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Investigation of the Enyzmatic Interactions Between Roxithromycin and DNA Topoisomerase I and II

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ABSTRACT

Topoisomerase enzymes are meticulously researched and targeted through their interaction mechanisms in the development of anticancer and antibiotic drugs. The reasons underlying this unique priority are that topoisomerase enzymes are indispensable for the cell's own life dynamics, in the synthesis of RNA and proteins in the cell, or more importantly, for the preservation of genetic material. These enzymes are similar in terms of structure and functionality in many living groups, and by having relatively similar activities, they enable the creation of controlled DNA breaks and the reunification of DNA fragments in the cell. In fact, they are a kind of problem solver regarding the use of narrow spaces with their activities that enable the separation of long DNA molecules, which can be considered as gigantic in size, depending on the cell area in which they are located. Topoisomerase inhibitors are widely used and highly valuable in clinical practice. However, there remains an urgent need to identify new molecules targeting these enzymes. To address this, diverse methodological approaches should be explored and evaluated. Drug repurposing has become an increasingly valuable approach in modern therapeutics, as it allows existing drugs to be evaluated for new targets and disease applications, potentially reducing development time and costs. Roxithromycin, a well-established antibiotic, shows promising potential for repurposing as an anticancer agent due to its ability to interact with multiple molecular targets. Its pharmacological properties and safety profile make it an attractive candidate for investigation, and emerging evidence suggests that it may exert direct effects on key cellular enzymes involved in DNA replication and cell survival, supporting its potential application in cancer therapy. Given the growing body of evidence supporting the anticancer properties of certain antibiotics, we evaluated whether Roxithromycin has different targets than those known through its potential anticancer effects and interactions with topoisomerase enzymes. Here, we present the first evidence that Roxithromycin directly inhibits topoisomerase II, as confirmed through detailed topoisomerase enzyme assay analyses, highlighting a previously unrecognized mechanism that may contribute to its potential anticancer activity.

Keywords: Topoisomerase enzymes, Anti-cancer drugs, Roxithromycin, Antibiotics.

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Introduction

In the biological vaults called cells, our existence, encoded by DNA strands, determines our resistance to various diseases. By storing and transferring those traces of self, we transfer our unique hereditary memories to new cells. DNA topoisomerase enzymes are the power elements that manage the diplomatic relations of DNA molecules that break and then combine in the narrow space in the cell. The mechanisms of action of topoisomerase enzymes that govern conformational changes on DNA have long been of interest in the medical field. These enzymes are targets for antibiotic and anticancer drug development due to their unique abilities to act as transcription factors under specific conditions, in addition to their direct replication dependence. The breaking-joining activities of DNA topoisomerase enzymes help to create superhelical structures in the DNA molecule and, when necessary, to relax these structures and to create various cellular events such as replication and transcription. Preventing these enzymes from creating temporary breaks on DNA can have a devastating effect on the cell [1, 2]. While antibiotics can be developed by inhibiting bacterial topoisomerases for various bacterial infections, anticancer drugs are designed by inhibiting human topoisomerase enzymes in a similar way. Most importantly, the use of drugs such as the type 2 topoisomerase enzyme inhibitor etoposide, especially for the regression of aggressive tumors, shows how critically important the inhibition of topoisomerase enzymes is [3]. Anticancer drugs such as Topotecan, a topoisomerase inhibitor whose medical use is increasingly widespread, are promising. Topotecan has been reported to be effective in the treatment of pediatric tumors such as retinoblastoma [4]. A study investigating the long-term efficacy of topotecan in the treatment of eye retinoblastoma reported that the drug provided vitreous seed regression and eye salvage [5]. According to a phase study report in which Bevacizumab, Irinotecan or Topotecan were tested together with Temozolomide in patients with neuroblastoma, an improvement in progression-free survival was observed when irinotecan

or topotecan was added [6]. Convection-enhanced delivery of topotecan has shown positive results in phase studies for the treatment of glioma [7]. Carboplatin, when administered in combination with etoposide, has been offered as a second-line chemotherapy option in patients with recurrent small cell lung cancer [8].

