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CYTOTOXIC ACTIVITY OF EPETRABOROLE AGAINST CANCER CELL LINES

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Abstract

Objective: Antibiotic repurposing represents a promising strategy in oncology, utilizing well-characterized pharmacophores to exploit conserved vulnerabilities in cancer cells. This study aimed to evaluate the cytotoxic potential of epetraborole (EP), a benzoxaborole-class leucyl-tRNA synthetase inhibitor, against various cancer cell types.

Methods: The cytotoxic effects of EP were assessed in five cancer cell lines: malignant mesothelioma (CARM-L12 TG3), osteosarcoma (MG63), and colorectal carcinomas (HT-29, SW-48, and 2A3). MTT cell viability assays were conducted to determine dose- and time-dependent responses following 72-hour exposure. Antibacterial activity was verified by determining the minimum inhibitory concentration (MIC) against *Escherichia coli.*

Results: EP exhibited significant cytotoxic activity in a dose- and time-dependent manner. The compound showed highest potency against CARM-L12 TG3 (IC50= 0.408 ± 1.1 µg/mL) and MG63 (IC_{50} = 0.875 ± 1.6 µg/mL) cell lines, while colorectal carcinoma cells were less sensitive (IC50 > 3 µg/mL). Antibacterial testing confirmed EP's bioactivity, with an MIC value of 0.5 µg/mL against *E. coli*.

Conclusion: Epetraborole demonstrates strong cytotoxic potential, particularly against mesenchymal-derived cancers such as mesothelioma and osteosarcoma. These results suggest that EP is a promising candidate for repurposing in oncology, targeting malignancies dependent on protein synthesis machinery.

Keywords: *Epetraborole (AN3365), Cytotoxicity, Cancer cell lines, IC50, Benzoxaborole, MTT.*

Introduction

Cancer remains a leading global cause of mortality, characterized fundamentally by dysregulated cellular proliferation and evasion of growth control mechanisms.[1] While localized disease is often managed effectively with surgery and radiotherapy, advanced or metastatic malignancies necessitate systemic therapies, including chemotherapy and molecularly targeted agents.[2] However, the clinical utility of these treatments is frequently limited by intrinsic or acquired drug resistance and significant toxicities.[3] This persistent therapeutic challenge has spurred interest in drug repurposing strategies, particularly exploring the potential anticancer properties of existing antimicrobial agents beyond their primary indications.[4]

Certain classes of antibiotics have demonstrated direct cytotoxic or cytostatic effects on cancer cells, independent of their antibacterial activity. Mechanisms include induction of apoptosis, inhibition of mitochondrial function, disruption of proteostasis, and interference with DNA repair.[5–7] Notable examples include tetracyclines targeting mitochondrial ribosomes[8], fluoroquinolones inhibiting topoisomerases[9], and the ionophore salinomycin targeting cancer stem cells.[10] This evolving field highlights the potential for identifying novel anticancer candidates within existing antibiotic pharmacopeia.

Boron-containing compounds represent a distinct class with emerging therapeutic potential in oncology. Beyond the established Boron Neutron Capture Therapy (BNCT)[11], small-molecule boron-based drugs, particularly benzoxaboroles, have gained attention due to their unique physicochemical properties.[12] The electron-deficient boron atom facilitates reversible interactions with biological nucleophiles, enabling high-affinity binding to specific targets like enzymes involved in key metabolic pathways.[13] Benzoxaboroles have shown promise in treating infectious diseases[14], and inflammatory conditions[13], but their exploration in oncology is more recent.

A critical vulnerability of cancer cells lies in their altered metabolic state, often characterized by an increased dependence on exogenous amino acids to support rapid proliferation and biomass accumulation.[15,16] This heightened reliance renders enzymes crucial for amino acid utilization, such as aminoacyl-tRNA synthetases (aaRSs), potential therapeutic targets.[17] Leucyl-tRNA synthetase (LARS) is particularly relevant due to its documented overexpression in lung cancer and genetic alterations in other malignancies.[18,19] The structural and functional analogy between the catalytic domains of bacterial and mammalian aaRSs provides a rationale for exploring bacterial aaRS inhibitors against cancer cells.

