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Evaluation of the interactions of the Sunitinib malate with topoisomerase enzymes by *in vitro* enzyme and molecular docking analyses

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Abstract: Today, we live with the fact that anti-cancer drugs, which are in medical use and have been identified to have many mechanisms of action, only allow the treatment of a certain number of cancer types, and despite the research of many molecules with potential anti-cancer properties, not all types of cancer can be treated. Tyrosine kinase inhibitors (TKIs) mostly destroy certain types of cancer cells and many TKIs are currently being investigated in phase stages. Determining their use for various types of cancer is especially important for cases of acquired resistance in cancer. In our study, we investigated whether Sunitinib malate molecule, a multi-target receptor tyrosine kinase inhibitor, targets the topoisomerase I enzyme in addition to its known targets. In our study, we investigated whether Sunitinib malate molecule, a multi-target receptor tyrosine kinase inhibitor, targets the topoisomerase I enzyme in addition to its known targets. The interactions of Sunitinib malate with topoisomerase enzyme I were evaluated by in vitro enzyme activity tests, and Sunitinib malate was shown to inhibit topoisomerase I enzyme in a concentration-dependent manner, and when used in combination with Camptothecin, the potential for inhibition effects was evaluated by in vitro enzyme assays and molecular docking analysis.

Keywords: topoisomerase enzymes; cancer; sunitinib; tyrosine kinase inhibitor; molecular docking.

1. Introduction

The DNA molecule is protected inside the cell by being wrapped around itself with tight folds, and it shows structural-conformational changes only when necessary, within these narrow spaces provided by these folds, called the superhelical structure. The DNA molecule can relax by loosening its supercoil form with the help of topoisomerase enzymes in processes that require the opening of the DNA helix. Metabolic events, which we call these processes, may require many processes to ensure the transfer of the information that the DNA molecule preserves and conveys to new generations as if it were a museum of ancient heredity. Many enzymes are involved in transferring this information, which the DNA

molecule stores by avoiding changes and sometimes being subjected to changes, through replication (Pommier *et al.*, 2022). Among these enzymes, topoisomerase enzymes ensure the welfare of the cell by carrying out the cell's diplomacy in the chaos caused by the young DNA population that needs to be transferred to new cells, by gently breaking and recombining the DNA molecule in their own unique ways. Lack of the unique functional mechanisms of topoisomerase enzymes or their inoperability causes uncontrolled DNA breaks and other disruptions due to DNA breakage, which are irreparable for the living being. That's exactly why these enzymes are chosen as targets in both antibiotic and anticancer research, and their popularity is not decreasing (Champoux 2001, Pommier *et al.*, 2010).

Sunitinib inhibits all receptor tyrosine kinases, including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), although it is not targeted, perturbing the AMPK pathway (Gorini *et al.*, 2018; Boumezzourh *et al.*, 2023). Disruption of kinase signaling pathways often leads to diseases including cancer, and kinase inhibitors for cancer are among the most successful anticancer drugs in use (Skok *et al.*, 2020). Kinases regulate topoisomerase II (Topo II) enzymes by phosphorylation, and unfortunately, phosphorylation of Topo II reduces the effectiveness of many anticancer drugs. The reason for this is that phosphorylation accelerates the rebinding step by up to two times after the topoisomerase enzyme breaks the DNA strands and reduces the stress on the DNA. The levels of DNA breaks caused by Etoposide and m-AMSA are also reduced at the same rate, making the topo II enzyme less sensitive to topo II poisons (Pommier *et al.*, 2010). According to an intriguing study highlighting this information, the Fibroblast Growth Factor Receptor (FGFR) inhibitor Infigratinib re-sensitized imatinib-resistant gastrointestinal stromal tumor cells to topo II poisons (Boichuk *et al.*, 2018, Skok *et al.*, 2020). In light of the results obtained in this study, the fact that low concentrations of Doxorubicin and Etoposide can provide sufficient effect on re-sensitized cells becomes a second advantage, and perhaps it may be a feasible approach for the use of other topoisomerase poisons. Since Topo II and Epidermal Growth Factor Receptor (EGFR) tyrosine kinase can alter the expression of each other in various types of cancer, high EGFR levels are associated with the development of resistance of cancer cells to topoisomerase II inhibitors. In cancer types where EGFR is highly expressed, inhibition of Topo II and EGFR with drugs in combination may be promising for anticancer treatments (Skok *et al.*, 2020).