However, these drugs can also have dual or multiple targets. As an example, Doxorubicin can inhibit both topoisomerase enzymes and the pathways of some other enzymes at the same time [9, 10]. Doxorubicin is used in breast cancer, sarcomas and hematological malignancies, and it not only creates DNA breaks by intercalating DNA, but also affects the production of reactive oxygen species, apoptosis, senescence and autophagy in the cell, which will play a role in the DNA damage response by affecting the interactions of various enzymes [10]. Some topoisomerase inhibitor drugs are used for some types of cancer that have no cure and alternatives are few or not possible. The topoisomerase 2 inhibitor etoposide is frequently used, despite its toxic properties, to treat types of cancer such as malignant ovarian, testicular and small cell lung cancer, leukemia and lymphoma [11].

Topoisomerase inhibitors may also exhibit intercalative effects. It is possible with compounds that can create a relaxation effect similar to topoisomerase enzyme activity by showing intercalative effect in DNA structure [12]. These compounds can also show various therapeutic effects by interacting with DNA. In order to determine the interactions of topoisomerase enzymes with DNA substrate, it should also be determined whether the compounds show intercalative effects. In this way, it can be shown to what extent the compound interacts with DNA or DNA-enzyme complexes. Molecules that interact with DNA-enzyme complexes are also called topoisomerase poisons. However, many compounds that can form both intercalative and reversible DNA-enzyme-compound complexes have anticancer properties and their activities are considered quite successful [13, 14].

One of the most appropriate approaches to evaluate drugs in clinical use can also be seen as investigating their as yet unknown indications. It is no longer surprising to come across articles where this approach is referred to as repurposing or repositioning. Fluoroquinolones have long been used as antibiotics, and the repositioning of fluoroquinolones into anticancer molecules has attracted considerable attention due to their anti-metastatic potential [15]. A variety of antibiotic compounds act as antitumor agents and therefore there is a growing interest in the literature to control antibiotics for cancer treatments [15, 16]. Since molecular docking studies are very helpful in identifying these molecules, a study in this conceptual approach analyzed the interactions of 138 antibiotics with human topoisomerase II enzyme and reported that the most promising candidates were investigated for their antitumor activity against three cancer cell lines. Consequently, while azithromycin, spiramycin, and clarithromycin are predicted to have anticancer properties, Topoisomerase II enzyme inhibition testing was performed to determine whether they have specific targeting properties, and Topoisomerase II inhibition was observed for erythromycin and roxithromycin [17].

Roxithromycin (ROX), a macrolide antibiotic structurally and pharmacologically similar to erythromycin, has demonstrated potential anticancer properties through multiple mechanisms, including anti-angiogenic effects and modulation of key signaling pathways involved in tumor progression. Notably, roxithromycin has been reported to inhibit the Raf/MEK/ERK pathway and suppress the constitutive activation of nuclear factor kappa B (NF-κB) in hepatocellular carcinoma models[18-20] . However, although roxithromycin is reported to be better tolerated, it is considered to have a lower potential for clinically significant drug interactions compared to erythromycin [21, 22]. In recent years, the potential of roxithromycin as an anticancer agent has garnered increasing scientific interest, particularly in the context of cancers such as hepatocellular carcinoma, breast cancer, and melanoma, with studies focusing on its anti-angiogenic effects, modulation of oncogenic signaling pathways, and inhibition of tumor growth and metastasis [19]. Within this framework, the interaction between roxithromycin and topoisomerase enzymes constitutes the primary focus of our investigation. Our findings highlight not only the versatility of existing drugs but also the critical role of systematic evaluation in uncovering novel therapeutic opportunities.

