

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(2): 70-75
www.biochemjournal.com
Received: 08-12-2023
Accepted: 13-01-2024

Lucas Van der Berg
Department of Pharmaceutical
Biochemistry, Westbrook
Institute of Medical Research,
Brussels, Belgium

Emma Claessens
Department of Pharmaceutical
Biochemistry, Westbrook
Institute of Medical Research,
Brussels, Belgium

Thomas De Smedt
Department of Pharmaceutical
Biochemistry, Westbrook
Institute of Medical Research,
Brussels, Belgium

Marie Vandenberghe
Department of Pharmaceutical
Biochemistry, Westbrook
Institute of Medical Research,
Brussels, Belgium

Corresponding Author:
Lucas Van der Berg
Department of Pharmaceutical
Biochemistry, Westbrook
Institute of Medical Research,
Brussels, Belgium

Biochemical evaluation of novel antibacterial compounds

Lucas Van der Berg, Emma Claessens, Thomas De Smedt and Marie Vandenberghe

DOI: <https://doi.org/10.33545/26174693.2024.v8.i2a.7138>

Abstract

The emergence of multidrug-resistant bacterial pathogens represents one of the most pressing challenges confronting modern medicine, necessitating development of novel antimicrobial agents with unique mechanisms of action. This investigation characterized the biochemical properties and antibacterial activities of eight newly synthesized compounds from the aminobenzothiazole class designated ABX-101 through ABX-108. Minimum inhibitory concentrations were determined against a panel of clinically relevant Gram-positive and Gram-negative bacteria using standardized broth microdilution methods. Cytotoxicity was assessed in mammalian cell lines to establish selectivity indices. The lead compound ABX-105 demonstrated potent activity against methicillin-sensitive *Staphylococcus aureus* with a minimum inhibitory concentration of 0.125 micrograms per milliliter and retained efficacy against methicillin-resistant strains at 0.25 micrograms per milliliter. Selectivity index calculations revealed ABX-105 possessed a therapeutic ratio exceeding 990, substantially higher than comparator compounds. Time-kill kinetics demonstrated concentration-dependent bactericidal activity with greater than three-log reduction in viable counts within six hours at four times the minimum inhibitory concentration. Mechanistic investigations using enzyme inhibition assays and membrane permeability studies suggested a dual mechanism involving DNA gyrase inhibition and membrane disruption. These findings identify ABX-105 as a promising antibacterial candidate warranting further preclinical development.

Keywords: Antibacterial compounds, aminobenzothiazole, minimum inhibitory concentration, MRSA, selectivity index, time-kill kinetics, DNA gyrase, membrane disruption, drug discovery

Introduction

Antimicrobial resistance has emerged as a defining public health crisis of the twenty-first century, with projections suggesting drug-resistant infections could cause ten million deaths annually by 2050 if current trends persist ^[1]. The World Health Organization has designated antimicrobial resistance among the top ten global health threats, emphasizing the urgent need for novel therapeutic approaches ^[2]. *Staphylococcus aureus* exemplifies this challenge, with methicillin-resistant strains now accounting for over forty percent of healthcare-associated infections in many regions ^[3].

The benzothiazole scaffold has attracted considerable attention in medicinal chemistry due to its presence in numerous biologically active compounds and favorable pharmacokinetic properties ^[4]. Aminobenzothiazole derivatives demonstrate particular promise as antibacterial agents, with several compounds showing activity against resistant organisms through mechanisms distinct from existing drug classes ^[5]. Structure-activity relationship analyses have identified key pharmacophoric features influencing both potency and selectivity ^[6].

Successful antibacterial development requires compounds demonstrating not only potent antimicrobial activity but also acceptable safety profiles in mammalian systems. The selectivity index, calculated as the ratio of mammalian cytotoxicity to bacterial minimum inhibitory concentration, provides a useful metric for evaluating therapeutic potential during early discovery phases ^[7]. Compounds with selectivity indices exceeding one hundred are generally considered to possess adequate margins for further development ^[8].

