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Molecular Docking of 4-*Tert*-buthyl-bis-(2,6-thiomorpholin-4-ylmethyl)-1-phenol (LQM319) on Fas Receptor (CD95)

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ABSTRACT

The balance between cell proliferation and cell growth characterizes tissue homeostasis on one side and cell death on the other side. Fas receptor-mediated apoptosis is a control mechanism for tissue homeostasis, and avoiding this death pathway predisposes to many human diseases, including cancer. Current therapies for this disease are invasive and do not have the desired effect in the control of the disease. In this context, the search for new drugs that contribute to a better treatment is gaining more relevance. 4-*tert*-butyl-bis-(2,6-thiomorpholin-4-ylmethyl)-1-phenol (LQM319) [1,2] is a drug currently in preclinical stage, and we have shown that it has a hypertensive effect, similar to captopril, in a hypertensive rat model. Different studies have shown that some chemicals that are used as antihypertensive agents have an antineoplastic effect against certain types of cancer, as is the case of hydralazine [3], and captopril [4], among others [5]. On the other hand, it has been reported that morpholine derivatives may activate Fas (CD95)-mediated apoptosis. The aim of the present study was to show the interaction between CD95 (receptor) and thiomorpholine derivatives (ligand) using molecular modeling and docking studies, and to elucidate the possible action mechanism of 4-*tert*-butyl-bis-(2,6-thiomorpholin-4-ylmethyl)-1-phenol.

Keywords: Fas Receptor (CD95); Fas Ligand (FasL); Docking

1. Introduction

In response to stressful stimuli, cells usually mount a cellular stress response to ensure cell survival [6]. Under physiological conditions, this stress response limits tissue damage [7]. Apoptosis, or programmed cell death, is an evolutionarily conserved event that eliminates unwanted cells produced during animal development [8,9], and an important mechanism for eliminating tumorigenic cells. Apoptosis plays an important role in homeostasis, immune response, and elimination of abnormal cells [10]. Fas (CD95/Apo-1) has a central role in the physiological regulation of programmed cell death [11]. Apoptosis involves the activation of the pathways that lead to cell suicide by a characteristic sequence of events in which the cell becomes more compact, membrane blebbing occurs, chromatin becomes condensed, and DNA is fragmented [12]. Therefore, apoptosis evasion could be an important step possibly contributing to tumorigenesis.

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Apoptosis can be triggered by various stimuli from both outside and inside the cell; e.g., ligation of cell surface receptors, DNA damage, treatment with cytotoxic drugs or irradiation, and lack of survival signals [7]. A membrane receptor complex is formed in the apoptosis extrinsic pathway, followed by ligation of a member of the tumor necrosis factor receptor (TNFR) family [13]. Fas receptor and its ligand (FasL) are a pair of plasma membrane proteins whose interaction triggers one of the pathways for apoptosis. This intracellular cascade of events requires the Fas-associated death domain protein and the formation of death-inducing signaling complex, leading to caspase-8 activation and cell apoptosis [14,15]. The molecular pathway that regulates apoptosis will help in the investigation of novel cancer chemotherapeutic targets [16] offering, in turn, an opportunity to discover and develop new drugs [17].

Moreover, morpholine compounds based on phosphate derivatives, such as Amprenavir, are a new step towards the development of potent mimetic compounds of HIV-1

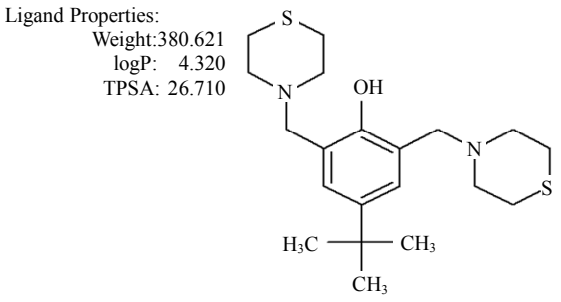
protease inhibitors. It was also found that benzomorpholine is a PPARγ agonist, used in the treatment of Type II Diabetes (Rybczynski *et al*., 2004). On the other hand, evidence suggests that morpholine compounds have an antiproliferative effect [18-21]. LQM319 is a thiomorpholine compound that, as we showed in a previous report, has antihypertensive activity, similar to captopril [1]. Recent studies suggest that it also has an antiproliferative effect.

With this kind of evidence suggesting that the morpholine compound has biological activity, the aim of this study was to explore the potential mechanism by which LQM319 (**Figure 1**) could inhibit proliferation in transformed cells mediated by CD95.