Epetraborole (EP) is a benzoxaborole antibiotic developed to inhibit bacterial leucyl-tRNA synthetase (LeuRS)[20], primarily targeting Gram-negative pathogens like *Burkholderia pseudomallei* and nontuberculous *Mycobacteria.*[14,21,22] Its mechanism involves the unique boron-mediated trapping of $tRNA^{Leu\ 23}$ effectively halting protein synthesis. Given the critical role of LARS in cancer biology and the metabolic vulnerabilities of cancer cells, we hypothesized that EP might exert cytotoxic effects on human cancer cells by potentially targeting LeuRS or exploiting cancer-specific translational dependencies.

Therefore, this study presents the first comprehensive ex vivo evaluation of the cytotoxic potential of epetraborole against a panel of cancer cell lines, including malignant mesothelioma, osteosarcoma, and colorectal carcinoma models. We aimed to determine the sensitivity profiles of these cells to EP and establish a foundation for further investigation into its potential as a repurposed anticancer agent targeting protein synthesis machinery.

Methods

Chemicals and reagents

Epetraborole (EP; MedKoo Biosciences, Cat#319569, USA) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, USA) to prepare a 10 mg/mL stock solution, stored at -20°C. Key reagents included: MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Dulbecco's Modified Eagle Medium (DMEM; high glucose), Luria-Bertani (LB) agar, and DMSO (all from Sigma-Aldrich, USA); penicillin/streptomycin and Dulbecco's phosphate-buffered saline (dPBS) (Capricorn Scientific, Germany); fetal bovine serum (FBS) and Trypsin-EDTA (Gibco™, Thermo Fisher Scientific, USA). All solutions were prepared under sterile conditions.

Cell lines and bacterial strain

Cancer cell lines included: HT-29 (colon adenocarcinoma, ATCC® HTB-38™), SW-48 (colorectal adenocarcinoma, ATCC® CCL-231™), CARM-L12 TG3 (malignant mesothelioma), MG63 (osteosarcoma, ATCC® CRL-1427™), and 2A3 (squamous cell carcinoma). *Escherichia coli* K12 MG1655 served for antibacterial validation. All cell lines were obtained from the American Type Culture Collection (ATCC, USA) and maintained according to provider specifications.

Determination of epetraborole's antibacterial efficacy

To assess the efficacy of the antibiotic, a spot test was conducted using *E. coli* strain. Initially, the bacterial strain was recovered from a -80°C stock and grown overnight at 37°C on an LB agar plate. A single colony was then inoculated into 5 mL of LB and cultured overnight at 37°C with shaking at 200 rpm to reach the stationary phase. The cells were subsequently pelleted, washed with phosphate-buffered saline (PBS), and the OD_{600} was adjusted to 0.5. Serial 2-fold dilutions (from 1:1 to 1:16) were prepared and spotted onto LB agar plates containing varying concentrations of epetraborole (EP; MedKoo Biosciences, Cat#319569)—0, 0.25, 0.5, 1, 2, and 4 µg/mL (dissolved in DMSO). The plates were incubated at 37°C for 5 days, with daily imaging performed using a digital camera for result evaluation.[24, 25]

Cell culture conditions and cytotoxicity assessment (MTT assay)

The experimental setup utilized a panel of cancer cell lines, including colon cancers HT-29 and SW-48, malignant mesothelioma CARM-L12 TG3, osteosarcoma MG63, and squamous cell carcinoma 2A3. All cell lines were maintained in DMEM, supplemented with 10–20% FBS, L-glutamine, and antibiotics (100 U/mL). The cell cultures were then subjected to incubation at a temperature of 37°C within a controlled atmosphere comprising 95% CO_2 and 5% O_2.

