BOR DERGİSİ
JOURNAL OF BORON
https://dergipark.org.tr/boron

Antimicrobial potential of boron-containing compounds: Antibacterial, antifungal, and antimycobacterial activities

Pınar Guner [1,*], **Tülin Askun** [1], **Aylin Er** [1]

[1]*Balikesir University, Sciences and Arts Faculty, Biology Department, Balikesir, 10000, Türkiye*

ARTICLE INFO

Article history:
Received May 15, 2025
Accepted July 23, 2025
Available online September 31, 2025

Research Article

DOI: 10.30728/boron.1693877

Keywords:
Antibacterial activity
Antifungal activity
Antimycobacterial activity
Boron-containing compounds
Minimum inhibitory concentration

ABSTRACT

The global rise in multidrug-resistant (MDR) pathogens necessitates the discovery of new antimicrobial agents. Boron-containing compounds (BCCs) are increasingly studied for their broad-spectrum biological activities. The current study aimed to investigate the antibacterial, antifungal, and antimycobacterial activities of four different BCCs (Zinc borate, boric acid, borax, and Etidot-67) by determining their minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC). For the first time, the antimycobacterial activity of BCCs was evaluated against both reference and clinical strains.

All tested compounds exhibited notable antimicrobial activity. Among them, boric acid and zinc borate showed strong antibacterial effects, particularly against *Staphylococcus aureus* and *Salmonella typhimurium* at 64 µg/mL. Borax displayed the most potent antimycobacterial activity, with a MIC of 64 µg/mL against *Mycobacterium tuberculosis* H37Ra (MT-H37Ra). Antifungal tests revealed boric acid to be highly effective against *Candida albicans* and *Saccharomyces cerevisiae*, with MIC values as low as 8-16 µg/mL. These findings suggest that BCCs, especially borax and boric acid, may serve as viable candidates for the development of alternative antimicrobial therapies. However, further *in vivo* studies, toxicological assessments, and mechanistic investigations are necessary to support their clinical application.

1. Introduction

The global burden of infectious diseases has grown considerably in recent years, largely due to the diminishing efficacy of both conventional and novel antibiotics. A major driver of this trend is antimicrobial resistance (AMR), fueled by microbial mutations that allow pathogens to evade antibiotic action [1, 2]. Multidrug-resistant (MDR) infections spread through mechanisms such as gene transfer, poor hygiene in healthcare environments, and increased global travel. The World Health Organization (WHO) reports that a significant portion of infections are contracted via contaminated surfaces in public areas [2].

In 2019, bacterial infections were the direct cause of approximately 1.27 million deaths globally and were associated with nearly 5 million deaths overall [3]. Fungal pathogens also represent a critical health concern, with approximately 6.5 million cases of invasive fungal diseases annually, resulting in 3.8 million deaths, 2.5 million of which are directly attributed to fungal infections. For example, chronic pulmonary aspergillosis affects around 1.84 million individuals with an 18.5% mortality rate, while Candida infections lead to nearly 1 million deaths each year. Tuberculosis (TB) remains among the most prevalent infectious diseases, causing about 1.25 million deaths globally in 2023 despite long-term eradication efforts [4]. If left unaddressed, MDR infections are projected to cause up to 10 million deaths annually by 2050 [5, 6].

Boron is a rare element with significant biological activity in higher organisms [7]. It has been suggested for pharmaceutical use due to its ability to enhance cellular function and metabolism [8]. Boron plays a key role in immune response, bone maintenance, and brain function; deficiencies can impair physiological processes [9]. Compounds incorporating boron have exhibited diverse biological properties, notably antibacterial, antifungal, antiviral, anticancer, and enzyme-inhibitory activities. These bioactivities are generally linked to mechanisms such as disruption of enzymatic function, impairment of cellular membranes, and inhibition of biofilm development [10, 11]. Despite their considerable potential, the antimicrobial effects of boron-based compounds such as calcium metaborate, sodium metaborate tetrahydrate, zinc borate, sodium tetrafluoroborate, sodium tetraborate, potassium tetrafluoroborate, ammonium pentaborate tetrahydrate, sodium perborate monohydrate, and

*Corresponding author: pvpcelik@hotmail.com

ammonium tetrafluoroborate have been insufficiently studied, particularly against MDR pathogens including *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* sp. [12-14]. In recent years, the growing interest in the role of boron in drug design has significantly expanded the therapeutic potential of boron-containing compounds (BCCs). Within this context, five Food and Drug Administration (FDA) approved boron-containing drugs, bortezomib (Velcade), tavaborole (Kerydin), ixazomib (Ninlaro), crisaborole (Eucrisa), and vaborbactam (in combination with meropenem in Vabomere), have heightened attention towards boron as a promising candidate in drug development processes [15].

Boron is utilized not only as a therapeutic agent but also in various pharmaceutical applications such as protective groups and drug delivery systems. While efforts to optimize targeting strategies towards tumor cells continue, the scope of boron usage has notably broadened with the development of diverse chemical scaffolds, including diazaborines with antimicrobial activity, peptidic boronic acids serving as proteasome inhibitors in cancer therapy, benzoxaboroles acting as leucyl-tRNA synthetase inhibitors, and cyclic boronates employed as β-lactamase inhibitors to combat antimicrobial resistance [15]. Boronic acids, in particular, have shown promise as β-lactamase inhibitors that restore antibiotic efficacy against resistant strains such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterobacter aerogenes, Neisseria gonorrhoeae, A. baumannii, P. aeruginosa*, and *K. pneumoniae* [16-18]. Boric acid, a weak acid exhibiting a trigonal planar structure, stands out for its antibiofilm activity of up to 93% against yeast species such as *Candida albicans* [19]. Borax, due to its high solubility in water, can inhibit biofilms formed by bacteria such as *S. aureus* and *P. aeruginosa* by 65-72% [13]. Zinc borate, widely used in industry, exhibits antimicrobial activity against *C. albicans* and *S. aureus* [20]. Etidot-67, characterized by high solubility and synergistic combination potential, is a promising borate salt for antibacterial applications [21].

The current study aims to investigate the antimicrobial potential of four boron compounds (zinc borate, boric acid, borax, and Etidot-67) by determining their minimum inhibitory concentrations (MIC) and bactericidal/fungicidal (MBC/MFC) concentrations against ten bacterial strains, six fungal species, and four *Mycobacterium* isolates. Notably, this work presents the first assessment of their antimycobacterial activity.

