

# Modelling and docking analysis of a tumor target protein BAG3

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## Abstract:

BCL2 associated anthanogene (BAG)s are class of proteins linked to tumorigenesis and apoptosis. Therefore, it is of interest to design and develop potent inhibitors for BAG. Hence, we report the optimal structure-based docked features of sesamol with BAG3 with the acceptable ADMET properties for consideration in the context of Glioblastoma Multiforme treatment and therapy.

**Keywords:** Glioblastoma, BAG3, tumorigenesis, sesamol, apoptosis.

## Background:

Glioblastoma is the most aggressive and occurring brain tumor in humans [1]. The BCL2 associated anthanogene BAG3 proteins is a survival protein that has been shown to be stimulated during cell

response to stressful conditions, such as high temperatures and heavy metals [2] and have been found to be involved in the HSF/HSP70/BAG3 pathway which confer resistance in glioma cells to apoptosis [3]. BCL2 associated anthanogene (BAG) s are a

cross-functional class of proteins biochemically implicated to have varied physiological roles within the ambit of tumorigenesis and apoptosis with highly conserved genes [4]. The BCL2 associated anthanogene domains (BAG) across species are commonly conserved regions close to the C terminal in BAG family and this has been implicated to orchestrate interaction with the ATPase domain, which allows phosphorylation [5], located on the HSP70/HSC70 molecular chaperones [6-7]. BAG3 may regulate the chaperone activity of HSP70 [8-9] and other signalling pathways involved in cancer development through its domains [9-12]. The anti-apoptotic BAG3 protein has been found to be capable of maintaining metastatic cell survival [13, 14] and it's over expression can promote cell proliferation with up regulation of auto-phagy genes [15]. The Nuclear factor-kappa B (NF-kB)-inducing kinase (NIK), in the NF-kB pathway has been identified to mediate the up regulation of BAG3 which gives an explanation to rhabdomyosarcoma cells resistance to treatment [16] and this up regulation of BAG3 is not a general feature of apoptotic cells [17]. The BAG3 is up regulated in colon cancer cells [18]. The expression of BAG3 can be negatively regulated [19-20], blockage of the PI3K/AKT pathway can reduce levels of

expression of BAG3 [21]. However, BAG3 knockdown was found not to interfere with the stabilization of anti-apoptosis-related proteins in retinoblastoma cells [22] and also decreases insulin cell content with increase secretion involved in diabetes pathogenesis [23]. For over 40 centuries, Sesame orientale, a potential oilseed plant, has been grown globally [24]. The S. orientale contains phytochemicals whose therapeutic roles have been validated both in the in vitro and in vivo studies, which further established their anti-hypertensive, anti-estrogenic, hepatoprotective, and anti-cancer properties [25-29]. Anti-cancer characteristics of Sesamin were attributed to its pro-apoptotic, anti-metastatic, anti-angiogenic, anti-inflammatory, and anti-auto phagocytic activities and thus portrayed to play critical roles in a number of signal transduction pathways that orchestrate angiogenesis, proliferation, apoptosis, and oxidative stress [15]. Pharmacological intervention by interfering with the BAG3 function to reactivate apoptotic cell death in glioma can be useful for future therapy on glioblastoma [3]. Therefore, it is of interest to design and develop potent inhibitors for BAG with improved binding features.

