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Synthesis and Biological Studies of New 2-Benzoxazolinone Derivatives as Antibacterial Agents

Jūratė Šiugždaitė [1], Raimundas Lelešius [1], Birutė Grybaitė [2], Rita Vaickelionienė [2] and Vytautas Mickevičius [2,*]

[1] Department of Pathobiology, Lithuanian University of Health Sciences, Tilžės Street 18, 47181 Kaunas, Lithuania; jurate.siugzdaite@lsmu.lt (J.Š.); raimundas.lelesius@lsmu.lt (R.L.)
[2] Department of Organic Chemistry, Kaunas University of Technology, Radvilėnų Road 19, 50254 Kaunas, Lithuania; birute.grybaite@ktu.lt (B.G.); rita.vaickelioniene@ktu.lt (R.V.)
* Correspondence: vytautas.mickevicius@ktu.lt

Abstract: In the present study, new series of benzoxazolin-2-one linked to a variety of hydrazones and azoles were synthesized and assessed for their antibacterial properties against different bacterial microorganisms. All the synthesized target compounds were characterized by ^{1}H NMR, ^{13}C NMR and IR spectroscopy, and elemental analysis as well. The antibacterial activity of the synthesized compounds was evaluated according to the bacteriostatic and bactericidal activity against the tested pathogen strains by determining the minimum inhibition (MIC) and minimum bactericidal (MBC) concentrations and MBC/MIC ratios. The MIC was evaluated by the broth dilution and the MBC was evaluated by plating methods. The in vitro analysis suggested that some compounds, namely, amide, 5-chlorobenzimidazole, hydrazones with a 3-chloro substitution on the additional phenyl ring, and hydrazones with 2-furyl and 5-nitro-2-furyl substituents, demonstrated wide antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Salmonella* Enteritidis. The most sensitive strains appeared to be Gram-negative *E. coli* and Gram-positive *B. subtilis*, while *S. aureus* showed some resistance. The most resistant pathogen was found to be *S. enteritidis*. The remaining compounds demonstrated moderate to low antibacterial potential. The research results have shown that benzoxazolinone-based derivatives are suitable for the development of a library of compounds and can be used in the future development of antibacterial drugs against various Gram-positive and Gram-negative pathogens, which is of great importance in therapy practice.

Keywords: benzoxazolinone; *β*-amino acids; hydrazones; heterocycles; bacteria; antibacterial activity

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1. Introduction

The global problem of infectious and often fatal diseases caused by Gram-positive and Gram-negative pathogens is currently the main task of scientists, which must be solved as soon as possible. Bacterial infections are generally easier to treat than viral infections because there is a large army of antimicrobial agents that work against bacteria. However, due to the widespread and often excessive use of antibiotics, bacterial resistance to antimicrobial agents is a rapidly growing problem with devastating consequences [1].

The widespread drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis* (MRSE), vancomycin-resistant *Staphylococcus aureus* (VRSA), extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*, and drug-resistant tuberculosis (DR-TB) have already reached a frightening degree and cause considerable mortality among the world's population [2]. According to the latest estimates of the World Health Organization (WHO), in 2019, 1.27 million deaths were directly attributed to drug-resistant infections worldwide, while by 2050, up to 10 million people in the world can die every year [3]. AMR poses a threat to humans, animals, plants, and the environment. It affects us all.

To solve this problem, it is necessary not only to raise the level of sanitation and hygiene and awareness of the use of antibiotic substances but also to search for new

effective drugs with the fewest side effects. And this is where the chemistry of heterocycles comes in handy. Among them, benzoxazole pharmacophore, consisting a two fused oxazole and a benzene rings, occupies an important place as a promising target for medicinal chemistry, being a starting material for the synthesis of a number of biological and pharmacological active substances [4] which were found to exhibit antifungal [5,6], antituberculosis [7,8], anticancer [9–11], antileishmanial [12], anti-inflammatory-analgesic [13,14], and antibacterial [15–20] properties, and so forth [21–26]. Benzoxazole scaffolds have had a significant impact on drug discovery, and a variety of benzoxazole-based drugs are market-available nowadays [27–29]. Among benzoxazoles, the 2(3*H*)-benzoxazolone derivatives have been described as having diverse applications in medicinal chemistry [30] with large-scale therapeutic activities which include analgesic, anti-inflammatory, antimicrobial [31], cholinesterase inhibitors [32], antinociceptive [33], anti-HIV, antileishmanial, anticancer, antioxidant, antidepressant, and neurodegenerative [34] effects. Some pharmacologically active benzoxazole-2-one derivatives [35–39] are shown in Figure 1.

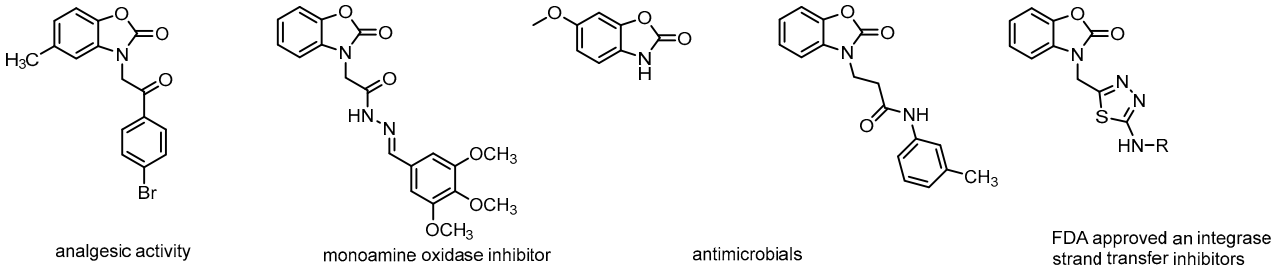


Figure 1. Some benzoxazole-2-ones with therapy efficacy.