2. Materials and Methods

2.1. Boron Compounds

The boron compounds utilized in this research included Etidot-67 (disodium octaborate tetrahydrate, $Na_2B_8O_{13}.4H_2O$), zinc borate (Eti-ZnBor, $2ZnO{\cdot}3B_2O_{13}.5H_2O$), borax (disodium tetraborate decahydrate, $Na_2[B_4O_5(OH)_4].8H_2O$), and boric acid (H_3BO_3). All substances were procured from the Eti Maden Bandırma Boron and Acid Factory (Türkiye).

For antimicrobial assays, the samples were weighed; their weight was found to be 10.24 mg. They were dissolved in 0.5% Dimethyl Sulfoxide (DMSO, Merck 116743.1000, USA) to prepare the stock solution. The stock solution concentration was 1024 µg/mL. The sterilization of compounds dissolved in DMSO was performed using a syringe filter (Merck Millex™-GS Sterile Syringe Filter Unit, MCE, 0.22 µm, USA) to ensure compatibility with culture conditions.

2.2. Microorganisms

In this study, microbial strains were selected from clinically significant and/or drug-resistant species commonly associated with infectious diseases. A total of ten bacterial strains were employed: *Bacillus cereus* (ATCC 10876), *S. aureus* (ATCC 538), *Salmonella typhimurium* (ATCC 14028), *K. pneumoniae* (ATCC 31488), *Proteus vulgaris* (ATCC 6897), methicillin-resistant *Staphylococcus aureus* (ATCC 33592), *Streptococcus agalactiae* (ATCC 23956), *Serratia marcescens* (ATCC 13880), *Enterococcus faecalis* (ATCC 29212), and *Escherichia coli* (ATCC 8739). Six fungal species used in the experiments included *Candida albicans* (ATCC 10239), *Saccharomyces cerevisiae* (ATCC 9763), *Aspergillus flavus* (ATA41-17), *Aspergillus ochraceus* (MUCL 39534), *Aspergillus niger* (TA47-3), and *Fusarium proliferatum* (TA18-2). Additionally, tests were conducted against avirulent *Mycobacterium tuberculosis* H37Ra (MT-H37Ra, ATCC 25177) and virulent H37Rv (MT-H37Rv, ATCC 25618) strains. Two additional strains (Strain-1 and Strain-2) were sourced from the tuberculosis laboratory at Balıkesir Chest Diseases Hospital in 2022.

Bacterial stock cultures were maintained on nutrient agar (NA, Merck 105450, USA), fungal stock cultures on malt extract Agar (MEA, Merck 105398, USA), and mycobacterial stock cultures in middlebrook 7H9 broth base (MBB, Millipore, M0178, USA), all stored at 4°C in a refrigerator (Vestel, S6540B, Türkiye).

2.3. Antimicrobial Sensitivity Assays

2.3.1. Antibacterial and antifungal activities

Antibacterial and antifungal MIC determinations were performed following the guidelines outlined in the Clinical and Laboratory Standards Institute (CLSI) protocols-M07 for bacteria and M27 for fungus [22, 23]. Mueller-Hinton Broth (MHB, Millipore 70192, USA) was used for bacterial testing, whereas Sabouraud Dextrose Broth (SDB, Merck 108339, USA) was employed for fungal assays. The inoculum suspensions were prepared in accordance with the 0.5 Mc Farland standard (GBL, 0471, Türkiye) ($1.5x10^8$ CFU/mL), utilising 24 h fresh cultures of the microorganisms

under investigation. The inoculum suspension was prepared using microorganisms in a solution of 0.85% w/v NaCl (Sigma-Aldrich, S9888, USA) [24, 25]. In the study, 100 µL of medium (NA) was added to sterile microplates (PrimeSurface, 96U-MS-9096UZ, USA). A sample at a concentration of 1024 µg/mL (100 µL) was added only to the first well. While the volume in the first well was 200 µL (sample solution plus medium), the volumes in the subsequent wells were 100 µL (medium only). The 200 µL solution was mixed three times using an automatic pipette (Sartorius Multichannel 5-100 µL, BM8-100R, Germany) and transferred from the first well to the second well. Serial dilutions were performed to achieve final concentrations ranging from 1 to 512 µg/mL in the assay wells. Row 12 was positive, and row 1 was the negative control. A 10 µL of microbial suspension as inoculum was added to all wells except row 1. Negative controls comprised the compound and medium without the addition of microorganisms, while positive controls included the respective test organisms in the medium. All experiments were conducted in triplicate. Microplates were incubated in an incubator (NUVE, FN 300, Türkiye) at 37°C for bacterial cultures and at 28°C for fungal strains for 24 hours.

Following this period, Thiazolyl Blue Tetrazolium Bromide (20 µL) (TBTB, Sigma M2128, USA) dissolved in water (10 mg/mL) was added to each well, and the microplates were incubated at 37°C for an additional 4 hours. TBTB is a yellowish solution and is converted to water-insoluble formazan of dark blue color by mitochondrial dehydrogenases of living cells. The absence of a color change, confirmed by TBTB staining, was interpreted as the lack of microbial viability. The use of colorimetric methods enables the acquisition of visually and quantitatively interpretable results based on color changes, minimizing the need for instrumentation and reducing costs. These methods also offer significant advantages, such as rapid response time, naked-eye detectability, and practical applicability. However, the presence of compounds such as pigments or reducing agents that can mask the indicator dye may lead to false positive or negative results. Since color change is associated with microbial metabolic activity, bacteriostatic effects may sometimes be mistaken for bactericidal ones [26]. In this study, the solutions used were colorless, and their bactericidal effects were confirmed through MBC/MFC tests.

For determining MBC/MFC, 20 µL samples from wells with no apparent growth were transferred into fresh wells containing 80 µL of newly prepared MHB for bacterial samples or SDB for fungal samples and incubated under the same respective conditions. After incubation, color change in positive and negative control wells was checked with a TBTB indicator. The lowest concentration without bacterial and fungal growth was accepted as MBC/MFC [27]. Iespor (IE, Ibrahim Etem, IM/IV, Türkiye) served as the antibacterial reference compound, whereas Amphotericin B (AmB, Sigma A2942, USA) functioned as the antifungal standard. For this purpose, 0.2 mg of antibiotics were dissolved in 10 mL of distilled water to prepare a stock solution at a concentration of 20 µg/mL. The working concentration range was set between 0.01 and 10 µg/mL.