The MTT assay was the selected as the methodology for the evaluation of the cytotoxic impact of EP on the aforementioned cancer cell lines. The cells were distributed into 96-well plates at a density of 4000 cells per well, using their respective culture media, then left to incubate for 24 hours under standard conditions (37°C, 5% CO_2, 95% humidity). Following the initial incubation, the culture medium was carefully removed and replaced with fresh medium containing varying concentrations of EP, ranging from 0.125 to 8 µg/mL (specifically, 0.125, 0.25, 0.5, 1, 2, 4,

and 8 µg/mL). The treated cells were then subjected to an incubation period, with the duration being either 48 or 72 hours. Subsequent to the process of incubation, the medium was disposed of, and 100 µL of MTT reagent (5 mg/mL in physiological solution) was added to each well a dark environment. Cells were incubated with MTT for 2–4 hours at 37°C, 95% humidity, and 5% CO_2, with the duration adjusted based on cell growth characteristics. Subsequent to the MTT incubation, the MTT-containing medium was removed, and 100 µL of DMSO was added to each well to solubilize the formazan crystals. The absorbance at 575 nm was then measured using a Microplate Reader (Thermo Scientific), and the resulting data were subjected to analysis.[26,27]

Statistical analyzes

We analyzed the cell viability data, presenting it as the mean ± SEM from three separate experiments. Statistical variations between samples were assessed using one-way ANOVA in GraphPad Prism 5 (GraphPad Software Inc., San Diego, USA).[28]

Results

Validation of epetraborole's antibacterial activity

Prior to cytotoxicity assessment, the functional integrity of epetraborole (EP) was confirmed using *Escherichia coli* K12 MG1655. As shown in Figure 1, EP exhibited potent antibacterial activity with complete growth inhibition at 0.5 µg/mL. This minimum inhibitory concentration (MIC) aligns with established literature for EP's activity against Gram-negative bacteria[14], validating the compound's bioactivity for subsequent experiments.

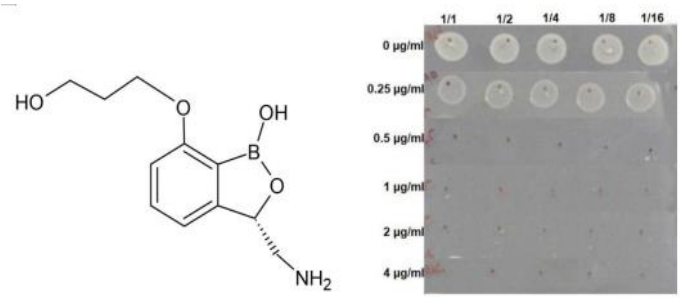

Figure 1. The structure and antibacterial activity of epetraborole. Spot test analysis demonstrating dose-dependent growth inhibition across EP concentrations (0–4 µg/mL). Complete suppression occurred at ≥0.5 µg/mL against *E. coli* K12 MG1655.

Cytotoxic activity of epetraborole on cancer cell lines

The cytotoxic effects of EP on several cancer cell lines were determined using the MTT assay after 48 and 72 hours of treatment.

As demonstrated in Figure 2, a concentration-dependent reduction in cell viability was observed for all of the cell lines that were examined.

The results of the study demonstrated a dose-dependent cytotoxic effect across all cancer cell lines. Specifically, after a 72-hour treatment period, EP significantly reduced the viability of the CARM-L12 TG3 and MG63 cell lines. The most potent effect was observed in CARM-L12 TG3 cells, with an IC50 value of 0.408 ± 1.1 µg/mL (Figure 2A), and in MG63 cells, with an IC50 value of 0.875 ± 1.6 µg/mL after 72 hours (Figure 2B). While the other cell lines exhibited a cytotoxic response, their IC50 values were elevated, signifying reduced sensitivity to the compound (Figure 2C, D, E).

The concentration required to inhibit 50% of cell growth (IC50) was calculated for each cell line, and these values are summarized in Table 1. The markedly lower IC50 value for the CARM-L12 TG3 cell line suggests its exceptional sensitivity to EP treatment, thus rendering it the most responsive cell line in this study.