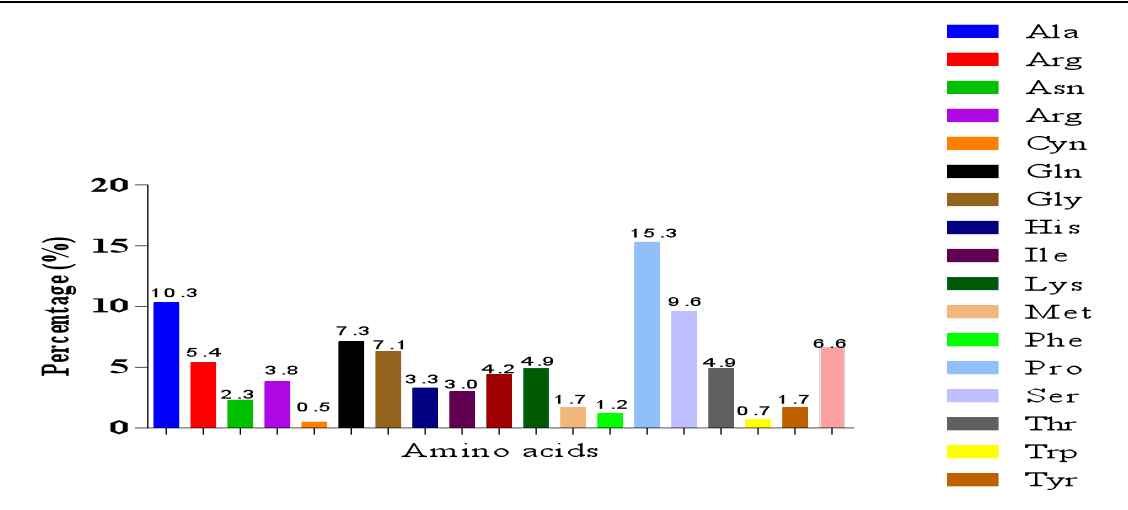


Figure 1: Composition of the 575 amino acids residues with (Pro) having the highest concentration followed by (Cyn) having the lowest concentration

Table 1: Domains in BAG3 and their amino acid sequence ranges

Table with 6 columns: Domain, Source, ID, Start, End, Length of amino acid sequence. It lists domains WW and BAG with their respective sources (PFam, SAMRT, PROSITE) and amino acid sequence ranges.

Materials and Methods:

Ligand selection and preparation:

The twenty-nine phyto-chemicals of Sesame orientale used in this study were retrieved from FoodB (foodb.ca) [30] and PubChem compound database [31] in MOL SDF format which was converted to PDBQT file using PyRx tool to generate atomic coordinates, and energy minimization was carried out using the optimization algorithm at force field set at Universal Force Field (UFF) has required on PyRx. The standard used, Obatoclax Mesylate, a synthetic inhibitor of the bcl-2 family of proteins with potential pro-apoptotic and antineoplastic activities was also retrieved from the PubChem database and converted also to PDBQT file using PyRx tool.

**Table 2:** Top two templates with their corresponding sequence identity

Template	Name of Template	GMQE	Identity	Method	Oligo State	Found by
IUK5.1. A	BAG3	0.11	93.88%	NMR	Monomer	BLAST
IUK5.1. A	BAG3	0.10	98.77%	NMR	Monomer	BLAST

**Table 4:** Binding affinity of sesamol, sesamin, and justisol using AutoDock Vina scoring function algorithm.

Compounds	Fred Docking Score	SwissDock Score	AutoDock Vina
Sesamol	-4.31	-7.39	-6.8
Sesamin	-4.0	-6.85	-6.8
Justisol	-3.92	-7.24	-6.8

## Homology modeling of B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) target protein:

The crystal structure of Human BAG3 was unavailable in the PDB databank; hence homology modeling was used to generate the 3D structure needed for this study. Human BAG3 protein FASTA query sequences (NP\_004272.2) were retrieved from NCBI and PDB databases. The human BAG3 was subjected to the Basic Local Alignment Search Tool algorithm (BLASTP). The multiple sequence alignments (MSA) were carried out using CLUSTAL W [32] and a phylogenetic tree was constructed while the identical and similar amino acids are shaded or colored. The relevance of this technique is to analyze the sequence similarities of BAG3 across different organisms. In order to find the conserved region, for this purpose, we used the Cunsurf server (<http://consurf.tau.ac.il/>). Two templates with the same ID IUK5.1.A but different sequence identity of 93.88 % and 98.77% were used for homology modeling using a SWISS-MODEL web server ([swissmodel.expasy.org](http://swissmodel.expasy.org)). These tools were used to generate a 3D structure of the homology-modeled protein.

## Optimization and Refinement of B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) modeled protein:

The generated homology model was uploaded on 3Drefine webserver, this makes use of iterative optimization of hydrogen bonding network in addition to atomic-level energy minimization on the optimized protein model using a composite physics and knowledge-based force fields for efficient protein structure refinement [33].

