

Potential inhibitory action of cafestol on apoptosis proteins: An *in-silico* study

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ABSTRACT

Coffee has been found to reduce cell proliferation and induce apoptosis, which is significant in terms of protecting against cancer. However, the molecular mechanism of this effect is yet to be fully elucidated. The aim of this study was to evaluate the affinity between 12 key proteins involved in apoptosis and four of the main compounds derived from coffee: caffeine, cafestol, caffeic acid, and chlorogenic acid, to contribute to the understanding of the effect of coffee on apoptosis. Molecular docking was performed between proteins and ligands using Autodock Tools in Autodock Vina. The best protein-ligand complexes were then submitted to molecular modeling simulations in GROMACS 2018.2. The best interactions in molecular docking analysis were BCL2 with cafestol, BCL2L1 with cafestol, and BAX with cafestol. Molecular modeling simulations show that cafestol binds more strongly within the binding pockets of BCL2 and BAX, while the BCL2L1-cafestol complex is not very stable. These results imply that cafestol competes for binding with BCL2 and BAX. Binding of cafestol could inhibit the anti-apoptotic activity of BCL2 and promote the pro-apoptotic activity of BAX, thus favoring apoptosis. This is especially relevant in the context of cancer cells, where the expression of BCL2 can be increased. We propose a model in which the binding of cafestol with BCL2 favors a pro-apoptotic effect, and as such is worth further scrutiny as an anticancer strategy.

Key words: Apoptosis; cafestol; cancer; coffee; molecular modeling.

1 INTRODUCTION

In recent decades, considerable efforts have been made to research dietary products as chemopreventive and therapeutic agents for different types of cancer, and by 2012, 60% of clinically used anticancer drugs were of plant origin (Zhang et al., 2008). Coffee, one of the most consumed natural beverages worldwide, is of particular interest as it has a high content of bioactive compounds with antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (Ludwig et al., 2014). A great number of epidemiological studies have been conducted over the last decade revealing a significant protective effect against cancer (Poole et al., 2017), as well as *in vitro* and *in vivo* studies that show that coffee reduces cell proliferation and induces apoptosis (Moreno-Ceballos et al., 2019). Compounds found in coffee, such as caffeine, chlorogenic acid, cafestol, and caffeic acid have been shown in *in vitro* studies to have effects on cell proliferation, viability, migration potential, and apoptosis (Buldak et al., 2018). However, the molecular mechanisms are yet to be fully elucidated.

Apoptosis is a series of steps that lead to programmed cell death. It is an important process for the regulation of cell growth and proliferation and is a natural barrier to the development of cancer. Apoptosis can be activated by two pathways, intrinsic and extrinsic. Both pathways converge in the activation of caspases 8 and 9, which leads to the activation of cellular proteolysis inducing cell disassembly (Kashyap; Garg; Goel, 2021). Apoptosis is controlled by pro- and anti-

apoptotic proteins of the BCL2 family. Proteins such as BCL2, BCL2L1, BCL2L2, MCL1, and BCL2A1 are inhibitors of apoptosis by binding to BAX and BAK1 proteins, which are pro-apoptotic proteins found in the outer mitochondrial membrane. When BAX and BAK1 are activated, they alter the integrity of the outer mitochondrial membrane, allowing the release of pro-apoptotic proteins such as cytochrome C. This in turn, triggers the activation of caspases that lead to cellular proteolysis (Adams; Cory, 2007).

In cancer cells, attenuation and evasion of apoptosis are necessary for their survival and for tumorigenesis to thrive. Cancer cells evade apoptosis via mechanisms that include inducing the overexpression of anti-apoptotic proteins such as BCL2 and BCL2L1, and the under-expression of pro-apoptotic proteins such as BAX (Delbridge et al., 2016). Therefore, the aim of this study was to evaluate the affinity between 12 of the key proteins involved in apoptosis and four of the major compounds derived from coffee: caffeine, cafestol, caffeic acid, and chlorogenic acid, in order to contribute to the understanding of the effect of coffee on apoptosis.

2 MATERIAL AND METHODS

2.1 Selection and preparation of protein structures

According to literature review (Qian et al., 2022; Thomas et al., 2013; Elena-Real et al., 2018), the following

key apoptotic proteins were selected: Apoptosis regulator BCL2, BCL2-like protein 1, Cytochrome c, apoptotic protease-activating factor 1, Caspase 2, Caspase 3, Caspase 7, Myc proto-oncogene protein, Cellular tumor antigen p53, apoptosis regulator BAX, BCL2-binding component 3, and E3 ubiquitin-protein ligase MDM2 (Table 1). Structures were downloaded from RCSB PDB Protein Data Bank (<https://www.rcsb.org/>) or PDBe Protein Data Bank Europe (<https://www.ebi.ac.uk/pdbe/>), filtering for *Homo sapiens*, resolution < 3 Å, no mutations present in the structure, and free R-value < 0.25.

Table 1: Apoptosis proteins included in this study.