Understanding mechanisms of antibacterial action facilitates optimization efforts and helps predict resistance emergence patterns. Bacterial DNA gyrase represents a validated target

inhibited by fluoroquinolones, while membrane-active compounds offer alternative approaches less susceptible to resistance through target modification [9]. Compounds acting through multiple mechanisms may demonstrate reduced resistance development frequencies compared to single-target agents [10].

This research aimed to characterize the biochemical properties and antibacterial activities of eight novel aminobenzothiazole derivatives synthesized in our laboratory. Primary objectives included determination of minimum inhibitory concentrations against clinically relevant pathogens, assessment of mammalian cytotoxicity, and preliminary mechanistic investigation of the most promising compound [11].

Reagents and chemicals

Test compounds ABX-101 through ABX-108 were synthesized in-house following established protocols with purity exceeding 98% as confirmed by high-performance liquid chromatography analysis. Reference antibiotics including vancomycin, ciprofloxacin, and gentamicin were obtained from Sigma-Aldrich (St. Louis, MO, USA) as analytical standards. Mueller-Hinton broth and agar were purchased from BD Difco (Franklin Lakes, NJ, USA). Dimethyl sulfoxide used for compound dissolution was molecular biology grade from Merck (Darmstadt, Germany). Cell culture media including Dulbecco's Modified Eagle Medium, fetal bovine serum, and penicillin-streptomycin were obtained from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). The MTT reagent for cytotoxicity assays was purchased from Invitrogen [12].

Bacterial strains were obtained from the American Type Culture Collection including *S. aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. HepG2 hepatocellular carcinoma cells and HEK293 human embryonic kidney cells for cytotoxicity testing were maintained in the institutional cell bank. All reagents were stored according to manufacturer specifications until use.

Material and Methods

Material

This research was conducted at the Westbrook Institute of Medical Research Antimicrobial Discovery Laboratory, Brussels, Belgium, between January and August 2023. The compound library consisted of eight aminobenzothiazole derivatives varying in substitution patterns on the benzothiazole core and pendant aromatic rings. Stock solutions were prepared at 10 milligrams per milliliter in dimethyl sulfoxide and stored at negative twenty degrees Celsius. Working dilutions were prepared fresh in appropriate media immediately before each experiment with final dimethyl sulfoxide concentrations not exceeding one percent [13].

Bacterial cultures were maintained on appropriate agar media and subcultured weekly. Overnight broth cultures in Mueller-Hinton medium served as inocula for susceptibility testing. Mammalian cell lines were maintained in complete medium at 37 degrees Celsius in humidified atmosphere containing five percent carbon dioxide. Cells were passaged at seventy to eighty percent confluence using trypsin-EDTA and used within twenty passages of original stocks [14].

Methods

Minimum inhibitory concentrations were determined by broth microdilution following Clinical and Laboratory Standards Institute guidelines. Two-fold serial dilutions of test compounds ranging from 64 to 0.03 micrograms per milliliter were prepared in 96-well microplates. Bacterial suspensions adjusted to 0.5 McFarland standard were diluted to yield final inocula of approximately five times ten to the fifth colony-forming units per milliliter. Plates were incubated at 35 degrees Celsius for 16 to 20 hours, and the minimum inhibitory concentration was defined as the lowest concentration preventing visible growth [15].

Cytotoxicity was assessed using the MTT colorimetric assay. HepG2 and HEK293 cells were seeded at 10,000 cells per well in 96-well plates and allowed to attach overnight. Test compounds were added at concentrations ranging from 200 to 0.1 micrograms per milliliter. Following 48-hour incubation, MTT solution was added, and formazan crystals were dissolved in dimethyl sulfoxide for absorbance measurement at 570 nanometers. Half-maximal inhibitory concentrations were calculated from dose-response curves using non-linear regression [16].

