Abstract: Rapidly growing antimicrobial resistance among clinically important bacterial and fungal pathogens accounts for high morbidity and mortality worldwide. Therefore, it is critical to look for new small molecules targeting multidrug-resistant pathogens. Herein, in this paper we report a synthesis, ADME properties, and in vitro antimicrobial activity characterization of novel thiazole derivatives bearing β-amino acid, azole, and aromatic moieties. The in silico ADME characterization revealed that compounds 1–9 meet at least 2 Lipinski drug-like properties while cytotoxicity studies demonstrated low cytotoxicity to Vero cells. Further in vitro antimicrobial activity characterization showed the selective and potent bactericidal activity of 2a–c against Gram-positive pathogens (MIC 1–64 µg/mL) with profound activity against S. aureus (MIC 1–2 µg/mL) harboring genetically defined resistance mechanisms. Furthermore, the compounds 2a–c exhibited antifungal activity againstazole resistant A. fumigatus, while only 2b and 5a showed antifungal activity against multidrug resistant yeasts including C. auris. Collectively, these results demonstrate that thiazole derivatives 2a–c and 5a could be further explored as a promising scaffold for future development of antifungal and antibacterial agents targeting highly resistant pathogenic microorganisms.

Keywords: azole; thiazoles; β-amino acids; antimicrobial activity

1. Introduction

Increasing antimicrobial resistance (AR) results in high morbidity and mortality worldwide [1]. The highest impact of AR is often more evident in developing countries, although AR remains a major global healthcare challenge. The World Health Organization in 2015 released the action plan aimed to fight the antimicrobial resistance among bacterial and fungal pathogens, although despite the efforts the resistance remains one of the leading threats [1,2]. Therefore, it is crucial to explore novel scaffolds leading to the development of future antimicrobial compounds.

Heterocycles being the widest division of organic chemistry occupy a unique place not only in nature but provide large-scale biologically active synthetic compounds with wide variety of properties and this number is permanently increasing. The majority of pharmaceuticals are from four-, up to eight-membered heterocycles or their fused derivatives with one or more the same or different heteroatoms in the structure. Straightforward synthesis...
and the large scale of biological properties of heterocyclic compounds is the best offer for
the designing and development of the biologically active molecules [3].

The importance of heterocycles is well illustrated by their presence in many natural
drugs such as atropine, quinine, theophylline, reserpine, papaverine, morphine, lodopyridone, and many others [4–6], and green pigment chlorophyll, hemoglobin, antibiotic penicillin are heterocyclic compounds.

One or several nitrogen heteroatoms containing cycles have been largely described to have many pharmacological actions such as anticancer [7], antiviral [8], anti-inflammatory [9], antidiabetic [10], antihypertensive [11], antitubercular [12], bronchodilator, antimicrobial [13], anticonvulsant [14], are also used in the treatment of Alzheimer’s disease [15] etc.

Oxygen-containing heterocycles as those of the nitrogen analogues are widely present in various kinds of natural products, such as carbohydrates, polyketides, peptides, and terpenoids, which show large potential of diverse pharmacological properties [16–22]. Nature-derived O-heterocycle pharmaceuticals salinomycin and Taxol are used to treat cancer, artemisinin is effective antimalarial agent, digoxin is a cardiac glycoside, and has inotropic effects in addition to effects on cardiac output, and codeine and morphine are opioid drugs [23].

Other heterocycles, especially S-heterocycles, are no less attractive than N-analogues. Those derivatives are known to possess anticancer, anti-diabetic, anti-depressant, bronchodilator, anti-platelet, diuretic, anti-inflammatory, antiviral, anti-ulcer, and many other properties [24,25]. The high volatility and reactivity of sulfur determines that many sulfur-containing compounds also are used in flavoring of food products [26].

The importance of N-, O-, and S-containing heterocyclic compounds is obvious; however, the value of other heterocycles cannot be ruled out. Organoselenium and organophosphorus compounds indicate the power in the construction of molecules of medicinal interest and represent an important class of compounds with large potential for pharmaceutic applications [27,28].

Azoles have been long known as an important core in designing of various pharmaceutica l agents. They can also be used as intermediates for the synthesis pharmaceuticals, for example, ambrisentan, a drug for the treatment of pulmonary hypertension [29]. The synthetic azole antimycotics constitute the largest group of antifungal agents currently widely used in clinical practice [30]. The antifungal activity of azoles against clinically important fungi is the fungal cell wall directed and mediated by the inhibition of ergosterol synthesis [31]. Due to strong and broad-spectrum antifungal activity,azole antifungal drugs are a first-line choice for the treatment and prevention of invasive fungal infections.

The overuse of azoles in the clinical field as well as the use of azole moieties containing herbicides in agriculture lead to the development of antifungal resistance among the clinical and environmental strains [32]. Therefore, novel compounds with selective antifungal activity and good tolerability are needed to overcome the rapidly growing problem.

Various antifungal azoles such as miconazole, clotrimazole, and ketoconazole demonstrate fungistatic activity. Azoles can inhibit fungal growth and virulence but not directly target the viability. Therefore, azoles alone are often used to treat superficial mycoses [30]. Newer generation of azole containing antifungals was developed and successfully demonstrated to be active against systemic fungal infections using both animal models and clinical trials [33]. Systemic triazoles such as fluconazole, itraconazole, isavuconazole, posaconazole, and voriconazole were developed and used to treat infections caused by Candida, Aspergillus, and other yeasts and mold [34]. Despite that, increasing resistance and resistance-associated treatment failures are now often observed [35–37].

Azole moiety-containing compounds also have promising anticonvulsant [38], antimicrobial [39–42], antiurease [43], anti-inflammatory [44], and antioxidant [45], as well as analgesic [46] properties. The combining of steroid with azole pharmacophore generated potential lead compounds with superior anticancer properties [47].

The various azole-based molecules, such as benzimidazole, imidazoles, pyrazole, triazole, thiazole, and others with a wide variety of functionalizations and coordination modes, can be an effective tool for the preparation of metal complexes [48]. Azoles in
combination with metals provide a new alternative of efficient drugs, even against drug-resistant pathogens [49–51]. Although the complexes may demonstrate different antimicrobial activity [52–54], anticancer activity of such compounds is more predominant. Therefore, it is crucial to consider various pharmacological, excretion, metabolism, and toxicity properties while designing novel compounds [55].

Profound biological activity ofazole derivatives makes them as attractive building blocks to develop novel antimicrobial candidates for the further pre-clinical development [55–60]. In this paper we describe the synthesis and in vitro antimicrobial activity characterization of novel thiazole derivatives bearing N-acyl hydrazone, pyrrole, pyrazole, and triazole.

2. Results

2.1. Synthesis

In this study, the prepared compound 1 [61,62] was used as a starting precursor for the preparation of thiazoles 2a–c by the Hantsch method by combining thioureido acid 1 with the corresponding acetoephones (Scheme 1). The structures of the obtained compounds 2 were confirmed by the data of the 1H and 13C NMR spectra (Supplementary material, Figures S3–S8). Using the reactivity of carboxylic group, the esterification of compound 2 and subsequent hydrazinolysis of the resulting ester 3 was performed. In the NMR spectra for ester 3, proton signals of the methoxy group are visible as an intense singlet at 3.54 ppm (1H), and signal of carbon of the same group resonate at 51.43 (13C) ppm. The spectra of the obtained hydrazide 4 showed the characteristic signals for the CONHNH2 fragment – two singlets at 9.10 and 4.17 ppm (1H), respectively, and the resonance line at 170.03 ppm for carbon of the C=O group (13C).

Scheme 1. Synthesis of thiazoles 2–5. 2a, Ar = 4-FC6H4; 2b, Ar = 4-ClC6H4; 2c, Ar = 4-BrC6H4; 5a, R = C6H5; 5b, R = 4-FC6H4; 5c, R = 4-ClC6H4; 5d, R = 4-BrC6H4; 5e, R = 4-O2NC6H4; 5f, R = 4-Me2NC6H4; 5g, R = 4-MeC6H4; 5h, R = thien-2-yl; 5i, R = 5-nitrothien-2-yl; 5j–k, R1 = H; 5j, R = R1 = Me; 5k, R = Et, R1 = Me. Reagents and conditions: (a) 10% Na2CO3, MeOH, 2-bromo-4'-substituted acetoephene, Δ, 2 h (a,b), 4 h (c), 30% AcOH to pH 6, 68–88%; (b) MeOH, H2SO4, Δ, 5 h, 10% Na2CO3, 80%; (c) N2H4, H2O, 2-PrOH, Δ, 18 h, H2O, 73%; (d) RCHO, AcOH, 2-PrOH, Δ, 2 h (5a–i), Me2CO (5j) or MEK (5k), Δ, 5 h, 68–95%.