2.3.2. Antimycobacterial activity test

Antimycobacterial susceptibility testing for *M. tuberculosis* was carried out following the guidelines outlined in the MGIT (Mycobacteria Growth Indicator Tube) protocol and the NCCLS-M24-A standard [28]. Cultivation of the strains was performed at 37°C using 4 mL of MBB, which was enriched with 0.5 mL of OADC supplement (Middlebrook, 515840) (oleic acid, albumin, dextrose, and catalase) and 0.1 mL of PANTA antibiotic mixture (BACTEC MGIT 960) (polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, and azlocillin). Inoculum made from a positive BACTEC MGIT tube (4 mL) was used one day after the tube became positive (Day 1) and up to the fifth day. Day 1 and Day 2 positives were used directly for susceptibility testing, while Day 3-Day 5 positives were diluted 1:5 (1 mL positive broth into 4 mL sterile saline) and used for inoculum. MGIT (4 mL), containing modified MBB, was used to grow the strains at 37°C. Microorganism growth in the tubes was tested daily starting from the second day of incubation using a fluorescence reader (MicroMGIT, BD-445923, USA) equipped with long-wavelength UV light.

The Microplate Presto Blue Assay (MPBA) was employed to assess antimycobacterial potential. The medium in MGIT tubes prepared as mentioned above was put into each well (100 µL), and a sample (100 µL) at the concentration of 1024 µg/mL was added to the first well only. The volume of the first well was 200 µL (sample solution and medium), while the others were 100 µL (only medium). 200 µLvolume of the solution was transferred from the first well to the second well by mixing three times with an automatic pipette. The tested compound concentrations varied between 1 and 512 µg/mL, and all assays were replicated three times. The experiments also included positive and negative controls. Row 12 was positive, and row 11 was the negative control. A 10 µL of mycobacterial suspension as inoculum was added to all wells except row 11. Then the microplates were incubated at 37°C.

Post-incubation, 20 µL of Presto Blue Reagent (Thermo Fisher Scientific, A12361, USA) was dispensed into each well. A persistent blue color indicated bacterial inhibition, while a shift to pink denoted active bacterial proliferation. MIC was identified as the lowest concentration without a visible color transition to pink. For MBC assessment, 20 µL samples from wells with no apparent growth were transferred into fresh wells containing 80 µL of newly prepared MBB. Following additional incubation at 37°C, the presence or absence of bacterial activity was determined using the same colorimetric method. The MBC was defined as the minimum concentration at which bacterial viability was no longer observed [27].

Rifampicin (RIF, Sigma-Aldrich 557303, USA) served as the reference antibiotic in these evaluations. To prepare the stock solution, 0.2048 mg of the antibiotic was dissolved in 10 mL of distilled water, resulting in a final concentration of 20.48 µg/mL. The concentration range of the working solutions was set between 10.24 µg/mL and 0.02 µg/mL.

3. Results

3.1. Antibacterial Activity

BCCs demonstrated varying degrees of antibacterial efficacy across the ten tested strains. Gram (+) bacteria, particularly *S. aureus* and *E. faecalis*, showed the highest susceptibility. In the case of S. aureus, borax and Etidot-67 had the lowest MIC values (32 µg/mL), whereas borax was most effective in terms of MBC (64 µg/mL). For *B. cereus*, both boric acid and Etidot-67 had the lowest MIC (64 µg/mL) and MBC (256 µg/mL) values, suggesting balanced antimicrobial performance. With *S. agalactiae*, borax had the lowest MIC (64 µg/mL), and boric acid, zinc borate, and borax exhibited the lowest MBC (256 µg/mL). Against MRSA, borax and Etidot-67 showed better MIC performance (128 µg/mL), while borax also yielded the lowest MBC (128 µg/mL), indicating notable bactericidal activity. *E. faecalis* was most susceptible to borax, which presented both the lowest MIC (32 µg/mL) and MBC (64 µg/mL) values. For *P. vulgaris*, all four compounds exhibited identical MIC values (128 µg/mL); *E. coli*, borax and Etidot-67 demonstrated the most effective MIC values (64 µg/mL), while zinc borate and boric acid had the lowest MBC (256 µg/mL). Against *K. pneumoniae*, Etidot-67 displayed the lowest MIC (64 µg/mL) and the lowest MBC (128 µg/mL). In *S. marcescens*, boric acid and borax again had the lowest MIC (64 µg/mL) and MBC (128 µg/mL) values. Lastly, for *S. typhimurium*, Etidot-67, zinc borate, and boric acid had the most effective MIC (64 µg/mL), while borax and Etidot-67 showed the lowest MBC values (128 µg/mL). These findings are detailed in Table 1 and visualized in Figure 1, where MIC and MBC values are compared across Gram-positive (+) and Gram-negative (-) strains.

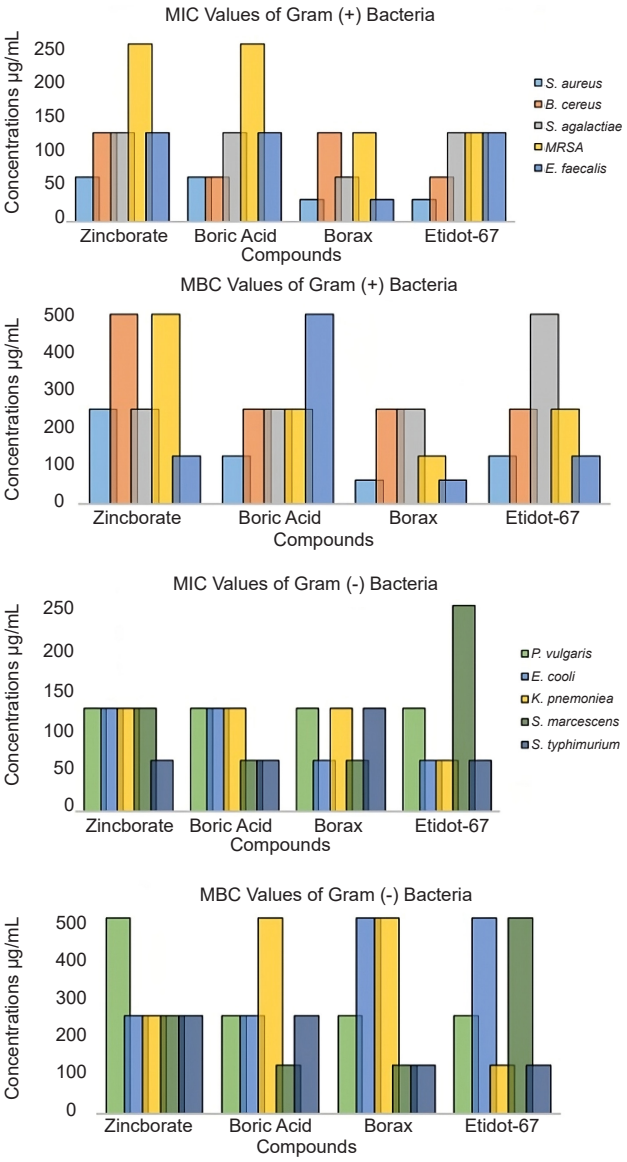

Figure 1. MIC and MBC values of the Gram (-) and Gram (+) bacteria (µg/mL).