In addition to those mentioned above, synthetic benzoxazoles and their naturally occurring counterparts were found to be biologically active [40,41] and can be potential materials for drug design. Thus, benzoxazole derivatives are an excellent basis for the search and development of new antimicrobial compounds.

Considering the above-documented therapeutic efficacy including the broad antimicrobial properties of benzoxazole derivatives and our previous successful studies in the synthesis and discovery of effective antimicrobial agents among azole- and hydrazone-containing compounds [42–45] led us to the selection of this scaffold and its combinations with hydrazone and azole moieties in the molecules for our further studies. To achieve set goals, a new series of compounds with an integrated benzoxazolin-2-one scaffold were developed to discover the antibacterial potential against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella* Enteritidis pathogens. Notably, the synthesized compounds have shown auspicious antibacterial activity, making them a promising avenue in the search for novel antibacterial pharmaceuticals.

2. Materials and Methods

2.1. Synthesis

General procedures. Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification or processing. TLC plates were used to check the reaction course and purity of the compounds (Silica gel with F254 nm, Merck KGaA, Darmstadt, Germany). All melting points were measured with a B-540 melting point analyzer (Büchi Corporation, New Castle, DE, USA) and were uncorrected. ^{1}H and ^{13}C NMR spectra were determined in DMSO-*d6* (the δ for ^{1}H NMR is 2.50 ppm, and the δ for ^{13}C NMR is 39.52 ppm) on a Brucker Avance III (400, 101 MHz) spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) at 25 °C. Chemical shifts were expressed in (δ) ppm. The spectral data are incorporated as follows: chemical shift, multiplicity, integration, coupling constant (Hz), and assignment. IR spectra (ν, cm^{-1}) were obtained on a Perkin–Elmer Spectrum BX FT–IR spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) (KBr pellets). For microanalysis (C, H, and N), the Elemental Analyzer CE-440 was

used, and the results were found within an acceptable range (±0.3%) in comparison with the calculated values. Hydrazine-containing compounds were stored in tightly closed dark glass containers below 25 °C, avoiding direct sunlight, heat, sparks, flames, or contact with air.

Methyl 3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanoate (**2**): A mixture of acid **1** (11.28 g, 54 mmol), methanol (300 mL), and conc. H_2SO_4 (1 mL) was heated under reflux for 8 h. After the solvent evaporation, the residue was neutralized with 5% aqueous Na_2CO_3 solution to pH 8, and the mixture was stirred and extracted with ethyl ether (3 × 100 mL). The combined ether layers were dried with sodium carbonate, and the solvent was evaporated under reduced pressure.

Yield 9.67 g (81%), mp 58–60 °C (from hexane);

^{1}H-NMR (400 MHz): δ 2.80 (t, 2H, *J* = 6.8 Hz, CH_2CO), 4.06 (t, 2H, *J* = 6.8 Hz, CH_2N), 3.56 (s, 3H, OCH_3), 7.12 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.21 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.32 (t, 2H, *J* = 7.0 Hz, H_{ar});

^{13}C-NMR (101 MHz): δ 31.51, 37.79 (CH_2CO, CH_2N), 51.58 (OCH_3), 109.44, 109.59, 122.19, 123.83, 130.79, 141.96, 153.55, 171.06 (Car, CO);

IR (KBr): ν_{max} = 1757, 1718 (C=O) cm^{-1}.

Anal. Calcd for $C_{11}H_{11}NO_4$ (221.21), %: C, 59.73; H, 5.01; N, 6.33. Found: C, 59.57; H, 4.85; N, 6.20.

2.1.1. 3-(2-Oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**3**)

To a solution of ester **2** (9 g, 6.5 mmol) in propan-2-ol (30 mL) hydrazine hydrate (3.77 g, 75.3 mmol) was added dropwise, and the mixture was heated under reflux for 5 h. The crystallization of the product took place during the reaction. After the completion of the reaction, the reaction mixture was left in the refrigerator overnight, and then the formed precipitate was filtered off, washed with cold propan-2-ol, and recrystallized from propan-2-ol.

Yield 8.25 g (91%), mp 185–190 °C;

^{1}H-NMR (400 MHz): δ 2.50 (t, 2H, *J* = 6.8 Hz, CH_2CO), 4.02 (t, 2H, *J* = 6.8 Hz, CH_2N), 4.16 (br s, 2H, NH_2), 7.12 (t, 1H, *J* = 7.7 Hz, H_{ar}), 7.21 (t, 1H, *J* = 7.7 Hz, H_{ar}), 7.26 (d, 1H, *J* = 7.8 Hz, H_{ar}), 7.32 (d, 1H, *J* = 7.8 Hz, H_{ar}), 9.11 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 31.64, 38.58 (CH_2CO, CH_2N), 109.49, 109.55, 122.12, 123.85, 130.95, 141.93, 153.57, 168.83 (Car, CO);

IR (KBr): ν_{max} = 3332, 3315 (NH_2, NH), 1746 (C=O) cm^{-1}.

