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Pharmacological Evaluation of a Phenyl Boronic Acid Derivative Having Antibacterial Activity Against *Staphylococcus aureus:* Synthesis, biological activity, and ADME Studies

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Abstract

Boron-containing compounds have attracted considerable attention due to their unique chemical reactivity, biological versatility, and low toxicity. In this study, a phenylboronic acid (PBA) derivative (3-((3,5-ditert-butyl-2-hydroxybenzylidene)amino)phenyl)boronic, 3,5-Tb-Bmin) was synthesized and characterized using elemental analysis and spectroscopic techniques. The *in vitro* antibacterial activity of the compound 3,5-Tb-Bmin against *Staphylococcus aureus* was determined by the broth microdilution method (BMD) . The results revealed that the synthesized boron derivative exhibited significant inhibitory activity, suggesting its potential as a promising antibacterial agent. Furthermore, ADME (Absorption, Distribution, Metabolism, and Excretion) analysis was performed to assess the pharmacokinetic behavior and drug-likeness of the compound. The results indicated favorable oral bioavailability, acceptable lipophilicity, and compliance with Lipinski's rule of five. These findings demonstrate that the synthesized boronic acid derivative could serve as a potential lead compound for the development of novel boron-based antibacterial drugs.

Keywords: Phenylboronic acid, Boron Schiff Base, *Staphylococcus aureus*, Antibacterial activity, ADME

1. Introduction

Arylboronic acids represent an important class of compounds due to their wide range of applications. Although these compounds have been known for more than a century, their properties and potential uses are still under investigation. Significant areas of interest include the synthesis of biaryl compounds, the development of molecular receptors, and the evaluation of their biological activities [1].

The nature and position of substituents on the phenyl ring significantly influence the acidity, binding behavior, and biological activity of boronic acids. Phenylboronic acid (PBA) has attracted attention in drug delivery studies. Kitano et al. reported a glucose-responsive polymer complex containing a PBA moiety that functions as a novel drug delivery system [2].

Recent studies have highlighted the therapeutic potential of boronic acids in antiviral, antibacterial, and anticancer applications. Many antimicrobial agents contain halogen atoms, which enhance activity through their electronegativity and ability to form hydrogen-bond interactions. Therefore, halogenation remains a powerful strategy for modulating the properties of bioactive compounds [3].

Boronic acids are chemically stable, reactive, and exhibit low toxicity. As bioisosteres of carboxylic acids, they belong to the same periodic group as carbon. Their ability to bind saccharides makes them valuable for studying biological systems and identifying metabolites associated with diabetes. As mild Lewis acids, boric acids are widely used in organic synthesis and cross-coupling reactions due to their stability and ease of handling. In addition, they serve as functional groups in anticancer, antiviral, and antibacterial agents. Halogenated boric acids have been reported to exhibit antimicrobial and antibiofilm activity against Vibrio species [4]. The biological activities of boron derivatives play a key role in the design of new therapeutic agents, largely depending on the chemical behavior of the boron atom [5]. Many boron-containing compounds are promising candidates for for the development of antimicrobial drugs. A clinically approved cyclic boronic acid derivative has been used to treat urinary tract infections by inhibiting β-lactamase enzymes, which are central to bacterial resistance [6]. Therefore, this study aims to investigate the *in vitro* antibacterial activity of phenylboronic acid (PBA) derivative against *Staphylococcus aureus*.