Table 1. Antibacterial activity of BCCs (µg/mL).

	Zincborate		Boric Acid		Borax		Etidot-67		IE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
P. vulgaris	128	512	128	256	128	256	128	256	0.31	0.31
E. coli	128	256	128	256	64	512	64	512	0.31	0.62
K. pnemoniae	128	256	128	512	128	512	64	128	0.31	0.62
S. aureus	64	256	64	128	32	64	32	128	0.31	0.31
B. cereus	128	512	64	256	128	256	64	256	10	10
S. marcescens	128	256	64	128	64	128	256	512	0.31	1.25
S. agalactiae	128	256	128	256	64	256	128	512	0.31	0.62
MRSA	256	512	256	256	128	128	128	256	10	20
E. faecalis	128	128	128	512	32	64	128	128	0.62	0.31
S. typhimurium	64	256	64	256	128	128	64	128	0.31	1.25

3.2. Antifungal Activity

The antifungal activities of four BCCs were evaluated against six fungal species based on their MIC and MFC. For *C. albicans*, boric acid and borax exhibited the lowest MIC values (16 µg/mL), while all compounds demonstrated similar MFC values (128 µg/mL). In *S. cerevisiae*, boric acid and borax again showed the most effective MIC values (8 µg/mL), with boric acid presenting the lowest MFC (64 µg/mL). For *A. flavus*, boric acid (16 µg/mL MIC and 64 µg/mL MFC) and borax (32 µg/mL MIC and 64 µg/mL MFC) showed notable activity. In *A. niger*, borax displayed the lowest MIC (16 µg/mL) and MFC (32 µg/mL) values, indicating the strongest antifungal activity among the tested compounds. For *F. proliferatum*, all compounds shared the same MIC value (64 µg/mL), but boric acid had the lowest MFC (64 µg/mL). Lastly, in *A. ochraceus*, zinc borate and borax showed the lowest MIC values (32 µg/mL), while all compounds showed high MFC values (256-512 µg/mL). Among the compounds tested, zinc borate exhibited the least antifungal efficacy, while AmB served as a positive control with MIC values between 0.15 and 2.5 µg/mL. Detailed antifungal data are provided in Table 2 and summarized graphically in Figure 2.

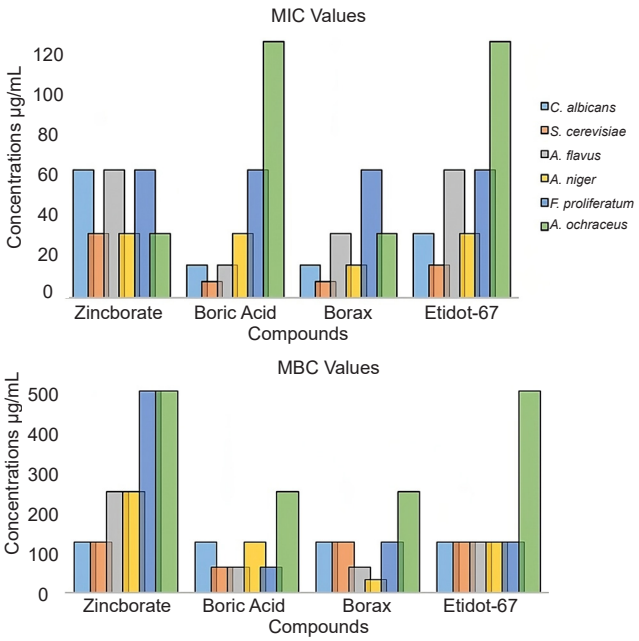

Figure 2. MIC and MBC values of the fungal strains.

3.3. Antimycobacterial Activity

This study represents the first report on the antimycobacterial effects of BCCs. All four tested compounds inhibited the growth of both reference and clinical strains. For MT-H37Ra, borax exhibited the strongest antimycobacterial activity, with the lowest MIC (64 µg/mL) and MBC (128 µg/mL) values. The other compounds (zinc borate, boric acid, and Etidot-67) shared identical MIC (128 µg/mL) and MBC (256 µg/mL) values. In the case of MT-H37Rv, borax presented the lowest MIC value (128 µg/mL), while the other three compounds showed higher MICs (256 µg/mL). For Strain-1, borax was again the most effective, demonstrating the lowest MIC (64 µg/mL) and MBC (128 µg/mL). Zinc borate and boric acid displayed identical MIC/MBC values of 128/256 µg/mL. In Strain-2, all compounds exhibited the same MIC (128 µg/mL). However, boric acid and Etidot-67 had the highest MBC (512 µg/mL), while borax and zinc borate shared a lower MBC value of 256 µg/mL. Rifampicin, the reference drug, exhibited superior efficacy with MIC values between 0.32 and 5.12 µg/mL across the strains. These findings are summarized in Table 3 and illustrated in Figure 3, which highlights the comparative activity of the tested compounds.

Table 2. Antifungal activity of BCCs (µg/mL).

	Zincborate		Boric Acid		Borax		Etidot-67		IE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
C. albicans	64	128	16	128	16	128	32	128	0.31	0.62
S. cerevisiae	32	128	8	64	8	128	16	128	0.15	0.62
A. flavus	64	256	16	64	32	64	64	128	1.25	2.5
A. niger	32	256	32	128	16	32	32	128	1.25	5
F. proliferatum	64	512	64	64	64	128	64	128	2.5	10
A. ochraceus	32	512	128	256	32	256	128	512	1.25	2.5

Table 3. Antimycobacterial activity of BCCs (µg/mL).