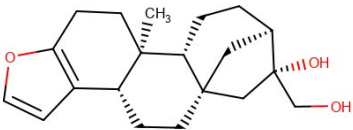
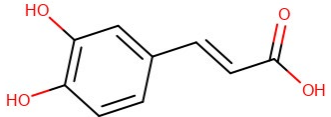
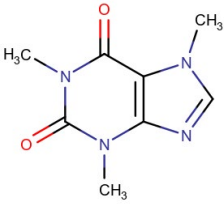
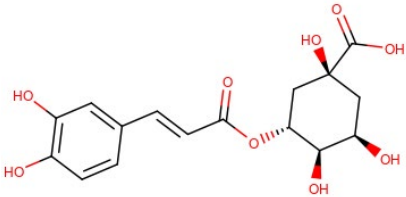
Gene	Protein	Uniprot	PDB code
<i>BCL2</i>	Apoptosis regulator BCL2	P10415	6O0K
<i>BCL2L1</i>	BCL2-like protein 1	Q07817	3SPF
<i>CYCS</i>	Cytochrome c	P99999	3ZCF
<i>APAF1</i>	Apoptotic protease-activating factor 1	O14727	1Z6T
<i>CASP2</i>	Caspase-2	P42575	1PYO
<i>CASP3</i>	Caspase-3	P42574	1NMS
<i>CASP7</i>	Caspase-7	P55210	1F1J
<i>MYC</i>	Myc proto-oncogene protein	P01106	1NKP
<i>TP53</i>	Cellular tumor antigen p53	P04637	2AC0
<i>BAX</i>	Apoptosis regulator BAX	Q07812	4S0O
<i>BBC3</i>	BCL2-binding component 3	Q9BXH1	4BPJ
<i>MDM2</i>	E3 ubiquitin-protein ligase MDM2	Q00987	4OGN

The protein structures were initially prepared using the software UCSF Chimera (<https://www.rbvi.ucsf.edu/chimera/>), where additional chains and inhibitors in the structure were eliminated, and energy minimization was applied to the structures to allow a better geometry of the receptor-ligand, this process was carry out using Amber99sb-ildn force field (Lindorff-Larsen et al., 2010) using 100,000 steps with the *steepest descent* method included in GROMACS software (Valdés-Tresanco et al., 2021). Heteroatoms (except cofactors) were then eliminated using Autodock Tools (<https://autodock.scripps.edu/adit/>). Finally, polar hydrogen atoms and Kollman charges were added according to docking protocol, and AD4 atom type was assigned.

2.2 Selection and preparation of ligand structures

The following bioactive compounds in coffee were selected as ligands: cafestol, caffeic acid, caffeine, and chlorogenic acid. Structures were downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) (Table 2).

Table 2: Coffee compounds used in this study.

Name	PubChem CID	Structure
Cafestol	108052	
Caffeic acid	689043	
Caffeine	2519	
Chlorogenic acid	1794427	

The ligand structures were prepared using the software MarvinSketch (<https://chemaxon.com/marvin/>), where the protonation state of each ligand was set to pH 7.4. Using software Avogadro 1.2 (<https://avogadro.cc/>), the force field was set to MMFF94 to minimize structure energy, and hydrogen atoms were added according to the previously set pH value.

2.3 Molecular docking

The molecular docking of the 12 apoptosis-related proteins with each of the four coffee compounds was conducted in three steps: blind and site-specific docking, validation of docking results, and interaction analysis. Blind docking was implemented with the aim of identifying potential allosteric sites, and directed docking was performed using active sites where the native ligands were localized.

Docking simulations were performed in triplicate for each of the 12 proteins against the four ligands, using Autodock Tools in Autodock Vina (<https://autodock.scripps.edu/>) with the default parameters. This was followed by site-specific docking, which was carried out similarly. Only affinity values < -8 kcal/mol were considered. Validation of the blind and site-specific

docking results was performed using Autodock 4.2, selecting output Lamarckian GA algorithm with default parameters.

The interactions that showed significant affinity values in the validation step were evaluated using BIOVIA Discovery Studio Visualizer (<https://www.3ds.com/products/biovia/discovery-studio>) and the interaction map for each result was obtained. Distance analysis was performed in UCSF Chimera to identify the most important bonds in each interaction. Hydrogen bonds must be $< 3 \text{ \AA}$.

2.4 Molecular modeling

Molecular modeling (MM) simulations were performed in GROMACS 2018.2 (van der Spoel et al., 2005) installed on an NMRbox server (Maciejewski et al., 2017) using the ligand-protein complexes with the best affinity scores. Molecular topologies of the selected ligands were generated using the semi-empirical quantum chemistry program SQM of ACPYPE (Fogh et al., 2005; Nilges et al., 2008; Rieping et al., 2007; Sousa Da Silva; Vranken, 2012; Vranken et al., 2005; Wang et al., 2006a, 2006b). All-atom MM simulations were performed with the Amber99-ILDN force field (Best; Hummer, 2009; Hornak et al., 2006; Lindorff-Larsen et al., 2010) and TIP3P water model (Jorgensen et al., 1998). Protein-ligand complexes were solvated with water in a cubic box with 1.0 nm of water, and Na^+ and Cl^- ions were added randomly to neutralize the system and produce a 0.15 M concentration.