Staphylococcus aureus is an important opportunistic pathogen that causes a wide range of infections in humans and animals [7]. It can lead to serious morbidity and mortality through a spectrum of clinical manifestations, including skin and soft tissue infections, pneumonia, endocarditis, and sepsis [7,8]. The widespread and uncontrolled use of antibiotics has resulted in the emergence of multidrug-resistant strains. In particular, methicillin-resistant *S. aureus* (MRSA) has become one of the major causative agents of both nosocomial and community-acquired infections worldwide [9]. The pathogenicity of *S. aureus* is primarily attributed to its diverse virulence factors, which facilitate adhesion to host tissues, invasion, and toxin production [8,10]. The increasing prevalence of antibiotic-resistant isolates has significantly limited treatment options and underscored the urgent need for new antimicrobial compounds that can overcome conventional resistance mechanisms [11]. In this context, the development of molecules with mechanisms of action different from those of existing antibiotics has gained increasing attention. Sulfonamide derivatives, in particular, exhibit broad-spectrum antimicrobial activity by interfering with folate metabolism and inhibiting key enzymatic processes such as dihydropteroate synthase activity [12,13]. Structural modification of these compounds can further enhance their antimicrobial efficacy. In our previous studies, various sulfonamides, sulfonyl hydrazones, sulfa drugs, Schiff bases and their metal complexes were synthesized; their spectroscopic properties, antimicrobial, anticancer, antidiabetic activities, and enzyme inhibition effects were studied [14-22]. In the present study, novel phenyl boronic acid derivative was synthesized and characterized spectroscopic methods . The synthesized compound was then tested *in vitro* against standard strain of bacteria *S. aureus*. The newly synthesized 3.5-Tb-Bim compound evaluated in this study possesses structural features that could enhance its antibacterial potential through improved interactions with bacterial targets. Therefore, it is anticipated that this compound could be a promising candidate for the development of new antimicrobial agents effective against *S. aureus*, including drug-resistant strains.

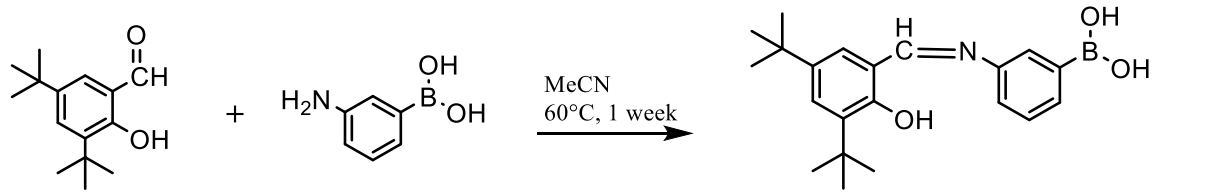
2.Materials and Methods

The starting reagents were purchased from commercial sources (Sigma-Aldrich Chemical Company (USA) and were used without further purification. The solvents were dried according to standard procedures. (3,5-ditert-butyl-2-hydroxybenzaldehyde and (3-aminophenyl)boronic acid were commercial products (Purum). All reactions were magnetically stirred and monitored by thin layer chromatography (TLC) using Merck silica gel and visualized by ultraviolet light. Melting points were determined in open capillary tubes on Buchi B-540. Proton nuclear magnetic resonance was determined with a 500-MHz Brucker Avance NEO Liquid NMR Spectrometry (Gazi Univ. Fundamental and Engineering Sciences Central Laboratory Application and Research Center – GUTMAM) using DMSO as solvent and TMS as internal standard. The

FTIR-ATR spectra were recorded (v, in cm^{-1}) using a Thermo Nicolet iD5 ATR spectrophotometer (Gazi Univ. Department of Chemistry).

2.1. General procedure for the synthesis of boron compounds

A solution of 1 mmol (3-aminophenyl)boronic acid (Bmin) in 5 ml of acetonitrile is prepared at room temperature and added to a solution of 1 mmol of the 3,5-ditert-butyl-2-hydroxybenzaldehyde (3,5Tb) in 5 ml of acetonitrile at room temperature. Organo boron compounds are synthesized by refluxing at 55-60 ° C for 1 week. Product formation during the reaction is monitored by thin-layer chromatography (TLC) using the hexane:ethyl acetate (1:1.5) solvent system. The crude product obtained at the end of the reaction is purified by crystallization and reprecipitation methods in appropriate solvent mixtures. Synthesis of the compound was carried out as seen in Figure 1



3,5-di-*tert*-butyl-2-hydroxybenzaldehyde

(3-aminophenyl)boronic acid

(3-((3,5-di-*tert*-butyl-2-hydroxybenzylidene)amino)phenyl)boronic acid

Figure.1. Synthesis of compound 3,5-Tb-Bmin

2.2 Antibacterial Activity Assay

The *in vitro* antibacterial activity of the synthesized compound was evaluated using the broth microdilution (BMD) method, as outlined in the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The Gram-positive strain *Staphylococcus aureus* ATCC 29213 was used in this study. A bacterial suspension was prepared at a final concentration of 5×10^5 CFU/mL. For bacterial growth, sterile 96-well microplates containing Mueller–Hinton broth (MHB; HiMedia Laboratories, India) were used. The compound was serially diluted in the concentration range of 10–0.02 mg/mL. Wells containing only bacteria and medium served as positive controls, whereas wells containing only medium served as negative controls. The plates were incubated at 37 °C for 24–48 h, and the minimum inhibitory concentration (MIC_{90}) value was determined as the lowest concentration of the compound that inhibited 90% of bacterial growth compared with the positive control. Sulfisoxazole and sulfamethoxazole were used as reference agents.