Then the obtained hydrazide 4 was used as a starting material for the synthesis of hydrazones 5 and azoles 6, 7, as well as 9, which was prepared through the thiosemicarbazide intermediate 8 (Scheme 2). Condensation of compound 4 with aromatic aldehydes or carbaldehydes in propan-2-ol and using a catalytic amount of acetic acid led to the formation of N'-benzylidene hydrazides 5a–i, while heating of hydrazide 4 at reflux with acetone or 2-butanol (MEK) yielded N’-(propan- or butan-2-ylidene)propaneydrazides 5j–k. The stereochemistry of the synthesized hydrazones was ascribed by 1H NMR spectra.
The NMR spectra of 5a–g exhibited double sets of signals of the CONH group protons which is caused by a restricted rotation around the amide bond. The splitting of the proton signals indicates the formation of the Z/E isomers mixture in DMSO-d₆ solutions, where usually Z-form predominates [63]. The intense ratio of the signals of the isomers was found to be 65:35. For compounds 5h, i, this ratio was observed 55:45 and 60:40, respectively. It is noteworthy that the substitution at the azomethine fragment in compounds 5j, k resulted in the formation of a mixture of geometrical isomers, the presence of which is clearly reflected in the ¹H NMR spectra of the compounds [64] (Supplementary Material, Figures S31–S34). Accordingly, hydrazones 5j, k in DMSO-d₆ solutions exist as a mixture of geometrical isomers and amide conformers. The ¹H NMR spectrum of derivative 5k (Supplementary Material, Figure S33) showed the splitting of the characteristic signals of the ethyl and methyl protons in the intervals of 0.76–1.01, 2.11–2.24 ppm for CH₃CH₂, and singlet peaks at 1.79, 1.83, and 1.87 ppm for CH₃ which were integrated for 3, 2, and 3 protons, respectively, as well as NH protons of the amide bond resonated as four singlets at 9.99, 10.07, 10.12, and 10.16 ppm which were totally integrated for 1 proton assigned for the amide (CONH) proton.

Scheme 2. Synthesis of azoles 6–9. Reagents and conditions: (a) 2-PrOH, 2,4-PD (dropwise), HCl, Δ, 9 h, 74%; (b) 2-PrOH, 2,5-HD (dropwise), AcOH, Δ, 3 h, 80%; (c) MeOH, PhNCS (dropwise), Δ, 2 h, H₂O, 93%; (d) 4% NaOH, Δ, 3 h, AcOH to pH 6, 83%.

In the next step of the study, considering the immense biological properties of various azoles, we chose to obtain several five-membered heterocyclic derivatives and to evaluate their biological properties. Condensation of hydrazide 4 with the corresponding diketone—2,4-pentanedione (2,4-PD) and 2,5-hexanedione (2,5-HD) catalyzed by hydrochloric or acetic acid, respectively, gave 3,5-dimethylpyrazole derivative 6 and 2,5-dimethylpyrrole 7 (Scheme 2). The formation of the appropriate desired ring was approved by the NMR spectra of these compounds. Characteristic intense singlets at 2.16, 2.36, and 6.26 ppm (¹H) and resonances at 15.79, 15.85, and 110.10 (¹3C) were assigned to the protons and carbons of the methyl groups and the CH fragment of the pyrazole cycle for compound 6, respectively. The presence of a pyrrole ring was proven by the signals at 1.94, 10.91, and 11.02 ppm (¹H, ¹3C, 2CH₃) as well as 5.61 and 102.90 ppm (¹H, ¹3C, CH-CH₃) which are typical for such a structure.

Triazole derivative 9 was obtained in a two-step manner, by addition of phenyl isothiocyanate to a solution of hydrazide 4 in methanol to give carbothioamide 8, which
was then subjected to a cyclization step in basic conditions (4% NaOH aqueous solution) to afford 1,2,4-triazole-3-thione compound 9 in an 83% yield. In the $^{13}$C NMR spectrum of a linear structure, compound 8 carbon signal of the C=S fragment resonated at 180.82 ppm, while in the spectrum of a cyclic-form 9, the resonance line of the C=S was observed upfield (167.90 ppm). Noteworthy, in the $^1$H NMR spectrum of triazole 9, the singlet of NH group proton was characteristically shifted downfield (13.70 ppm) in comparison with the spectrum of non-cyclic compound 8.

All the compounds were characterized by the $^1$H, $^{13}$C NMR, and IR spectroscopy and the data of elemental analysis. The NMR spectra are presented in the Supplementary material, Figures S1–S42.

2.2. Characterization of Absorption, Distribution, Metabolism, and Excretion (ADME) Properties

Generating poly-bioactive compounds with favorable bioavailability is paramount in medicinal chemistry. After successfully synthesizing and characterizing the compounds, we used in silico prediction tool based on Lipinski drug-likeness rule of 5 to characterize the ADME properties of the compounds 1–9 [65–67]. The compounds were considered as drug-like when they met the following criteria: lipophilicity: XLOGP3 between $-0.7$ and $+5.0$, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å$^2$, solubility: log $S$ not higher than 6, saturation: fraction of carbons in the sp$^3$ hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds. We first evaluated the physicochemical properties on compounds 1–9 (Table 1). Based on physicochemical properties, most of the analyzed compounds met at least 2 rules of drug-likeness (Table 1).

| Compound | MW     | No of Heavy Atoms | No of Aromatic Heavy Atoms | Fraction Csp$^3$ | Rotatable Bonds | H-Bond Acceptors | H-Bond Donors | Molar Refractivity | TPSA |
|----------|--------|-------------------|---------------------------|-----------------|----------------|------------------|---------------|-------------------|------|
| 1        | 315.39 | 22                | 12                        | 0.12            | 7              | 2                | 3             | 92.08             | 110.68 |
| 2a       | 433.5  | 31                | 23                        | 0.08            | 8              | 4                | 2             | 122.88            | 93.7  |
| 2b       | 449.95 | 31                | 23                        | 0.08            | 8              | 3                | 2             | 127.93            | 93.7  |
| 2c       | 494.4  | 31                | 23                        | 0.08            | 8              | 3                | 2             | 130.62            | 93.7  |
| 3        | 463.98 | 32                | 23                        | 0.12            | 9              | 3                | 1             | 132.25            | 82.7  |
| 4        | 463.98 | 32                | 23                        | 0.08            | 9              | 3                | 3             | 131.87            | 111.52 |
| 5a       | 552.09 | 39                | 29                        | 0.06            | 11             | 3                | 2             | 162.14            | 97.86 |
| 5b       | 570.08 | 40                | 29                        | 0.06            | 11             | 4                | 2             | 162.1             | 97.86 |
| 5c       | 586.53 | 40                | 29                        | 0.06            | 11             | 3                | 2             | 167.15            | 97.86 |
| 5d       | 630.99 | 40                | 29                        | 0.06            | 11             | 3                | 2             | 169.84            | 97.86 |
| 5e       | 597.09 | 42                | 29                        | 0.06            | 12             | 5                | 2             | 170.97            | 143.68 |
| 5f       | 595.16 | 42                | 29                        | 0.12            | 12             | 3                | 2             | 176.35            | 101.1 |
| 5g       | 566.12 | 40                | 29                        | 0.09            | 11             | 3                | 2             | 167.11            | 97.86 |
| 5h       | 558.12 | 38                | 28                        | 0.07            | 11             | 3                | 2             | 160.02            | 126.1 |
| 5i       | 603.11 | 41                | 28                        | 0.07            | 12             | 5                | 2             | 168.84            | 171.92 |
| 5j       | 504.05 | 35                | 23                        | 0.15            | 10             | 3                | 2             | 147.27            | 97.86 |
| 5k       | 518.07 | 36                | 23                        | 0.18            | 11             | 3                | 2             | 152.08            | 97.86 |
| 6        | 528.07 | 37                | 28                        | 0.14            | 9              | 3                | 1             | 153.08            | 91.29 |
| 7        | 542.09 | 38                | 28                        | 0.13            | 10             | 2                | 2             | 158.85            | 90.43 |
| 8        | 599.17 | 41                | 29                        | 0.06            | 13             | 2                | 4             | 173.39            | 141.65 |
| 9        | 581.15 | 40                | 34                        | 0.06            | 9              | 2                | 2             | 168.15            | 122.1 |

Abbreviations: TPSA—total polar surface area, MW—molecular weight.

Compounds 1–9 demonstrated good drug-like properties based on the numbers of hydrogen bond acceptors and donors (Table 1) while only compounds 1–2c met molecular refractivity criteria.
After characterizing the physicochemical properties of the synthesized compounds, we further determined the pharmacokinetic, excretion, and metabolism profiles of compounds 1–9 (Table 2).