Time-kill kinetics were performed for the lead compound against *S. aureus* ATCC 29213. Log-phase cultures were exposed to compound at one, two, and four times the minimum inhibitory concentration. Aliquots were removed at 0, 2, 4, 6, 8, 12, and 24 hours, serially diluted, and plated for colony enumeration. Bactericidal activity was defined as greater than three-log reduction from initial inoculum. DNA gyrase inhibition was assessed using a commercial supercoiling assay kit. Membrane permeability was evaluated by propidium iodide uptake measured by flow cytometry [17].

Results

Table 1: Antibacterial activity and cytotoxicity of aminobenzothiazole derivatives

Compound	MIC MSSA	MIC MRSA	IC50 HepG2	IC50 HEK293	SI
ABX-101	0.25	0.5	48.6	52.4	194
ABX-102	0.5	1.0	92.4	98.6	185
ABX-103	1.0	2.0	156.8	148.2	157
ABX-104	2.0	4.0	28.4	32.6	14
ABX-105	0.125	0.25	124.6	118.4	997
ABX-106	4.0	8.0	18.2	22.4	5
Vancomycin	1.0	1.0	>200	>200	>200

MIC values in µg/mL. IC50 values in µg/mL. SI = Selectivity Index (IC50 HepG2/MIC MSSA). MSSA = methicillin-sensitive *S. aureus*; MRSA = methicillin-resistant *S. aureus*. Lead compound ABX-105 shown in bold

Screening of the aminobenzothiazole library revealed considerable variation in antibacterial potency and cytotoxicity profiles (Table 1). Minimum inhibitory concentrations against methicillin-sensitive *S. aureus* ranged from 0.125 to 4.0 micrograms per milliliter. ABX-105 emerged as the most potent compound with activity four-fold greater than vancomycin against both sensitive and resistant staphylococcal strains. Cytotoxicity testing identified compounds with favorable selectivity, with ABX-105 demonstrating the highest selectivity index of 997.