The ability to estimate and understand protein-small molecule interactions is crucial in biology and drug development. This prescient ability allows scientists to investigate deeply biological processes, establishing the groundwork for pharmaceutical research and innovation. By accurately predicting these interactions, scientists gain valuable biological insights. They can detect subtle protein-small molecule interactions, revealing the complex molecular choreography of cellular functions. This foresight helps scientists identify therapeutic targets, opening up new drug and treatment possibilities. This predictive capability is crucial to understanding cellular mechanisms, according to the scientific community. Predictive modeling of protein-small molecule interactions helps us understand complex biological processes like cellular signaling pathways, protein regulation, and disease pathways. It guides the search for new medical treatments and breakthroughs (Guclu *et al.*, 2023, Kaya *et al.*, 2023). Drug development requires accurate protein-small molecule interaction prediction. Researchers may determine drug targets and improve drug formulations with this precision. Mastery of these interactions allows scientists to control biological pathways and create highly customized drugs that fine-tune protein activities to achieve therapeutic goals.

2. Methodology

All of the compounds were purchased in lyophilized form. Sunitinib malate (Selleck Chemicals GmbH) and Camptothecin (Sigma-Aldrich) were dissolved in 100% Dimethyl Sulfoxide (DMSO).

2.1 Enzyme activity tests

Relaxed form of supercoiled DNA substrates was runned by horizontal electrophoresis (5V/cm) in 1xTAE buffer on a 1% agarose gel, in the absence of EtBr. The compounds were dissolved in 100% DMSO.

2.2 DNA intercalation tests

Whether the Sunitinib malate compound intercalated DNA was determined in a range of different concentrations (1mM to 0.001mM). The conditions of this test are exactly the same as the DNA relaxations tests, the only difference from these conditions is that the enzyme is not added. Under these conditions, in the absence of enzymes, it can be determined whether the DNA molecule has undergone a topological change only if it interacts with the chemical.

2.3 Supercoiled DNA relaxation assays

Supercoiled plasmid DNA relaxation test was carried out in a total of 20 µL volume of buffer (72 mM KCl, 5 mM MgCl2, 5 mM DTT, 5 mM spermidine and 0.1% bovine serum albumin), with 0.5 µg of supercoiled DNA (Takara) and 1 unit of human topoisomerase I enzyme (Inspiralis). One unit of topoisomerase enzyme is defined as the amount of enzyme that relaxes 0.5 µg of supercoiled DNA in 30 minutes at 37°C. Supercoiled DNA becomes less supercoiled and assumes a topological form as relaxed supercoils after the unwinding activity of topoisomerase I. When applied to an agarose gel, the less supercoiled DNA band appears further back in the gel due to the DNA unwinding activity of the enzyme (Nitiss, 1998). Inhibition of relaxation activity means that the activity of the enzyme is inhibited and it loses its ability to create temporary breaks on DNA, and to reassemble these breaks after the DNA relaxation phase (Champoux 2001, Peixoto *et al*., 2010, Senarisoy *et al.*, 2013).

2.4 Determination of drug combination effects on topoisomerase I enzyme

Camptothecin (CPT), a known topoisomerase inhibitor (Xu and Her, 2015), is used in our tests so that its inhibitory effects can be compared with Sunitinib. Coaddition of control compound (CPT) and Sunitib malate (SUN) compound at concentrations below the minimum enzyme inhibition concentrations was determined to observe the combinatorial effect. The inhibition effect of combinational application on topoisomerase I enzyme was examined by using different combinations together. With this test, minimal concentrations were selected for both chemicals, and the concentration range of combinations that had an inhibitory effect when used together was determined.