## Validation and quality estimation of Optimized and Refined B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) modeled protein:

**Table 3:** Best model indicating 3D<sup>refine</sup> Score, Molprobability, RMSD, RWplus, GDT-HA, and GDT-TS after energy minimization

Models	3D <sup>refine</sup> Score	GDT-TS	GDT-HA	RMSD	MolProbability	RWPlus
Best Model	3686.66	1.0000	1.0000	0.242	2.534	-15538.68

**Table 5:** Interacting residues of the compounds with BAG3

Ligands	Interacting Residues	Ligand and Protein atoms involved in H-bonding	Hydrophobic and electrostatic interaction
Sesamol	ARG <sup>479</sup> , ARG <sup>480</sup> , GLN <sup>487</sup> , VAL <sup>483</sup> , ARG <sup>484</sup>	O; ARG <sup>479</sup> :HH11	VAL <sup>483</sup> ; ARG <sup>484</sup> , ARG <sup>480</sup> , ASP <sup>465</sup>
Sesamin	ARG <sup>476</sup> , ARG <sup>479</sup> , ARG <sup>480</sup> , VAL <sup>483</sup> , ARG <sup>484</sup> , GLN <sup>487</sup> , PRO <sup>469</sup>	O; GLN <sup>487</sup> :HE22	ARG <sup>480</sup> , VAL <sup>483</sup> , ARG <sup>484</sup> , ARG <sup>476</sup> , PRO <sup>469</sup>
Justisol	ASP <sup>465</sup> , VAL <sup>483</sup> , LEU <sup>461</sup> , GLN <sup>487</sup> , LEU <sup>490</sup>	LEU <sup>490</sup> :O	LEU <sup>461</sup> , LEU <sup>490</sup> , VAL <sup>483</sup> , ASP <sup>465</sup>

**Table 6:** Sesamol ADME analysis

Physicochemical properties	Chemical Formula	Molecular Weight (g/mol)	H-bond Acceptors	H-bond Donors	Molar Refractivity	Lipophilicity
Sesamol	C <sub>20</sub> H <sub>18</sub> O <sub>7</sub>	370.35	7	0	91.52	2.74

The optimized BAG3 model was validated using RAMPAGE, PROSESS [34], and PDBsum server [35]. The quality estimation of the modeled protein was carried out using ERRAT, VADAR, and SAVES servers, and ProSA-web. The resolution of the optimized modeled Human BAGE3 was calculated using ResProx server and visualized using Discovery Studio 3.0.

## Submission of the model in the protein model database:

The model generated for human BAG3 was successfully submitted in the protein model database (PMDb) having PMDB ID: PM0082220 [36].

## Molecular docking using PyRx:

For our analysis, we used the PyRx, AutoDock Vina exhaustiveness search docking function. After the minimization process, the grid box resolution of human BAG3 was centered at -1.7888 x -6.9655 x 0.9312 along the x, y and z center axes respectively at the grid dimension of 33.1350 x 53.635 x 33.906 Å to define the binding site [37].

## Drug likeness and ADME-Toxicity:

The Canonical smiles of the Sesamol (PubChem CID: 101746) were gotten from the PubChem Database and were used to analyze the ADMET properties of the Sesamol using the SwissADME server [38]. ADME (absorption, digestion, metabolism, and excretion) and toxicity (mutagenic, tumorigenic, irritant) properties determine the biological effect of drugs. Using the Variable Nearest Neighbor ADMET (vNN-ADMET) server <https://vnnadmet.bhsai.org/vnnadmet/home.xhtml>, sesamol biological effects on health were determined. The vNN-ADMET web server is equipped with prebuilt ADMET models and these models assess properties like the cytotoxicity, mutagenicity, drug-drug interactions, and likelihood of causing liver injury [39-40].

## Lead optimization:

### Validation of docking results:

The docking protocol was further validated using docking for Obloclax and the hits to the binding site of human B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) modeled protein using Fred and SwissDock scoring function.