	Zincborate		Boric Acid		Borax		Etidot-67		IE	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MT-H37Ra	128	256	128	256	64	128	128	256	0.64	5.12
MT-H37Rv	256	512	256	512	128	512	256	512	0.32	2.56
Strain-1	128	256	128	256	64	128	256	512	0.64	0.64
Strain-2	128	256	128	512	128	256	128	512	5.12	10.24

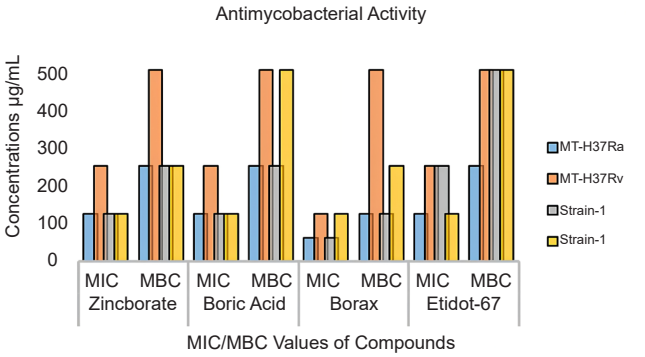

Figure 3. MIC and MBC values of the mycobacterial strains.

4. Discussion

Boron is an essential micronutrient for living organisms, although it functions effectively only within a narrow physiological concentration range [29]. At elevated levels, boron can exert toxic effects through various mechanisms, including interference with vital cellular processes [30]. Its high affinity for ribose, a key structural component of molecules such as ATP, NADH, NADPH, and RNA, underlies its central role in cellular metabolism and energy transfer [31]. However, excessive boron levels may disrupt protein synthesis, impair mitochondrial function, and hinder processes such as cell division and development [32]. In addition to its metabolic roles, boron has been shown to affect quorum sensing, an essential microbial communication system, which becomes dysregulated in the presence of boron overload [31, 32]. BCCs also interact with diverse enzymes and contribute to the integrity and functionality of biological membranes [33]. Nevertheless, at toxic concentrations, boron may compromise membrane stability, alter membrane structure, and disrupt transport mechanisms across cellular barriers [34]. In light of these biological properties, the present study supports the notion that four different BCCs can influence microbial viability through multiple pathways. The observed inhibitory activity across bacterial, fungal, and mycobacterial strains was confirmed through MIC and MBC/MFC assays. BCCs may act not only through direct antimicrobial mechanisms but also potentially by targeting fundamental cellular structures and communication pathways. These compounds can interfere with ribose-dependent metabolic pathways, disrupt protein synthesis and mitochondrial function, impair quorum sensing mechanisms, and destabilize biological membranes [31, 33]. These multifaceted mechanisms act synergistically, contributing to the broad-spectrum antimicrobial activity observed in this study.

Several studies have explored the antimicrobial potential of boron compounds [35]. Yılmaz [36] reported that the MIC values of boric acid were 7.60 mg/mL against *E. coli* and *Pseudomonas aeruginosa* and 3.80 mg/mL against *S. aureus*. In contrast, the current study demonstrated significantly lower MIC values, with boric acid showing inhibitory effects at 64 µg/mL

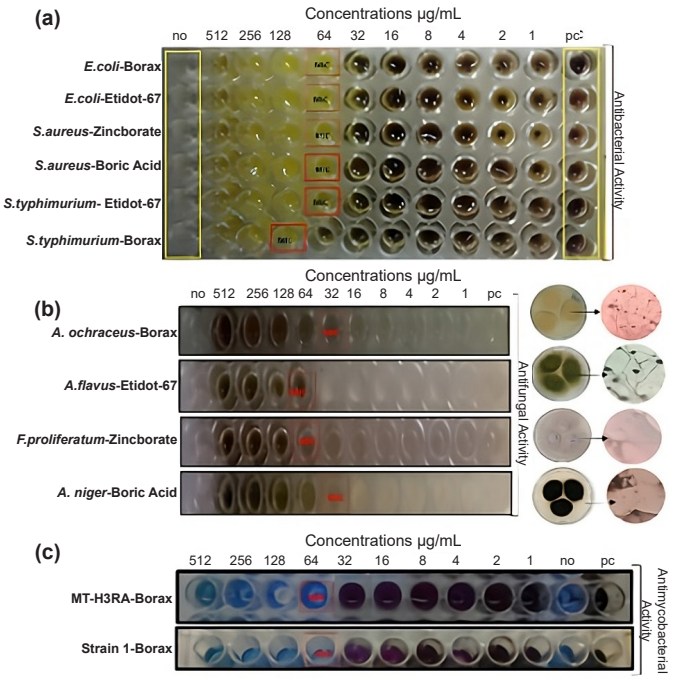

Figure 4. Illustration of the microdilution method using a 96-well microplate for selected strains: a) Antibacterial activity of BCCs; b) Antifungal activity of BCCs; c) Antimycobacterial activity of BCCs.

against *S. aureus, S. marcescens, S. typhimurium*, and *B. cereus*, indicating a higher antimicrobial efficacy under the tested conditions. Zinc contributes to wound healing by promoting collagen deposition, stimulating fibroblast proliferation, enhancing epithelial formation, and increasing keratinocyte migration [37]. Moreover, its antimicrobial activity mediated through disruption of bacterial membranes and degradation of biofilms not only inhibits bacterial growth but also complements the antibacterial and anti-inflammatory properties of boron, thereby enhancing the overall therapeutic potential of BCCs [38]. The present study observed an inhibitory concentration of zinc borate at 64 µg/mL against *S. aureus* and *S. typhimurium*, which suggests a potentially stronger antimicrobial effect in this experimental context compared to the findings of Boran et al. [38], where the MIC for *S. aureus* was reported as 0.5 mg/mL. In this study, both *S. aureus* and MRSA strains were used to evaluate the efficacy of BCCs. The inclusion of the MRSA strain, which is known for its multidrug resistance, enabled the assessment of the antimicrobial potential of BCCs against resistant bacterial forms. For all tested BCCs, the MIC and MBC values obtained for MRSA were significantly higher compared to those for *S. aureus*, indicating reduced susceptibility. Nevertheless, the BCCs demonstrated measurable inhibitory activity against the MRSA strain as well, suggesting their potential as alternative agents in the treatment of resistant infections.