2. Material and Methods

2.1. Molecular Docking

A structural model of the catalytic domain of CD95 was constructed using Sybyl, with the published magnetic resonance structure of CD95, and the modeling template was obtained from the Protein Data Bank. The active site was determinate using Sybyl and MOE software. In both cases, the protein active site was similar. LQM319 was modeled with Molecular Operating Environment (MOE) ver. 2010.10. A conformational analysis was performed using the Sybyl conformational analysis command, and the most stable ligand structure was docked into the CD95 binding pocket using the Sybyl DOCKING Protocol. The CD95 catalytic domain was downloaded from the protein data bank (www.pdb.org) with PDB-ID 3EZQ and 2.73 Å resolution, and the pocket was determined by MOE's Site-Finder. The molecular structures studied by Masahiko *et al*. were modeled, minimized, and docked with the CD95 catalytic domain using MOE. For the conformational analysis, we used the stochastic method to avoid local minima for each molecule; whereas for the docking process, we used the protocol for rigid-rigid docking, followed by rigid-flexible and flexible-flexible docking methods. Finally, the enzyme-ligand complex was visualized with MOE ver. 2010.10.



[4-*tert*-buthyl-bis-(2,6-thiomorpholin-4-ylmethyl)-1-phenol]

Figure 1. Chemical characteristics and structure of thiomorpholine compound.

3. Results and Discussion

Angiotensin-converting enzyme (ACE) is involved in hypertension, heart failure, myocardial infarction and diabetic nephropathy. Angiotensin is a vasoconstrictive peptide that directly influences the pathophysiology of coronary artery disease, playing a pivotal role in blood pressure regulation [22]. Captopril is a specific competetive inhibitor of angiotensin-converting enzyme, which is responsible for the conversion of angiotensin I to angiotensin II, and it has been suggested that it could inhibit cancer cells [23]. Captopril showed potent inhibition of Fas-induced apoptosis in a human lung epithelial cell line, and therefore it is also known as a possible lung fibrogenesis blocker [24]. This finding suggests that the conversion of ANG I to ANG II might be involved in Fas-induced apoptosis by ACE [23]. In this context, we used molecular docking between captopril and Fas receptor with MOE ver. 2010.10. The results showed a possible arrangement between captopril (ligand) and CD95 (receptor).

Based on previous reports suggesting that a group of morpholine compounds can modulate the synthesis of CD95 and that this regulation could trigger apoptosis [19,21], and on a docking study conducted by Masahiko, *et al*., showing a favorable interaction between morpholine compounds and the receptor CD95 (**Table 1** and **Figures 2(a)** and **(b)**), our hypothesis is that the LQM319 compound can bind to the CD95 receptor (also known as Fas receptor), and that this interaction could activate apoptosis in cancer cells. To check this hypothesis, docking studies were performed using SYBYL ver. 7.0. and MOE ver. 2010.10. The results showed a favorable interaction between the ligand (LQM319) and the CD95 active site. The amino acids that are more relevant to the molecular recognition process are: Lys 215, Tyr 216, Asp 301, Ser 304, Asn 308 (**Figures 2(a)** and **(b)**).

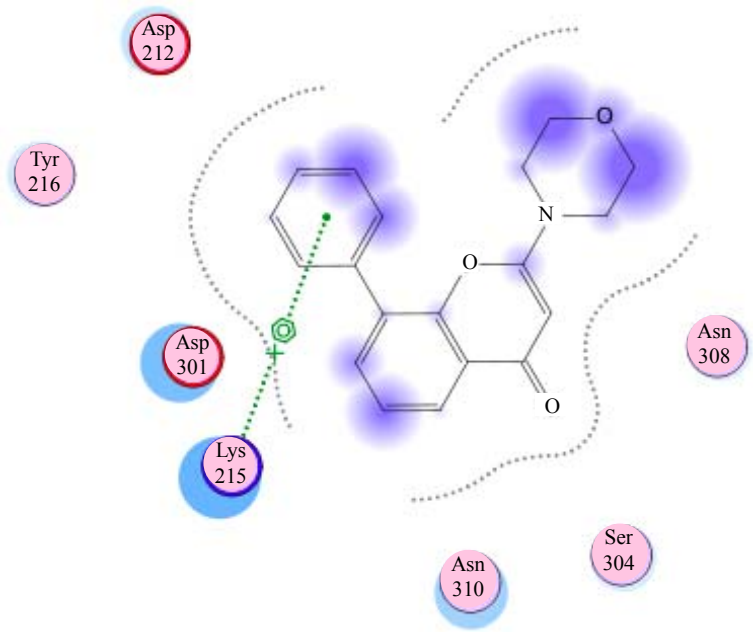
According to our results, the LQM319 compound has a great affinity (pKI) for Fas receptor (**Table 2**); the calculated efficiency is favorable, and the Gibbs free energy is negative. Also, based on this data, we can propose that interaction between the CD95 receptor and the LQM319 compound is possible and could induce programmed cell death or apoptosis.