Methods

All of the compounds used in our experiments were purchased in lyophilized form. Roxithromycin and Camptothecin (Sigma-Aldrich) were dissolved in 100% Dimethyl Sulfoxide (Sigma-Aldrich).

DNA Intercalation Analyses

Intercalating compounds, which insert between DNA base pairs, can alter the superhelical conformation of the DNA helix. Therefore, the ability of a compound to generate relaxed DNA forms-mimicking the relaxation activity of topoisomerase enzymes-can be used as an indicator of its intercalating properties. In our study, in order to evaluate the interactions of compounds with topoisomerase I-II enzymes more accurately, the interactions of the compound with the substrate DNA used in optimized experiments were examined in the presence and absence of the enzyme [23].

Topoisomerase I Enzyme/Relaxation Tests

Topoisomerase enzymes create controlled breaks in a way that will relax supercoiled DNA, and thus the relaxed DNA in different topological forms is observed as a band left behind in the agarose gel. Since inhibitory compounds do not allow the enzyme to work, DNA molecules in a relaxed form cannot be formed at the end of the reaction. With this approach, we examined how the applied compound affects the enzyme activity. In the presence

and absence of the test compound, 1 unit of human topoisomerase I enzyme (Inspiralis) relaxed 0.5 µg of supercoiled plasmid DNA (Inspiralis) in a total volume of 20 µL of buffer (72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine and 0.1% bovine serum albumin (BSA)). One unit of enzyme activity is defined as the amount required to relax 0.5 µg of supercoiled plasmid DNA substrate at 37°C for 30 minutes. This assay was conducted using supercoiled plasmid DNA as the substrate to evaluate topoisomerase I activity [23-25].

Topoisomerase II Enzyme/Decatenation Analyses

Topoisomerase II enzyme activity can be assessed by monitoring the structural differences between catenated and decatenated DNA products generated upon completion of the reaction. The presence of inhibitory compounds in the reaction mixture results in reduced enzyme activity, thereby enabling the identification of compounds that inhibit topoisomerase II. In decatenation assays, the enzymatic activity of 1 unit of human topoisomerase II (Inspiralis) was evaluated in the presence and absence of the test compound by incubating the enzyme with 0.2 µg of catenated kinetoplast DNA (kDNA) (Inspiralis) substrate at 37 °C for 30 minutes, in a final reaction volume of 20 µl containing 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, and 0.5 mM dithiothreitol [23, 26].

Results

DNA Intercalation Tests

Comparative analysis of the compound's interaction with supercoiled DNA in the presence and absence of the enzyme confirmed that the compound did not inhibit supercoiled DNA in the absence of enzymatic activity. Our results demonstrated that the compound did not induce DNA intercalation and the absence of DNA-compound interaction suggests that the compound exerts its effects specifically through interaction with the enzyme in the topoisomerase enzyme assays (Figure 1, lane 5).