2.5 Molecular docking

Blind docking of proteins and ligands is a powerful technique used to investigate the specific locations where receptors bind and the orientations of ligand binding (Liu *et al.*, 2020, Liu *et al*., 2022). This method has garnered significant support in the fields of pharmacology and biology, and the application

of Molegro (Bitencourt-Ferreira, and de-Azevedo, 2019) software was made for the express purpose of achieving this goal.

3. Results and Discussion

3.1 Topoisomerase enzyme assays

In this study, whether the Sunitinib malate molecule is intercalative or not was examined under identical conditions using relaxation assay without the presence of topoisomerase I enzyme. According to the results of the test, it was shown that the Sunitinib malate compound used in high concentrations may have an intercalative effect on DNA (Figure 1). It was observed that the Sunitinib malate molecule showed intercalative activity starting at 1mM concentration and gradually decreasing up to 0.001mM concentration, as the supercoiled DNA bands, which were able to move in a way that changed their positions in the gel, were left behind due to its topological structure. DNA intercalating agents reverse the rotation direction of the DNA molecule, first reducing the number of supercoils and then increasing them in the opposite direction, making the DNA molecule supercoiled in a similar way to before (Peixoto *et al.* 2010, Webb and Ebeler 2003, Webb *et al.*, 2008). This analysis shows that Sunitinib is capable of unwinding supercoiled DNA and appears to have relaxation activity even in the absence of topoisomerase I enzyme. It may be possible that the rotations in the other direction increase between intermediate concentrations of the compound and then superhelical structures form again (Peixoto *et al.*, 2010).

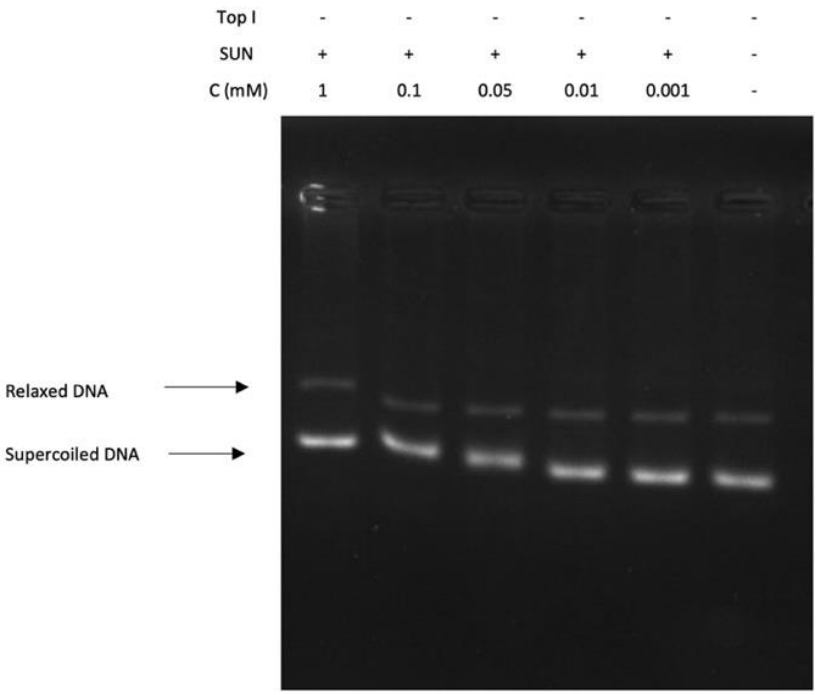

Figure 1. Suninitib shows concentration-dependent intercalative effects on DNA. All in the presence of 0.5 µg supercoiled DNA, 1; SUN (1mM), 2; SUN (0.1mM), 3; SUN (0.05mM), 4; SUN (0.01mM), 5; SUN (0.001mM), 6; 0.5 µg supercoiled DNA.

To evaluate the efficacy of the compound Sunitinib in comparison with the activity of CPT, a known topoisomerase I enzyme inhibitor, we determined the minimal concentration value of the compound CPT that inhibits the topoisomerase I enzyme. CPT inhibits Topoisomerase I at the concentration range of 0.05 mM – 1 mM (Figure 2).