On the other hand, we studied whether Fas receptor

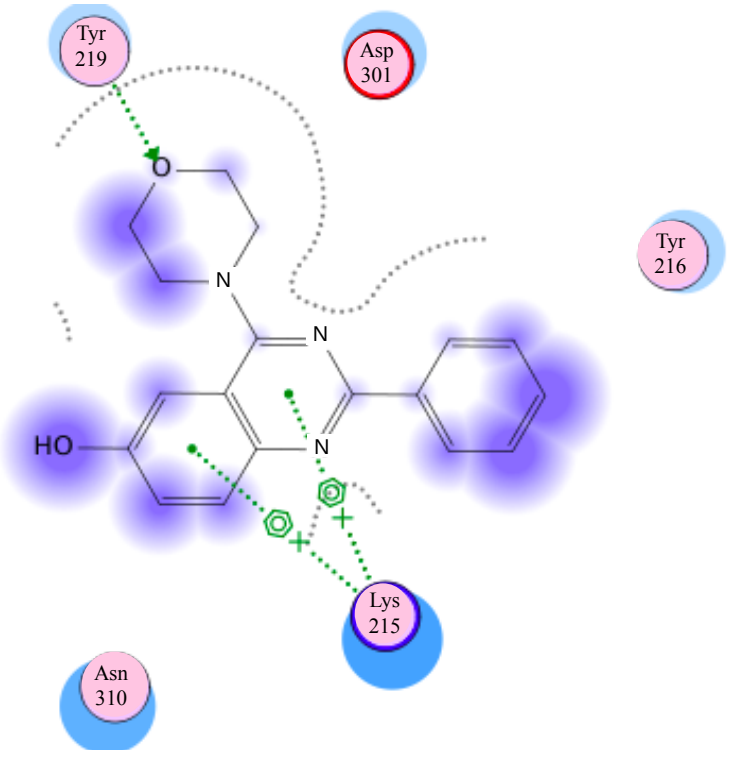
Table 1. Table comparing the affinity and energy of the compounds studied.

Compound	energy	affinity
LY294002	−11.982	5.258
4-morpholino-2-phenylquinazolin-6-ol	−10.992	5.838

Energies from complexes with CD95 and morpholine compounds reported by Masahiko, computed with MOE.



(a)



(b)

Figure 2. (a) Interaction of LY294002 with CD95; (b) Interaction of 4-morpholino-2-phenylquinazolin-6-ol with CD95.

Table 2. Table showing the energy and affinity of LQM319 compound.

Compound	energy	affinity
LQM319	5.5826 ± 0.144	−14.9332 ± 1.574

Energy and affinity computed for the complex LQM319 and FAS receptor.

could interact with captopril, an ACE inhibitor, since it has been reported by other studies to suppress rat hepatic fibrosis induced by pig serum [24] by blocking the renin angiotensin system (RAS) via angiotensin I converting enzyme (ACE) inhibition, thus reducing growth of colorectal cancer (CRC) and liver metastases in a mouse

model [25]. Captopril also induces Fas-mediated apoptosis of alveolar epithelial cells [23], but the results indicate that there are some interactions between the amino acids of the CD95 receptor pocket and captopril. The Gibbs free energy reaches a value of −10.08 kcal/mol, which indicates lower affinity between the two molecules (**Figures 3** and **4**).

4. Conclusion

The molecular docking results suggest a favorable interaction between the CD95 receptor (Apo-1 or Fas) and LQM319 (ligand) in the pocket of Fas receptor. Also, interactions between the CD95 receptor and the morpholine compounds reported by Masahiko were favorable, just as suggested by experimental studies. This helped us demonstrate that docking studies conducted with LQM319 compound using the MOE program are reliable. We initially modeled LQM319's most stable conformation and reconstructed the CD95 pocket. The results indicate that the compound had a higher affinity for this protein (**Table 1**) than captopril and the morpholine compounds (LY294002, 4-morpholino-2-phenylquinazolin-6-ol). All the interactions that the complex showed prior to the docking study helped us to stabilize the entire system, as shown by the energy in table 2. This suggests a more favorable complex between CD95 and LQM319 than that reported for captopril and the Masaiko morpholine compounds, and it might explain the antiproliferative effect of LQM319.