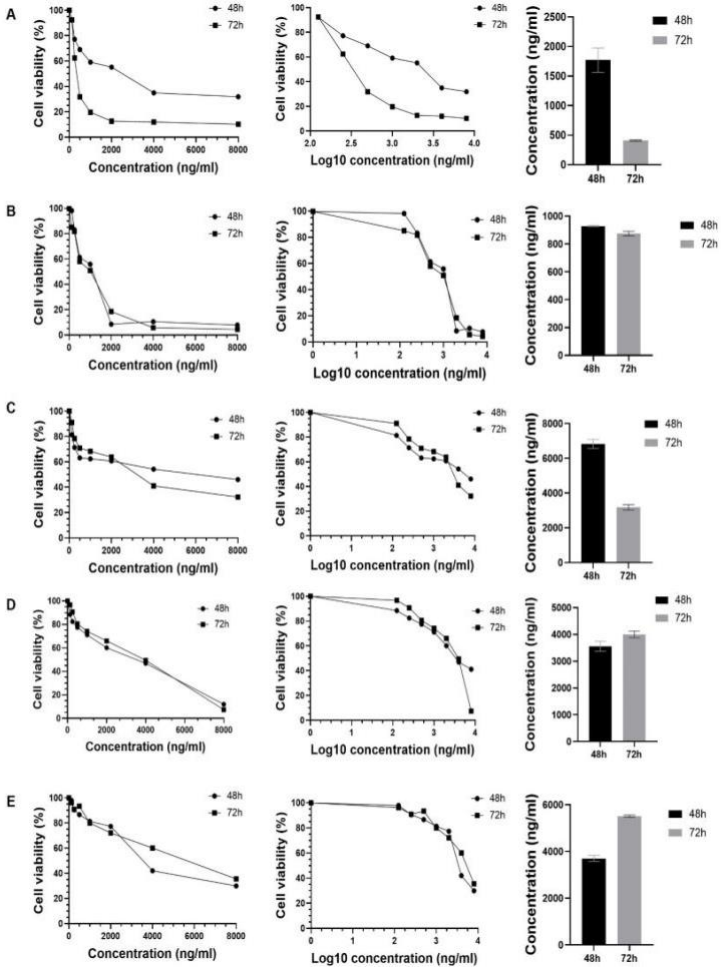

Figure 2. The cytotoxic effect of Epetraborole (EP) on selected cancer cell lines. The experiment involved exposing the cells (A-CARM-L12 TG3), (B- MG63), (C- SW48), (D- HT29), (E-2A3) to EP for 48 and 72 hours. Results showed a significant decrease in cell viability after exposure to EP. The graphs on the left represent percent viability, while the graphs in the middle show log 10% viability. The bar graphs on the right illustrate the calculated IC50 values. The abbreviations for the cell lines are shown on the figure. The data is presented as the mean ± SEM of three independent experiments, with three replicates in each experiment.

Table 1. IC50 values for the cytotoxic effect of Epetraborole on five mammalian cell lines [a].

Cell line	EP (48h) µg/mL	EP (72h) µg/mL
CARM-L12 TG3	1.769 ± 20.3	0.408 ± 1.1
MG63	0.928 ± 0.4	0.875 ± 1.6
SW-48	6.829 ± 2.62	3.186 ± 1.56
HT-29	3.557 ± 1.85	4.009 ± 1.24
2A3	3.699 ± 12.64	5.514 ± 4.67

[a] IC50 values were determined following the incubation for 48h hours and 72 hours with epetraborole. The data is presented as the mean ± SEM of three independent experiments, with three replicates in each experiment.

Discussion

Antibiotics have long been recognized for their antimicrobial properties, but growing evidence suggests broader applications in oncology.[4] While traditional anticancer antibiotics like anthracyclines and mitomycins have been used for decades[29,30], recent discoveries highlight untapped potential in novel antibiotic classes targeting unique molecular pathways. The field has since expanded from early agents such as doxorubicin, epirubicin, and mitomycin to more recent discoveries, including salinomycin and fluoroquinolones, reflecting the growing importance of antibiotics in modern oncology.[31] Among these, epetraborole (EP) represents a promising candidate as a leucyl-tRNA synthetase (LARS) inhibitor, though its anticancer effects remain unexplored. Our study addresses this gap by demonstrating EP's potent cytotoxicity across cancer cell lines, with remarkable activity against malignant mesothelioma (CARM-L12 TG3) and osteosarcoma (MG63). Notably, this work provides the first evidence of EP's anticancer properties and the first evaluation of any antibiotic in CARM-L12 TG3 and 2A3 squamous cell carcinoma models, opening new avenues for antibiotic repurposing in oncology.