### Table 2. Pharmacokinetic and drug excretion properties of thiazole derivatives bearing β-amino acid and aromatic moieties.

| Compound | Log Kp (cm/s) | GI Absorption | BBB Permeant | P-Gp Substrate | Inhibition of Cytochrome P450 System |
|----------|---------------|---------------|--------------|----------------|-------------------------------------|
|          |               |               |              |                | CYP1A2 | CYP2C19 | CYP2C9 | CYP2D6 | CYP3A4 |
| 1        | −6.56         | High          | No           | No             | No | No | Yes | Yes | No | No |
| 2a       | −4.85         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 2b       | −4.57         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 2c       | −4.8          | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 3        | −4.43         | Low           | Yes          | No             | No | Yes | Yes | Yes | Yes |
| 4        | −5.37         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5a       | −4.21         | Low           | No           | No             | No | Yes | No | Yes | Yes |
| 5b       | −4.25         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5c       | −3.98         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5d       | −4.21         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5e       | −4.61         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5f       | −4.39         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5g       | −4.04         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5h       | −4.24         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5i       | −4.4          | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 5j       | −4.94         | Low           | No           | Yes            | No | Yes | Yes | Yes | Yes |
| 5k       | −4.7          | Low           | No           | Yes            | No | Yes | Yes | Yes | Yes |
| 6        | −4.25         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 7        | −4.22         | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 8        | −4.6          | Low           | No           | No             | No | Yes | Yes | Yes | Yes |
| 9        | −4.14         | Low           | No           | No             | No | No | No | Yes | Yes |

Abbreviations: log Kp (cm/s)—skin permeability; P-gp—P glycoprotein 1; BBB—brain–blood barrier; GI—gastrointestinal; CYP1A2—cytochrome P450 1A2; CYP2C19—cytochrome P450 2C19; CYP2C9—cytochrome P450 2C9; CYP2D6—cytochrome P450 2D6; CYP3A4—cytochrome P450 3A4.

All compounds demonstrated good, predicted skin permeability suggesting the possibility to use the most potent compounds as possible topical antimicrobial agents. All compounds except thioureido acid were predicted to be non-absorbable in the gastrointestinal tract.

P glycoprotein 1-mediated drug transport is an important parameter often limiting cellular drug intake. Compounds 3 (ester), 5j (aliphatic hydrazone), and 5k (aliphatic hydrazone) were predicted to be a substrates of P glycoprotein 1. Finally, structure-dependent interactions with cytochrome metabolism system components were predicted for the novel thiazole derivatives bearing β-amino acid and aromatic moieties. Further in vitro and in vivo studies are needed to validate the in silico ADME results.

### 2.3. Antimicrobial Activity of Compounds 1–9

After characterizing in silico ADME properties of the compounds 1–9 and demonstrating the good skin permeability of the compounds, we further hypothesized weather the compounds could be used as topical antimicrobials targeting skin colonizing multidrug-resistant microbial pathogens.

We used a panel of bacterial (Staphylococcus aureus, Enterococcus spp., Klebsiella pneumoniae, Stenotrophomonas maltophilia, Pseudomonas aeruginosa) and fungal (Candida auris, Candida albicans, Aspergillus fumigatus) pathogens to evaluate the antimicrobial activity of the thiazole derivatives bearing β-amino acid and aromatic moieties.

First, we screened our compounds on Gram-positive and Gram-negative bacterial pathogens harboring genetically defined resistance mechanisms. Only compounds 2a–c demonstrated an antimicrobial activity against Gram-positive bacterial pathogens. The
highest antimicrobial activity was observed against S. aureus (MIC 1–64 µg/mL) while weak antibacterial activity (MIC 32–64 µg/mL) was observed when Enterococcus spp. were used for the assay (Table 3). Compounds 2a (4F-C₆H₄) and 2b (4Cl-C₆H₄) demonstrated the highest antibacterial activity against S. aureus with genetically defined resistance mechanisms (MIC 1–2 µg/mL), moreover compound 2a was also efficient against multidrug-resistant Enterococcus spp. (MIC 32–64 µg/mL).

Table 3. The antibacterial activity expressed as minimal inhibitory concentration (MIC, µg/mL) of compounds 1–9 and comparator drugs (vancomycin, daptomycin, and meropenem) against panel of multidrug-resistant Gram-positive and Gram-negative bacterial pathogens harboring different resistance profiles.

| Compound | Gram-Positive | Grammyegative |
|----------|---------------|---------------|
|          | S. a MRSA | S. a MSSA | S. a VRSA | E. fec | E. fae VREF | E. raffi | K. pn NDM-1 | S. m R | P. ae AmpC |
| 1        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 2a       | 2 | 2 | 2 | 32 | 32 | 64< | 64< | 64< | 64< |
| 2b       | 64 | 32 | 32 | 32 | 32 | 64< | 64< | 64< | 64< |
| 3        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 4        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5a       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5b       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5c       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5d       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5e       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5f       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5g       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5h       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5i       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5j       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 5k       | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 6        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 7        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 8        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| 9        | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< | 64< |
| Vancomycin | 2 | 0.5 | 64< | 0.5 | 32 | 32 | n.a | n.a | n.a |
| Daptomycin | 0.5< | 0.5< | 0.5 | 1 | 2 | 2 | n.a | n.a | n.a |
| Meropenem | 4 | 1 | 4 | n.a | n.a | 32 | 16 | 16 | 16 |

Abbreviations: S. a MRSA—MRSA Staphylococcus aureus USA 300 TCH-1516, S. a MSSA—Staphylococcus aureus ATCC 29212, S. a VRSA Staphylococcus aureus USA 100 DE-11, E. fec—Enterococcus faecalis ATCC 29212, E. fae—Enterococcus faecium ATCC 51299 VREF, E. raff—Enterococcus raffinosus, K. pn NDM-1—Kp. pneumoniae NDM-1 producing strain, S. m—Multidrug-resistant Stenotrophomonas maltophilia, P. ae AmpC—Inducible AmpC overexpressing Pseudomonas aeruginosa, n.a.—not applicable.

Notably, the antimicrobial activity of compounds 2a, b against methicillin resistant S. aureus (MRSA) was similar to vancomycin (MIC 2 µg/mL). Strikingly, 2b demonstrated profound activity against vancomycin resistant S. aureus (Table 3). Furthermore, none of the compounds exhibited antimicrobial activity against any Gram-negative bacterial pathogen that was used for the study, suggesting that the novel thiazole derivatives bearing 4-CI-C₆H₄ and 4-FC-C₆H₄ substitutions are critical for the selective, Gram-positive bacteria directed antibacterial activity. Numerous thiazoles have been previously explored as antifungal agents [68–70]. Therefore, we further evaluated the antifungal activity of compounds 1–9 against azole resistant A. fumigatus harboring L98H, TR34 and F495I, L98H, S297T, and TR34 mutation in CYP51A protein conferring resistance to azole antifungals [71,72].

Compounds 2a–c demonstrated the structure-dependent antifungal activity on azole-resistant A. fumigatus while low antifungal activity was observed on wild type (Wt), pan-susceptible A. fumigatus (Table 4). These results suggest that thiazole derivatives might be targeting upstream or downstream of mutated CYP51A pathway, due to the lack of susceptible phenotype in Wt A. fumigatus strain with pan-susceptible phenotype. Therefore 2a–c scaffold could be potentially further optimized to generate the compounds targeting azole-resistant A. fumigatus.
Table 4. The antifungal activity expressed as minimal inhibitory concentration (MIC, µg/mL) of compounds 1–9 and comparator drugs (voriconazole, itraconazole) against azole-resistant *Aspergillus fumigatus* with genetically defined resistance mechanisms.

| Compound | *A. fumigatus* Azole Resistance Phenotype |
|----------|----------------------------------------|
|          | L98H, TR34 | F495I, L98H, S297T, TR34 | Wt |
| 1        | 64<       | 64<       | 64< |
| 2a       | 32        | 32        | 64< |
| 2b       | 32        | 32        | 64< |
| 2c       | 64        | 64<       | 64  |
| 3        | 64<       | 64<       | 64< |
| 4        | 64<       | 64<       | 64< |
| 5a       | 64<       | 64<       | 64< |
| 5b       | 64<       | 64<       | 64< |
| 5c       | 64<       | 64<       | 64< |
| 5d       | 64<       | 64<       | 64< |
| 5e       | 64<       | 64<       | 64< |
| 5f       | 64<       | 64<       | 64< |
| 5g       | 64<       | 64<       | 64< |
| 5h       | 64<       | 64<       | 64< |
| 5i       | 64<       | 64<       | 64< |
| 5j       | 64<       | 64<       | 64< |
| 5k       | 64<       | 64<       | 64< |
| 6        | 64<       | 64<       | 64< |
| 7        | 64<       | 64<       | 64< |
| 8        | 64<       | 64<       | 64< |
| 9        | 64<       | 64<       | 64< |

Voriconazole | 8 | 1 | 0.5 |
Itraconazole  | 2 | 16< | 1 |

We further decided to step forward and evaluate the in vitro antifungal activity of compounds 1–9 against extensively resistant strains of *Candida* including multidrug-resistant priority pathogen *Candida auris* (Table 5) [73,74].

When compounds 1–9 were screened against azole resistant *Candida auris, C. duobushaemulonii, C. krusei*, and *C. albicans*, only compounds 2b and 5a exhibited antifungal activity against multidrug-resistant yeasts.

Compound 2b showed antifungal activity against *C. auris* and *C. duobushaemulonii* but not *C. krusei* or *C. albicans* suggesting that the 4-ClC₆H₄ substituent is critical for the antifungal activity against *C. auris*. Interestingly, the addition of phenyl substituent (compound 5a) resulted in decreased antifungal activity against *C. auris* and *C. duobushaemulonii* in comparison to compound 2b but restored activity against *C. krusei* (Table 5).