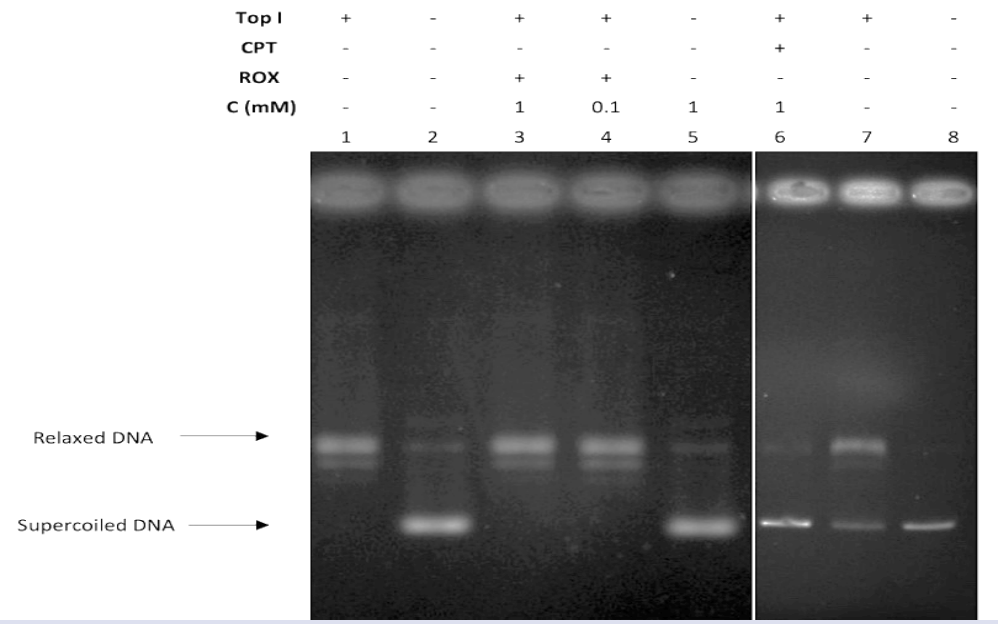

Figure 1. DNA intercalation property of roxithromycin and its inhibitory effects on topoisomerase I enzyme.

Supercoiled plasmid DNA was incubated with Roxithromycin in the absence (lanes 2, 8) and presence (lanes 1, 7) of topoisomerase I enzyme. Roxithromycin did not induce relaxation of supercoiled DNA in the absence of enzyme (lane 5), indicating no DNA intercalation. Camptothecin (CPT) was used as a positive control for enzyme inhibition (lane 6). Roxithromycin was tested at 1 mM and 0.1 mM for its effect on topoisomerase I relaxation activity (lanes 3, 4, respectively).

Topoisomerase I Enzyme Relaxation Analyses

Our analyses demonstrated that Roxithromycin, at a concentration of 1 mM (Figure 1, lane 3), did not inhibit the relaxation activity of topoisomerase I under the tested experimental conditions, as evidenced by the persistence of relaxed DNA bands comparable to those observed in the positive enzyme control. In contrast, under identical assay conditions, the control compound camptothecin (CPT) exhibited a pronounced inhibitory effect on topoisomerase I activity at the same concentration (Figure 1, lane 6), resulting in the retention of supercoiled DNA, which reflects effective enzyme inhibition.

Topoisomerase II Enzyme Decatenation Assays

The inhibitory effect of Roxithromycin on topoisomerase II enzyme activity was evaluated using decatenation assays, in which kinetoplast DNA (kDNA) was employed as the substrate. The results demonstrated that Roxithromycin significantly reduced the enzymatic

activity of topoisomerase II by approximately 50% under the tested conditions (Figure 2).

This partial inhibition suggests that Roxithromycin interferes with the enzyme's ability to decatenate DNA, thereby preventing the resolution of intertwined DNA strands. These findings highlight the potential of Roxithromycin as a moderate inhibitor of topoisomerase II, warranting further investigation into its mechanism of action and possible therapeutic applications.