Table 7: ADME-Tox of Sesamolin using vNN-ADMET

Table with 11 columns: Query, Liver Toxicity, Metabolism (Cyp Inhibitors: HLM, 1A2, 3A4, 2D6), Membrane transporters (BBB, P-gp), Others (hErg Blocker, MMP, AMES, MRTD (mg/day)).

\*HLM = Human Liver Microsomal Stability, Cyp1A2 = Cytochrome p450 1A2, Cyp3A4 = Cytochrome p450 3A4, Cyp2D6 = Cytochrome p450 2D6, BBB = blood brain barrier, P-gp = glycoprotein, MMP = metallo matrix protein, MRTD = maximum recommended therapeutic dose

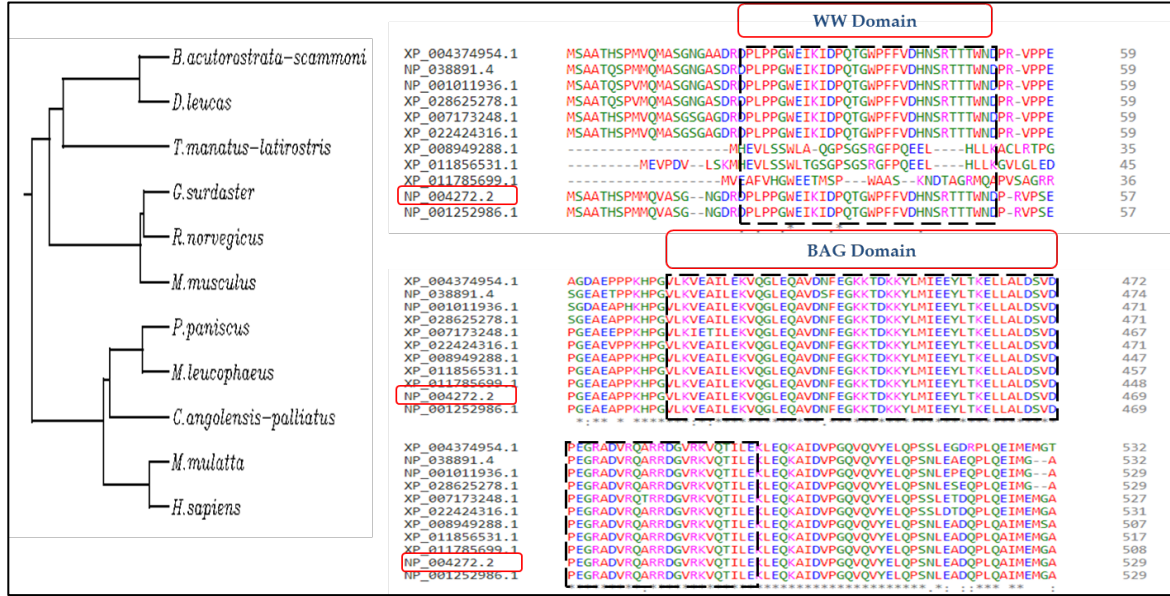


Figure 2: Phylogenetic tree and multiple sequence alignment of B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) co-chaperone from different species indicating the BAG and WW domain