Collectively, these data demonstrate the wide spectrum antimicrobial activity of compounds 2a–c and 5a. The structure–activity relationship study (SAR) revealed that the 4-ClC₆H₄ and the 4-FC₆H₄ substituents are critical for the antibacterial and antifungal activity, while additional substitution in phenyl ring can affect the antifungal potency and species spectrum against clinically important and multidrug-resistant yeasts.
Table 5. The antifungal activity expressed as minimal inhibitory concentration (MIC, \(\mu g/mL\)) of compounds 1–9 and comparator drugs (fluconazole, itraconazole) of 1–9 against extensively multidrug-resistant Candida spp.

| Compound | C. auris 381 | C. auris 382 | C. auris 383 | C. auris 384 | C. duobushaemulonii 394 | C. krusei 397 | C. albicans 1214 |
|----------|--------------|--------------|--------------|--------------|-------------------------|-------------|----------------|
| 1        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 2a       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 2b       | 32           | 32           | 32           | 32           | 16                       | \(64<\)    | \(64<\)        |
| 2c       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 3        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 4        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5a       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5b       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5c       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5d       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5e       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5f       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5g       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5h       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5i       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5j       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 5k       | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 6        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 7        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 8        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| 9        | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)      | \(64<\)                  | \(64<\)    | \(64<\)        |
| Fluconazole | 4          | 16           | 64<          | 64<          | 8                        | 64          | 32             |
| Itraconazole | 0.125     | 0.5          | 0.5          | 0.5          | 1                        | 1           | 1              |

2.4. The Cytotoxicity Evaluation of Compounds 1–9

After characterizing the in vitro antimicrobial activity of we further characterized the in vitro cytotoxic activity of compounds 1–9 and compared it to the cytotoxicity of two clinically approved drugs used for treatment of the infections caused by Gram-positive pathogens. We used non-cancerous Vero African green monkey kidney cells to measure the cytotoxicity of the compounds 1–9 as well as vancomycin (Van) and daptomycin (Dap) that served as a control. The compounds 1–9 demonstrated the structure-dependent cytotoxic activity on Vero cells, although the compound induced toxicity was similar or lower than comparator drugs, that was comparable to the activity of vancomycin and daptomycin (Figure 1). Vancomycin (Van) exhibited the highest cytotoxic activity on Vero cells, by reducing the viability to 56% after 48-h exposure (Figure 1). Daptomycin demonstrated lower cytotoxicity to Vero cells with resulting 76.7% viability after 48-h exposure.

![Figure 1](image_url)  
Figure 1. The cytotoxicity evaluation of compounds 1–9 in Vero African green monkey kidney cells. The Vero cells were exposed with fixed (100 \(\mu g/mL\)) concentration of each compound or vancomycin (Van) and daptomycin (Dap) that served as a comparator drug. The cells were incubated with test compounds for 48 h and the resulting viability was measured using MTT assay. Data provided in the graphs are mean ± SD from 3 experimental replicas.
The compound 5b bearing 4-fluorophenyl substituent demonstrated the highest cytotoxicity to Vero cells by decreasing the viability to 54.8%. Notably, the most promising compounds 2a–c exhibiting good antimicrobial activity demonstrated similar or lower cytotoxicity in comparison to Van or Dap (Figure 1). The 48-h exposure with compound 2a resulted in 61.2% viability while compounds 2b and 2c resulted in 75.1% and 72.3% viability respectively (Figure 1). These results demonstrate that the most promising compounds 2a–c are exerting similar or lower cytotoxicity profiles than standard care drugs (Van or Dap).

2.5. The Compounds 2a–c Demonstrates Bactericidal Activity on Staphylococcus aureus with Genetically Defined Resistance Mechanisms

We further characterized the S. aureus-directed in vitro antimicrobial activity of the most promising compounds 2a–c against representative strains with genetically defined resistance mechanisms. By using the time kill assay, we characterized the mode and kinetics of compounds-mediated S. aureus killing in vitro by exposing the bacteria at MIC concentration of the compounds or control drug (vancomycin; Van) (Figure 2). The time-kill assay demonstrated that compounds 2a–c can rapidly kill the S. aureus in comparison to untreated control (UC) (p < 0.05). The compounds did not demonstrate the killing dependence on already pre-existing S. aureus resistance mechanisms. The most potent compound 2a bearing the 4-fluorophenyl moiety demonstrated the highest bactericidal activity against S. aureus and was able to rapidly kill MSSA, MRSA, and VRSA in 6 h, suggesting the importance of 4-fluorophenyl substitution. The 2a bactericidal activity against MSSA and MRSA was comparable to vancomycin (p < 0.05) (Figure 2). Moreover, the compound 2a exhibited a significantly better bactericidal activity against VRSA in comparison to vancomycin (p < 0.05). Compound 2b was able to completely kill the MSSA in 24 h but did not fully cleared MRSA and VRSA S. aureus. The compound 2c demonstrated the time-depended antimicrobial activity against all S. aureus strains but failed to fully clear the viable bacteria. These data demonstrate that the in vitro antimicrobial activity of novel thiazole derivatives 2a, b is mostly bactericidal and highly depended on the halophenyl substitution.

Figure 2. The in vitro time-kill studies of most potent compounds 2a–c against Staphylococcus aureus harboring genetically defined resistance mechanisms. The representative S. aureus strains (MRSA, MSSA, VRSA) were exposed at MIC concentration of compounds 2a–c or vancomycin for 24 h. The viable bacterial numbers in treatment groups or untreated control (UC) were calculated after dilution and plating. Data provided in the graphs are mean ± SD from 3 experimental replicas.

2.6. The Activity of the Compounds 2a–c on the Staphylococcus aureus Biofilm Integrity

We further hypothesized that rapid killing effect of the most promising compounds 2a–c could be further explored as a S. aureus biofilm disrupting strategy. We exposed the S. aureus biofilms with increasing concentrations of compounds 2a–c or vancomycin (Van) for 24 h and evaluated the effect on S. aureus biofilm integrity by using crystal violet assay. The compound 2a, b was able to disrupt the S. aureus biofilm integrity at the MIC or 4X MIC concentrations for all S. aureus strains. The compound 2a demonstrated the highest biofilm disrupting activity that was comparable to vancomycin (Figure 3). Collectively, these results demonstrate that novel thiazole derivatives 2a–c can alter S. aureus biofilm
integrity, while compound 2a demonstrates the highest biofilm disrupting activity that is comparable or greater to vancomycin (Figure 3).

Figure 3. The activity of compounds 2a–c on the Staphylococcus aureus biofilm integrity. The S. aureus biofilms were exposed with increasing concentrations of compounds 2a–c or vancomycin (Van) for 24 h. After exposure with the test compounds, the biofilm integrity was evaluated by crystal violet assay. Data provided in the graphs are mean ± SD from 3 experimental replicas.

3. Materials and Methods

3.1. Synthesis

Reagents and solvents were obtained from Sigma–Aldrich (St. Louis, MO, USA) and used without further purification. The reaction course and purity of the synthesized compounds were monitored by TLC using aluminum plates precoated with Silica gel with F254 nm (Merck KGaA, Darmstadt, Germany). Melting points were determined with a B-540 melting point analyzer (Büchi Corporation, New Castle, DE, USA) and were uncorrected. NMR spectra were recorded on a Bruker Avance III (400, 101 MHz) spectrometer (Bruker BioSpin AG, Fällanden, Switzerland). Chemical shifts were reported in (δ) ppm relative to tetramethylsilane (TMS) with the residual solvent as internal reference (DMSO-δ6, δ = 2.50 ppm for 1H and δ = 39.5 ppm for 13C). Data were reported as follows: chemical shift, multiplicity, coupling constant (Hz), integration, and assignment. IR spectra (ν, cm⁻¹) were recorded on a Perkin–Elmer Spectrum BX FT–IR spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) using KBr pellets. Elemental analyses (C, H, N) were conducted using the Elemental Analyzer CE-440 (Exeter Analytical, Inc., Chelmsford, MA, USA); their results were found to be in good agreement (±0.3%) with the calculated values.

3-(1-(4-(phenylamino)phenyl)thioureido)propanoic acid (1). A solution of thioxo tetrahydro pyrimidinone [35] (0.1 mol, 29.7 g) in aqueous 10% sodium hydroxide (200 mL) was boiled, then left to cool down to room temperature, filtered off, and the obtained filtrate was acidified with acetic acid to pH 6. The formed precipitate was filtered off, washed with water, and dried to give the title compound 1 (white solid, yield 20.2 g (64%), m.p. 195 °C (decomp.).

1H-NMR (400 MHz, DMSO-δ6): δ = 2.55 (t, J = 7.9 Hz, 2H, CH2CO), 4.18 (t, J = 7.9 Hz, 2H, NCH2), 6.45–7.68 (m, 11H, H Ar + NH2), 8.35 (s, 1H, NH), 12.24 (s, 1H, COOH) ppm.