Anal. Calcd for $C_{10}H_{11}N_3O_3$ (221.22), %: C, 54.30; H, 5.01; N, 19.00. Found: C, 53.10; H, 4.77; N, 18.80.

2.1.2. 3-(2-Oxobenzo[d]oxazol-3(2*H*)-yl)-N-(4-sulfamoylphenyl)propanamide (**4**)

To a mixture of acid **1** (1.04 g, 5 mmol), 4-aminobenzenesulfonamide (1.03 g, 6 mmol), DMSO (15 mL), triethyl amine (1.52 g, 15 mmol, dropwise), and HBTU (2.84 g, 7.5 mmol) were added, and the mixture was stirred for 20 h at room temperature. After the completion of the reaction (TLC), the mixture was diluted with water (20 mL), and the formed precipitate was filtered off, washed with water, and recrystallized from propan-2-ol.

Yield 1.41 g (78%), mp 252–254 °C;

^{1}H-NMR (400 MHz): δ 2.85 (t, 2H, *J* = 6.6 Hz, CH_2CO), 4.13 (t, 2H, *J* = 6.6 Hz, CH_2N), 7.11 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.21 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.24 (s, 2H, NH_2), 7.28–7.36 (m, 2H, H_{ar}) 7.67 (d, 2H, *J* = 8.5 Hz, H_{ar}), 7.73 (d, 2H, *J* = 8.5 Hz, H_{ar}), 10.39 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 34.45, 38.25 (CH_2CO, CH_2N), 109.51, 109.60, 112.43, 118.69, 122.16, 123.82, 126.69, 127.44, 130.97, 138.38, 141.77, 141.96, 153.63, 169.20 (Car, CO);

IR (KBr): ν_{max} = 3374, 3304, 3260 (NH_2, NH), 1754 (C=O) cm^{-1}.

Calcd for $C_{16}H_{15}N_3O_5S$ (361.37), %: C 53.18; H 4.18; N 11.63. Found, %: C 53.00; H 4.01; N 11.41.

2.2. General Procedure for the Preparation of Benzimidazoles **5**–**7**

A mixture of carboxylic acid **1** (2 g, 9.65 mmol), the corresponding benzene-1,2-diamine (19.3 mmol), and 17.5% aqueous hydrochloric acid solution (25 mL) was heated at reflux for 72 h. Afterwards, it was cooled and neutralized with 5% Na_2CO_3 to pH 8. The formed precipitate was filtered off, washed with plenty of water, and recrystallized from propan-2-ol.

2.2.1. 3-(2-(1H-Benzo[d]imidazol-2-yl)ethyl)benzo[d]oxazol-2(3*H*)-one (**5**)

Yield 0.66 g (27%), mp 204–206 °C;

^{1}H-NMR (400 MHz): δ δ 3.26 (t, 2H, *J* = 7.5 Hz, CH_2CO), 4.28 (t, 2H, *J* = 7.5 Hz, CH_2N), 6.97–7.20 (m, 4H, H_{ar}), 7.22 (d, 1H, *J* = 7.5 Hz, H_{ar}), 7.31 (d, 1H, *J* = 7.5 Hz, H_{ar}), 7.47 (dd, 2H, *J* = 5.8, 3.1 Hz, H_{ar}), 12.30 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 26.98, 40.45 (CH_2CO, CH_2N), 109.14, 109.62, 114.63, 121.41, 122.15, 123.76, 138.75, 141.97, 141.96, 151.52, 153.60 (Car, CO);

IR (KBr): ν_{max} = 3070 (NH), 1769 (C=O), 1487 (C=N) cm^{-1}.

Calcd for $C_{16}H_{13}N_3O_2$ (279.30), %: C 68.81; H 4.69; N 15.05. Found, %: C 68.62; H 4.43; N 14.85.

2.2.2. 3-(2-(5-Fluoro-1H-benzo[d]imidazol-2-yl)ethyl)benzo[d]oxazol-2(3*H*)-one (**6**)

Yield 0.4 g (27%), mp 218–220 °C;

^{1}H-NMR (400 MHz): δ δ 3.24 (t, 2H, *J* = 7.0 Hz, CH_2CO), 4.26 (t, 2H, *J* = 7.0 Hz, CH_2N), 6.97 (t, 1H, *J* = 9.1 Hz, H_{ar}), 7.15–7.18 (m, 2H, H_{ar}), 7.20 (d, 1H, *J* = 7.5 Hz, H_{ar}), 7.27 (s, 1H, H_{ar}), 7.31 (d, 1H, *J* = 7.8 Hz, H_{ar}), 7.45 (br s, 1H, H_{ar}), 12.46 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 27.00, 40.39 (CH_2CO, CH_2N), 97.46, 103.96, 109.09, 109.36, 109.62, 111.56, 118.82, 122.16, 123.75, 130.82, 134.08, 141.95, 152.82, 153.58, 158.25 (d, *J* = 233.4 Hz) (Car, CO);

IR (KBr): ν_{max} = 3074 (NH), 1769 (C=O, 1488 (C=N) cm^{-1}.

Calcd for $C_{16}H_{12}FN_3O_2$ (297.29), %: C 64.64; H 4.07; N 14.13. Found, %: C 64.39; H 3.90; N 13.89.