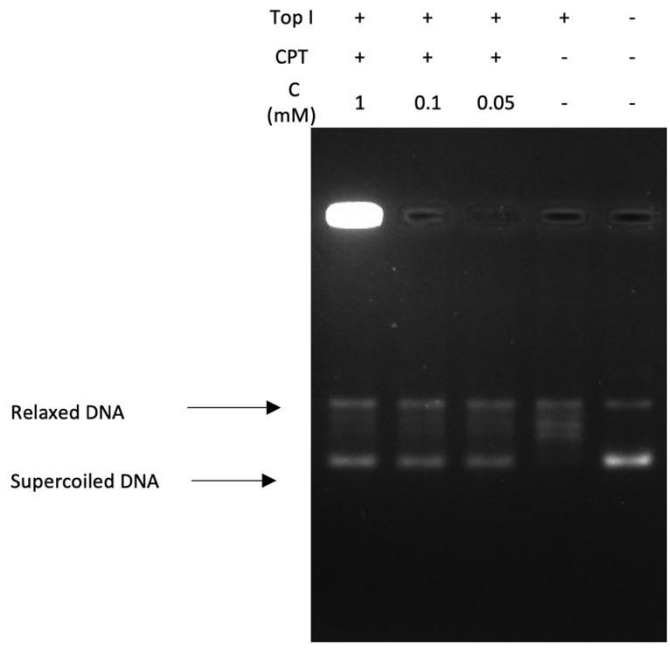

Figure 2. CPT inhibits Topoisomerase I at the concentration range of 0.05mM-1mM. All in the presence of 0.5 µg supercoiled DNA, 1; CPT (1mM), 2; CPT (0.1mM), 3; CPT (0.05mM), 4; 0.5 µg supercoiled DNA and topoisomerase I, 5; 0.5 µg supercoiled DNA.

It was analyzed whether the Sunitib molecule inhibited the topoisomerase I enzyme and a concentration range similar to the concentration range in which the effect of the CPT molecule was observed was determined. Suninitib inhibited Topoisomerase I in the concentration range of 0.001mM-1 mM (Figure 3).