13C-NMR (101 MHz, DMSO-δ6): δ = 32.69 (CH2CO), 50.91 (NCH2), 117.47, 117.61, 118.05, 120.86, 128.74, 128.92, 129.20, 129.59, 129.67, 133.00, 143.23, 143.89, 172.97, 182.18 (CAr, C=O, C=S) ppm.

IR (KBr): νmax = 1704 (C=O); 3287 (NH); 3407 (OH) cm⁻¹.

Calcd. for C16H17N3O2S, %: C 60.93; H 5.43; N 13.32. Found, %: C 60.74; H 5.52; N 13.17.

General procedure for the preparation of thiazoles 2a–c. A suspension of compound 1 (2.2 mmol) in aqueous 5% sodium carbonate solution (10 mL) was dissolved in methanol (6 mL), and the appropriate acetophenone (2.7 mmol) was added. The mixture was refluxed for 2 h (a, b) or 4 h (c), the cooled down and acidified with diluted (30%) acetic acid to pH 6. The formed precipitate was filtered off, washed with water and recrystallized from propan-2-ol to give the title compounds 2a (white solid, yield 0.65 g, 68%, m. p. 124–125 °C), 2b (white solid, yield 0.87 g, 88%, m. p. 130–132 °C), and 2c (white solid, yield 0.87 g, 80%, m.p. 120–122 °C).
3-((4-(4-Fluorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanoic acid (2a). \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta = 2.62 \ (J = 7.4 \ Hz, 2H, CH_2CO), 4.11 \ (J = 7.4 \ Hz, 2H, NCH_2), 6.88 \ (J = 7.3, 1H, H_{Ar}), 7.08–7.17 \ (m, 5H, SCH, H_{Ar}), 7.22–7.30 \ (m, 4H, H_{Ar}), 7.44 \ (d, J = 8.5 \ Hz, 2H, H_{Ar}), 7.88 \ (d, J = 8.5 \ Hz, 2H, H_{Ar}), 8.39 \ (s, 1H, NH) \) ppm.

\(^1\)C-NMR (101 MHz, DMSO-\(d_6\)): \(\delta = 32.78 \ (CH_2CO), 48.75 \ (NCH_2), 102.21 \ (SCH), 115.24, 115.45, 116.99, 117.56, 120.40, 127.56, 127.64, 128.59, 129.25, 131.39, 135.83, 142.73, 143.26, 149.38, 160.35, 162.77 \) ppm.

IR (KBr): \(\nu_{max} = 1518 \ (C=O); 1710 \ (C=O); 3051–3105 \ (NH); 3389 \ (OH) \) cm\(^{-1}\).

Calcd. for C\(_{32}\)H\(_{20}\)F\(_3\)N\(_2\)O\(_5\): %: C 66.50; H 4.63; N 9.69. Found, %: C 66.73; H 4.60; N 9.40.

3-((4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanoic acid (2b). \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta = 2.62 \ (J = 7.4 \ Hz, 2H, CH_2CO), 4.11 \ (J = 7.4 \ Hz, 2H, NCH_2), 6.88 \ (J = 7.3, 1H, H_{Ar}), 7.05–7.16 \ (m, 5H, SCH, H_{Ar}), 7.16–7.39 \ (m, 4H, H_{Ar}), 7.44 \ (d, J = 8.5 \ Hz, 2H, H_{Ar}), 7.88 \ (d, J = 8.5 \ Hz, 2H, H_{Ar}), 8.39 \ (s, 1H, NH) \) ppm.

\(^1\)C-NMR (101 MHz, DMSO-\(d_6\)): \(\delta = 32.99 \ (CH_2CO), 48.86 \ (NCH_2), 103.24 \ (SCH), 117.00, 117.57, 120.41, 127.34, 128.54, 128.60, 129.26, 131.81, 133.66, 135.79, 142.73, 143.28, 149.19, 170.10, 173.07 \) ppm.

IR (KBr): \(\nu_{max} = 1514 \ (C=O); 1698 \ (C=O); 3031–3106 \ (NH); 3376 \ (OH) \) cm\(^{-1}\).

Calcd. for C\(_{32}\)H\(_{20}\)ClN\(_2\)O\(_5\): %: C 64.07; H 4.48; N 9.34. Found, %: C 64.41; H 4.46; N 9.57.

3-((4-Bromophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanoic acid (2c). \(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta = 2.44, 2.57 \ (2H, J = 7.6 \ Hz, CH_2CO), 4.09 \ (J = 7.6 \ Hz, 2H, NCH_2), 6.75–7.34 \ (m, 10H, SCH, H_{Ar}), 7.58 \ (d, J = 8.2 \ Hz, 4H, H_{Ar}), 7.81 \ (d, J = 8.2 \ Hz, 4H, H_{Ar}), 8.33, 8.36 \) ppm.

\(^1\)C-NMR (101 MHz, DMSO-\(d_6\)): \(\delta = 33.27 \ (CH_2CO), 49.04 \ (NCH_2), 103.28 \ (SCH), 116.99, 117.48, 117.54, 120.28, 120.38, 127.64, 128.60, 129.20, 129.25, 131.44, 134.00, 142.73, 143.24, 149.22, 170.10, 173.21 \) ppm.

IR (KBr): \(\nu_{max} = 1513 \ (C=O); 1707 \ (C=O); 3031–3285 \ (NH); 3372 \ (OH) \) cm\(^{-1}\).

Calcd. for C\(_{34}\)H\(_{22}\)BrN\(_2\)O\(_5\): %: C 58.30; H 4.08; N 8.50. Found, %: C 58.22; H 4.21; N 8.61.

Methyl 3-((4-(4-Fluorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanoate (3). To a boiling solution of compound 2b (11.1 mmol, 5 g) in methanol (100 mL), a catalytic amount of sulfuric acid (0.6 mL) was added, and the reaction mixture was refluxed for 5 h. Then the volatile fractions were evaporated under reduced pressure, the residue was poured with aqueous 5% sodium carbonate solution (150 mL) and boiled. After cooling, the formed precipitate was filtered off, washed with water and recrystallized from the mixture of methanol and water (1:1) to give the title compound 4 (white solid, yield 4.12 g, 80%, m.p. 73–74 °C).

\(^1\)H-NMR (400 MHz, DMSO-\(d_6\)): \(\delta = 2.75 \ (t, J = 7.0 \ Hz, 2H, CH_2CO), 3.54 \ (s, 3H, OCH_3), 4.17 \ (t, J = 7.0 \ Hz, 2H, CH_2CO), 6.89 \ (t, J = 7.3 \ Hz, 1H, H_{Ar}), 7.08–7.20 \ (m, 6H, SCH, H_{Ar}), 7.24–7.29 \ (m, 4H, H_{Ar}), 7.46 \ (d, J = 8.2 \ Hz, 2H, H_{Ar}), 7.88 \ (d, J = 8.2 \ Hz, 2H, H_{Ar}) \) ppm.

\(^1\)C-NMR (101 MHz, DMSO-\(d_6\)): \(\delta = 32.28 \ (CH_2CO), 48.52 \ (NCH_2), 51.43 \ (OCH_3), 103.49 \ (SCH), 116.97, 117.60, 120.47, 127.34, 128.55, 128.61, 129.26, 131.89, 133.45, 135.51, 142.65, 143.43, 148.97, 170.10, 170.60 \) ppm.

IR (KBr): \(\nu_{max} = 1596 \ (C=N); 1734 \ (C=O); 3107–3193 \ (NH) \) cm\(^{-1}\).

Calcd. For C\(_{22}\)H\(_{20}\)F\(_2\)N\(_2\)O\(_5\): %: C 64.72; H 4.78; N 9.06. Found, %: C 64.67; H 4.79; N 9.17.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanoylhydrazide (4). To a solution of ester 3 (20 mmol, 7.12 g) in propan-2-ol (150 mL), hydrazine monohydrate (60 mmol, 3 g) was added dropwise and the mixture was heated at reflux for 18 h. After completion of the reaction the mixture was cooled down and diluted with water (100 mL). The obtained precipitate was filtered off, washed with water, and recrystallized from propan-2-ol to give the title compound 4 (yellow solid, yield 7.52 g, 73%, m.p. 104–105 °C).
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General procedure for the preparation of hydrazones 5a–i. To a boiling solution of hydrazide 4 (5 mmol, 2.32 g) in propan-2-ol (35 mL) the corresponding aromatic aldehyde (6 mmol) and a catalytic amount of acetic acid (0.4 mL) were added, and the mixture was heated at reflux for 2 h after, cooled down, the formed solid was filtered off, washed with propan-2-ol, diethyl ether, and recrystallized from propan-2-ol to give the appropriate title compound 5.

N-benzylidene-3-((4-(4-chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanehydrazide (5a). White solid, yield 2.24 g, 81%, m.p. 162–164 °C.