2.3. In silico ADME Analysis

Absorption, distribution, metabolism, and excretion (ADME) are key pharmacokinetic parameters that play a critical role in the early stages of drug discovery and development. To reduce time and cost, computational (in silico) models have been developed to predict these properties. In this study, the pharmacokinetic and drug-likeness properties of investigated boron compound was predicted using web-based tool: SwissADME [23,24].

3. Results and Discussion

The synthesis of the 3,5-Tb-Bmin compound was accomplished as described in the experimental section (Fig1). The obtained boronic acid containing compound was identified as (3-((3,5-ditert-butyl-2-hydroxybenzylidene)amino)phenyl)boronic, 3,5-Tb-Bmin). The compound was characterized using

spectroscopic techniques. The elemental analysis results (C, H, N) agreed with the theoretically calculated values, confirming the proposed molecular formulas. Furthermore, the sharp melting points observed provide additional evidence of the compound's purity. The structures of boron compound was given in Figure 1. Anal. Calcd. for $C_{21}H_{28}BNO_3$: C, 71.40; H, 7.99; N, 3.96; Found: C, 70.97; H, 7.25; N, 3.16.

3.1 Characterization of compounds

3.1.1. FT-IR spectra

Examination of the FTIR-ATR spectrum of the 3,5-Tb-Bmin compound revealed a strong band at 1621 cm^{-1} corresponding to the ν(C=N) stretching vibration, confirming the presence of the imine linkage. The broad absorption observed around 3614 cm^{-1} was attributed to the ν(O–H) stretching vibration of the $B(OH)_2$ group. The medium-intensity bands at 2955, 2906, and 2868 cm^{-1} were assigned to aliphatic ν(C–H) stretching vibrations. In addition, the bands at 1360 cm^{-1}, 1201 cm^{-1}, and 1025 cm^{-1} were attributed to ν(B–O), ν(C–O), and ν(B–C) stretching vibrations, respectively.

3.1.2. NMR spectra

The ^{1}H-NMR spectra of the 3,5-Tb-Bmin were recorded in DMSO-d_6 at room temperature. The singlet signal observed at 8.82 ppm corresponds to the imine (–CH=N–) protons, strongly confirming the successful formation of the Schiff base. The singlet signal at δ 14.11 ppm is assigned to the phenolic OH proton, while the singlet at δ 8.18 ppm corresponds to two protons of the $B(OH)_2$ group. The multiplet signals observed in the range of 6.91–7.85 ppm is attributed to aromatic protons. In addition, the strong signals at δ 1.30 and 1.39 ppm correspond to the resonance of nine protons belonging to the *t*-Bu group, indicating the presence of the aliphatic $–C(CH_3)_3$ moiety.

3.2 Biological Activity

3.2.1. Antibacterial Activity

The MIC_{90} value of the 3,5-Tb-Bim compound against *S aureus* ATCC 29213 was determined to be 1.25 mg/mL. This value indicates approximately a four-fold higher inhibitory activity compared with the reference sulfonamide agents sulfisoxazole (5.00 mg/mL) and sulfamethoxazole (5.00 mg/mL) (Figure 2). These findings demonstrate that 3,5-Tb-Bim possesses a strong inhibitory potential, particularly against Gram-positive bacteria.