The antifungal activity of BCCs was evaluated against a range of fungal strains, with promising results against both yeast and filamentous fungi. This indicates a differential efficacy of BCCs, where they

are more effective against yeast-type fungi compared to filamentous species. The reduced effectiveness against filamentous fungi may be attributed to differences in cell wall composition, fungal morphology, or resistance mechanisms inherent to these species [39]. Zinc borate exhibited the least antifungal efficacy among the compounds tested, as indicated by its higher MIC and MFC values across the fungal strains. Notably, MIC values ranged between 32 and 64 µg/mL, while MFC values extended up to 512 µg/mL. This finding suggests that the presence of zinc in the borate compound may not be as potent in combating fungal infections as other boron-based compounds, such as boric acid and borax. Furthermore, the antifungal effects of zinc borate appear to vary among fungal species. For instance, *S. cerevisiae, A. niger* and *A. ochraceus* showed lower MICs (32 µg/mL), whereas *F. proliferatum* required higher concentrations for both inhibitory and fungicidal effects. However, further investigation into the mechanisms of action of zinc borate may provide insights into its specific antifungal properties or potential synergistic effects when combined with other compounds. Boric acid has long been recognized for its antifungal properties, with its fungitoxicity attributed primarily to the disruption of carbohydrate metabolism, which impairs fungal growth and reproduction. In *S. cerevisiae*, boric acid interferes with cytoskeletal organization at the bud neck, disrupting septum formation and resulting in abnormal chitin-rich cell walls that prevent proper cell separation, ultimately leading to the formation of cell chains and aggregates [40]. This structural stress induces compensatory chitin synthesis as part of the fungal stress response. Additionally, boric acid inhibits β-glucosidase activity in several fungal species [41], further impairing essential metabolic functions. Its antifungal efficacy has also been demonstrated clinically; for instance, a 5% ethanol-based boric acid solution has proven effective against *Aspergillus* and *Candida* species in the treatment of otomycosis [42]. Moreover, boric acid and other BCCs have shown effectiveness against azole-resistant *C. albicans* strains [43].

In our study, the antimycobacterial activities of various BCCs were evaluated against both reference and clinical strains. Among the tested compounds, borax demonstrated the most potent activity against the reference strain MT-H37Ra, with a MIC of 64 µg/mL. Additionally, zinc borate, boric acid, and Etidot-67 exhibited notable inhibitory effects against the same strain, each with an MIC value of 128 µg/mL. Borax was more effective against clinical strains compared to the other compounds, showing an MIC value of 64 µg/mL against Strain-1. The observed MIC and MBC values demonstrated that boron compounds possess both bacteriostatic and bactericidal potential. To the best of our knowledge, this is the first study to report the anti-mycobacterial potential of BCCs against both reference and patient-derived *M. tuberculosis* strains. While previous studies have highlighted the promising antimicrobial properties of boronic acids, particularly in the context of β-lactamase inhibition and cell wall targeting in *M. tuberculosis*. Boronic acids have been shown to exert selective activity through mechanisms such as oxaborole tRNA-trapping or glycan binding in the unique mycobacterial cell envelope [44]. Boric acids are capable of forming bonds with cis-1,2- and 1,3-diols in carbohydrates, and the incorporation of multiple boric acid moieties on a single scaffold can result in a synergistic enhancement of binding affinity [45]. To address the challenge posed by the impermeable cell envelope of *M. tuberculosis*, Guy et al. [46] developed multivalent boronic acid constructs aimed at selectively binding to the structurally distinct glycans of the M. tuberculosis cell envelope. Supporting these findings, Adamska et al. [47] demonstrated that thymine derivatives modified with boron clusters, particularly those containing 7,8-dicarba-nido-undecaborate and 1,2-dicarba-closo-dodecaborane moieties, exhibited strong inhibition of both *M. tuberculosis* thymidylate kinase (TMPK) and mycobacterial growth. Taken together, these results highlight the therapeutic promise of boron-based compounds as dual-action agents with both enzymatic and whole-cell inhibitory effects against M. tuberculosis [47].

5. Conclusions

The findings of this research indicate that BCCs (zinc borate, boric acid, borax, and Etidot-67) possess significant in vitro antimicrobial properties against a wide array of pathogenic microorganisms, encompassing both Gram-positive (+) and Gram-negative (-) bacteria, fungi, and *Mycobacterium* species. Among the tested compounds, borax and boric acid exhibited the most potent antimicrobial effects, showing low MIC and MBC/MFC values, especially against *S. aureus, C. albicans, S. cerevisiae* and *M. tuberculosis* strains. These results underline the significant in vitro efficacy of BCCs, particularly borax, as a promising antimicrobial agent. The observed antimicrobial performance, especially the ability to inhibit multidrug-resistant strains like MRSA and MT-H37Rv, highlights the therapeutic potential of BCCs as alternative or adjunct antimicrobial candidates. Notably, this study also reports, for the first time, the antimycobacterial potential of BCCs, opening a novel avenue in the search for anti-TB agents from boron chemistry. These findings support the growing body of evidence suggesting that BCCs could serve as alternative or adjunct antimicrobial agents, especially in the face of escalating multidrug resistance.

In particular, for BCCs to be considered as effective antimicrobial drug candidates, in vivo efficacy studies, toxicological profiling, pharmacokinetic and pharmacodynamic analyses, as well as the condition in which they show stronger therapeutic effects when used in combination with other drugs compared to when used alone (synergistic interactions), need to be investigated.

In conclusion, BCCs represent a promising and underexplored class of antimicrobial agents with activity against a wide range of clinically relevant pathogens. However, in order to advance from experimental data to practical applications, it is crucial to bridge the gap between in vitro observations and in vivo validation through multidisciplinary research efforts. Future studies focusing on safety, efficacy, and the mechanism of action will be pivotal in unlocking the full therapeutic potential of boron chemistry in combating antimicrobial resistance.

6. Author Contribution Statement

Pinar Guner: Methodology, laboratory work, graphic design, original draft writing.

Tulin Askun: Methodology, data analysis, writing analysis, original draft writing.

Aylin Er: Methodology, data analysis, writing analysis, original draft writing.