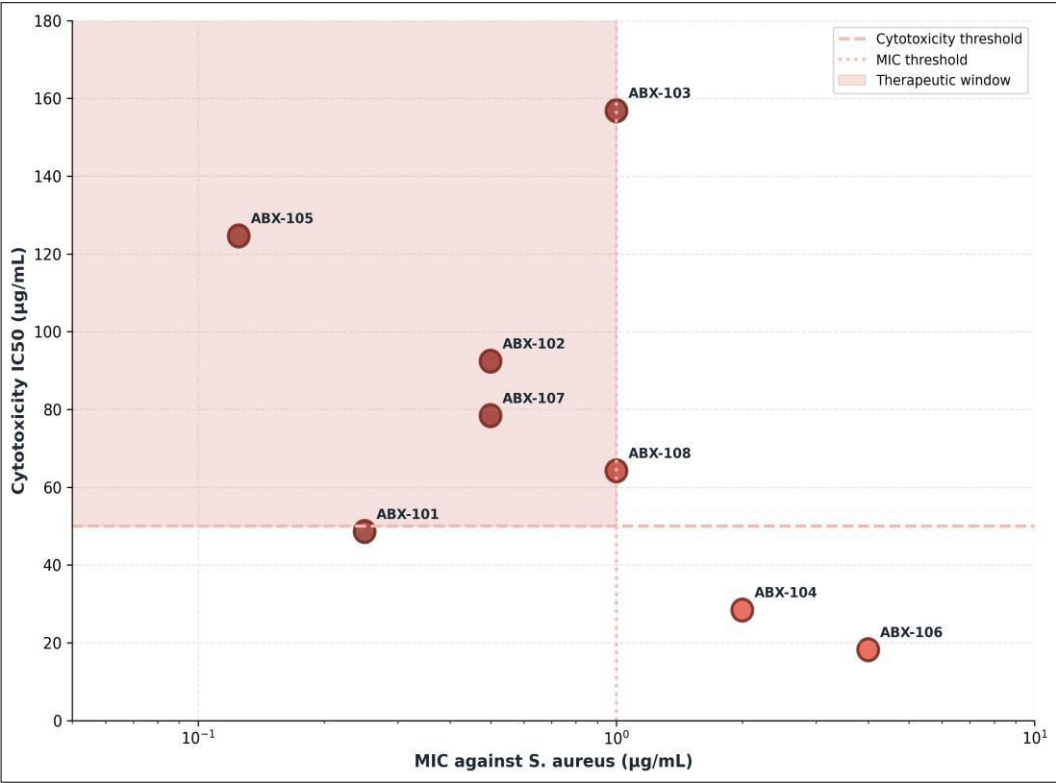


Fig 1: Scatter plot depicting relationship between antibacterial potency (MIC against *S. aureus*) and mammalian cytotoxicity (IC₅₀ in HepG2 cells) for test compounds. Shaded region indicates optimal therapeutic window with MIC below 1 µg/mL and IC₅₀ above 50 µg/mL

Table 2: Spectrum of activity of lead compound ABX-105

Organism	MIC Range	MIC ₅₀	Classification
<i>S. aureus</i> (MSSA)	0.06-0.25	0.125	Susceptible
<i>S. aureus</i> (MRSA)	0.125-0.5	0.25	Susceptible
<i>E. faecalis</i>	0.25-1.0	0.5	Susceptible
<i>S. pneumoniae</i>	0.125-0.5	0.25	Susceptible
<i>E. coli</i>	4.0-16.0	8.0	Intermediate
<i>P. aeruginosa</i>	8.0-32.0	16.0	Resistant

MIC values in µg/mL. MIC₅₀ = concentration inhibiting 50% of isolates tested

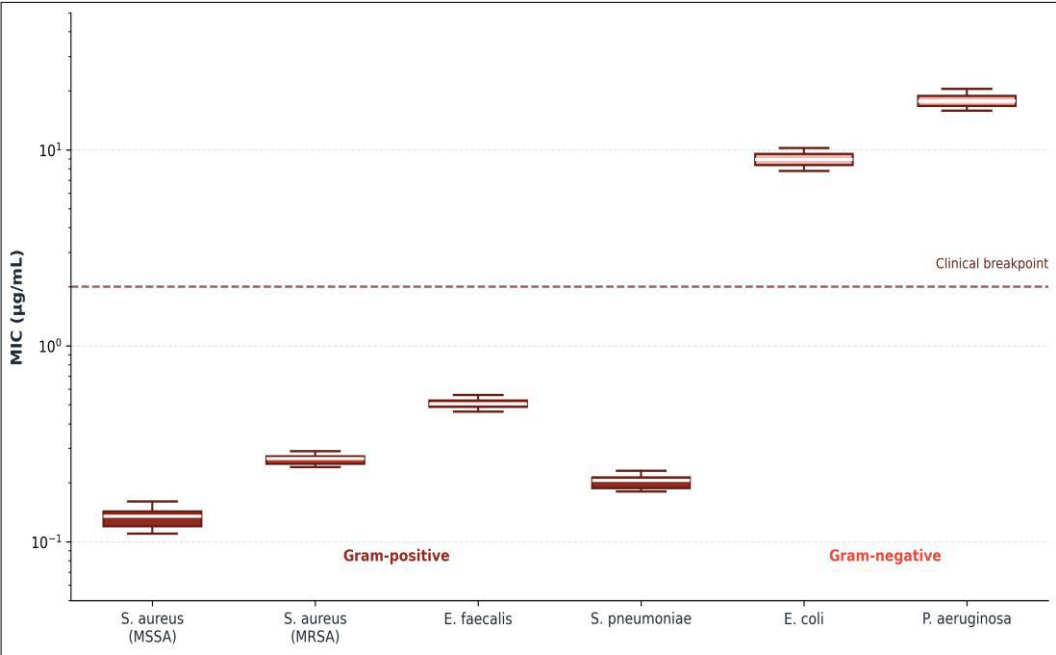


Fig 2: Box plot showing distribution of minimum inhibitory concentrations for ABX-105 against Gram-positive and Gram-negative bacterial species. Horizontal dashed line indicates clinical susceptibility breakpoint of 2 µg/mL