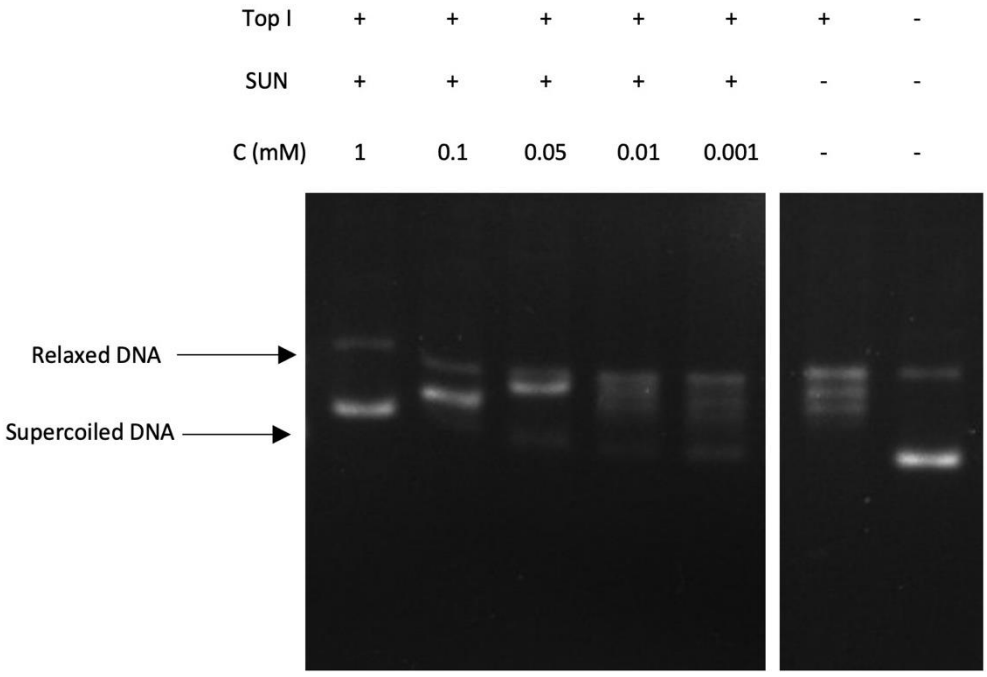

Figure 3. Suninitib inhibits Topoisomerase I at the concentration range of 0.01mM-1mM. All in the presence of 0.5 µg supercoiled DNA, 1; SUN (1mM), 2; SUN (0.1mM), 3; SUN (0.05mM), 4; SUN 0.01mM), 5; SUN (0.001mM), 6; 0.5 µg supercoiled DNA and topoisomerase I, 7; 0.5 µg supercoiled DNA.

By applying the Sunitinib molecule in combination with the CPT molecule, it was analyzed whether anticancer drugs used together at lower doses inhibit the topoisomerase I enzyme. Sunitinib inhibited CPT and Topoisomerase I combinatorically in the concentration range of 1mM to 0.01 mM (Figure 4).

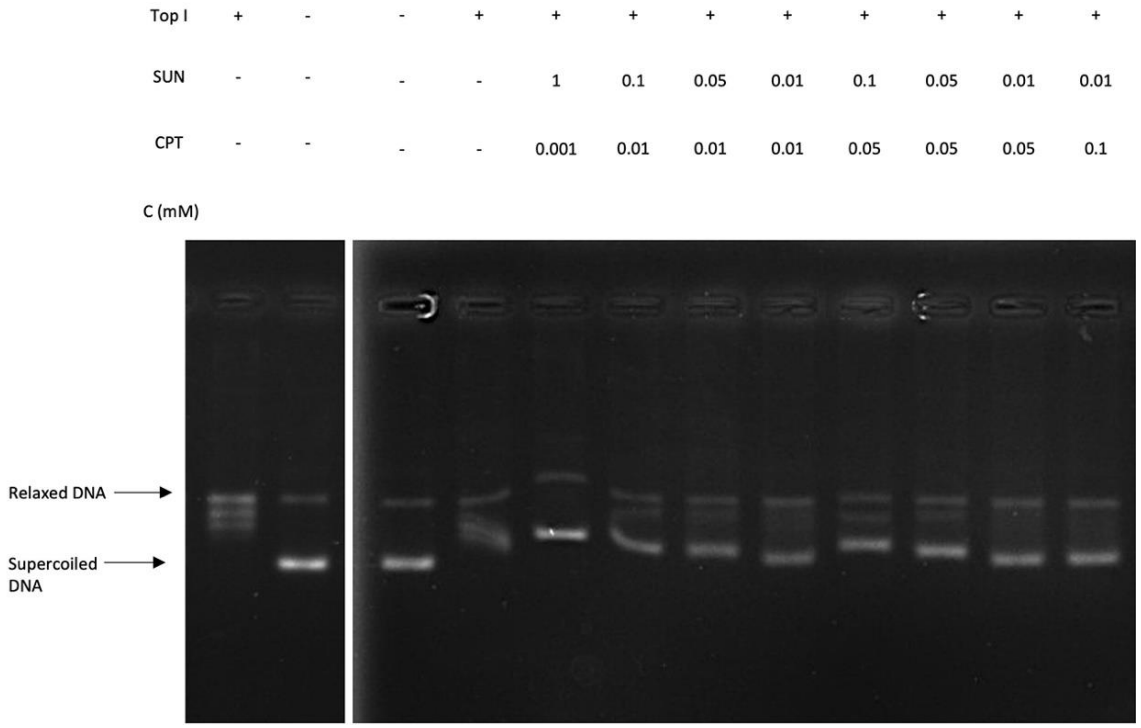

Figure 4. The combinational application of Suninitib and CPT inhibits the topoisomerase I. All in the presence of 0.5 µg supercoiled DNA, 1; 0.5 µg supercoiled DNA and topoisomerase I, 2; 0.5 µg supercoiled DNA, 3; 0.5 µg supercoiled DNA, 4; 0.5 µg supercoiled DNA and topoisomerase I, 5; CPT (0.001mM) and SUN (1mM), 6; CPT (0.01mM) and SUN (0.1mM), 7; CPT (0.01mM) and SUN (0.05mM), 8; CPT (0.01mM) and SUN (0.01mM), 9; CPT (0.05mM) and SUN (0.1mM), 10; CPT (0.05mM) and SUN (0.05mM), 11; CPT (0.05mM) and SUN (0.01mM), 12; CPT (0.1mM) and SUN (0.01mM).