1H-NMR (400 MHz, DMSO-d6): δ = (Z/E) 65/35), 2.67, 3.10 (2t, J = 7.1, 7.4 Hz, 2H, CH₂CO), 4.16–4.32 (m, 2H, NCH₂), 6.84–6.91 (m, 1H, HAr), 7.08–7.17 (m, 6H, SCH, HAr), 7.21–7.35 (m, 5H, HAr), 7.41 (dd, J = 16.7, 8.2 Hz, 2H, HAr), 7.52–7.73 (m, 2H, HAr), 7.86–8.14 (m, 3H, HAr+CH=N), 8.38, 8.42 (2s, 1H, NH), 11.37, 11.46 (2s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 30.99, 33.02, 48.99 (CH₂CO, NCH₂), 103.25 (SCH), 116.88, 116.95, 117.54, 117.65, 120.45, 126.63, 126.97, 127.31, 128.47, 128.64, 128.67, 129.23, 129.60, 129.89, 131.74, 131.79, 133.61, 133.64, 134.18, 135.78, 135.85, 142.67, 142.77, 143.33, 146.06, 149.18, 166.65, 169.98, 170.09, 172.47 (C₅Ar, CH=N, C=O) ppm. IR (KBr): δmax = 1513 (C=N); 1660 (C=O); 3031–3180 (NH) cm⁻¹.

Calcd. for C₃H₂5ClN₅O₅, %: C 67.44; H 4.75; N 12.69. Found, %: C 66.57; H 4.67; N 12.46.

N'-benzylidene-3-((4-(4-chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)-N'-((fluorobenzylidene)propanehydrazide (5b). White solid, yield 2.39 g, 84%, m.p. 178–180 °C.

1H-NMR (400 MHz, DMSO-d6): δ = (Z/E) 65/35), 2.67, 3.10 (2t, J = 7.1, 7.4 Hz, 2H, CH₂CO), 4.16–4.32 (m, 2H, NCH₂), 6.84–6.91 (m, 1H, HAr), 7.08–7.17 (m, 6H, SCH, HAr), 7.21–7.35 (m, 5H, HAr), 7.41 (dd, J = 16.7, 8.2 Hz, 2H, HAr), 7.52–7.73 (m, 2H, HAr), 7.86–8.14 (m, 3H, HAr+CH=N), 8.38, 8.42 (2s, 1H, NH), 11.37, 11.46 (2s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 30.99, 33.02, 48.99 (CH₂CO, NCH₂), 103.26 (SCH), 115.54, 115.75, 116.84, 116.95, 117.54, 117.66, 120.48, 127.30, 128.47, 128.58, 128.65, 128.68, 128.77, 129.23, 130.78, 131.77, 133.60, 133.63, 135.82, 141.60, 142.62, 142.67, 143.35, 144.93, 149.19, 161.53, 163.49, 166.68, 170.08, 172.44 (C₅Ar, CH=N, C=O) ppm. IR (KBr): δmax = 1509 (C₅Ar); 1652 (C=O), 3030–3068 (NH) cm⁻¹.

Calcd. for C₃H₂5ClN₅O₅, %: C 65.31; H 4.42; N 12.29. Found, %: C 65.17; H 4.58; N 12.05.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)-N'-((chlorobenzylidene)propanehydrazide (5c). White solid, yield 2.58 g, 88%, m.p. 201–203 ºC.

1H-NMR (400 MHz, DMSO-d6): δ = (Z/E) 65/35), 2.68, 3.10 (2t, J = 7.1, 7.4 Hz, 2H, CH₂CO), 4.15–4.30 (m, 2H, NCH₂), 6.85–6.92 (m, 1H, HAr), 7.12–7.68 (m, 15H, SCH, HAr), 7.84–8.13 (m, 2H, HAr+CH=N), 8.37, 8.42 (2s, 1H, NH), 11.42, 11.52 (2s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 30.99, 33.02, 48.99 (CH₂CO, NCH₂), 103.28 (SCH), 116.80, 116.94, 117.54, 117.70, 120.40, 120.50, 123.70, 123.77, 128.20, 128.47, 128.58, 128.67, 128.70, 128.82, 129.29, 131.78, 133.10, 133.26, 133.62, 134.04, 134.31, 135.77, 141.46, 142.59, 143.38, 144.72, 149.19, 166.76, 169.95, 170.07, 172.50 (C₅Ar, CH=N, C=O) ppm. IR (KBr): δmax = 1509 (C₅Ar); 1656 (C=O) 3029–3065 (NH) cm⁻¹.

Calcd. for C₃H₂5ClN₅O₅, %: C 63.48; H 4.30; N 11.94. Found, %: C 63.17; H 4.50; N 11.81.

N'-((bromobenzylidene)-3-((4-(4-chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanehydrazide (5d). White solid, yield 2.78 g, 88%, m.p. 182–184 ºC.

1H-NMR (400 MHz, DMSO-d6): δ = (Z/E) 70/30), 2.68, 3.09 (2t, J = 7.1, 7.5 Hz, 2H, CH₂CO), 4.16–4.29 (m, 2H, NCH₂), 6.86–6.91 (m, 1H, HAr), 7.10–7.45 (m, 14H, SCH, HAr),
3-((4-Chlorophenyl)thiazol-2-yl)-N-(4-nitrobenzylidene)-(4-(phenylamino)phenyl)amino)propanehydrazide (5e). White solid, yield 2.38 g, 80%, m.p. 205–207 °C.

3-((4-Chlorophenyl)thiazol-2-yl)-N′-(4-dimethylamino)benzylidene)-(4-(phenylamino)phenyl)amino)propanehydrazide (5f). White solid, yield 2.38 g, 80%, m.p. 191–193 °C.

3-((4-Chlorophenyl)thiazol-2-yl)-N′-(4-(methylbenzylidene)-(4-(phenylamino)phenyl)amino)propanehydrazide (5g). White solid, yield 2.69 g, 95%, m.p. 176–178 °C.

1H-NMR (400 MHz, DMSO-d_6): δ = (Z/E 65/35), 2.63, 3.07 (2t, J = 7.0, 7.3 Hz, 2H, CH_2CO), 2.85, 2.95 (2s, 6H, 2CH_3), 4.15–4.28 (m, 2H, NCH_2), 6.56, 6.72 (2d, J = 8.4 Hz, 2H, H_Ar), 6.87 (t, J = 7.2 Hz, 1H, H_Ar), 7.11–7.46 (m, 13H, SCH, H_Ar), 7.80–8.00 (m, 3H, H_Ar, N=CH), 8.38, 8.44 (2s, 1H, NH), 11.67, 11.75 (2s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d_6): δ = 30.83, 32.47, 48.96 (CH_2CO, NCH_2), 103.33 (SCH), 116.86, 117.53, 117.60, 120.46, 123.82, 123.96, 127.29, 127.36, 127.46, 127.86, 128.47, 128.63, 129.21, 131.79, 133.60, 135.74, 140.46, 142.59, 143.35, 147.46, 149.18, 169.96, 172.91 (C_Ar, CH=N, C=O) ppm.

IR (KBr): ν_max = 1513 (C=N); 1666 (C=O); 3076 (NH) cm⁻¹.

Calcd. for C_{33}H_{31}N_6O_5S, %: C 62.36; H 4.22; N 14.08. Found, %: C 62.25; H 4.25; N 13.97.

1H-NMR (400 MHz, DMSO-d_6): δ = (Z/E 65/35), 2.25, 2.32 (2s, 3H, CH_3), 2.67, 3.08 (2t, J = 6.9, 7.5 Hz, 2H, CH_2CO), 4.22 (q, J = 6.4 Hz, 2H, NCH_2), 6.88 (t, J = 7.1 Hz, 1H, H_Ar), 7.08–7.17 (m, 6H, SCH, H_Ar), 7.22–7.55 (m, 9H, H_Ar), 7.84–8.10 (m, 3H, H_Ar, N=CH), 8.37, 8.42 (2s, 1H, NH), 11.28, 11.38 (2s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d_6): δ = 29.66, 31.09, 32.89, 49.01 (CH_3, CH_2CO, NCH_2), 103.25 (SCH), 116.85, 116.95, 117.54, 117.62, 120.43, 126.59, 126.95, 127.32, 127.38, 128.49, 128.58, 128.67, 129.23, 129.28, 129.35, 131.47, 131.74, 133.64, 135.81, 139.31, 142.64, 142.86, 143.34, 149.19, 169.95, 172.28 (C_Ar, CH=N, C=O) ppm.

IR (KBr): ν_max = 1511 (C=N); 1668 (C=O); 3055–3169 (NH) cm⁻¹.

Calcd. for C_{33}H_{31}N_6O_5S, %: C 66.60; H 5.25; N 14.12. Found, %: C 66.29; H 5.22; N 13.88.