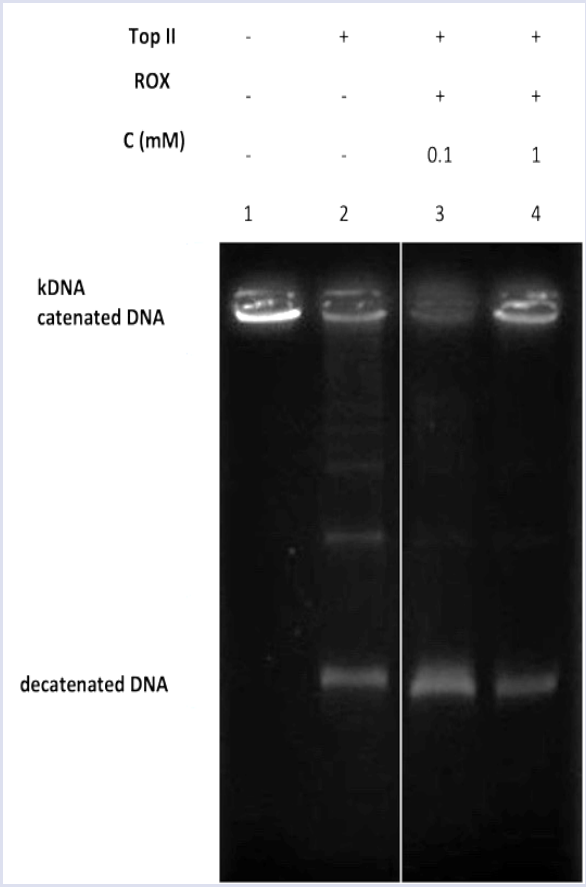

Figure 2. Inhibition of the Roxithromycin on DNA topoisomerase II enzymes.

Application of Roxithromycin in the absence of enzyme (negative control, lane 1); in the presence of enzyme and kDNA (positive control, lane 2); in the presence of enzyme with 0.1 mM Roxithromycin (lane 3); and in the presence of enzyme with 1 mM Roxithromycin (lane 4).

Discussion

Drug repurposing has become an increasingly valuable strategy in modern medicine. Many drugs, originally developed for specific diseases, possess diverse chemical and biological properties that allow them to interact with multiple molecular targets. This multiplicity of targets means that a single compound may have therapeutic potential beyond its original use. Therefore, systematically evaluating existing drugs across different targets and disease contexts can uncover new applications, accelerate drug development, and reduce the time and cost associated with bringing effective therapies to patients. In light of the findings of this study, roxithromycin was shown to inhibit topoisomerase enzymes. Topoisomerase enzyme activities were assessed through a series of inhibition assays, utilizing plasmid DNA as the substrate in topoisomerase I relaxation assays and kinetoplast DNA (kDNA) from *Crithidia fasciculata* in topoisomerase II decatenation assays, selected for its knotted double-stranded DNA structure analogous to human DNA. This strategic use of structurally relevant model DNA not only facilitated the evaluation of enzyme–drug interactions but also provides a valuable framework for identifying and characterizing novel topoisomerase inhibitors. The inhibitory effect of roxithromycin on topoisomerase II has previously been investigated in a single study [17] although the researchers employed a different DNA substrate from the structurally relevant model DNA used in our experiments. Importantly, despite this difference, our findings are consistent with and further support the results of that study, while also demonstrating the advantage of employing a DNA model closely resembling human DNA to enhance the biological relevance of enzyme inhibition analyses. This approach not only strengthens the translational potential of our findings but also provides a methodological framework for future studies aiming to evaluate novel topoisomerase inhibitors with greater clinical applicability. These findings highlight the potential of roxithromycin derivatives as candidates for anticancer drug development, particularly in malignancies dependent on topoisomerase II activity or exhibiting resistance to current topoisomerase-targeting therapies. Future research should focus on elucidating the molecular mechanisms underlying roxithromycin's enzyme inhibition, optimizing derivative compounds for improved potency and selectivity, and evaluating their therapeutic efficacy and safety in preclinical cancer models. In line with these results, the anticancer potential of roxithromycin was further supported by its inhibitory effects on topoisomerase enzymes, suggesting a mechanism of action distinct from its established antibacterial properties. The development of more potent roxithromycin derivatives targeting topoisomerase II may represent a promising strategy for the discovery and synthesis of novel anticancer therapeutics, particularly for malignancies such as breast cancer and leukemia, which are characterized by topoisomerase II overexpression or resistance to existing inhibitors.

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Pommier Y., Sun Y.,. Huang S.Y. N, and Nitiss J.L., Roles of eukaryotic topoisomerases in transcription, replication and genomic stability, *Nat Rev Mol Cell Biol*., 11 (2016) 703-721.