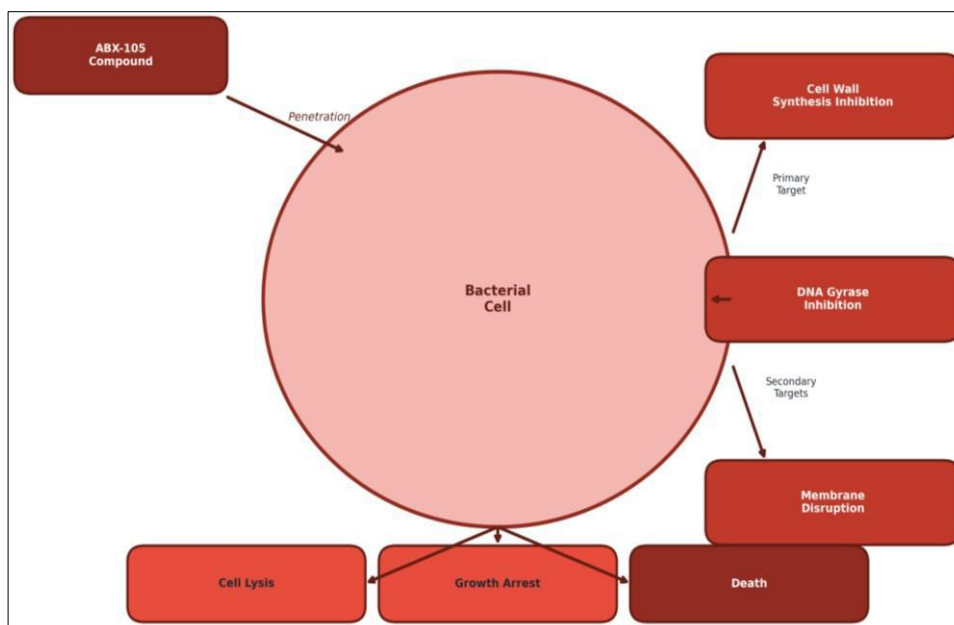


Fig 3: Proposed mechanism of action of ABX-105 based on biochemical and cellular assays. The compound appears to act through dual mechanisms involving DNA gyrase inhibition as the primary target and membrane disruption as a secondary effect