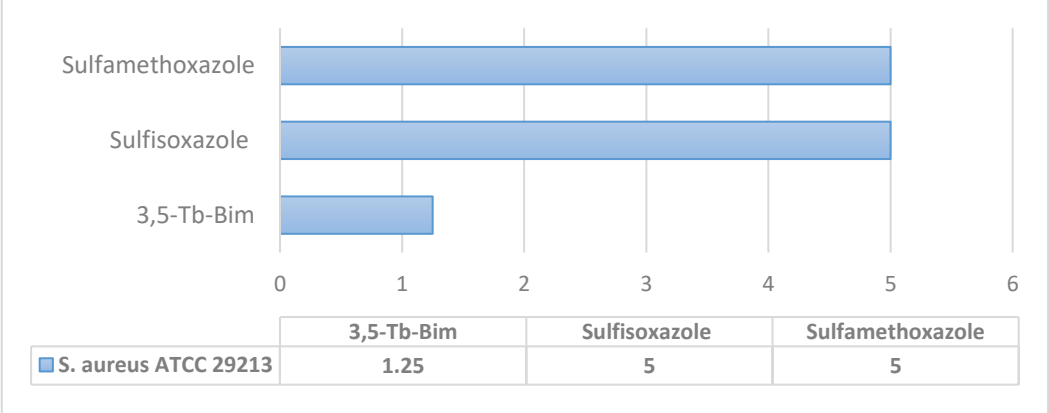


Figure 2. Minimum inhibitory concentration (MIC) values of 3,5-Tb-Bim and reference compounds against *S. aureus* ATCC 29213.

S. aureus is one of the leading pathogens in healthcare-associated infections due to its high virulence factors, biofilm-forming ability, and multidrug resistance [7-9]. The increasing prevalence of resistant strains has markedly reduced the efficacy of current antibiotics, necessitating the development of alternative therapeutic strategies. Therefore, the investigation of new compounds effective against *S. aureus* remains of significant clinical importance.

In this study, the antibacterial activity of the newly synthesized 3,5-Tb-Bim compound against *S. aureus* ATCC 29213 was evaluated using the broth microdilution method. The compound demonstrated more potent inhibitory activity than the reference sulfonamide agents, indicating that structural modification may have enhanced its antimicrobial potential. According to the assay results, the MIC_{90} value of 3,5-Tb-Bim was 1.25 mg/mL, which was approximately four times lower than that of sulfisoxazole and sulfamethoxazole. This finding suggests that the compound possesses a strong inhibitory potential, particularly against Gram-positive bacteria, and may interact effectively with cellular targets involved in bacterial growth.

Previous studies have reported that structural modification of sulfonamide derivatives can increase their affinity for dihydropteroate synthase, thereby improving antibacterial potency [12]. The present findings are consistent with these observations, suggesting that 3,5-Tb-Bim may act through similar molecular mechanisms.

In conclusion, the 3,5-Tb-Bim compound exhibited significant antibacterial activity against *S. aureus* ATCC 29213, highlighting its potential as a promising candidate for the development of new antibacterial agents targeting Gram-positive pathogens.

3.3. Drug-Likeness Assessment and ADME-Based in Silico Profiling

The bioavailability radar is a visual tool that illustrates the oral drug-likeness profile of a compound, highlighting the optimal physicochemical space (pink area). As shown in Figure 3, the synthesized compounds fall within this acceptable region by satisfying critical criteria, including flexibility, insolubility, lipophilicity, saturation, molecular size, and polarity [25].

The basic parameters used in the drug similarity analysis of the synthesized boron compound are presented in Table 1. The results boron compound with the Lipinski, Muegge, Ghose, Veber, and Egan rules [26-29]. These rule sets, each with different threshold values, represent the basic criteria for assessing the similarity of a compound to other drugs. Furthermore, the Lipinski, Veber, Egan, Ghose, and Muegge drug similarity criteria for the commonly used drugs bortezomib, ixazomib, Tavaborole, and vaborbactam were also calculated and are presented in Table 1. As presented in Table 1, the blood-brain barrier (BBB) parameter refers to the degree to which a compound can cross the central nervous system (CNS) [30,31]. In CNS-related treatments such as those for tumors or neurodegenerative diseases, a high BBB value is desirable for efficacy. Conversely, for non-CNS targets, lower BBB permeability is preferred to minimize off-target effects. This study demonstrates that the boron compound has a balanced BBB permeability profile.