1H-NMR (400 MHz, DMSO-d_6): δ = (Z/E 65/35), 2.65, 3.00 (2t, J = 7.1, 7.3 Hz, 2H, CH_2CO), 4.18–4.28 (m, 2H, NCH_2), 6.87 (t, J = 7.3 Hz, 1H, H_Ar), 7.08–7.17 (m, 6H, SCH, H_Ar), 7.23–7.32 (m, 4H, H_2Ar), 7.56–7.45 (m, 3H, H_2Ar), 7.52, 7.63 (2d, J = 5.1 Hz, 1H, H_2Ar), 7.84–7.92 (m, 2H, H_2Ar), 8.14, 8.36 (2s, 1H, N=CH), 8.38 (s, 1H, NH), 11.32, 11.40 (2s, 1H, NH) ppm.
13C-NMR (101 MHz, DMSO-d$_6$): $\delta = 30.84, 33.00, 48.62, 48.87$ (CH$_2$CO, NCH$_2$), 103.23, 103.35 (SCH), 116.94, 117.04, 117.48, 117.55, 120.35, 127.34, 127.75, 127.84, 128.02, 128.44, 128.49, 128.60, 128.68, 129.23, 129.93, 130.67, 131.73, 131.79, 133.60, 133.64, 135.78, 137.87, 139.06, 139.08, 141.30, 142.66, 142.73, 143.24, 143.28, 149.17, 166.51, 170.07, 172.09 (C$_{Ar}$, CH=$N$, C=O) ppm.

IR (KBr): $\nu_{max} = 1510$ (C=N); 1687 (C=O), 3029–3116 (NH) cm$^{-1}$.

Calcd. for C$_{56}$H$_{45}$N$_{19}$O$_{17}$S: C 64.67; H 5.56; N 13.92. Found, %: C 64.56; H 5.23; N 13.86.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)-N'-(5-nitrothiophen-2-yl)methylenepropanehydrazide (5f). White solid, yield 2.20 g, 73%, m.p. 186–188 $^\circ$C.

H-NMR (400 MHz, DMSO-d$_6$): $\delta = (Z/E$ 60/40), 2.69, 3.06 (2t, $J = 7.0, 7.1$ Hz, 2H, CH$_2$CO), 4.24 (q, $J = 7.4$ Hz, 2H, NCH$_2$), 6.87 (t, $J = 7.3$ Hz, 1H, H$_{Ar}$), 7.05–7.54 (m, 13H, SCH, H$_{Ar}$), 7.82–7.91 (m, 2H, H$_{Ar}$), 8.04, 8.09 (2d, $J = 4.3, 4.4$ Hz, 1H, H$_{Ar}$), 8.13 (s, 0.6H, N=CH), 8.36, 8.37, 8.40 (3s, 1H, NH+0.4H, N=CH), 11.76, 11.81 (2s, 1H, NH) ppm.

IR (KBr): $\nu_{max} = 1508$ (C=N); 1681 (C=O), 3028–3107 (NH) cm$^{-1}$.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-((phenylamino)phenyl)amino)-N'-(benzaldehyde)propanehydrazide (5j). A mixture of hydrazide (5 mmol, 2.32 g) and the corresponding ketone (acetone or 2-butanone) (35 mL) was heated at reflux for 5 h. After completion of the reaction, the mixture was cooled down, diluted with water (35 mL), and left in refrigerator for 24 h. Then the formed precipitate was filtered off, washed with acetone, diethyl ether and recrystallized from acetone to give the title compound 5j. Product 5k was separated from the reaction mixture by evaporating the volatile fractions under reduced pressure, diluting the residue with diethyl ether, filtering the obtained solid, and recrystallizing it from 2-butanone to give the title compound 5k.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)-N’-(propan-2-ylidene)propanehydrazide (5i). White solid, yield 2.20 g, 73%, m.p. 76–78 $^\circ$C.

1H-NMR (400 MHz, DMSO-d$_6$): $\delta = (Z/E$ 60/40), 1.80, 1.85, 1.89 (3s, 6H, 2CH$_3$), 2.66, 2.95 (2t, $J = 7.3, 7.5$ Hz, 2H, CH$_2$CO), 4.17 (t, $J = 7.5$ Hz, 2H, NCH$_2$), 6.88 (t, $J = 7.3$ Hz, 1H, H$_{Ar}$), 7.09–7.17 (m, 5H, SCH, H$_{Ar}$), 7.23–7.31 (m, 4H, H$_{Ar}$), 7.44 (d, $J = 8.3$ Hz, 2H, H$_{Ar}$), 7.84–7.93 (m, 2H, H$_{Ar}$), 8.37, 8.38 (2s, 1H, NH), 10.02, 10.06 (2s, 1H, NH) ppm.

IR (KBr): $\nu_{max} = 1513$ (C=N); 1667 (C=O), 3031–3107 (NH) cm$^{-1}$.

Calcd. for C$_{56}$H$_{45}$N$_{19}$O$_{17}$S: C 64.34; H 5.20; N 13.89. Found, %: C 64.56; H 5.28; N 13.94.

N’-(butan-2-ylidene)-3-((4-(4-chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenyl)amino)propanehydrazide (5k). White solid, yield 1.76 g, 88%, m.p. 79–81 $^\circ$C.

1H-NMR (400 MHz, DMSO-d$_6$): $\delta = (Z/E$ 60/40), 0.76–1.01 (m, 3H, CH$_2$CH$_3$), 1.79, 1.83, 1.87 (3s, 3H, CH$_3$), 2.11–2.24 (m, 2H, CH$_2$CH$_3$), 2.67, 2.96 (2t, $J = 7.4, 7.5$ Hz, 2H, CH$_2$CO), 4.13–4.24 (m, 2H, NCH$_2$), 6.89 (t, $J = 7.3$ Hz, 1H, H$_{Ar}$), 7.10–7.16 (m, 5H, SCH, H$_{Ar}$), 7.27 (t, $J = 7.8$ Hz, 4H, H$_{Ar}$), 7.44 (d, $J = 8.2$ Hz, 2H, H$_{Ar}$), 7.88 (t, $J = 8.2$ Hz, 2H, H$_{Ar}$), 8.37, 8.38 (2s, 1H, NH), 9.99, 10.07, 10.12, 10.16 (4s, 1H, NH) ppm.

IR (KBr): $\nu_{max} = 1512$ (C=N); 1667 (C=O), 3031–3099 (NH) cm$^{-1}$.

Calcd. for C$_{33}$H$_{26}$N$_{14}$O$_{14}$S: C 57.75; H 3.84; N 13.93. Found, %: C 57.59; H 3.85; N 13.76.
Calcd. for C_{28}H_{28}ClN_{5}O_{2}, %: C 64.91; H 5.45; N 13.52. Found, %: C 64.57; H 5.37; N 13.88.

3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenylamino)-1-(3,5-dimethyl-1H-pyrazol-1-yl)propan-1-one (6). To a solution of hydrazide 4 (5 mmol, 2.32 g) in propan-2-ol (35 mL), 2.5-pentanedione (10 mmol, 1 g) and hydrochloric acid (2 drops) were added dropwise and the mixture was refluxed for 9 h, then cooled down, and the formed precipitate was filtered off washed with propan-2-ol and recrystallized from propan-2-ol to give the title compound 7 (white solid, 1.95 g, 74%, m.p. 105–106 °C).

1H-NMR (400 MHz, DMSO-d_{6}): δ = 1.94, 2.01 (2s, 6H, 2CH_{3}), 2.77 (t, J = 6.9 Hz, 2H, CH_{2}CO), 4.25 (t, J = 6.8 Hz, 2H, NCH_{2}), 5.61 (s, 2H, CH-CH), 6.89 (t, J = 7.1 Hz, 1H, H_{Ar}), 7.12–7.34 (m, 10H, SCH, H_{Ar}), 7.45 (d, J = 8.1 Hz, 2H, H_{Ar}), 7.91 (d, J = 8.1 Hz, 2H, H_{Ar}), 8.37, 8.40 (2s, 1H, NH), 10.68 (s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d_{6}): δ = 10.91, 11.02 (2CH_{3}), 31.75 (CH_{2}CO), 48.66 (NCH_{2}), 102.90 (CH-CH), 103.46 (SCH), 116.99, 117.45, 117.55, 120.43, 126.69, 127.36, 128.36, 128.49, 128.54, 129.25, 131.84, 133.61, 135.73, 142.69, 143.32, 149.13, 151.25, 152.84, 170.16, 180.82 (C_{Ar}, C=N, C=O) ppm.

IR (KBr): ν_{max} = 1513 (C=N); 1647 (C=O), 3031–3110 (NH) cm⁻¹.

Calcd. for C_{28}H_{28}ClN_{5}O_{2}, %: C 66.03; H 4.60; N 13.34.

2-(3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenylamino)-N-(5-(2-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenylamino)-N-(2,5-dimethyl-1H-pyrrol-1-yl)propanoyl)-N-phenylhydrazine-1-yl)propanamide (7). To a solution of hydrazide 4 (5 mmol, 2.32 g) in propan-2-ol (45 mL), 2,5-hexanedione (9 mmol, 1.03 g) and acetic acid (dropwise, 0.75 mL) were added and the mixture was refluxed for 3 h, then cooled down and diluted with water (30 mL). The formed crystalline solid was filtered off, washed with water and recrystallized from propan-2-ol to give the title compound 7 (white solid, 2.17 g, 80%, m.p. 146–147 °C).