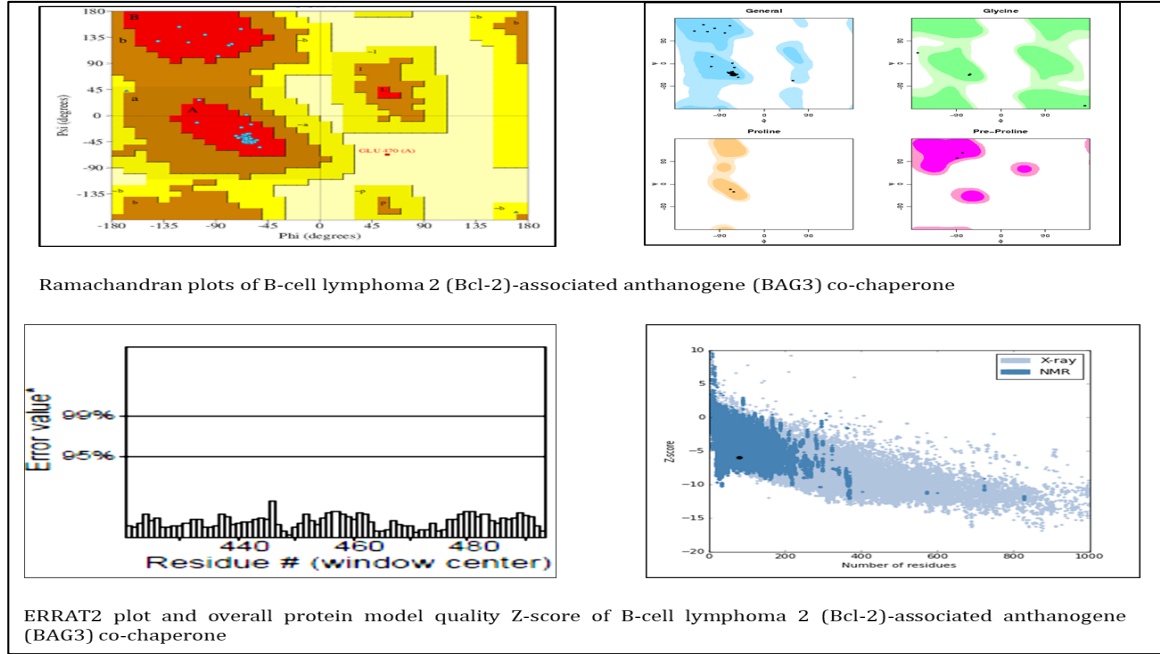
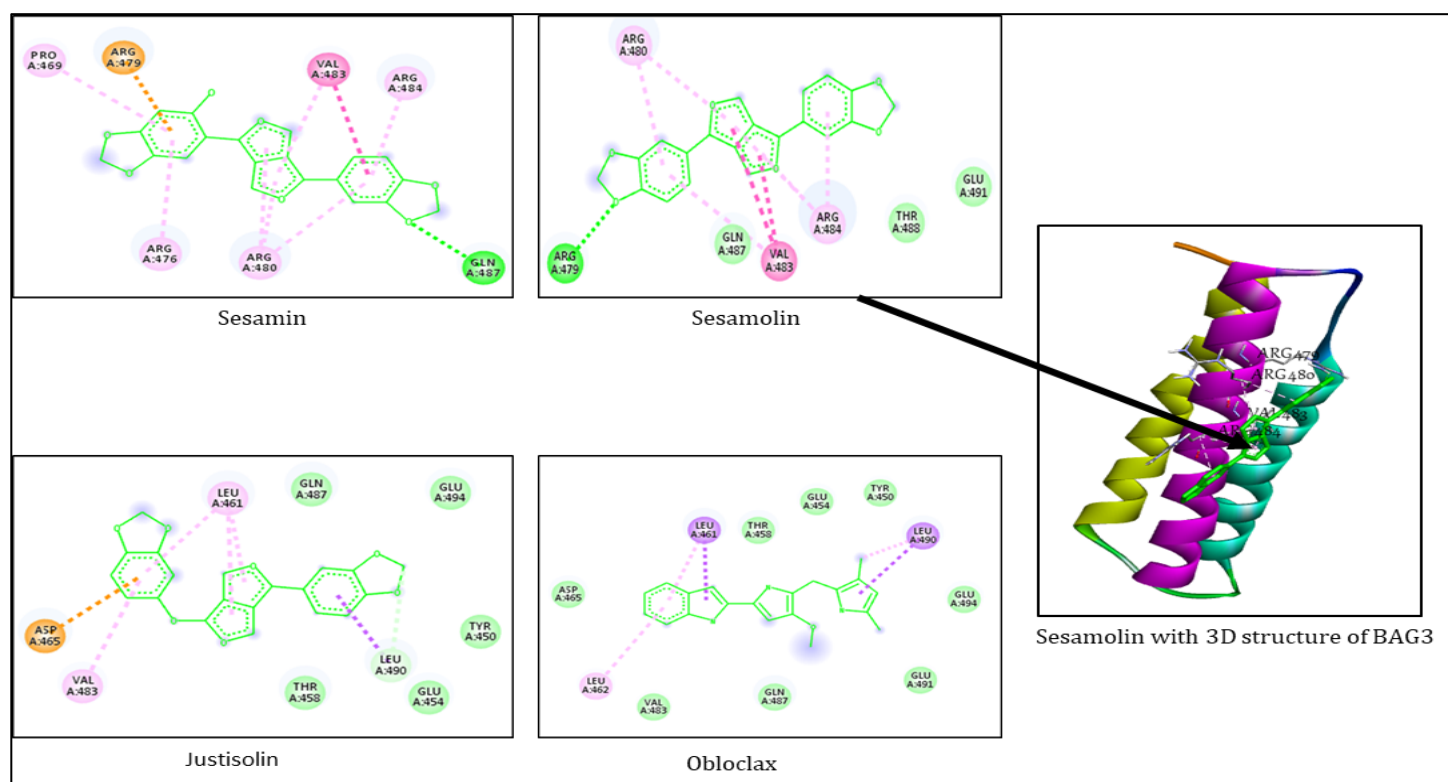