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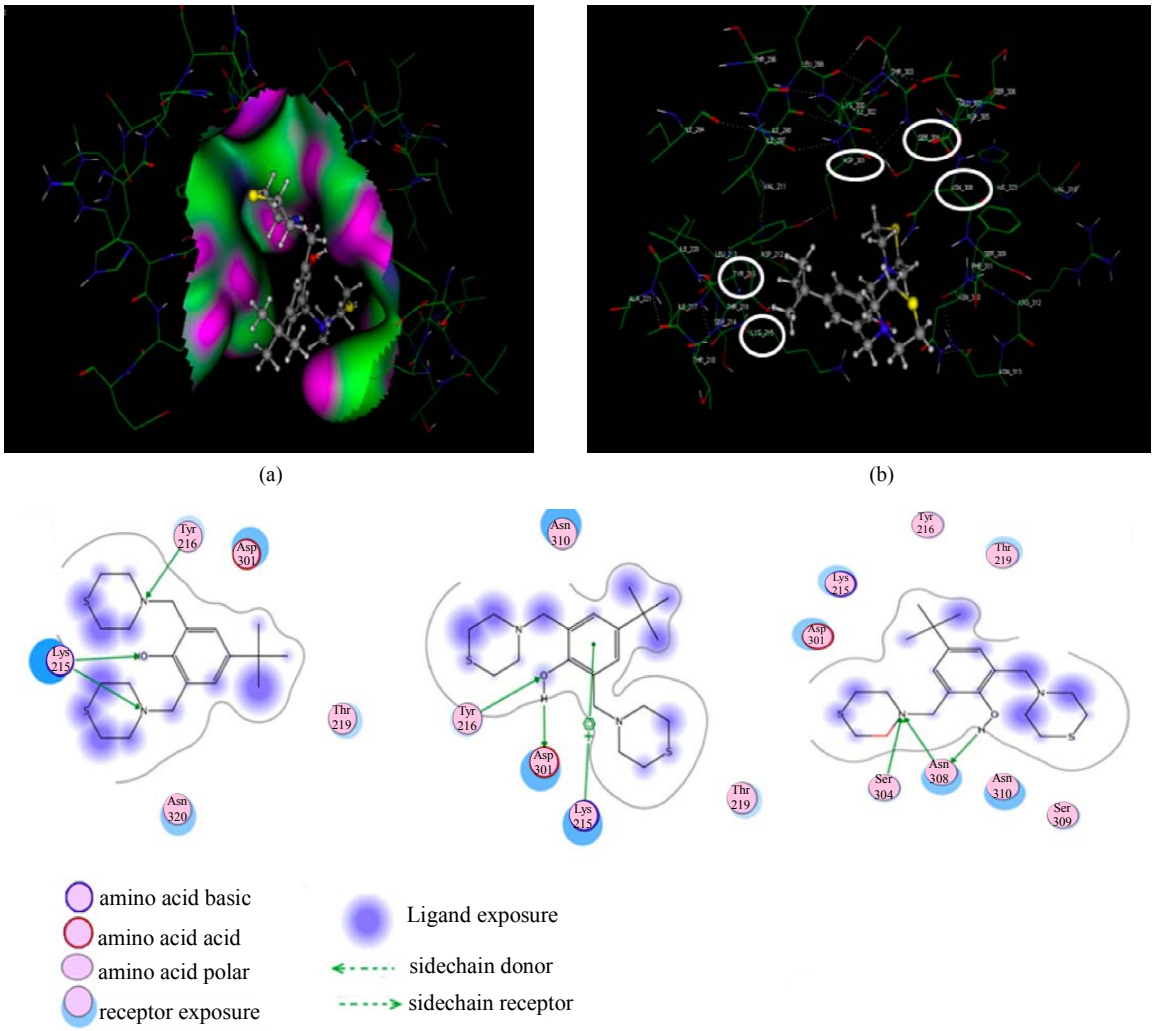

Figure 3. Molecular Docking. Interactions between amino acids of active site of receptor CD95 and LQM319. The image (a) corresponding to formation of surface trought Gaussian contact of the CD95 receptor and LQM319 (ligand) the color green corresponding to hydrophobic region, the color violet corresponding to H-Bonding and the blue color corresponding to mild Polar. The images (b) notes the Amino acids of CD95 receptor that interaction with the LQM319. (c) Representation of three docking between receptor CD95 and LQM319.