The rationale for investigating EP stems from its unique mechanism. As a LARS inhibitor, EP disrupts protein synthesis-a vulnerability shared by bacteria and mitochondria due to their evolutionary relationship. This mitochondrial targeting aligns with known anticancer mechanisms of other antibiotics: tetracyclines inhibit 30S ribosomal subunits[32], chloramphenicol blocks 50S peptidyl transferase[33], and anisomycin interferes with aminoacyl-tRNA transfer.[34] However, EP's specificity for LARS may offer distinct advantages. Our findings support this premise, with EP exhibiting dose-dependent cytotoxicity and low IC50 values (e.g., 0.875 ± 1.6 µg/mL in MG63 osteosarcoma at 72h), surpassing reported efficacies of ciprofloxacin (40 µg/mL)[35] and anisomycin (10–40 µM)[36] in similar models.

Critically, EP's potency extends to colorectal cancer (CRC) cells—a relevant finding given recent debates about antibiotics' dual roles in CRC. Despite this promise, some antibiotics have been associated with a potential risk for colorectal cancer (CRC), which remains a global health concern. CRC accounts for over 600,000 deaths annually[37] and was responsible for approximately one million fatalities in 2020.[38] Specific antibiotics, such as metronidazole, ciprofloxacin, and rifaximin, are commonly prescribed for treating colitis and intestinal lesions.[39,40] While epidemiological studies associate prolonged antibiotic use with increased CRC risk[36,41-44], certain antibiotics paradoxically show direct antitumor effects. For instance, ciprofloxacin inhibits mitochondrial DNA synthesis in CRC cells[9], and rifaximin suppresses angiogenesis.[45] Further investigations have highlighted the varied mechanisms through which antibiotics exert their anticancer effects. An evaluation of monensin on human colorectal cancer cells found it to be a potent inhibitor of cell proliferation, migration, and cell cycle progression, while also inducing apoptosis.[46] Similarly, in an in vitro study on HT29 cells, two tetracycline compounds, DOXY and COL-3, were shown to inhibit proliferation in a dose-dependent manner, inducing mitochondria-mediated apoptosis through both caspase-dependent and caspase-independent pathways.[47] Our data reveal EP's activity in HT-29 (IC50 3.557 µg/mL at 48h) and SW-48 (IC50 3.186 µg/mL at 72h) lines, suggesting its effects may outweigh potential risks when used therapeutically rather than chronically. This dichotomy underscores the importance of mechanism-driven antibiotic repurposing.

The implications for osteosarcoma are particularly compelling. Scientific literature has characterized osteosarcoma (OS) as a tumor composed of malignant mesenchymal cells originating in the bone stroma.[48] The general incidence of this tumor is reported as 2-3 cases per million per year, but it peaks significantly during adolescence, reaching annual rates of 8–11 cases per million.[49] In the context of potential treatments, the effects of certain antibiotics on OS cells have been investigated. For instance, a study exploring the possible effects of ciprofloxacin on bone tissue utilized the MG-63 human osteosarcoma cell line. With MG63 cells showing high sensitivity to EP (IC50 <1 µg/mL), our results outperform prior studies testing 20 antibiotics on osteosarcoma, including ciprofloxacin (100 µg/mL) and tetracycline (200 µg/mL).[50] This potency mirrors Gao et al.'s findings with another LARS inhibitor (IC50 66.8 µM in U2OS cells), reinforcing LARS as a druggable target.

Conclusion

Based on these findings, Epetraborole demonstrates significant cytotoxic potential against a variety of human cancer cell lines, particularly the CARM-L12 TG3 and MG63 osteosarcoma lines, where it shows a notably high potency. Its mechanism, which involves inhibiting leucyl-tRNA synthetase, aligns with the known anticancer effects of other antibiotics that interfere with protein synthesis. A comparison of its low IC50 values to the effective concentrations of other antibiotics highlights Epetraborole's superior efficacy. This evidence positions Epetraborole as a promising and powerful candidate for further investigation in cancer therapy.

Conflict of Interest

Authors declared no conflict of interest.

Compliance of Ethical Statement

Ethics committee approval is not required for the study.

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Author Contributions

A.B.: Data curation; formal analysis; investigation; methodology; project administration; software; writing—original draft; B.Ç.: Conceptualization; funding acquisition; project administration; writing—review and editing.