[2] Wang, J.C., Cellular roles of DNA topoisomerases: A molecular perspective, *Nat Rev Mol Cell Biol.,* (6) (2002) 430-440.

[3] Pommier Y., Leo E., Zhang H., and Marchand C., DNA topoisomerases and their poisoning by anticancer and antibacterial drugs, *Chem Biol.,* 17 (5) (2010) 421-433.

[4] Nadelmann J., Francis J.H., Brodie S.E., Muca E., and Abramson D.H., Is intravitreal topotecan toxic to retinal function?, *Br J Ophthalmol.*, 105 (7) (2021) 1016-1018.

[5] Sen M., Rao R., Mulay K., Reddy V.A.P., and Honavar S.G., Intravitreal Topotecan for Vitreous Seeds in Retinoblastoma, *Ophthalmology*, 131 (10) (2024) 1215-1224.

[6] Moreno L, Weston R, Owens C, Valteau-Couanet D, Gambart M, Castel V, Zwaan CM, Nysom K, Gerber N, Castellano A, Laureys G, Ladenstein R, Rössler J, Makin G, Murphy D, Morland B, Vaidya S, Thebaud E, van Eijkelenburg N, Tweddle DA, Barone G, Tandonnet J, Corradini N, Chastagner P, Paillard C, Bautista FJ, Gallego Melcon S, De Wilde B, Marshall L, Gray J, Burchill SA, Schleiermacher G, Chesler L, Peet A, Leach MO, McHugh K, Hayes R, Jerome N, Caron H, Laidler J, Fenwick N, Holt G, Moroz V, Kearns P, Gates S, Pearson ADJ, Wheatley K., Innovative Therapies for Children with Cancer (ITCC) and European Association for Neuroblastoma Research (SIOPEN). Bevacizumab, Irinotecan, or Topotecan Added to Temozolomide for Children With Relapsed and Refractory Neuroblastoma: Results of the ITCC-SIOPEN BEACON-Neuroblastoma Trial, *J Clin Oncol.,* 42 (10) (2024) 1135-1145.

[7] Upadhyayula P.S., Spinazzi E.F., Argenziano M. G., Canoll P., and Bruce J.N., Convection Enhanced Delivery of Topotecan for Gliomas: A Single-Center Experience, *Pharmaceutics*, 13 (1) (2020) 39.

[8] Baize N, Monnet I, Greillier L, Geier M, Lena H, Janicot H, Vergnenegre A, Crequit J, Lamy R, Auliac JB, Letreut J, Le Caer H, Gervais R, Dansin E, Madroszyk A, Renault PA, Le Garff G, Falchero L, Berard H, Schott R, Saulnier P, Chouaid C; Groupe Français de Pneumo-Cancérologie 01–13 investigators, Carboplatin plus etoposide versus topotecan as second-line treatment for patients with sensitive relapsed small-cell lung cancer: an open-label, multicentre, randomised, phase 3 trial, *Lancet Oncol.,* 21 (9) (2020) 1224-1233.

[9] Singh V., Afshan T., Tyagi P., Varadwaj P.K., and Sahoo A.K., Recent development of multi-targeted inhibitors of human topoisomerase II enzyme as potent cancer therapeutics, *Int J Biol Macromol.*, 226, (2023) 473-484.

[10] Kciuk M., Gielecińska A., Mujwar S., Kołat D., Kałuzińska-Kołat Ż., Celik I., Kontek R., Doxorubicin-An Agent with Multiple Mechanisms of Anticancer Activity, *Cells*, 12 (4) (2023) 659.

[11] Matias-Barrios V.M. and Dong X., The Implication of Topoisomerase II Inhibitors in Synthetic Lethality for Cancer Therapy, *Pharmaceuticals*, 16 (1) (2023) 94.

[12] Avendaño C. and Menéndez J.C., DNA Intercalators and Topoisomerase Inhibitors, in *Medicinal Chemistry of Anticancer Drugs*, (2008), 199-228.