Energy was minimized for each protein-ligand complex using the steepest-descent method. Complexes were then equilibrated using the Berendsen thermostat and Parrinello-Rahman barostat (Berendsen et al., 1998; Parrinello; Rahman, 1980), and finally, 100 ns of MM simulations were performed on the systems. Root mean square deviation (RMSD) was analyzed during the complete MM simulation and binding free energy of the protein-ligand complex was calculated based on the Molecular Mechanics/Generalized-Born Surface Area using gmx_MMPBSA (Valdés-Tresanco et al., 2021) during the last 30 ns.

2.5 ADME analysis

For the purposes of discussion and after the simulations, an ADME (chemical absorption, distribution, metabolism, and excretion) analysis was performed for cafestol using SwissADME online tool (Daina et al., 2017).

3 RESULTS

Blind docking was the first step in determining the potential protein-ligand interactions. In blind docking, the software algorithm finds the optimal conformation of the ligand with the entire protein, allowing to identify binding sites of the ligand within the protein. The second step was site-specific docking, which is possible when the binding pocket

of the protein is known or predicted. For this, some of the protein structures downloaded from Protein Data Bank had a ligand or inhibitor bound, the corresponding binding site was selected in these cases. For other proteins that did not have a ligand or inhibitor in the crystallographic structure, it was carried out a literature search to find the reported binding site. Table 3 shows the results of blind and site-specific docking. The compounds that showed potential binding were cafestol and chlorogenic acid with proteins BCL2, BCL2L1, APAF1, CASP2, BAX, BBC3, and MDM2. Only affinity values < -8 kcal/mol were considered, and results are shown as the average of the affinities of the three experiments run for each protein-ligand combination.

Table 3: Results of blind and site-specific docking.

Blind Docking			
Protein	Ligand	Affinity (kcal/mol)	SD
BCL2	Cafestol	-7.47	0.15
	Chlorogenic acid	-7.27	0.06
BCL2L1	Cafestol	-8.07	0.06
	Chlorogenic acid	-7.07	0.12
APAF1	Cafestol	-8.13	0.23
	Chlorogenic acid	-8.97	0.12
CASP2	Cafestol	-8.2	0
	Chlorogenic acid	-7.97	0.23
BAX	Cafestol	-7.13	0.12
BBC3	Cafestol	-8.33	0.06
	Chlorogenic acid	-7.57	0.06
MDM2	Cafestol	-7.57	0.06
Site-Specific Docking			
Protein	Ligand	Affinity (kcal/mol)	SD
BCL2	Cafestol	-7.4	0
BCL2L1	Cafestol	-8.2	0
APAF1	Cafestol	-7.73	0.55
	Chlorogenic acid	-8.9	0.1
BAX	Cafestol	-7.2	0
BBC3	Cafestol	-7.4	0
	Chlorogenic acid	-7.6	0
MDM2	Cafestol	-7.6	0
	Chlorogenic acid	-7.07	0.06

Affinity values are in kcal/mol, SD: standard deviation. Best affinities are highlighted in bold.

Validation of the blind and site-specific docking results was performed in Autodock 4.2, selecting output Lamarckian GA algorithm with default parameters. The blind and site-specific docking results were validated in Autodock 4.2, and the results of this validation step are shown in Table 4.

Table 4: Validation through redocking of blind and site-specific docking.

Blind Docking Validation		
Protein	Ligand	Affinity (kcal/mol)
BCL2	Chlorogenic acid	-8.24
	Cafestol	-7.37
BCL2L1	Caffeine	-4.59
	Cafestol	-8.41
BAX	Cafestol	-8.8
BBC3	Cafestol	-7.48
	Chlorogenic acid	-7.52
MDM2	Cafestol	-7.04
Site-Specific Docking Validation		
Protein	Ligand	Affinity (kcal/mol)
BCL2	Chlorogenic acid	-7.2
	Cafestol	-8.02
BCL2L1	Caffeine	-4.68
	Cafestol	-8.45
APAF1	Cafestol	-8.09
	Chlorogenic acid	-7.76
BAX	Cafestol	-8.8

Complexes highlighted in bold are those that had values < -8 in both software.

The interactions in both validations were BCL2 with cafestol, BCL2L1 with cafestol, and BAX with cafestol, so these interactions were evaluated using BIOVIA Discovery Studio Visualizer as shown in Figure 1. Within the validation analysis was included BCL2L1 with caffeine as a negative control of the validation, as this complex did not have a significant affinity value in the initial docking experiment.

Molecular docking studies suggest that cafestol interacts with proteins BCL2, BCL2L1 and BAX.

To determinate which target exhibits the best affinity for this ligand, MM simulations were carried out with the top models of protein-ligand complexes obtained in the docking studies. To assess the dynamic stability of the complexes, The RMSD plots for the three protein-ligand complexes are shown in Figure 2. This analysis indicates that cafestol demonstrates greater stability within the binding pockets of BCL2 and BAX, while the RMSD values of the Cafestol-BCL2L1 complex suggest low stability.

Figure 3 shows the hydrogen bond (HB) analysis between protein and ligand during the MM simulations described above. Cafestol forms a maximum of three HB with BAX and BCL2, whereas the BCL2L1-cafestol complex forms only two HB. These preliminary results suggest that BCL2L1 is the least likely target for cafestol.