References

1. Rajaraman R, Guernsey DL, Rajaraman MM, Rajaraman SR. Stem cells, senescence, neosis and self-renewal in cancer. *Cancer Cell Int*. 2006;6:25. doi:10.1186/1475-2867-6-25.
2. K E Kanady., W U Shipley., A L Zietman., et al. Treatment strategies using transurethral surgery, chemotherapy, and radiation therapy with selection that safely allows bladder conservation for invasive bladder cancer. *Semin Surg Oncol*. 1997;13(5):359-364. doi:10.1002/(sici)1098-2388(199709/10)13:5<359::aid-ssu10>3.0.co;2-i.
3. Lordick F, Hacker U. Chemotherapy and Targeted Therapy. In: *Imaging of Complications and Toxicity following Tumor Therapy*. 2014;3–15.
4. Xia D, Yang X, Liu W, et al. Over-expression of CHAF1A in Epithelial Ovarian Cancer can promote cell proliferation and inhibit cell apoptosis. *Biochem Biophys Res Commun*. 2017;486(1):191-197. doi:10.1016/j.bbrc.2017.03.026.

5. Pfab C, Schnobrich L, Eldnasoury S, Gessner A, El-Najjar N. Repurposing of Antimicrobial agents for cancer therapy: What do we know? *Cancers (Basel).* 2021;13(13):3193. doi:10.3390/cancers13133193
6. Karamanolis NN, Kounatidis D, Vallianou NG, et al. Unraveling the anti-cancer mechanisms of antibiotics: Current insights, controversies, and future perspectives. *Antibiotics.* 2024;14(1):9. doi:10.3390/antibiotics14010009.
7. Bano N, Parveen S, Saeed M, et al. Drug repurposing of selected antibiotics: an emerging approach in cancer drug discovery. *ACS Omega.* 2024;25:26762–26779. doi:10.1021/acsomega.4c00617.
8. Škrtić M, Sriskanthadevan S, Jhas B, et al. Inhibition of Mitochondrial Translation as a Therapeutic Strategy for Human Acute Myeloid Leukemia. *Cancer Cell.* 2011;20(5):674–688. doi:10.1016/j.ccr.2011.10.015
9. Herald C, Occur M, Ganslmayer M, et al. Ciprofloxacin induces apoptosis and inhibits proliferation of human colorectal carcinoma cells. *Br J Cancer.* 2002;86(3):443–448. doi:10.1038/sj.bjc.6600079
10. Gupta PB, Onder TT, Jiang G, et al. Identification of Selective Inhibitors of Cancer Stem Cells by High-Throughput Screening. *Cell*. 2009;138(4):645–659. doi:10.1016/j.cell.2009.06.034.
11. Das BC, Adil Shareef M, Das S, Nandwana NK, Das Y, Saito M, Weiss LM. Boron-Containing heterocycles as promising pharmacological agents. *Bioorg Med Chem.* 2022;63:116748. doi:10.1016/j.bmc.2022.116748.
12. Cardenas CA. Review of boron-based compounds: Advancing cancer therapy and beyond. *Clin Oncol Case Rep.* 2023;6:2–3.
13. Dhawan B, Akhter G, Hamid H, et al. Benzoxaboroles: New emerging and versatile scaffold with a plethora of pharmacological activities. *J Mol Struct.* 2022;1252. doi:10.1016/j.molstruc.2021.132057
14. Mendes RE, Alley MRK, Sader HS, et al. Potency and spectrum of activity of AN3365, a novel boron-containing protein synthesis inhibitor, tested against clinical isolates of enterobacteriaceae and nonfermentative gram-negative bacilli. *Antimicrob Agents Chemother.* 2013;57(6):2849-2857. doi:10.1128/AAC.00160-13.
15. Medina MÁ, Márquez J, Núñez de Castro I. Interchange of amino acids between tumor and host. *Biochem Med Metab Biol*. 1992;48(1):1-7. doi:0.1016/0885-4505(92)90041-V
16. Shikano N, Ogura M, Okudaira H, et al. Uptake of 3-[125I]iodo-α-methyl-l-tyrosine into colon cancer DLD-1 cells: characterization and inhibitory effect of natural amino acids and amino acid-like drugs. *Nucl Med Biol.* 2010;37(2):197-204. doi:10.1016/j.nucmedbio.2009.10.011
17. Lamb RF. Amino acid sensing mechanisms: An Achilles heel in cancer? *FEBS J.* 2012;279(15):2624-2631. doi:10.1111/j.1742-4658.2012.08659.x
18. Passarelli MC, Pinzaru AM, Asgharian H, et al. Leucyl-tRNA synthetase is a tumour suppressor in breast cancer and regulates codon-dependent translation dynamics. *Nat Cell Biol.* 2022;24(3):307-315. doi:10.1038/s41556-022-00856-5.
19. Gao G, Yao Y, Li K, Mashausi DS, Li D, Negi H, et al. A human leucyl-tRNA synthetase as an anticancer target. *Onco Targets Ther.* 2015;8:2933-2942. doi:10.2147/OTT.S88873.
20. Dibek E, Babayeva A, Sezer Kürkçü M, et al. Bor içeren bazı biyoaktif bileşikler. *J Boron.* 2020;5(1):29-39. doi:10.30728/boron.604069
21. Ganapathy US, Gengenbacher M, Dick T. Epetraborole is active against mycobacterium abscessus. *Antimicrob Agents Chemother*. 2021;65(10):e0115621. doi:10.1128/AAC.01156-21.