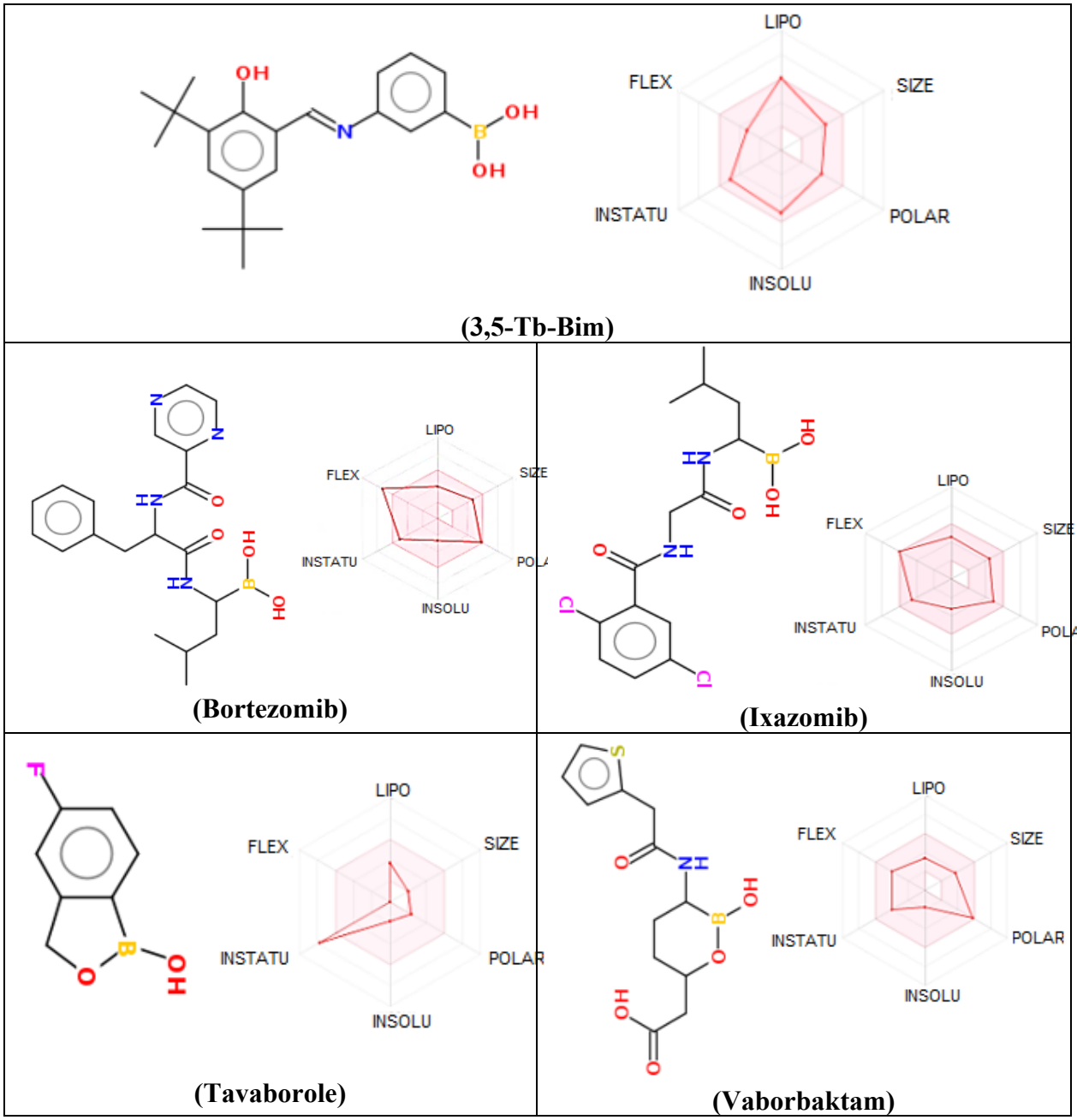


Figure 3. Radar plots related to physicochemical properties of molecules (Criteria: LIPO: -0.7 <XLOGP3< +5.0, SIZE: 150 g/mol <MW< 500 g/mol; POLAR: 20 Å^2<TPSA<130 Å^2; INSOLU: 0<log S<6; FLEX: 0< rotatable bonds < 9; INSTATU: 0.25< Csp^3 < 1).