3.2. Molecular Docking Analyses

The precise three-dimensional coordinates 1ZXM (Crystal Structure of Homo Sapiens Topoisomerase) were successfully retrieved from the Protein Data Bank (PDB) database (Lunelli *et al.*, 2011). Following that, the drug molecules underwent a comprehensive evaluation to ascertain their potential interactions with specific protein structures, as explained in Figure 5.

The obtained docking scores yielded valuable information regarding the binding strengths of these proteins. Predicting the interactions between proteins and molecules is crucial for understanding various biological processes, deciphering protein functions, and aiding in the development of drugs. The findings are given a significant boost by the fact that the Sunitinib was able to form hydrogen bonds with 1ZXM chains while the docking process was being carried out. This ability aligns perfectly with the exceptionally favorable docking score values that were observed in other places (Liu *et al.*, 2020, Trott and Olson, 2009).

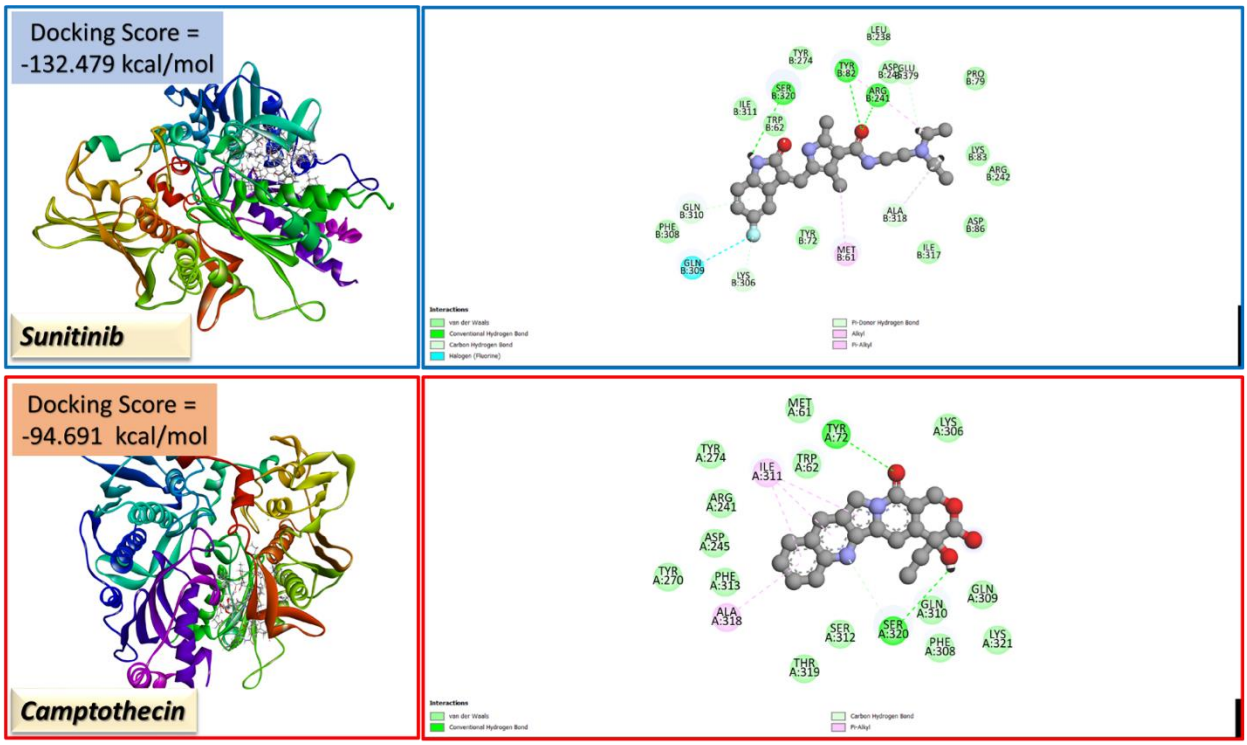

Figure 5. Corresponding 3D and 2D docking poses for interaction of the: Sunitinib and Camptothecin with the pdb id: 1ZXM.