22. Cummings JE, Lunde CS, Alley MRK, Slayden RA. Epetraborole, a leucyl-tRNA synthetase inhibitor, demonstrates murine efficacy, enhancing the in vivo activity of ceftazidime against Burkholderia pseudomallei, the causative agent of melioidosis. *PLoS Negl Trop Dis.* 2023;17(11): e0011795. doi:10.1371/journal.pntd.0011795.
23. Abayeva A, Dibek E, Sünnetçi Akkoyunlu D, et al. The effect of epetraborole on the transcriptome and proteome profiles of an Escherichia coli strain overexpressing leuS, Leucyl-tRNA Synthetase. *Front Life Sci RT.* 2024;5:48–58. doi:10.51753/flsrt.1416938.
24. Dibek E, Sezer M, Akguc N. Genomic library construction of a boron tolerant bacterium in Escherichia coli and selection by boron. *Tjmbb.* 2017;2:21–29.
25. Babayeva A, Çöl B. Genome-wide Screening of the Escherichia coli Keio Knockout Collection Identifies Genetic Determinants of Epetraborole Hypersusceptibility. *Eur J Clin Microbiol Infect Dis.* 2025;44(9):2167-2182. doi:10.1007/s10096-025-05183-9.
26. Babayeva A, Dibek E, Kıvrak İ, Çöl B. The cytotoxic effects of Turkish bee venom (Apis mellifera) on selected cancer cell lines. *Int J Pept Res Ther.* 2024;30(5):53. doi:10.1007/s10989-024-10631-9.
27. Kürkçü MS, Genç D, Çöl B. Caffeic Acid's Influence on the Viability and Apoptosis of a Diverse Array of Cancer Cell Lines. *JARNAS. 2025;11(2):95-106. doi:10.28979/jarnas.1645815*
28. Swift ML. GraphPad prism, data analysis, and scientific graphing. *J Chem Inf Comput Sci.* 1997;37.
29. Saeidnia S. Anticancer Antibiotics. *In: New Approaches to Natural Anticancer Drugs.* 2014;51–66.
30. Cragg GM, Newman DJ. Natural Products Drug Discovery and Development at the United States National Cancer Institute. *In: Drug Discovery and Traditional Chinese Medicine.* 2001.
31. Gao Y, Shang Q, Li W, Guo W, Stojadinovic A, Mannion C, Man YG, Chen T. Antibiotics for cancer treatment: A double-edged sword. *J Cancer.* 2020;11(17):5135-5149. doi:10.7150/jca.47470.
32. Brodersen DE, Clemons WM, Carter AP, et al. The structural basis for the action of the antibiotic's tetracycline, pactamycin, and hygromycin B, on the 30S ribosomal subunit. *Cell.* 2000;103. doi:10.1016/S0092-8674(00)00216-6.
33. Kostopoulou ON, Kourelis TG, Mamos P, et al. Insights into the chloramphenicol inhibition effect on peptidyl transferase activity, using two new analogs of the drug. *Open Enzyme Inhib.* 2011;4:1-10. doi:10.2174/1874940201104010001.
34. Grollman AP. Inhibitors of protein biosynthesis. II. Mode of action of anisomycin. *J Biol Chem.* 1967;242(13):3226-3233. doi:10.1016/s0021-9258(18)95953-3.
35. Miclau T, Edin ML, Lester GE, et al. Effect of ciprofloxacin on the proliferation of osteoblast-like MG-63 human osteosarcoma cells in vitro. *J Orthop Res.* 1998;16(4):509-512. doi:10.1002/jor.1100160417.
36. Cao Y, Wu K, Mehta R, et al. Long-term use of antibiotics and risk of colorectal adenoma. *Gut.* 2018;67(4):672-678. doi:10.1136/gutjnl-2016-313413.
37. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. American Cancer Society. *CA Cancer J Clin.* 2011;61(2):69-90. doi:10.3322/caac.20107.
38. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer statistics for the year 2020: An overview. *Int J Cancer.* 2021;149(4):778-789. doi:10.1002/ijc.33588.
39. Bernstein CN, Eliakim A, Fedail S, et al. World Gastroenterology Organisation global guidelines inflammatory bowel disease: Update August 2015. *J Clin Gastroenterol.* 2016;50(10):803-818. doi:10.1097/MCG.0000000000000660
40. Nitzan O, Elias M, Peretz A, Saliba W. Role of antibiotics for treatment of inflammatory bowel disease. *World J Gastroenterol.* 2016;22(3):1078-1087. doi:10.3748/wjg.v22.i3.1078.
41. Wang JL, Chang CH, Lin JW, et al. Infection, antibiotic therapy and risk of colorectal cancer: A nationwide nested case-control study in patients with Type 2 diabetes mellitus. *Int J Cancer.* 2014;135(4):956-967. doi:10.1002/ijc.28738.
42. Dik VK, van Oijen MGH, Smeets HM, Siersema PD. Frequent use of antibiotics is associated with colorectal cancer risk: Results of a nested case-control study. *Dig Dis Sci.* 2016;61(1):255-264. doi:10.1007/s10620-015-3828-0.
43. Crockett SD, Nagtegaal ID. Terminology, molecular features, epidemiology, and management of serrated colorectal