The HB analysis of cafestol with BAX and BCL2 reveals significant differences (Figure 4). Cafestol interacts with BAX at two sites; the primary interaction involves an HB between the oxygen atom from the furan moiety and the protonated amine of Gln28. The secondary HB is between the methylenhydroxy substituent and the peptide bond of Leu47, though it is weaker than the primary interaction. For BCL2, cafestol interacts through hydroxyl substituents in one region of the molecule forming HBs with the side chains of Arg110 and Glu114. These bonds are generally stronger due to the charged nature of these residues.

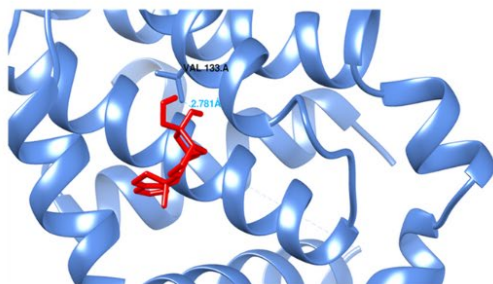
Finally, binding free energy (BE) values for the three complexes were calculated using the gmx_MMPBSA protocol. For these calculations, the last 30 ns were considered after the systems stabilization. The BE values for cafestol with BAX, BCL2, and BCL2L1 were -37.20 ± 2.73 , -30.15 ± 4.41 , and -20.23 ± 2.60 kcal/mol, respectively. These results support previous observations and confirm that BCL2L1 can be ruled out as a potential target of cafestol. Moreover, the BE values for BAX and BCL2 with cafestol show that the BAX complex is the most stable, though the BCL2 complex has a comparable BE value.

The fact that cafestol demonstrates greater stability within the binding pockets of BCL2 and BAX, as indicated by the RMSD values, hydrogen bond and binding free energy calculations, suggests that these proteins could serve as potential molecular targets for this ligand. This stability reflects a preferential affinity for these proteins, which may be associated with the ability of this ligand to interact specifically and persistently with their active sites. Consequently, it is reasonable to propose that cafestol might exhibit biological activity against BCL2 and BAX, paving the way for further exploration of its potential as a modulator of these proteins in future experimental studies.

4 DISCUSSION

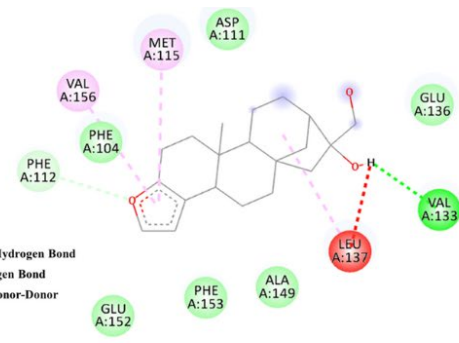
Together these results imply that cafestol could target both BAX (pro-apoptotic protein) and BCL2 (anti-apoptotic protein). Under normal circumstances, once a cell has been exposed to an apoptotic stimulus, cytosolic BAX undergoes a conformational change that causes it to migrate and insert into the mitochondrial membrane. This affects mitochondrial membrane permeabilization and the final effectors of apoptosis, such as cytochrome c, are released into the cytosol (Czabotar et al., 2013). BCL2 inhibits the oligomerization of BAX in the mitochondrial membrane and thereby inhibits mitochondrial permeabilization and the concurrent release of cytochrome c (Dlugosz et al., 2006) (Figure 5A). One of the mechanisms for cancer cell survival is the hyperactivation of BCL2, which blocks apoptosis. In

A. BCL2 with cafestol

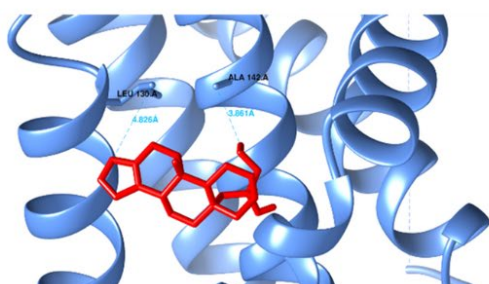


Interactions

- Van del Waals
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Unfavorable Donor-Donor
- Alkyl
- Pi-Alkyl

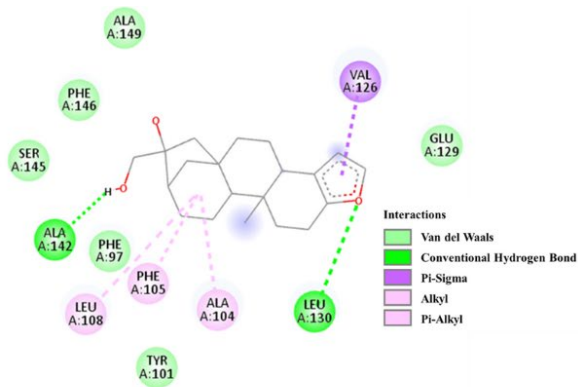


B. BCL2L1 with cafestol

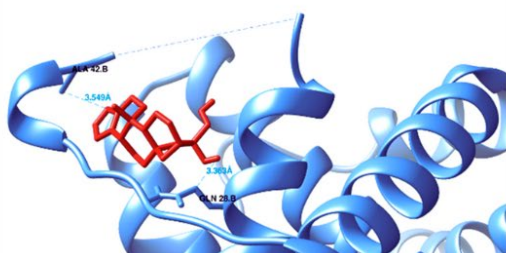


Interactions

- Van del Waals
- Conventional Hydrogen Bond
- Pi-Sigma
- Alkyl
- Pi-Alkyl



C. BAX with cafestol



Interactions

- Van del Waals
- Conventional Hydrogen Bond
- Carbon Hydrogen Bond
- Pi-Sigma
- Alkyl
- Pi-Alkyl