References

[1] Sugden, R., Kelly, R., & Davies, S. (2016). Combatting antimicrobial resistance globally. *Nature Microbiology*, *1*(10), 1-2. https://doi.org/10.1038/nmicrobiol.2016.187

[2] Otter, J. A., Yezli, S., & French, G. L. (2011). The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infection Control & Hospital Epidemiology*, *32*(7), 687-699. https://doi.org/10.1086/660363

[3] Antimicrobial Resistance Collaborators. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, *399*(10325), 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0

[4] World Health Organization. (2023). *Global tuberculosis report 2023*. https://www.who.int/publications/i/item/9789240083851

[5] Sefton, A. M. (2002). Mechanisms of antimicrobial resistance: Their clinical relevance in the new millennium. *Drugs*, *62*, 557-566. https://doi.org/10.2165/00003495-200262040-00001

[6] Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., ... & Cars, O. (2013). Antibiotic resistance-the need for global solutions. *The Lancet Infectious Diseases*, *13*(12), 1057-1098. https://doi.org/10.1016/S1473-3099(13)70318-9

[7] Scorei, R. (2012). Is boron a prebiotic element? A mini-review of the essentiality of boron for the appearance of life on earth. *Origins of Life and Evolution of Biospheres*, *42*, 3-17. https://doi.org/10.1007/s11084-012-9269-2

[8] Ali, F. S., Hosmane, N., & Zhu, Y. (2020). Boron chemistry for medical applications. *Molecules*, *25*(4), 828. https://doi.org/10.3390/molecules25040828

[9] Nielsen, F. H. (2017). Historical and recent aspects of boron in human and animal health. *Journal of Boron*, *2*(3), 153-160

[10] Song, S., Gao, P., Sun, L., Kang, D., Kongsted, J., Poongavanam, V., ... & Liu, X. (2021). Recent developments in the medicinal chemistry of single boron atom-containing compounds. *Acta Pharmaceutica Sinica B*, *11*(10), 3035-3059. https://doi.org/10.1016/j.apsb.2021.01.010

[11] Baker, S. J., Ding, C. Z., Akama, T., Zhang, Y. K., Hernandez, V., & Xia, Y. (2009). Therapeutic potential of boron-containing compounds. *Future Medicinal Chemistry*, *1*(7), 1275-1288. https://doi.org/10.4155/fmc.09.71

[12] Baker, S. J., Akama, T., Zhang, Y. K., Sauro, V., Pandit, C., Singh, R., ... & Maples, K. R. (2006). Identification of a novel boron-containing antibacterial agent (AN0128) with anti-inflammatory activity, for the potential treatment of cutaneous diseases. *Bioorganic & Medicinal Chemistry Letters*, *16*(23), 5963-5967. https://doi.org/10.1016/j.bmcl.2006.08.130

[13] Celebi, O., Celebi, D., Baser, S., Aydın, E., Rakıcı, E., Uğraş, S., ... & Abd El-Aty, A. M. (2024). Antibacterial activity of boron compounds against biofilm-forming pathogens. *Biological Trace Element Research*, *202*(1), 346-359. https://doi.org/10.1007/s12011-023-03768-z

[14] Esposito, S., & De Simone, G. (2017). Update on the main MDR pathogens: Prevalence and treatment options. *Le Infezioni in Medicina*, *25*(4), 301-310.

[15] Messner, K., Vuong, B., & Tranmer, G. K. (2022). The boron advantage: The evolution and diversification of boron's applications in medicinal chemistry. *Pharmaceuticals*, *15*(3), 264. https://doi.org/10.3390/ph15030264

[16] Silva, M. P., Saraiva, L., Pinto, M., & Sousa, M. E. (2020). Boronic acids and their derivatives in medicinal chemistry: synthesis and biological applications. *Molecules*, *25*(18), 4323. https://doi.org/10.3390/molecules25184323

[17] Jacobs, L. M., Consol, P., & Chen, Y. (2024). Drug discovery in the field of β-lactams: an academic perspective. *Antibiotics*, *13*(1), 59. https://doi.org/10.3390/antibiotics13010059

[18] Iqbal, Z., Sun, J., Yang, H., Ji, J., He, L., Zhai, L., ... & Yang, Z. (2022). Recent developments to cope the antibacterial resistance via β-lactamase inhibition. *Molecules*, *27*(12), 3832

[19] Syvolos, Y., Salama, O. E., & Gerstein, A. C. (2024). Constraint on boric acid resistance and tolerance evolvability in Candida albicans. *Canadian Journal of Microbiology*, *70*(9), 384-393. https://doi.org/10.1139/cjm-2023-0225

[20] Iyigundogdu, Z. (2023). Synergistic effects of zinc borate and graphene on enhanced thermal stability and antimicrobial properties of poly(methyl methacrylate). *Polymer Composites*, *44*(7), 3939-3951. https://doi.org/10.1002/pc.27367

[21] Celebi, D., Celebi, O., Baser, S., & Taghizadehghalehjoughi, A. (2025). Investigation of the antibacterial, antibiofilm and cytotoxic effects of boron compounds in a Streptococcus mitis infection model on HepG2 liver cell. *Journal of Research in Pharmacy*, *27*(6), 2277-2284. https://doi.org/10.29228/jrp.516

[22] Zimmer, B. L., Carpenter, D. E., Esparza, G., Alby, K., Bhatnagar, A. Ferrel, A. L., … & Yee, R. (2024). *Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (CLSI Standard No. M07). Clinical and Laboratory Standards Institute. https://clsi.org/shop/standards/m07/

[23] Alexander, B. D., Procop, G. W., Dufrense, P., Fuller, J., Ghannoum, M. A., Hanson, K. E., … & Zelanzy, A. M. (2017). *Refence Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts* (CLSI Standard No. M27). Clinical and Laboratory Standards Institute. https://clsi.org/shop/standards/m27/

[24] Güner, P., Aşkun, T., & Er, A. (2023). Evaluation of Anti-bacterial Activity Induced by Penicillium mallochii in the Hemolymph of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae). *International Journal of Nature and Life Sciences*, *7*(2), 79-88. https://doi.org/10.47947/ijnls.1362362

[25] El-Saadony, M. T., Sitohy, M. Z., Ramadan, M. F., & Saad, A. M. (2021). Green nanotechnology for preserving and enriching yogurt with biologically available iron (II). *Innovative Food Science & Emerging Technologies*, *69*, 102645. https://doi.org/10.1016/j.ifset.2021.102645

[26] Celik, C., Kalin, G., Cetinkaya, Z., Ildiz, N., & Ocsoy, I. (2023). Recent advances in colorimetric tests for the detection of infectious diseases and antimicrobial resistance. *Diagnostics*, *13*(14), 2427. https://doi.org/10.3390/diagnostics13142427