2.2.3. 3-(2-(5-Chloro-1H-benzo[d]imidazol-2-yl)ethyl)benzo[d]oxazol-2(3*H*)-one (**7**)

Yield 0.81 g (56%), mp 208–210 °C;

^{1}H-NMR (400 MHz): δ δ 3.25 (t, 2H, *J* = 6.9 Hz, CH_2CO), 4.26 (t, 2H, *J* = 6.9 Hz, CH_2N), 7.04–7.16 (m, 3H, H_{ar}), 7.20 (d, 1H, *J* = 7.5 Hz, H_{ar}), 7.31 (d, 1H, *J* = 7.8 Hz, H_{ar}), 7.40–7.61 (m, 2H, H_{ar}), 12.54 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 27.00, 40.39 (CH_2CO, CH_2N), 97.46, 103.96, 109.08, 109.61, 110.78, 112.23, 117.74, 119.57, 121.41, 121.75, 122.16, 123.75, 125.43, 126.03, 130.80, 133.08, 134.91, 134.96, 141.95, 144.24, 152.94, 153.40, 153.57 (Car, CO);

IR (KBr): ν_{max} = 3312 (NH), 1744 (C=O), 1483 (C=N) cm^{-1}.

Calcd for $C_{16}H_{12}ClN_3O_2$ (313.74), %: C 61.25; H 3.86; N 13.39. Found, %: C 60.98; H 3.70; N 13.13.

2.3. General Procedure for the Preparation of Hydrazones **8**–**23**

To a solution of hydrazide **3** (0.3 g, 1.4 mmol) in hot propan-2-ol (20 mL), the corresponding aromatic aldehyde (1.6 mmol) was added, and the mixture was heated under reflux for 3 h. After the reaction was completed, the mixture was cooled in a refrigerator, and the formed precipitate was filtered off, washed with plenty of propan-2-ol, and recrystallized from propan-2-ol.

2.3.1. *N*′-Benzylidene-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**8**)

Yield 0.35 g (81%), mp 170–172 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.10 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.01–4.19 (m, 2H, CH_2N), 7.11 (t, 1H, *J* = 6.8 Hz, H_{ar}), 7.22 (t, 1H, *J* = 6.8 Hz, H_{ar}), 7.26–7.46 (m, 5H, H_{ar}), 7.50–7.67 (m, 2H, H_{ar}), 7.94, 8.09 (2s, 1H, N=CH), 11.41 (s, 0.65H, NH), 11.48 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.40, 32.30 37.99, 38.24 (<u>C</u>H_2CO, CH_2N), 109.43, 109.55, 109.59, 122.12, 123.80, 126.71, 127.03, 128.73, 128.76, 129.77, 130.98, 134.03, 141.98, 143.30, 146.40, 153.60, 166.03, 171.86 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3183, 3064 (NH), 1772 (C=O), 1667 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{15}N_3O_3$ (309.32), %: C, 66.01; H, 4.89; N, 13.58. Found: C, 65.91; H, 4.63; N, 13.33.

2.3.2. *N*′-(4-Fluorobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**9**)

Yield 0.4 g (87%), mp 180–182 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.11 (2t, 2H, *J* = 6.8 Hz, CH_2CO), 4.13–4.20 (m, 2H, CH_2N), 7.12 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.18–7.31 (m, 3.65H, H_{ar}), 7.33, 7.36 (2d, 1.35H, *J* = 7.9 Hz, H_{ar}), 7.62, 7.72 (2dd, 2H, *J* = 8.2, 5.8 Hz, H_{ar}), 7.93, 8.10 (2s, 1H, N=CH), 11.42 (s, 0.65H, NH), 11.50 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.38, 32.29, 38.00, 38.24 (<u>C</u>H_2CO, CH_2N), 109.43, 109.54, 109.57, 109.59, 115.69 (d, J^2 = 21.9 Hz), 115.77 (d, J^2 = 21.9 Hz), 122.13, 123.82, 128.85 (d, J^3 = 8.5 Hz), 129.20 (d, J^3 = 8.5 Hz), 130.65 (d, J^4 = 2.9 Hz), 130.76 (d, J^4 = 2.9 Hz), 130.97, 141.97, 142.14, 145.29, 153.59, 162.88 (d, J^1 = 247.5 Hz), 166.05, 171.87 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3182, 3078 (NH), 1757 (C=O), 1662 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}FN_3O_3$ (327.32), %: C, 62.38; H, 4.31; N, 12.84. Found: C, 62.11; H, 4.17; N, 12.78.

2.3.3. *N*′-(2,4-Difluorobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**10**)

Yield 0.4 g (83%), mp 192–194 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.10 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.08–4.16 (m, 2H, CH_2N), 7.06–7.38 (m, 6H, H_{ar}), 7.74, 7.84 (2dd, 1H, *J* = 15.6, 8.3 Hz, H_{ar}), 8.07, 8.25 (2s, 1H, N=CH), 11.51 (s, 0.65H, NH), 11.62 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.35, 32.31, 37.95, 38.17 (<u>C</u>H_2CO, CH_2N), 104.13, 104.38, 104.64, 109.43, 109.52, 109.57, 112.35, 112.38, 112.47, 112.50, 112.56, 112.60, 112.68, 112.72, 118.38, 118.42, 118.48, 118.52, 122.14, 123.83, 127.59, 127.63, 127.69, 127.73, 127.82, 127.86, 127.92, 127.97, 130.95, 135.30, 138.42, 141.95, 153.58, 159.39, 161.82, 162.03, 164.31, 166.12, 171.98 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3183, 3091 (NH), 1783 (C=O), 1667 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{13}F_2N_3O_3$ (345.31), %: C, 59.13; H, 3.79; N, 12.17. Found: C, 58.89; H, 3.52; N, 11.92.