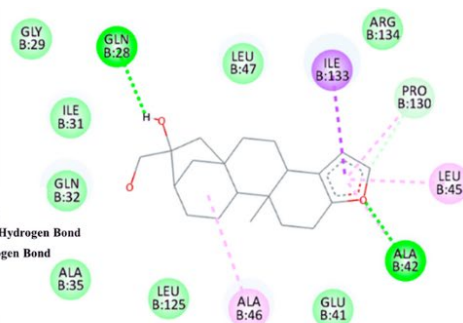


Figure 1: Interaction maps of BCL2 with cafestol (A), BCL2L1 with cafestol (B) and BAX with cafestol (C). Proteins are shown in blue and ligands in red. The most important bonds in each interaction are shown on the right.

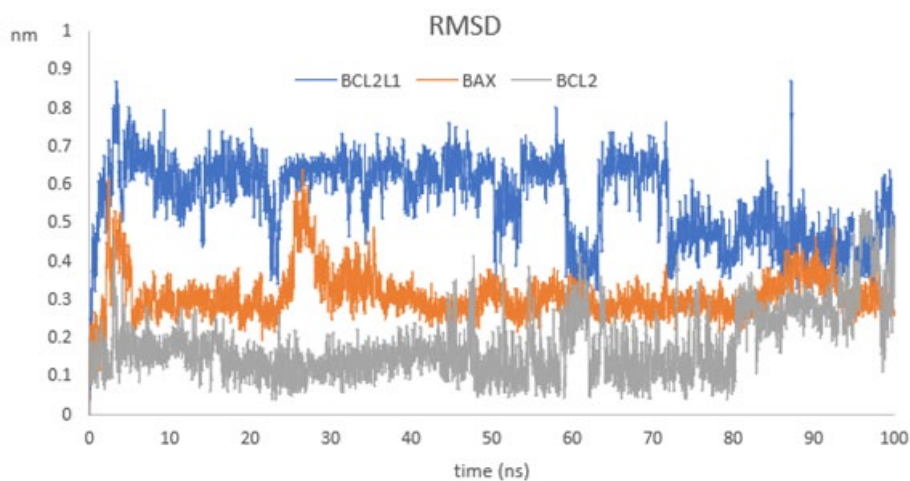


Figure 2: RMSD trajectories for cafestol complexes with proteins BCL2L1, BAX and BCL2.