Figure 3: Overall protein model quality check



**Figure 4:** Sesamol, Sesamin, Justisolin, and Obloclax (co-crystallized) ligands interaction with B-cell lymphoma 2 (Bcl-2)-associated anthanogene (BAG3) within the BAG domain

Using AutoDock Vina to study the protein-ligand binding, Sesamol, Sesamin, and Justisolin are the lead compounds in comparison to the standard drug, which is Obloclax used in this study as indicated in figure 4. Sesamol, Sesamin, and Justisolin all have the same docking score of  $-6.8 \Delta G$  kcal/mol compared to Obloclax which has a scoring function of  $-6.4 \Delta G$  kcal/mol. But at redocking of the three leads using Fred Docking Score, and SwissDock, Sesamol was indicated to be the consistent lead of the three as depicted in table 4 below. As indicated in figure 4, the amino acid residues VAL<sup>483</sup>; ARG<sup>484</sup>; ARG<sup>480</sup>; and ASP<sup>465</sup> are involved in hydrophobic and electrostatic interactions. While the amino acid residues involved in other forms of interactions with sesamin at the active site of BAG3 includes, ARG<sup>480</sup>; VAL<sup>483</sup>; ARG<sup>484</sup>; ARG<sup>476</sup> and PRO<sup>469</sup>. On the other hand, the residues involved in Justisolin hydrophobic and electrostatic interaction with the active site of BAG3 includes LEU<sup>461</sup>; LEU<sup>490</sup>; VAL<sup>483</sup>; and ASP<sup>465</sup>. Amino acid residues; ARG<sup>479</sup>, GLN<sup>487</sup>, and LEU<sup>490</sup> are the only residues involved in the formation of hydrogen bond interaction between Sesamol, Sesamin, and Justisolin with the active site of BAG3 respectively.

The drug-likeness analysis of Sesamol on the SwissADME shows that it doesn't violate the Lipinski's rule of five (rule of five include, Mol<sub>W</sub> < 500, Log P < 5, Donor H-bond ≤ 5, Acceptor H-bond ≤ 10 and Molar Refractivity (40-130). Therefore, sesamol is

considered druglike. The ADME-Tox of sesamol was predicted by the Variable Nearest Neighbor ADMET (vNN-ADMET) server method. The ADMET has an influence on the drug level and the kinetics of drug exposure to tissues. Sesamol shows positive predictions for ADMET endpoints, Human Liver Microsomal (HLM) stability test, can inhibit Cyp1A2, Cyp3A4, and also glycoprotein inhibition. Sesamol having the potential of being a therapeutic agent has a maximum recommended dose of 200 mg per day in clinical conditions.

## Conclusion:

We report the optimal structure-based docked features of Sesamol with BAG3 with the acceptable ADMET properties for consideration in the context of Glioblastoma Multiforme therapy.

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