2.3.4. *N*′-(2-Chlorobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**11**)

Yield 0.35 g (73%), mp 162–164 °C;

^{1}H-NMR (400 MHz): δ 2.73, 3.12 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.02–4.24 (m, 2H, CH_2N), 7.05–7.15 (m, 1H, H_{ar}), 7.18–7.52 (m, 6H, H_{ar}), 7.78, 7.91 (2d, 2H, *J* = 7.7 Hz, H_{ar}), 8.32, 8.47 (2s, 1H, N=CH), 11.60 (s, 0.65H, NH), 11.72 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.39, 32.34, 37.94, 38.14 (<u>C</u>H_2CO, CH_2N), 109.44, 109.53, 109.56, 122.12, 123.80, 126.59, 127.49, 129.81, 130.95, 131.16, 139.32, 141.96, 142.30, 153.59, 166.20, 172.04 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3182, 3069 (NH), 1770, 1760 (C=O), 1669 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}ClN_3O_3$ (343.77), %: C, 59.40; H, 4.11; N, 12.22. Found: C, 59.20; H, 3.93; N, 12.01.

2.3.5. *N*′-(3-Chlorobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**12**)

Yield 0.44 g (92%), mp 160–162 °C;

^{1}H-NMR (400 MHz): δ 2.73, 3.11 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.07–4.18 (m, 2H, CH_2N), 7.06–7.14 (m, 1H, H_{ar}), 7.18–7.62 (m, 6H, H_{ar}), 7.63, 7.70 (2s, 1H, H_{ar}), 7.91, 8.07 (2s, 1H, N=CH), 11.51 (s, 0.65H, NH), 11.61 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.37, 32.27, 38.00, 38.18 (CH_2CO, CH_2N), 109.39, 109.52, 109.57, 122.12, 123.80, 125.53, 125.66, 125.89, 126.33, 129.42, 130.59, 130.95, 133.61, 136.24, 141.72, 141.95, 153.59, 166.26, 172.04 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3182, 3066 (NH), 1780 (C=O), 1662 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}ClN_3O_3$ (343.77), %: C, 59.40; H, 4.11; N, 12.22. Found: C, 59.18; H, 3.94; N, 12.03.

2.3.6. *N*′-(4-Chlorobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**13**)

Yield 0.39 g (81%), mp 202–204 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.10 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.01–4.17 (m, 2H, CH_2N), 7.10 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.17–7.39 (m, 3H, H_{ar}), 7.42, 7.47 (2d, 2H, *J* = 8.3 Hz, H_{ar}), 7.57, 7.67 (2d, 2H, *J* = 8.3 Hz, H_{ar}), 7.91, 8.08 (2s, 1H, N=CH), 11.47 (s, 0.65H, NH), 11.55 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.36, 32.30, 37.97, 38.22 (CH_2CO, CH_2N), 109.42, 109. 53, 122.12, 123.81, 128.32, 128.66, 128.78, 128.84, 130.96, 132.97, 134.17, 141.96, 141.99, 145.09, 153.58, 166.14, 171.94 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3194, 3084 (NH), 1754 (C=O), 1672 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}ClN_3O_3$ (343.77), %: C, 59.40; H, 4.11; N, 12.22. Found: C, 59.18; H, 3.89; N, 12.01.

2.3.7. *N*′-(4-Bromobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2H)-yl)propanehydrazide (**14**)

Yield 0.48 g (89%), mp 210–212 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.10 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.06–4.18 (m, 2H, CH_2N), 7.11 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.17–7.40 (m, 3H, H_{ar}), 7.50, 7.57 (2d, 2H, *J* = 8.2 Hz, H_{ar}), 7.61 (s, 2H, H_{ar}), 7.90, 8.06 (2s, 1H, N=CH), 11.47 (s, 0.65H, NH), 11.55 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.35, 32.29, 37.96, 38.21 (CH_2CO, CH_2N), 109.44, 109. 54, 122.13, 122.94, 123.83, 128.57, 128.90, 130.96, 131.71, 131.77, 133.31, 141.96, 142.09, 145.16, 153.58, 166.14, 171.95 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3193, 3066 (NH), 1752 (C=O), 1671 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}BrN_3O_3$ (388.22), %: C, 52.60; H, 3.64; N, 10.82. Found: C, 52.39; H, 3.38; N, 10.59.

2.3.8. *N*′-(4-Nitrobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**15**)

Yield 0.43 g (86%), mp 232–234 °C;

^{1}H-NMR (400 MHz): δ 2.76, 3.14 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.06–4.22 (m, 2H, CH_2N), 7.10 (t, 1H, *J* = 7.8 Hz, H_{ar}), 7.16–7.37 (m, 3H, H_{ar}), 7.80, 7.91 (2d, 2H, *J* = 8.5 Hz, H_{ar}), 8.02, 8.19 (2s, 1H, N=CH), 8.19, 8.25 (2d, 2H, *J* = 8.5 Hz, H_{ar}), 11.71 (s, 0.65H, NH), 11.79 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.36, 32.33, 37.93, 38.14 (CH_2CO, CH_2N), 109.46, 109. 55, 109.58, 122.15, 123.85, 123.91, 127.59, 127.95, 130.95, 140.32, 140.91, 141.96, 143.93, 147.58, 153.59, 166.55, 172.33 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3182, 3079 (NH), 1752 (C=O), 1669 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{14}N_4O_5$ (354.32), %: C, 57.63; H, 3.98; N, 15.81. Found: C, 57.41; H, 3.71; N, 15.58.