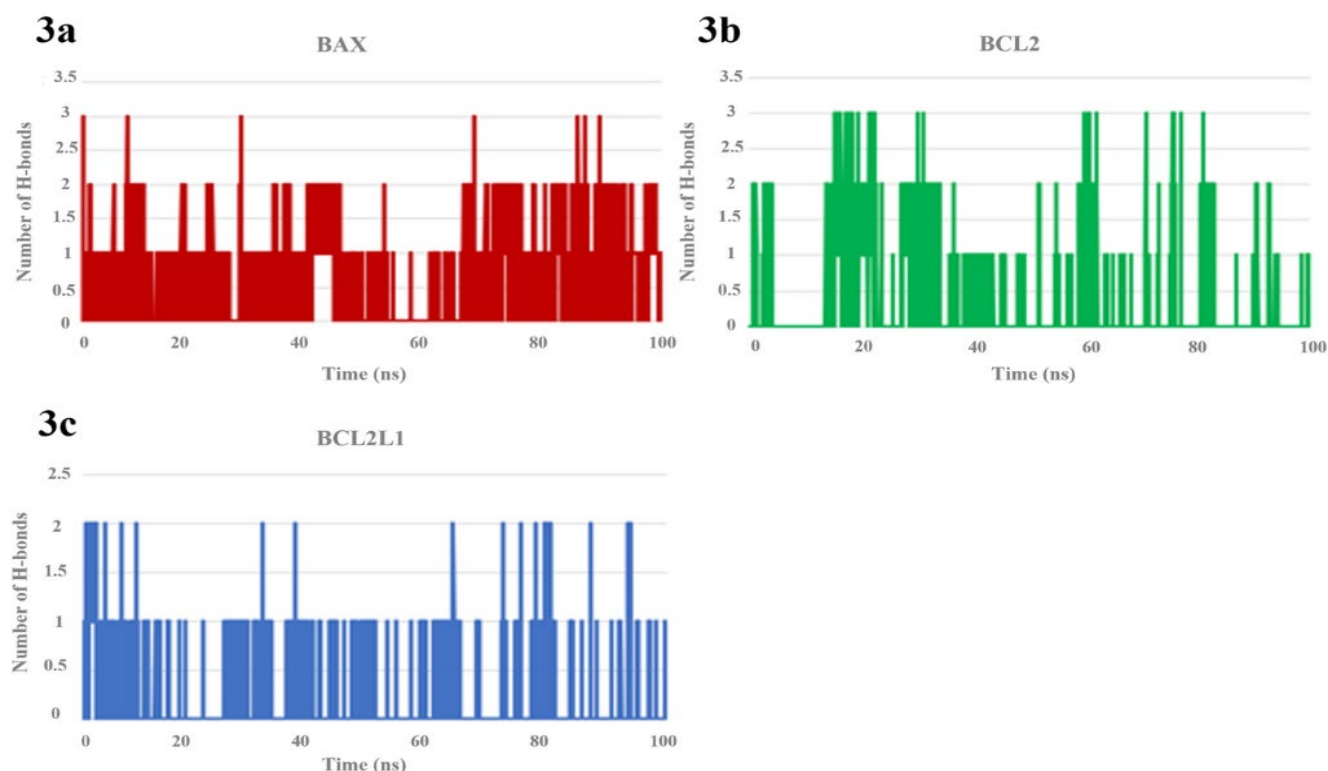


Figure 3: Hydrogen bond analysis for the complete MM simulations of cafestol with BAX (3a), BCL2(3b) and BCL2L1 (3c).

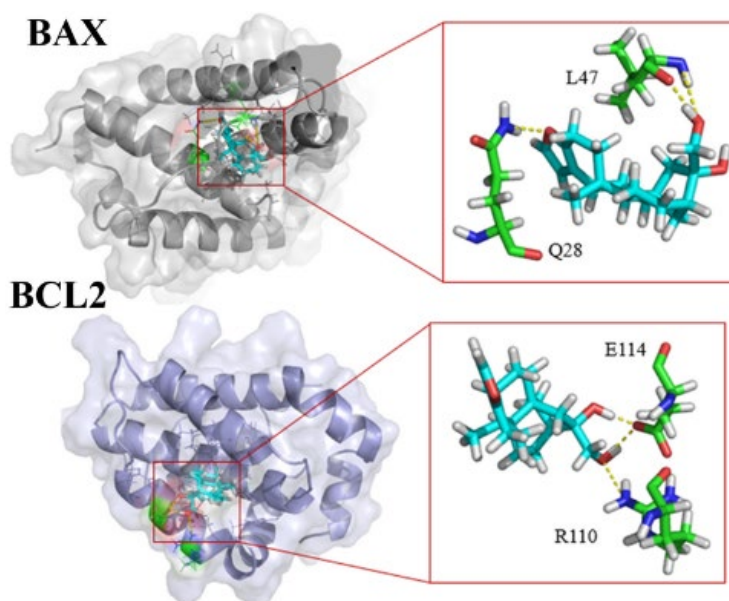


Figure 4: Hydrogen bonds among cafestol and BAX (up) and BCL2 (down).

this case, cafestol could have a regulatory role, meaning that when the hyperactivation of BCL2 takes place, cafestol can inhibit this protein and the cell can then activate apoptosis (Figure 5B). It is also possible that this could be via the activation of BAX. BCL2 is composed by 4 motif domains important for its molecular function: BH1, BH2, BH3 and BH4. According to the results shown here, cafestol interacts with the BH3 domain of BCL2 via Arg110. Interestingly,

the BH3 domain is also referred to as the ‘death domain’ and is likely key in suppressing BAX activity (Letai, 2005).

If cafestol crosses the cell membrane a competition for binding with both proteins (BCL2 and BAX) could take place. However, in the context of various types of cancer, there is a high probability that anti-apoptotic proteins are overexpressed and pro-apoptotic proteins underexpressed, as a mechanism for avoiding apoptosis. In this scenario, the

binding of cafestol would lean towards BCL2, inhibiting its ability to stop apoptosis and favoring its occurrence in cancer cells with BCL2 overexpression. This effect would not be the same in normal cells in which said overexpression is not present. Figure 5 schematizes this concept.

Not all molecules can be tested as potential candidates in drug discovery research, due to cost and time constraints. Therefore, *in-silico* analysis is valuable for refining the list of candidates that match the attributes of a drug used in medical therapy. To determine if cafestol is a “druggable” candidate, ADME analysis was performed as shown in Figure 6 and Table 5. In this analysis, cafestol appears to satisfy many of the properties required to be “druggable”. The druglikeness criteria like Lipinski rules, Ghose, Veber, Egan, and Muegge are fulfilled. There are zero alerts in the PAINS assay, which suggests that cafestol may be specific for its molecular targets. However, it has the potential to cross the blood-brain barrier

and to act as a substrate for P-gp, which should be taken into consideration.