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Properties	Compounds				
	3,5-Tb-Bim	Bortezomib	Ixazomib	Tavaborole	Vaborbaktam
Physicochemical properties					
Mol. Wt. (g/mol)	353.26	384.24	361.03	151.93	297.14
Fraction Csp3	0.38	0.37	0.43	0.14	0.50
No. of rotatable bonds	5	11	9	0	6
No. of H-bond acceptors	4	6	4	3	5
No. of H-bond donors	3	4	4	1	3
Molar refractivity	110.53	105.30	90.44	39.00	74.05
TPSA (Å²)	73.05	124.44	98.66	29.45	124.10
Lipophilicity					
Log Po/w (XLOGP3)	5.11	1.42	2.49	1.04	0.45
Log Po/w (WLOGP)	3.42	0.36	1.27	0.31	0.45
Log Po/w (MLOGP)	2.81	-0.80	1.24	0.65	-0.54
Consensus Log Po/w	2.96	0.22	1.16	0.54	0.11
Water solubility					
Log S (ESOL) /(mol/L)	-5.26 / $5.48x10^{-6}$	-2.71	-3.25	-1.84	-1.75
Class	M. Soluble	Soluble	Soluble	V. Soluble	V. Soluble
Log S (Ali) / (mol/L)	-6.39 / $4.09x10^{-7}$	-3.64	-4.21	-1.25	-2.62
Class	P. Soluble	Soluble	M. Soluble	V. Soluble	Soluble
Log S (SILICOS-IT)	-5.70/ $1.99x10^{-6}$	-4.73	-4.16	-2.14	-1.70
Class	M. Soluble	M. Soluble	M. Soluble	Soluble	Soluble
Pharmacokinetics					
GI absorption	High	High	High	High	High
BBB permeant	Yes	No	No	No	No
P-gp substrate	No	Yes	Yes	No	No
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No

CYP2C9 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	Yes	No	No	No	No
CYP3A4 inhibitor	Yes	No	No	No	No
Skinpermeation, cm/s)	-6.13	-7.64	-6.73	-6.49	-7.79
Drug-likeness					
Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
Ghose	Yes	Yes	Yes	No:3 violations:MW<160, MR<40, #atoms<20	Yes
Veber	Yes	No:1 violation: Rotors>10	Yes	Yes	Yes
Egan	Yes	Yes	Yes	Yes	Yes
Muegge	No:1 violation: XLOGP3>5	Yes	Yes	No:1 violation: MW<250	Yes
Bioavailability score	0.55	0.55	0.55	0.55	0.55
Medicinal chemistry					
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	2 heavy-metal, imalerts imin-1	1 alert heavy-metal	1 alert heavy-metal	1 alert heavy-metal	1 alert heavy-metal
Lead-likeness	No:2 violations: violations:MW>350, XLOGP3>3.5	No:2 violations:MW>350, Rotors>7	No:2 violations:MW>350, Rotors>7	No:1 violation: MW<250	Yes
Synthetic accessibility	3.47	3.61	2.80	2.77	3.62

Lipinski(Pfizer) filter: MW≤ 500; MLOGP ≤ 4.15; N or O ≤ 10; NH or OH ≤5; Ghose filter: 160 ≤ MW ≤ 480; -0.4 ≤ WLOGP ≤ 5.6; 40 ≤MR ≤130; 20 ≤ atoms ≤ 70
Veber (GSK) filter: Rotatable bonds ≤ 10; TPSA ≤ 140; Egan (Pharmacia) filter: WLOGP ≤ 5.88; TPSA ≤ 131.6
Muegge (Bayer) filter:200 ≤ MW ≤ 600; -0.2 ≤ WLOGP ≤ 5; TPSA ≤ 150; Num. Rings ≤7; Num. Carbon >4; Num. Heteroatoms > 1; Num. Otatable bonds ≤ 15; H-bond acc. ≤ 10; H-bond don. ≤ 5

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Boiled Egg diagrams were used to estimate both gastrointestinal absorption and BBB permeability (Figure 4). In these graphs, the yellow region represents compounds likely to cross the BBB. In contrast, the white area indicates the potential for strong GI absorption. Red dots (PGP−) represent compounds that are not substrates of the P-glycoprotein (P-gp) efflux transporter, while blue dots (PGP+) represent active substrates [32]. The presence of the boron compound in the yellow region, as well as the red dots, indicates a high BBB permeability potential.