2.3.9. *N*′-(4-(Dimethylamino)benzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanehydrazide (**16**)

Yield 0.43 g (88%), mp 188–190 °C;

^{1}H-NMR (400 MHz): δ 2.67, 3.06 (2t, 2H, *J* = 6.7, 6.9 Hz, CH_2CO), 2.94 (s, 6H, N(CH_3)$_2$), 4.06–4.15 (m, 2H, CH_2N), 6.55–6.85 (m, 2H, H_{ar}), 7.11 (t, 1H, *J* = 7.7 Hz, H_{ar}), 7.17–7.53 (m, 5H, H_{ar}), 7.80, 7.93 (2s, 1H, N=CH), 11.11 (s, 0.65H, NH), 11.17 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.43, 32.31, 38.03, 38.38, 39.75 (CH_2CO, CH_2N, 2CH_3), 109.41, 109.55, 109.61, 111.73, 121.38, 121.45, 122.10, 123.80, 127.99, 128.35, 130.99, 141.95, 141.98, 144.14, 147.23. 151.27, 151.46, 153.60, 165.33, 171.18 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3166, 3061 (NH), 1761 (C=O), 1666 (C=N) cm^{-1}.

Anal. Calcd for $C_{19}H_{20}N_4O_3$ (352.39), %: C, 64.76; H, 5.72; N, 15.90. Found: C, 64.53; H, 5.52; N, 15.73.

2.3.10. *N′*-(2-Chloro-5-nitrobenzylidene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanehydrazide (**17**)

Yield 0.44 g (82%), mp 202–204 °C;

^{1}H-NMR (400 MHz): δ 2.76, 3.15 (2t, 2H, *J* = 5.8 Hz, CH_2CO), 4.01–4.25 (m, 2H, CH_2N), 6.98–7.38 (m, 4H, H_{ar}), 7.78 (t, 1H, *J* = 8.1 Hz, H_{ar}), 8.18 (t, 1H, *J* = 9.8 Hz, H_{ar}), 8.30, 8.42, 8.46, 8.57 (4s, 2H, H_{ar}, N=CH), 11.77 (s, 0.65H, NH), 11.93 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.32, 32.34, 37.95, 38.08 ($\underline{C}H_2CO$, CH_2N), 109.39, 109. 57, 120.29, 121.97, 122.16, 123.74, 123.82, 124.91, 130.92, 131.45, 132.69, 137.52, 138.77, 141.88, 146.66, 153.57, 166.57, 172.28 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3104, 3085 (NH), 1778 (C=O), 1674 (C=N) cm^{-1}.

Anal. Calcd for $C_{17}H_{13}ClN_4O_5$ (388.76), %: C, 52.52; H, 3.37; N, 14.41. Found: C, 52.35; H, 3.13; N, 14.23.

2.3.11. 3-(2-Oxobenzo[d]oxazol-3(2*H*)-yl)-*N′*-(2,3,4-trimethoxybenzylidene) propanehydrazide (**18**)

Yield 0.43 g (77%), mp 150–154 °C;

^{1}H-NMR (400 MHz): δ 2.68, 3.07 (2t, 2H, *J* = 6.8 Hz, CH_2CO), 3.75, 3.78, 3.80, 3.82, 3.83 (5s, 9H, $3OCH_3$), 4.04–4.18 (m, 2H, CH_2N), 6.82, 6.89 (2d, 1H, *J* = 8.9 Hz, H_{ar}), 7.11, 7.22 (2t, 2H, *J* = 7.8 Hz, H_{ar}), 7.25–7.36 (m, 2H, H_{ar}), 7.39, 7.51 (2d, 2H, *J* = 8.8 Hz, H_{ar}), 8.12, 8.25 (2s, 1H, N=CH), 11.27 (s, 0.60H, NH), 11.41 (s, 0.40H, NH);

^{13}C-NMR (101 MHz): δ 30.42, 32.32, 38.00, 38.30 ($\underline{C}H_2CO$, CH_2N), 55.98, 60.46, 61.71, 108.62, 108.67, 109.43, 109.54, 120.22, 122.11, 123.81, 130.97, 139.17, 141.51, 141.96, 152.38, 152.50, 153.59, 154.87, 155.11, 165.63, 171.50 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3181, 3069 (NH), 1778 (C=O), 1671 (C=N) cm^{-1}.

Anal. Calcd for $C_{20}H_{21}N_3O_6$ (399.40), %: C, 60.14; H, 5.30; N, 10.52. Found: C, 59.95; H, 5.13; N, 10.34.