Although the role of cafestol has not been widely studied, the findings outlined in this paper are supported by several studies found in the literature. The potential regulatory role of cafestol in apoptosis was demonstrated in an *in vitro* study where cafestol caused a dose-dependent increase of apoptosis in Caki cells (a renal carcinoma cell line) (Choi et al., 2011). Cafestol increased the activation of caspases by down-regulating the expression of anti-apoptotic proteins BCL2, BCL2L1, MCL1, and cFLIP. A similar study in leukemia cell lines reported that cafestol caused an accumulation of cleaved caspase-3 and phosphatidylserine externalization in HL60 and KG1 cells, thus inducing apoptosis (Lima et al., 2017). BCL2 proteins are often overexpressed in leukemia cells (Letai et al., 2004), so it is possible that the effect of cafestol reported in Lima and colleagues is related to BCL2.

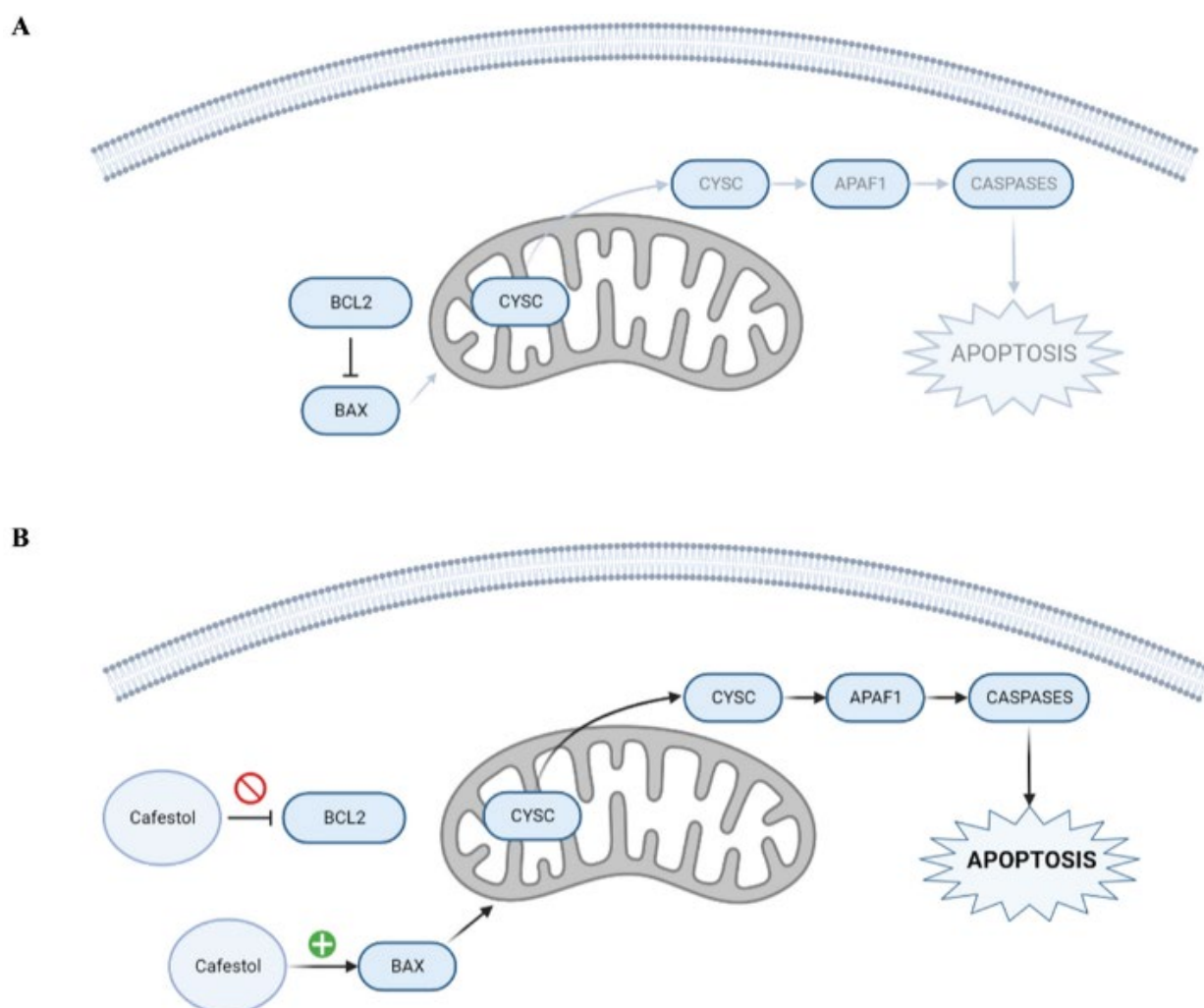


Figure 5: Proposed mechanism of the effect of cafestol on apoptosis via interaction with BCL2 and BAX. **A.** BCL2 inhibits the oligomerization of BAX in the mitochondrial membrane and thereby inhibits mitochondrial permeabilization and the concurrent release of CYSC. **B.** Cafestol competes for binding with BCL2 and BAX. Binding of cafestol could inhibit the anti-apoptotic activity of BCL2 and promote the pro-apoptotic activity of BAX, thus favoring apoptosis. Made in BioRender.