[27] Güner, P., & Aşkun, T. (2023). Anti-bacterial, anti-mycobacterial and anti-fungal properties of Punica granatum as natural dye. *European Journal of Biology*, *82*(1), 38-48. https://doi.org/10.26650/EurJBiol.2023.1239283

[28] Woods, G. L., Wengenack, G. N., Lin, G., Brown-Elliott, B. A., Cirillo, D. M., Conville, P. S., … & Turnidge S. D. (2018). *Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes* (CLSI Standard No. M24). Clinical and Laboratory Standards Institute. https://clsi.org/shop/standards/m24/

[29] Goldbach, H. E., & Wimmer, M. A. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure? *Journal of Plant Nutrition and Soil Science*, *170*(1), 39-48. https://doi.org/10.1002/jpln.200625161

[30] Reid, R. J., Hayes, J. E., Post, A., Stangoulis, J. R., & Graham, R. D. (2004). A critical analysis of the causes of boron toxicity in plants. *Plant, Cell & Environment*, *27*(11), 1405-1414. https://doi.org/10.1111/j.1365-3040.2004.01243.x

[31] Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B. L., & Hughson, F. M. (2002). Structural identification of a bacterial quorum-sensing signal containing boron. *Nature*, *415*(6871), 545-549. https://doi.org/10.1038/415545a

[32] Lowery, C. A., Salzameda, N. T., Sawada, D., Kaufmann, G. F., & Janda, K. D. (2010). Medicinal chemistry as a conduit for the modulation of quorum sensing. *Journal of Medicinal Chemistry*, *53*(21), 7467-7489. https://doi.org/10.1021/jm901742e

[33] Brown, P. H., Bellaloui, N., Wimmer, M. A., Bassil, E. S., Ruiz, J., Hu, H., ... & Römheld, V. (2002). Boron in plant biology. *Plant Biology*, *4*(2), 205-223. https://doi.org/ 10.1055/s-2002-25740

[34] Cakmak, I., Kurz, H., & Marschner, H. (1995). Short term effects of boron, germanium, and high light intensity on membrane permeability in boron deficient leaves of sunflower. *Physiologia Plantarum*, *95*(1), 11-8. https://doi.org/10.1111/j.1399-3054.1995.tb00801.x

[35] Soares, M. M. S. R., & Cury, A. E. (2001). In vitro activity of antifungal and antiseptic agents against dermatophyte isolates from patients with tinea pedis. *Brazilian Journal of Microbiology*, *32*(2), 130-134. https://doi.org/10.1590/S1517-83822001000200012

[36] Yilmaz, M. T. (2012). Minimum inhibitory and minimum bactericidal concentrations of boron compounds against several bacterial strains. *Turkish Journal of Medical Sciences*, *42*(2), 1423-1429. https://doi. org/10.3906/sag-1205-83.

[37] Wang, Y., Ying, T., Li, J., Xu, Y., Wang, R., Ke, Q., ... & Lin, K. (2020). Hierarchical micro/nanofibrous scaffolds incorporated with curcumin and zinc ion eutectic metal organic frameworks for enhanced diabetic wound healing via anti-oxidant and anti-inflammatory activities. *Chemical Engineering Journal*, *402*, 126273. https://doi.org/10.1016/j.cej.2020.126273

[38] Boran, R., Baygar, T., Saraç, N., Ayrıkçil, S., Yılmaz, D., & Uğur, A. (2023). Antimicrobial, antifibrinolytic, enzyme inhibitory and wound healing properties of zinc borate. *Journal of Boron*, *8*(3), 99-104. https://doi.org/10.30728/boron.1180847

[39] Anderson, T. M., Clay, M. C., Cioffi, A. G., Diaz, K. A., Hisao, G. S., Tuttle, M. D., ... & Burke, M. D. (2014). Amphotericin forms an extramembranous and fungicidal sterol sponge. *Nature Chemical Biology*, *10*(5), 400-406. https://doi.org/10.1038/nchembio.1496

[40] Schmidt, M., Schaumberg, J. Z., Steen, C. M., & Boyer, M. P. (2010). Boric acid disturbs cell wall

synthesis in Saccharomyces cerevisiae. *International Journal of Microbiology*, *2010*(1), 930465. https://doi.org/10.1155/2010/930465

[41] Ang, S. S., Salleh, A. B., Bakar, F. A., Yusof, N. A., Zaman, M. Z., & Heng, L. Y. (2011). Effect of boric acid on the growth and production of β-glucosidase in Paecilomyces variotii. *African Journal of Microbiology Research*, *5*(17), 2451-2454. https://doi.org/10.5897/AJMR11.078

[42] Del Palacio, A., Cuétara, M. S., Lopez-Suso, M. J., Amor, E., & Garau, M. (2002). Randomized prospective comparative study: Short-term treatment with ciclopiroxolamine (cream and solution) versus boric acid in the treatment of otomycosis. *Mycoses*, *45*(7-8), 317-328. https://doi.org/10.1046/j.1439-0507.2002.00737.x

[43] Larsen, B., Petrovic, M., & De Seta, F. (2018). Boric acid and commercial organoboron products as inhibitors of drug-resistant Candida albicans. *Mycopathologia*, *183*, 349-357. https://doi.org/10.1007/s11046-017-0209-6

[44] Krajnc, A., Lang, P. A., Panduwawala, T. D., Brem, J., & Schofield, C. J. (2019). Will morphing boron-based inhibitors beat the β-lactamases? *Current Opinion in Chemical Biology*, *50*, 101-110. https://doi.org/10.1016/j.cbpa.2019.03.001

[45] Arnaud, J., Audfray, A., & Imberty, A. (2013). Binding sugars: From natural lectins to synthetic receptors and engineered neolectins. *Chemical Society Reviews*, *42*(11), 4798-4813. https://doi.org/10.1039/c2cs35435g

[46] Guy, C. S., Gibson, M. I., & Fullam, E. (2019). Targeting extracellular glycans: Tuning multimeric boronic acids for pathogen-selective killing of Mycobacterium tuberculosis. *Chemical Science*, *10*(23), 5935-5942. https://doi.org/10.1039/c9sc00415g

[47] Adamska, A., Rumijowska-Galewicz, A., Ruszczynska, A., Studzińska, M., Jabłońska, A., Paradowska, E., ... & Olejniczak, A. B. (2016). Anti-mycobacterial activity of thymine derivatives bearing boron clusters. *European Journal of Medicinal Chemistry*, *121*, 71-81. https://doi.org/10.1016/j.ejmech.2016.05.030