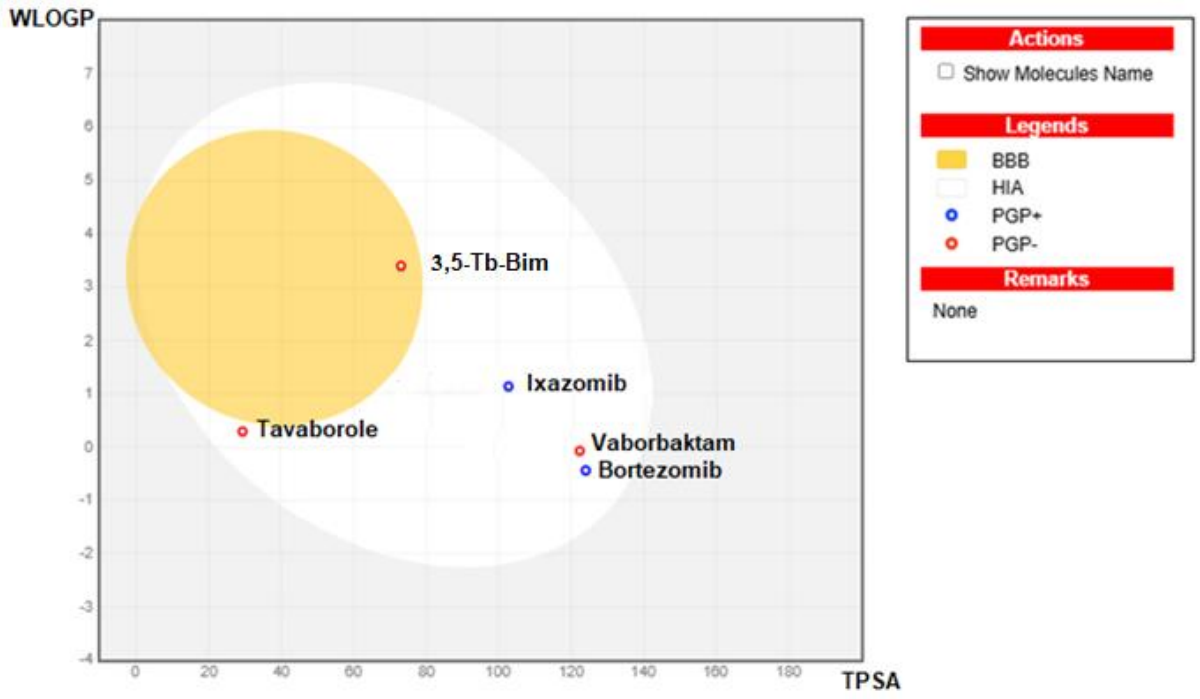

Figure 4. The BOILED-Egg plot of the predicted compound

Another crucial parameter is the inhibition of cytochrome P450 (CYP) enzymes. These enzymes, particularly CYP3A4 and CYP2D6, play a pivotal role in hepatic drug metabolism. Evaluating whether a compound is a substrate or inhibitor of these enzymes is essential for assessing the risks of drug-drug interactions. Based on Figure 4, boron compound is predicted to inhibit the CYP3A4 and CYP2D6 isoforms which may have implications for metabolism and potential drug interactions.

4. Conclusion

This study focused on the synthesis, antibacterial evaluation, and ADME analysis of phenyl boronic acid derivative against *Staphylococcus aureus*. The results demonstrated that the phenyl boronic acid derivative exhibited significant antibacterial activity, indicating its potential as an effective agent against Gram-positive bacteria. ADME analysis, which provides predictions about the pharmacokinetic behavior and drug similarity of the compound, supports that the synthesized boronic acid derivative can be a

potential lead compound for the development of new boron-based antibacterial drugs. The biocompatibility and chemical stability of phenyl boronic acid derivative further support its promise as a candidate for the development of new boron-containing antimicrobial agents. Overall, these findings highlight the potential of boronic acid derivatives as valuable scaffolds for future therapeutic applications, particularly in the design of novel drugs targeting resistant strains of *S. aureus*. However, this study has several limitations. First, the experiments were performed only on a single reference strain, and further validation with clinical isolates and Gram-negative bacteria is necessary. Additionally, the assays were conducted under *in vitro* conditions, and the *in vivo* efficacy and toxicity profile of the compound have not been examined. Moreover, the study focused solely on the antibacterial effects, without assessing its potential antibiofilm activity, which could provide additional insight into its therapeutic relevance. These limitations provide a foundation for future *in vivo* and mechanistic studies aimed at elucidating the antibacterial and possible antibiofilm properties of 3,5-Tb-Bim.

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