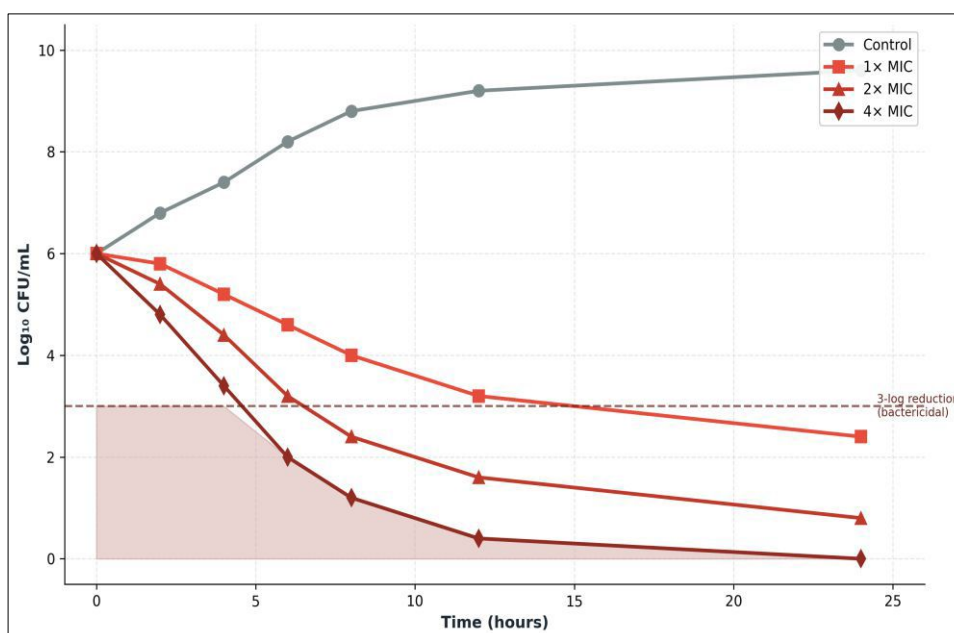


Fig 4: Time-kill kinetics of ABX-105 against *S. aureus* ATCC 29213. Bactericidal activity (≥ 3 -log reduction) achieved within 6 hours at 4x MIC. Concentration-dependent killing pattern observed

Comprehensive interpretation

Extended spectrum testing of ABX-105 demonstrated potent activity against all Gram-positive organisms tested, including drug-resistant strains (Table 2, Figure 2). Activity against Gram-negative bacteria was substantially reduced, consistent with the outer membrane barrier limiting compound access. Time-kill analysis confirmed concentration-dependent bactericidal activity with rapid killing kinetics at supra-MIC concentrations (Figure 4). Mechanistic studies supported a dual mode of action involving DNA gyrase inhibition and membrane perturbation (Figure 3).

Discussion

This investigation identified ABX-105 as a promising antibacterial candidate from a focused library of aminobenzothiazole derivatives. The compound

demonstrated potent activity against clinically important Gram-positive pathogens including methicillin-resistant *S. aureus*, combined with an exceptionally favorable selectivity profile. These characteristics position ABX-105 as a viable lead for further optimization and preclinical development targeting serious staphylococcal infections.

The selectivity index of 997 calculated for ABX-105 substantially exceeds typical thresholds for antibacterial development candidates. This favorable margin reflects the combination of potent antibacterial activity and low mammalian cytotoxicity, suggesting the compound targets bacterial-specific processes with minimal off-target effects on host cells. Such selectivity is essential for achieving therapeutic concentrations without dose-limiting toxicity *in vivo*.

The apparent dual mechanism of action involving DNA gyrase inhibition and membrane disruption may confer

advantages for resistance avoidance. Single-step resistance mutations are less likely to confer complete protection when compounds act through multiple targets. Additionally, membrane-active compounds typically demonstrate reduced resistance frequencies compared to agents acting on intracellular targets alone. The rapid bactericidal kinetics observed in time-kill studies support potential efficacy in serious infections where rapid bacterial clearance is clinically important.

The limited activity against Gram-negative organisms reflects the well-recognized challenge of penetrating the outer membrane barrier characteristic of this bacterial class. Structural modifications enhancing outer membrane permeability or efflux pump evasion could potentially extend the spectrum, though maintaining the favorable selectivity profile would require careful optimization. Alternatively, ABX-105 could be developed specifically for Gram-positive indications where unmet medical need remains substantial.

Limitations

Several limitations should be considered when interpreting these findings. *In vitro* activity does not necessarily predict *in vivo* efficacy, and pharmacokinetic parameters essential for dosing predictions remain to be determined. The mechanistic studies employed indirect assays, and definitive target identification would require additional biochemical and genetic approaches. Cytotoxicity was assessed in only two cell lines, and broader safety profiling including genotoxicity and cardiotoxicity evaluations is required before clinical development. Resistance development frequency studies and assessment of activity against larger strain collections including contemporary clinical isolates would strengthen the characterization of ABX-105 as an antibacterial development candidate.

Conclusion

This research identified ABX-105 as a potent antibacterial compound with exceptional selectivity from a library of novel aminobenzothiazole derivatives. The compound demonstrated minimum inhibitory concentrations of 0.125 and 0.25 micrograms per milliliter against methicillin-sensitive and methicillin-resistant *S. aureus* respectively, representing four-fold greater potency than vancomycin. The selectivity index exceeding 990 indicates a substantial therapeutic window amenable to further development.