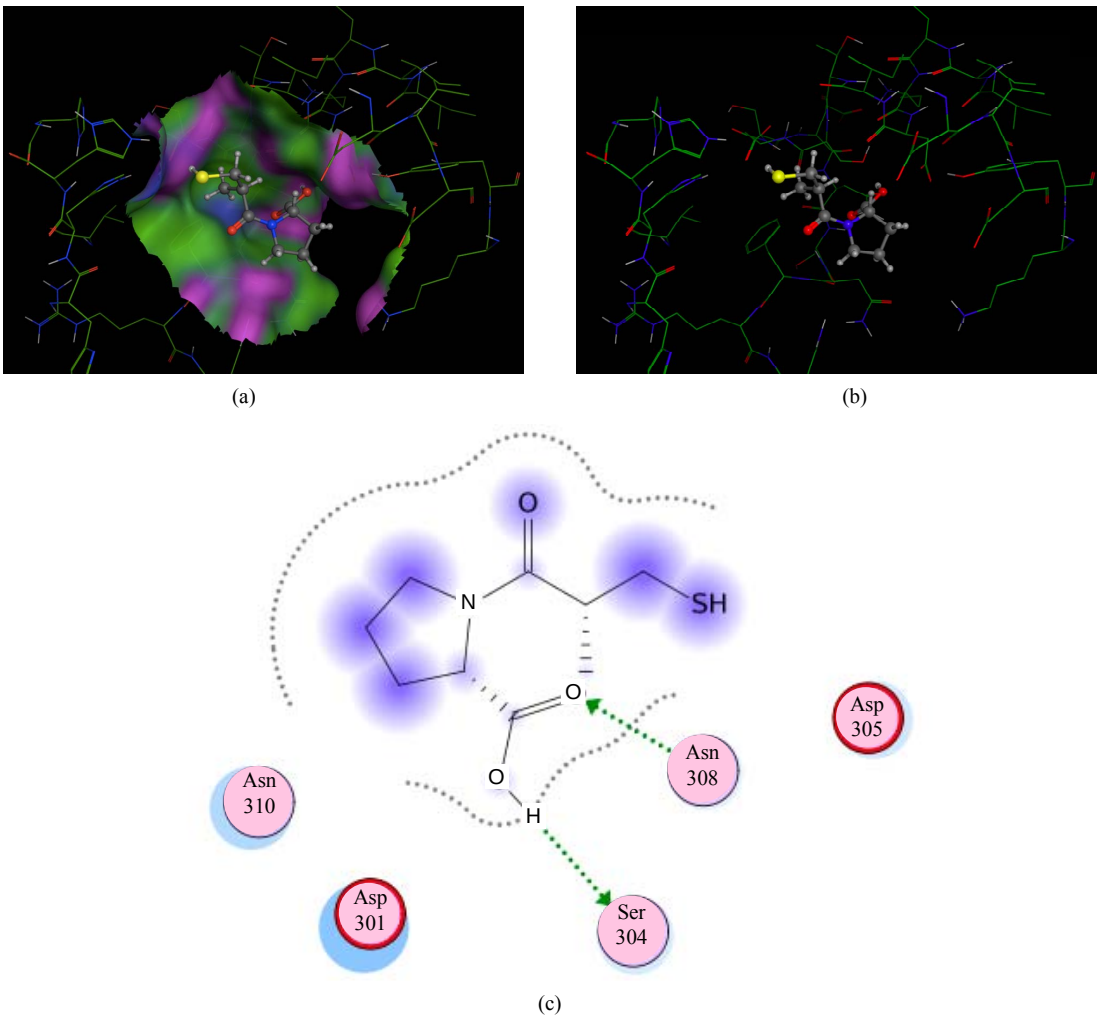

Figure 4. Molecular Docking. Between amino acids of active site of receptor CD95 and captopril. The image (a) corresponding to formation of surface trought Gaussian contact of the CD95 receptor and captopril (ligand) the color green corresponding to hydrophobic region, the color violet corresponding to H-Bonding and the blue color corresponding to mild Polar. The images (b) notes the Amino acids of CD95 receptor that interaction with the captopril. (c) Representation of the docking between receptor CD95 and captopril.

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