Figure 5 is a graphical representation of the strong binding affinity of the molecule to the 1M8W. It highlights a remarkable docking score that ranges from approximately -132 to -94 kcal/mol for both drug structures. This remarkable interaction between the molecule and the target proteins is predicated primarily on the molecule's inherent capability to form hydrogen bonds. These bonds are made possible by the electron pair that is present at the oxygen atoms (-C=O group) and the amino group (-NH) that is present in the drug. In addition to this, the molecule participates actively in -alkyl interactions via the aromatic rings that it possesses.

Combination therapies have been a topic of interest due to reasons such as predictable pharmacokinetics, reduced risks of drug interactions, as well as fewer complications in intellectual property rights and regulatory approval processes. Obstacles such as resistance to drugs in medical use and increased toxicity caused by long-term use of these drugs negatively affect existing cancer-fighting therapies (Mimeault *et al.*, 2007; Skok *et al.*, 2020; Syrios *et al.*, 2019). In order to prevent side effects that may occur in patients, cancer treatment is often applied in combination with more than one drug in some types of cancer and in cases where the treatment of cancer is difficult, creating a minimal chance of reducing side effects for the patient and strengthening the treatment. When we discuss alternative approaches in cancer therapies in recent years, it is aimed to improve the health status of patients, reach more comprehensive treatments, and not affect the quality of life of cancer treatment by designing safer and more effective drugs in chemotherapy applications (Cinelli 2019, Dastan et al., 2017, Pommier et al., 2010, Pommier and Thomas 2023, Van Ravenstein et al. 2022). It has been shown that multikinase inhibitors increase their anticancer effectiveness because they inactivate many targets, but of course, this may have disadvantages such as toxicity (Cella *et al.*, 2022, Roskoski 2023).

Sunitinib malate (Sutent®), has been determined as a selective inhibitor of multiple RTKs, due to its antitumour and antiangiogenic activities. Furthermore, PDGFR-α and -β, VEGF receptors (VEGFR-1, VEGFR-2 (Flk-1/KDR) and VEGFR-3), Fms- like tyrosine kinase-3 (FLT3), and the glial cell line-derived neutrophic factor receptor (RET), AXL are reported to be the targets of Sunitinib (Deeks and Keating 2006, Smidova *et al.*, 2021). Combinations of the Sunitinib molecule with different drugs are advantageous due to its multi-targeting feature, and it may be possible to reduce the effective dosage with these combinations. In our study, it has been demonstrated by in vitro enzyme analyzes that the Sunitinib malate compound can inhibit the topoisomerase I enzyme, thus we propose a new target for the compound. However, we have chosen a concentration range in which we can also demonstrate the DNA intercalating properties of the compound, supporting the limited data in the literature about this feature. It was reported that Sunitinib and pUC18 plasmid DNA interaction was analyzed by UV/VIS, CD and NMR analyses, and the Sunitinib intercalated the plasmid DNA (Kiss *et al.*, 2018). In parallel with this information in our study, DNA relaxation assays were performed without the addition of enzyme, and an intercalative effect was observed for the Sunitinib malate on supercoiled plasmid DNA.

Conclusion

Our results revealed that Sunitinib malate inhibited the topoisomerase I enzyme in a concentration-dependent manner under *in vitro* conditions. In light of this information, in addition to the known targets of this compound, it was revealed that it has the potential to target topoisomerase I enzymes and that this mechanism needs to be further investigated in *in vivo* conditions. These findings not only highlight the potential of the drug as a promising candidate for target protein binding, but they also provide insight into the intricate molecular mechanisms that are responsible for its strong binding to specific protein targets. This paves the way for additional exploration and development in the field of pharmaceutical research and opens up new possibilities.

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