2.3.12. 3-(2-Oxobenzo[d]oxazol-3(2*H*)-yl)-*N′*-(3,4,5-trimethoxybenzylidene) propanehydrazide (**19**)

Yield 0.46 g (82%), mp 192–194 °C;

^{1}H-NMR (400 MHz): δ 2.72, 3.10 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 3.68, 3.80 (2s, 9H, $3OCH_3$), 4.05–4.23 (m, 2H, CH_2N), 6.92, 6.95 (2s, 2H, H_{ar}), 7.06–7.34 (m, 4H, H_{ar}), 7.81, 8.02 (2s, 1H, N=CH), 11.40 (s, 0.60H, NH), 11.45 (s, 0.40H, NH);

^{13}C-NMR (101 MHz): δ 30.47, 32.24, 38.26 ($\underline{C}H_2CO$, CH_2N), 55.90, 55.93, 60.10, 104.02, 104.24, 109.29, 109.52, 109.57, 109.62, 122.09, 122.14, 123.75, 123.82, 129.54, 130.96, 128.96, 141.93, 141.97, 143.29, 146.40, 153.12, 153.14, 153.62, 165.95, 171.86 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3190, 3045 (NH), 1766 (C=O), 11,677 (C=N) cm^{-1}.

Anal. Calcd for $C_{20}H_{21}N_3O_6$ (399.40), %: C, 60.14; H, 5.30; N, 10.52. Found: C, 59.90; H, 5.09; N, 10.22.

2.3.13. 3-(2-Oxobenzo[d]oxazol-3(2*H*)-yl)-*N′*-(thiophen-2-ylmethylene) propanehydrazide (**20**)

Yield 0.31 g (71%), mp 164–166 °C;

^{1}H-NMR (400 MHz): δ 2.69, 3.03 (2t, 2H, *J* = 6.6, 6.8 Hz, CH_2CO), 4.10 (q, 2H, *J* = 6.8 Hz, CH_2N), 7.07–7.42 (m, 6H, H_{ar}), 7.59, 7.63 (2d, 1H, *J* = 4.9 Hz, H_{ar}), 8.13, 8.31 (2s, 1H, N=CH), 11.39 (s, 0.65H, NH), 11.43 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.25, 32.28, 37.86, 38.23 ($\underline{C}H_2CO$, CH_2N), 109.45, 109.56, 122.12, 123.81, 127.85, 128.34, 130.31, 130.93, 130.96, 138.47, 138.76, 141.61, 141.96, 141.98, 153.57, 153.60, 165.88, 171.41 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3187, 3100 (NH), 1767 (C=O), 1660 (C=N) cm^{-1}.

Anal. Calcd for $C_{15}H_{13}N_3O_3S$ (315.35), %: C, 57.13; H, 4.16; N, 13.33. Found: C, 56.87; H, 3.85; N, 13.08.

2.3.14. *N′*-((5-Nitrothiophen-2-yl)methylene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanehydrazide (**21**)

Yield 0.44 g (88%), mp 236–238 °C;

^{1}H-NMR (400 MHz): δ 2.75, 3.07 (2t, 2H, *J* = 6.7 Hz, CH_2CO), 4.03–4.21 (m, 2H, CH_2N), 7.11 (q, 1H, *J* = 7.5 Hz, H_{ar}), 7.18–7.41 (m, 3H, H_{ar}), 7.47, 7.53 (2d, 1H, *J* = 4.3 Hz, H_{ar}), 8.09 (dd, 1H, *J* = 10.4, 4.7 Hz, H_{ar}), 8.11, 8.36 (2s, 1H, N=CH), 11.82 (s, 0.65H, NH), 11.85 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.17, 32.308, 37.92, 38.04 ($\underline{C}H_2CO$, CH_2N), 109.44, 109.56, 122.16, 123.83, 129.06, 129.66, 130.49, 130.93, 136.62, 139.99, 141.99, 146.42, 150.43, 153.57, 166.64, 172.17 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3172, 3108 (NH), 1774 (C=O), 1657 (C=N) cm^{-1}.

Anal. Calcd for $C_{15}H_{12}N_4O_5S$ (360.34), %: C, 50.00; H, 3.36; N, 15.55. Found: C, 49.81; H, 3.05; N, 15.27.

2.3.15. *N′*-(Furan-2-ylmethylene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**22**)

Yield 0.31 g (74%), mp 158–160 °C;

^{1}H-NMR (400 MHz): δ 2.69, 3.03 (2t, 2H, *J* = 6.6, 6.9 Hz, CH_2CO), 4.10 (t, 2H, *J* = 6.8 Hz, CH_2N), 6.59 (s, 1H, H_{ar}), 6.81, 6.86 (2d, 1H, *J* = 2.7 Hz, H_{ar}), 7.05–7.44 (m, 4H, H_{ar}), 7.78, 7.80 (2s, 1H, H_{ar}), 7.84, 7.98 (2s, 1H, N=CH), 11.37 (s, 0.65H, NH), 11.42 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.17, 32.308, 37.92, 38.04 ($\underline{C}H_2CO$, CH_2N), 109.44, 109.56, 122.16, 123.83, 129.06, 129.66, 130.49, 130.93, 136.62, 139.99, 141.99, 146.42, 150.43, 153.57, 166.64, 172.17 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3176, 3129 (NH), 1761 (C=O), 1669 (C=N) cm^{-1}.

Anal. Calcd for $C_{15}H_{13}N_3O_4$ (299.29), %: C, 60.20; H, 4.38; N, 14.04. Found: C, 59.98; H, 4.14; N, 13.85.