1H-NMR (400 MHz, DMSO-d_{6}): δ = 1.61, 2.19 (2s, 6H, 2CH_{3}), 2.72–3.02 (m, 2H, CH_{2}CO), 4.05–4.35 (m, 2H, CH-CH), 6.88 (t, J = 6.8 Hz, 1H, H_{Ar}), 7.09–7.19 (m, 5H, SCH, H_{Ar}), 7.24–7.32 (m, 4H, H_{Ar}), 7.45 (d, J = 8.0 Hz, 2H, H_{Ar}), 7.88 (d, J = 8.0 Hz, 2H, H_{Ar}), 8.37 (s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d_{6}): δ = 15.79, 15.85 (2CH_{3}), 32.88 (CH_{2}CO), 48.57 (NCH_{2}), 103.16 (SCH), 110.10 (CH), 117.01, 117.49, 120.37, 127.28, 128.52, 129.24, 131.79, 133.65, 135.82, 142.74, 143.17, 149.10, 154.21, 168.29, 169.97 (C_{Ar}, C=N, C=O) ppm.

IR (KBr): ν_{max} = 1513 (C=N); 1647 (C=O), 3031–3110 (NH) cm⁻¹.

Calcd. for C_{30}H_{30}ClN_{5}OS, %: C 66.47; H 5.21; N 12.92. Found, %: C 66.36; H 5.31; N 12.75.

2-(3-((4-(4-Chlorophenyl)thiazol-2-yl)(4-(phenylamino)phenylamino)-N-(4-phenylthiazol-5-yl)carbenecarboxylic acid (8). To a solution of hydrazide 4 (5 mmol, 2.32 g) solution in methanol (100 mL), a solution of phenyl isothiocyanate (5 mmol, 0.6 mL) in methanol (5 mL) was added dropwise and the mixture was refluxed for 2 h. After completion of the reaction, the mixture was cooled down and diluted with water (80 mL). The formed solid was filtered off, washed with water, and recrystallized from propan-2-ol to give the title compound 8 (white solid, 2.79 g, 93%, m.p. 114–116 °C).

1H-NMR (400 MHz, DMSO-d_{6}): δ = 2.68 (t, J = 7.5 Hz, 2H, CH_{2}CO), 4.18 (t, J = 6.8 Hz, 2H, NCH_{2}), 6.89 (t, J = 7.2 Hz, 1H, H_{Ar}), 7.09–7.20 (m, 6H, SCH, H_{Ar}), 7.22–7.41 (m, 8H, H_{Ar}), 7.45 (d, J = 8.1 Hz, 2H, H_{Ar}), 7.90 (d, J = 8.1 Hz, 2H, H_{Ar}), 8.39 (s, 1H, NH), 9.54 (s, 1H, NH), 9.59 (br. s, 1H, NH), 9.99 (s, 1H, NH) ppm.

IR (KBr): ν_{max} = 1512 (C=N); 1681 (C=O), 3029–3108 (NH) cm⁻¹.

Calcd. for C_{30}H_{28}ClN_{5}OS_{2}, %: C 62.14; H 4.54; N 14.03. Found, %: C 61.98; H 4.64; N 13.91.
recrystallized from propan-2-ol to give the title compound 9 (white solid, 2.41 g, 83%, m.p. 119–121 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 2.93 (t, J = 6.8 Hz, 2H, CH₂), 3.98 (t, J = 6.8 Hz, 2H, NCH₂), 6.88 (t, J = 7.2 Hz, 1H, H₆), 7.05-7.15 (m, 7H, SCH, H₅), 7.25-7.32 (m, 4H, H₆), 7.43-7.53 (m, 5H, H₅), 7.77 (d, J = 8.2 Hz, 2H, H₆), 8.40 (s, 1H, NH), 13.70 (br. s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 23.97 (CH₂), 49.57 (NCH₂), 103.45 (SCH), 117.00, 117.58, 120.48, 127.35, 128.20, 128.38, 128.51, 129.26, 129.39, 131.84, 133.47, 133.73, 135.38, 142.63, 143.35, 149.18, 149.94, 167.70, 169.82 (C₆, C=N, C=S) ppm.

IR (KBr): νmax = 1090 (C=S); 1529 (C=N); 3037–3108 (NH) cm⁻¹.

Calcd. for C₃₁H₂₅ClN₆S₂, %: C 64.07; H 4.34; N 14.46. Found, %: C 63.93; H 4.32; N 14.78.

3.2. In Vitro Time-Kill Study

In vitro time-kill studies were performed using 5 mL of representative Staphylococcus aureus strains harboring genetically defined resistance mechanisms. The bacteria were cultured overnight in 10 mL of CAMHB. The inoculum was further diluted (1:10) with fresh CAMBH and incubated for 2 h to achieve the logarithmic growth phase. The bacterial inoculums were adjusted spectrophotometrically to reach approx. 10⁵ CFU/mL. Then the selected test compounds or vancomycin (control) were added to achieve the MIC concentration. The samples were further incubated at 37 °C with continuous agitation for 24 h. At the selected time points, the aliquots (100 µL) were taken, diluted, and plated in Tryptic Soy agar. The CFU were calculated after 24 h of incubation. The experiments were performed in triplicate.

3.3. Biofilm Formation Assay

The overnight culture of Staphylococcus aureus was adjusted with fresh media to reach OD₆₀₀ = 0.3 and was dispensed in flat bottomed 96-well microplates (100 µL). The biofilms were grown at 37 °C, 48 h and then the 2X dilutions of the compounds or vancomycin were added to each well to reach a final concentration of 0.5X MIC, MIC or 4X MIC of each compound or comparator drug. The biofilms were exposed to the test compounds for 24 h at 37 °C. After incubation, the media was gently removed, biofilms were washed 3 times using 200 µL of PBS, and then fixed for 3 h with 5% buffered paraformaldehyde. After fixation, the paraformaldehyde was removed, plates were washed 1 time with 200 µL of PBS and biofilms were stained using 0.5% crystal violet for 1 h at room temperature. After staining, plates were washed with deionized water and air-dried overnight. The biofilm absorbed crystal violet was solubilized using 30% acetic acid and quantified spectrophotometrically at OD 595 nm.

4. Conclusions

A series of thiazole derivatives bearing β-amino acid and aromatic moieties in the structure were synthesized, spectrally characterized, and evaluated for their antimicrobial activity using a panel of multidrug-resistant bacterial and fungal pathogens. The in vitro antimicrobial properties of the obtained compounds were evaluated against priority pathogens harboring genetically defined resistance mechanisms. The results revealed that thiazoles 2a–c with the 4-halophenyl fragment possess the profound bactericidal activity against Gram-positive pathogens, which was mostly evident with Staphylococcus aureus (MIC of 1–2 µg/mL) harboring defined resistance mechanisms (Pan-S, MRSA, and VRSA). The compounds demonstrated low cytotoxicity using Vero cell culture model suggesting the applicability for the further pre-clinical hit to lead optimization. The compounds also exhibited promising antifungal properties against azole-resistant fungal strain of Aspergillus fumigatus and 2a, b were found to be the best candidates. Further studies are needed to better understand the molecular basis of Gram-positive bacteria-directed bactericidal activity of thiazoles 2a–c and selective activity of compounds 2a, b against azole-resistant fungal pathogens as well as to validate the in vivo tolerance and bioavailability of the most active compounds.
Supplementary Materials: The following are available online, Figure S1: H-NMR of compound 1, Figure S2: C-NMR of compound 1, Figure S3: H-NMR of compound 2a, Figure S4: C-NMR of compound 2a, Figure S5: H-NMR of compound 2b, Figure S6: C-NMR of compound 2b, Figure S7: H-NMR of compound 2c, Figure S8: C-NMR of compound 2c, Figure S9: H-NMR of compound 3, Figure S10: C-NMR of compound 3, Figure S11: H-NMR of compound 4, Figure S12: C-NMR of compound 4, Figure S13: H-NMR of compound 5a, Figure S14: C-NMR of compound 5a, Figure S15: H-NMR of compound 5b, Figure S16: C-NMR of compound 5b, S17: H-NMR of compound 5c, Figure S18: C-NMR of compound 5c, Figure S19: H-NMR of compound 5d, Figure S20: C-NMR of compound 5d, Figure S21: H-NMR of compound 5e, Figure S22: C-NMR of compound 5e, Figure S23: H-NMR of compound 5f, Figure S24: C-NMR of compound 5f, Figure S25: C-NMR of compound 5g, Figure S26: C-NMR of compound 5g, Figure S27: H-NMR of compound 5h, Figure S28: C-NMR of compound 5h, Figure S29: H-NMR of compound 5i, Figure S30: C-NMR of compound 5i, Figure S31: H-NMR of compound 5j, Figure S32: C-NMR of compound 5j, Figure S33: H-NMR of compound 5k, Figure S34: C-NMR of compound 5k, Figure S35: H-NMR of compound 6, Figure S36: C-NMR of compound 6, Figure S37: H-NMR of compound 7, Figure S38: C-NMR of compound 7, Figure S39: H-NMR of compound 8, Figure S40: C-NMR of compound 8, Figure S41: H-NMR of compound 9, Figure S42: C-NMR of compound 9. Table S1: The list of fungal strains used for the study.

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