neoplasia. *Gastroenterology.* 2019;157(4):949-966.e4. doi:10.1053/j.gastro.2019.06.041.
44. Zhang J, Haines C, Watson AJM, et al. Oral antibiotic use and risk of colorectal cancer in the United Kingdom, 1989-2012: A matched case-control study. *Gut.* 2019;68(11):1971-1978. doi:10.1136/gutjnl-2019-318593.
45. Esposito G, Gigli S, Seguella L, et al. Rifaximin, a non-absorbable antibiotic, inhibits the release of pro-angiogenic mediators in colon cancer cells through a pregnane X receptor-dependent pathway. *Int J Oncol.* 2016;49(2):639-645. doi:10.3892/ijo.2016.3550.
46. Zhou Y, Deng Y, Wang J, et al. Effect of antibiotic monensin on cell proliferation and IGF1R signaling pathway in human colorectal cancer cells. *Ann Med.* 2023;55(1):2166980. doi:10.1080/07853890.2023.2166980.
47. Onoda T, Ono T, Dhar DK, et al. Tetracycline analogues (doxycycline and COL-3) induce caspase-dependent and -independent apoptosis in human colon cancer cells. *Int J Cancer.* 2006;118(5):1309-1315. doi:10.1002/ijc.21447.
48. Klein MJ, Siegal GP. Osteosarcoma: Anatomic and histologic variants. *Am J Clin Pathol.* 2006;125(4):555-581. doi:10.1309/UC6K-QHLD-9LV2-KENN
49. de Azevedo JWV, de Medeiros Fernandes TAA, Fernandes JV, et al. Biology and pathogenesis of human osteosarcoma. *Oncol Lett.* 2020;19(2):1099-1116. doi:10.3892/ol.2019.11229
50. Duewelhenke N, Krut O, Eysel P. Influence on mitochondria and cytotoxicity of different antibiotics administered in high concentrations on primary human osteoblasts and cell lines. *Antimicrob Agents Chemother.* 2007;51(1):54-63. doi:10.1128/AAC.00729-05.