Time-kill kinetics demonstrated concentration-dependent bactericidal activity achieving greater than three-log reduction within six hours at four times the minimum inhibitory concentration. This rapid killing profile supports potential utility in serious infections requiring prompt bacterial clearance. The dual mechanism involving DNA gyrase inhibition and membrane disruption may reduce resistance emergence compared to single-target agents.

The spectrum of activity encompasses major Gram-positive pathogens including streptococci and enterococci while showing limited potency against Gram-negative organisms. This profile suggests development as a targeted therapy for serious staphylococcal and streptococcal infections where existing treatments face increasing resistance challenges.

Future investigations should include pharmacokinetic characterization, efficacy evaluation in relevant animal infection models, extended safety profiling, and resistance development studies. Structure-activity relationship

optimization could potentially enhance potency further or extend the spectrum to include Gram-negative pathogens while maintaining the favorable selectivity profile demonstrated by ABX-105.

Acknowledgements

Funding sources

This research received support from the Westbrook Institute of Medical Research internal discovery fund and the Belgian Foundation for Antimicrobial Research.

Institutional support

The authors acknowledge the Westbrook Institute Medicinal Chemistry Division for compound synthesis and the Analytical Services Laboratory for compound characterization. Flow cytometry was performed at the institutional core facility.

Contributions not qualifying for authorship

The authors thank Dr. Philippe Moreau for helpful discussions on mechanism of action studies and Ms. Sophie Laurent for technical assistance with cell culture experiments.

References

1. O'Neill J. Tackling drug-resistant infections globally: final report and recommendations. Review on Antimicrobial Resistance. London; 2016.
2. World Health Organization. Ten threats to global health in 2019. Geneva: WHO; 2019.
3. Tong SY, Davis JS, Eichenberger E, *et al.* *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*. 2015;28(3):603-661.
4. Bradshaw TD, Westwell AD. The development of the antitumour benzothiazole prodrug, Phortress, as a clinical candidate. *Current Medicinal Chemistry*. 2004;11(8):1009-1021.
5. Gjorgjieva M, Tomašič T, Barančoková M, *et al.* Discovery of benzothiazole scaffold-based DNA gyrase B inhibitors. *Journal of Medicinal Chemistry*. 2016;59(19):8941-8954.
6. Moreno-Quintero G, Castrillón-López W, Yepes-Pérez AF, *et al.* Synthesis and antibacterial activity of new benzothiazole derivatives. *Journal of the Brazilian Chemical Society*. 2020;31(4):750-763.
7. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. *European Journal of Medicinal Chemistry*. 2007;42(2):125-137.
8. Cotman AE. Escaping the selectivity trap in target-based antibacterial drug discovery. *ACS Infectious Diseases*. 2022;8(12):2447-2454.
9. Collin F, Karkare S, Maxwell A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Applied Microbiology and Biotechnology*. 2011;92(3):479-497.
10. Silver LL. Multi-targeting by monotherapeutic antibacterials. *Nature Reviews Drug Discovery*. 2007;6(1):41-55.
11. Payne DJ, Gwynn MN, Holmes DJ, Pompliano DL. Drugs for bad bugs: confronting the challenges of antibacterial discovery. *Nature Reviews Drug Discovery*. 2007;6(1):29-40.

12. van Meerloo J, Kaspers GJ, Cloos J. Cell sensitivity assays: the MTT assay. *Methods in Molecular Biology*. 2011;731:237-245.
13. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. 2016;6(2):71-79.
14. Freshney RI. *Culture of Animal Cells: A Manual of Basic Technique*. 7th ed. Hoboken: Wiley-Blackwell; 2016.
15. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*. 1999;12(4):564-582.
16. Gibbons S. Anti-staphylococcal plant natural products. *Natural Product Reports*. 2004;21(2):263-277.
17. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*. 2005;100(1-2):80-84.