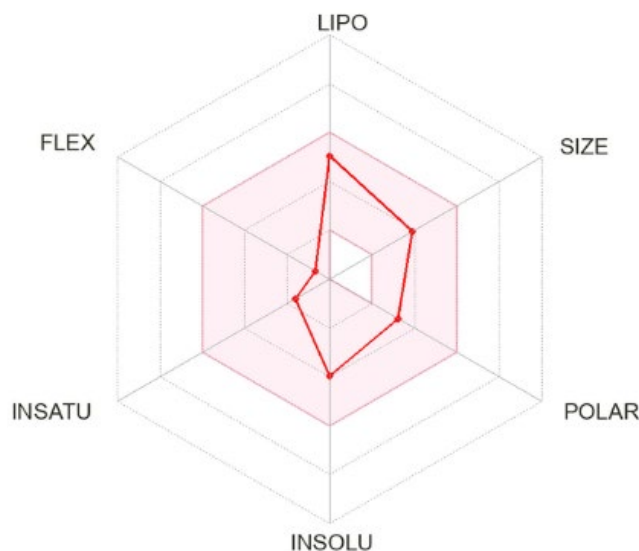


Figure 6: Radar chart of the ADME analysis of cafestol.

Table 5: ADME analysis of cafestol.

Physicochemical properties		Pharmacokinetics	
Molecular weight	316.43 g/mol	GI absorption	High
Num. heavy atoms	23	BBB permeant	Yes
Num. arom. heavy atoms	5	P-gp substrate	Yes
Fraction Csp ³	0.80	CYP1A2 inhibitor	No
Num. rotatable bonds	1	CYP2C19 inhibitor	No
Num. H-bond acceptors	3	CYP2C9 inhibitor	No
Num. H-bond donors	2	CYP2D6 inhibitor	Yes
Molar refractivity	89.32	CYP3A4 inhibitor	No
TPSA	53.60 Å ²	Log K _p (Skin permeation)	-5.90 cm/s
Lipophilicity		Water Solubility	
iLOGP	2.76	Log S (ESOL)	-3.96
XLOGP3	3.28	Solubility	3.44e-02 mg/ml; 1.09e-04 mol/l
WLOGP	3.64	Class	Soluble
MLOGP	2.89	Log S (Ali)	-4.08
SILICOS-IT	3.68	Solubility	2.63e-02 mg/ml; 8.31e-05 mol/l
Consensus Log P _{o/w}	3.25	Class	Moderately soluble
Druglikeness		Log S (SILICOS-IT)	-4.39
Lipinski	Yes; 0 violation	Solubility	1.30e-02 mg/ml; 4.11e-05 mol/l
Ghose	Yes	Class	Moderately soluble
Veber	Yes	Medical Chemistry	
Egab	Yes	PAINS	0 alert
Muegge	Yes	Brenk	0 alert
Bioavailability Score	0.55	Leadlikeness	Yes
		Synthetic accessibility	5.91

BBB: blood-brain barrier *logp*: the logarithm of the n-octanol/water distribution coefficient; *logs*: the logarithm of aqueous solubility value; TPSA: topological polar surface area; GI: Gastrointestinal.

The synergistic effect of cafestol with anticancer drugs has also been reported. ABT-737 is a cancer therapeutic agent that acts by inhibiting BCL2L1, BCL2 and BCL2L2. However, in several types of cancer cells with elevated expression of MCL1, cells are resistant to ABT-737-induced apoptosis. Woo and colleagues (Woo et al., 2014) reported that when Caki cells, human glioma, and human breast carcinoma cells were co-treated with cafestol and ABT-737, treatment resistance was overcome via downregulation of MCL1 expression and upregulation of BIM expression. A study of antileukemic drug cytarabine, reported that when HL60 and KG1 cells were co-treated with cafestol and cytarabine, cell viability reduced compared to treatment with cytarabine alone (Lima et al., 2017). Also, when drug resistant K562 and NB4 cells were treated with the highest dose of cafestol (150 μ M) treatment resistance was overcome. These reports suggest that cafestol has a great potential as an adjuvant treatment for cancer.

5 CONCLUSIONS

Molecular docking shows that cafestol has a high affinity for both BCL2 and BAX, and according to molecular modeling simulations the binding to these is stable. In the context of cancer cells, where the expression of BCL2 can be increased, we propose that the binding of cafestol with BCL2 promotes a pro-apoptotic effect. These findings are supported by *in vitro* studies previously published. Therefore, the regulatory role of cafestol on BCL2 as an anticancer strategy is worth further scrutiny.

6 ACKNOWLEDGEMENTS

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7 AUTHORS' CONTRIBUTION

Conceptual idea: Röthlisberger, S.; Lopera-Rodríguez, J.; Methodology design: Rendon-Rodríguez, J.; Lopera-Rodríguez, J.; Sanabria-Chanaga, E.; Data collection: Rendon-Rodríguez, J.; J.; Sanabria-Chanaga, E.; Data analysis and interpretation: Röthlisberger, S.; Lopera-Rodríguez, J.; Rendon-Rodríguez, J.; J.; Sanabria-Chanaga, E.; Writing and editing: Röthlisberger, S.; Lopera-Rodríguez, J.; Rendon-Rodríguez, J.; J.; Sanabria-Chanaga, E.

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