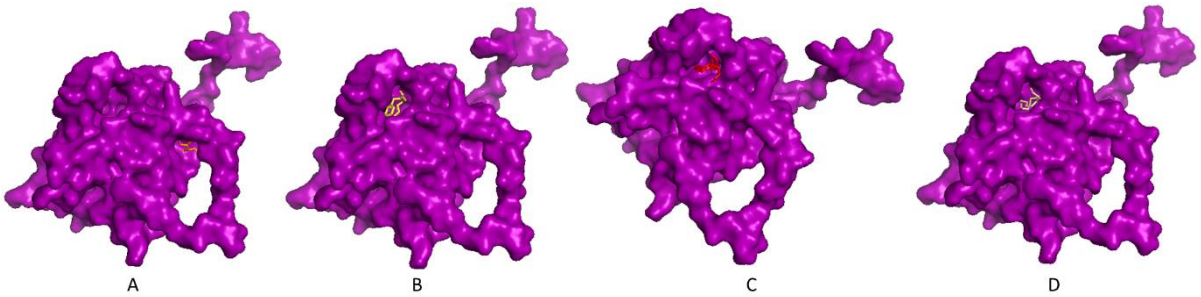

Fig 4: Bax protein with A) DL-norleucine B) 1-tyrosine butyl ester C) Arachidonic acid D) octadecenoic acid

Table 1: Binding Affinity

S.No	Docked Complexes	Binding values (kcal/mol)
1	2JCN+Tyrosine butyl ester	-6.1
2	2JCN+octadecenoic acid	-4.4
3	2JCN+arachidonic acid	-5.7
4	2JCN+norleucine	-5.4
5	2K7W+tyrosine butyl ester	-5.8
6	2K7W+octadecenoic acid	-5.6
7	2K7W+arachidonic acid	-5.9
8	2K7W+norleucine	-4.3
9	4AQ3+tyrosine butyl ester	-5.4
10	4AQ3+octadecenoic acid	-5.2
11	4AQ3+arachidonic acid	-6.4
12	4AQ3+norleucine	-4.3

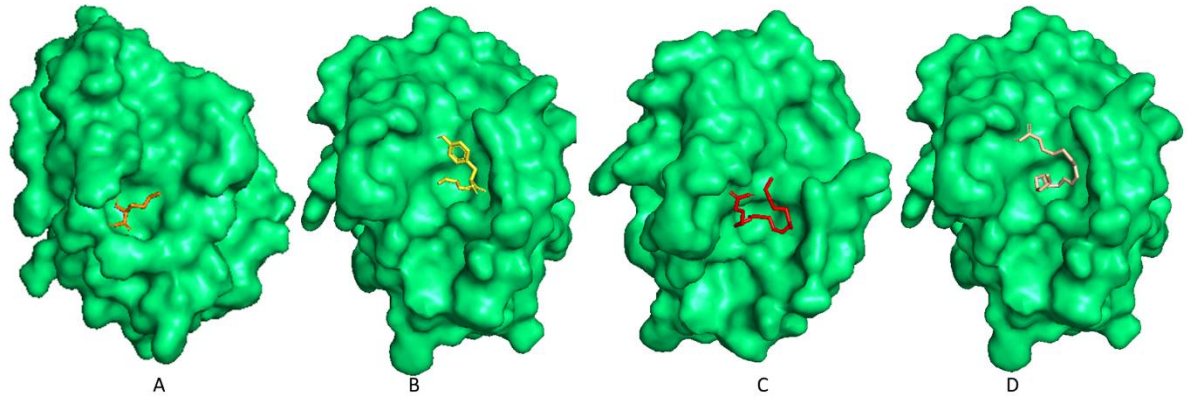

Fig 5: BCl2 protein with A) DL-norleucine B) 1-tyrosine butyl ester C) Arachidonic acid D) octadecenoic acid

Table 2: Hydrogen bonds & Hydrophobic interactions

S.No	Docked Complexes	Hydrogen bonds		Hydrophobic interactions	
		Residues	No of Bonds	Residues	No of Bonds
1	2JCN+Tyrosine butyl ester	R36	1	F40, L65, P67	4
2	2JCN+octadecenoic acid	Q32, R156	2	Q29, E32, T155, R156, V159, L163	8
3	2JCN+arachidonic acid	H43, Q47	2	F40, Q44, Q47, V61, Y41	7
4	2JCN+norleucine	R36, Q66	3	F40, L65	4
5	2K7W+tyrosine butylester	I66, G166	2	I66, V111, Y164, W170, V177	7
6	2K7W+octadecenoic acid	-	-	L63, I66, V111, F165, T167, V173, V177	12
7	2K7W+arachidonic acid	R65	2	I66, V111, Y164, F165, W170, V177	9
8	2K7W+norleucine	L25, G29, T56	3	L25, P51	2
9	4AQ3+tyrosine butylester	-	-	F63, Y67, V92, L96, F112	5
10	4AQ3+octadecenoic acid	L96, R105	3	F63, Y67, D70, F71, L96, R105, A108, F112	11
11	4AQ3+arachidonic acid	R105	2	F63, Y67, D70, F71, L96, R105, A108, F112	13
12	4AQ3+norleucine	R86, W135, E138	5	F89, V93, W135, Y139	4

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