[13] [Nitiss J.L., Investigating the biological functions of DNA topoisomerases in eukaryotic cells, *Biochim Biophys Acta*., 1400 (1-3) (1998) 63-81.

[14] Champoux J.J., DNA Topoisomerases: Structure, Function, and Mechanism, *Annu Rev Biochem.,* 70 (2001) 369-413.

[15] Yadav V. and Talwar P., Repositioning of fluoroquinolones from antibiotic to anti-cancer agents: An underestimated truth, Biomed Pharmacother., 111 (2019) 934-946.

[16] Elik A., Gokce G., Bostanci E., and Kiziloluk D., Development of Electrochemical DNA Sensor for The Determination of Anaplasma Phagocytophilum, *Cumhuriyet* Sci. J., 38 (3) (2017) 525-534.

[17] Farouk F, Elmaaty AA, Elkamhawy A, Tawfik HO, Alnajjar R, Abourehab MAS, Saleh MA, Eldehna WM, Al-Karmalawy AA., Investigating the potential anticancer activities of antibiotics as topoisomerase II inhibitors and DNA intercalators: *in vitro*, molecular docking, molecular dynamics, and SAR studies, *J Enzyme Inhib Med Chem.,* 38 (1) (2023) 2171029.

[18] Shinoda M., Macrolides as Immunomodulatory Agents Springer, in Progress in Inflammation Research 1st ed. Bruce K. Rubin, Masaharu Shinkai (Eds). (2024) 223-254.

[19] Yatsunami J., Tsuruta N., Fukuno Y., Kawashima M., Taniguchi S., and Hayashi S., Inhibitory effects of roxithromycin on tumor angiogenesis, growth and metastasis of mouse B16 melanoma cells, *Clin Exp Metastasis.*, 17 (2) (1999) 119-124.

[20] Ueno S, Aoki D, Kubo F, Hiwatashi K, Matsushita K, Oyama T, Maruyama I, Aikou T., Roxithromycin Inhibits Constitutive Activation of Nuclear Factor κB by Diminishing Oxidative Stress in a Rat Model of Hepatocellular Carcinoma, *Clin Cancer Res.*, 11 (15) (2005) 5645-5650.

[21] Gentry L.O., Roxithromycin, a new macrolide antibiotic, in the treatment of infections in the lower respiratory tract: an overview, *J Antimicrob Chemother.*, 20, (1987) 145-152.

[22] Young R.A., Gonzalez J.P., Sorkin E.M., Roxithromycin. A review of its antibacterial activity, pharmacokinetic properties and clinical efficacy, *Drugs*, 37 (1) (1989) 8-41.

[23] Nitiss J.L., Topoisomerase Assays, *Curr Protoc Pharmacol.*, 1, (1998) 3:Unit3.3.

[24] Hicyilmaz A.M., Nergiz K., Canturk P., Berisha A., Kaya S., Evaluation of the interactions of the Sunitinib malate with topoisomerase enzymes by in vitro enzyme and molecular docking analyses, *Maghr. J. Pure & Appl. Sci.*, 1, (2023) 13-22.

[25] Gok E., Unal N., Gungor B., Karakus G., Kaya S., Canturk P., Katin K.P., Evaluation of the Anticancer and Biological Activities of Istaroxime via Ex Vivo Analyses, Molecular Docking and Conceptual Density Functional Theory Computations, *Molecules*, 28 (22) (2023)

[26] Senkardeş S., Bolat I,, Şahinbey H,, Karakus S,, Erdoğan O,, Canturk P., Yözgüven S.Y., Çevik O., Design, synthesis and molecular modeling studies of thiosemicarbazide & thiazolyl-hydrazone derivatives as potential anticancer agents and topoisomerase inhibitors, *J Mol Struct.*, 1302, (2024) 137488.