2.3.16. *N′*-((5-Nitrofuran-2-yl)methylene)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanehydrazide (**23**)

Yield 0.41 g (85%), mp 230–232 °C;

^{1}H-NMR (400 MHz): δ 2.76, 3.08 (2t, 2H, *J* = 6.5, 6.8 Hz, CH_2CO), 4.12 (t, 2H, *J* = 6.8 Hz, CH_2N), 7.07–7.39 (m, 5H, H_{ar}), 7.75 (d, 1H, *J* = 3.8 Hz, H_{ar}), 7.89, 8.06 (2s, 1H, N=CH), 11.82 (s, 0.65H, NH), 11.87 (s, 0.35H, NH);

^{13}C-NMR (101 MHz): δ 30.35, 32.37, 37.73, 38.06 ($\underline{C}H_2CO$, CH_2N), 109.49, 109.52, 109.58, 114.58, 114.68, 115.38, 122.09, 122.17, 123.86, 130.91, 131.44, 134.36, 141.95, 151.48, 151.60, 151.70, 151.87, 153.59, 166.73, 172.21 (C_{ar}, N=CH, CO);

IR (KBr): ν_{max} = 3198, 3063 (NH), 1753 (C=O), 1690 (C=N) cm^{-1}.

Anal. Calcd for $C_{15}H_{12}N_4O_6$ (344.28), %: C, 52.33; H, 3.51; N, 16.27. Found: C, 52.17; H, 3.33; N, 16.03.

2.3.17. 3-(3-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-3-oxopropyl)benzo[d]oxazol-2(3*H*)-one (**24**)

To a solution of hydrazide **3** (0.3 g, 1.4 mmol) in hot in propan-2-ol (20 mL), pentane-2,4-dione (0.21 g, 2.1 mmol) and hydrochloric acid (2 drops) were added dropwise, and the mixture was heated under reflux for 2 h. After the completion of the reaction, the reaction mixture was cooled in a refrigerator, and the formed precipitate was filtered off, washed with cold propan-2-ol, and recrystallized from propan-2-ol.

Yield 0.21 g (53%), mp 164–166 °C;

^{1}H-NMR (400 MHz): δ 2.10, 2.41 (2s, 6H, $2CH_3$), 3.50 (t, 2H, *J* = 6.7 Hz, CH_2CO), 4.17 (t, 2H, *J* = 6.7 Hz, CH_2N), 6.14 (s, 1H, CH_{pyraz}), 7.11, 7.22 (2t, 2H, *J* = 7.8, 7.7 Hz, H_{ar}), 7.32 (t, 2H, *J* = 7.6 Hz, H_{ar}), 10.72 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 13.38, 13.98 ($2CH_3$), 33.07, 37.62 ($\underline{C}H_2CO$, CH_2N), 109.34, 109.53, 111.29, 122.14, 123.80, 130.90, 141.98, 143.17, 151.67, 153.58, 171.03 (C_{ar}, C_{pyrr}, CO);

IR (KBr): ν_{max} = 3221, 3070 (NH), 1759, 1722 (C=O), 1630 (C=N) cm^{-1}.

Anal. Calcd for $C_{15}H_{15}N_3O_3$ (285.30), %: C, 63.15; H, 5.30; N, 14.73. Found: C, 62.89; H, 5.15; N, 14.57.

2.3.18. *N*-(2,5-Dimethyl-1*H*-pyrrol-1-yl)-3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanamide (**25**)

To a solution of hydrazide **3** (0.3 g, 1.4 mmol) in hot in propan-2-ol (20 mL), hexane-2,5-dione (0.42 g, 2.8 mmol) and acetic acid (six drops) were added dropwise, and the mixture was heated under reflux for 10 h. After the completion of the reaction, the reaction mixture was cooled, and the formed precipitate was filtered off, washed with cold propan-2-ol, and recrystallized from propan-2-ol.

Yield 0.33 g (79%), mp 248–250 °C;

^{1}H-NMR (400 MHz): δ 1.77, 1.96 (2s, 6H, $2CH_3$), 2.82 (t, 2H, *J* = 6.4 Hz, CH_2CO), 4.13 (t, 2H, *J* = 6.4 Hz, CH_2N), 5.55, 5.70 (2s, 2H, CH-CH), 7.13, 7.22 (2t, 2H, *J* = 7.7 Hz, H_{ar}), 7.30, 7.33 (2d, 2H, *J* = 7.8 Hz, H_{ar}), 10.72 (s, 1H, NH);

^{13}C-NMR (101 MHz): δ 10.52, 10.88 ($2CH_3$), 31.05, 38.25 ($\underline{C}H_2CO$, CH_2N), 102.91, 104.06, 109.52, 109.76, 122.23, 123.81, 126.57, 127.03, 130.86, 141.98, 153.58, 169.13 (C_{ar}, C_{pyrr}, CO);

IR (KBr): ν_{max} = 3242, 3191 (NH), 1751 (C=O), 1658 (C=N) cm^{-1}.

Anal. Calcd for $C_{16}H_{17}N_3O_3$ (299.33), %: C, 64.20; H, 5.72; N, 14.04. Found: C, 63.97; H, 5.55; N, 13.83.

5-Oxo-1-(3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanamido)pyrrolidine-3-carboxylic acid (**26**) prepared via the procedure described in [46].

Yield 0.37 g (48%), mp 204–206 °C (Ref. [46], 213–214 °C).

2.4. Biology

2.4.1. Preparation of Bacterial Cultures

The bacteria strains of the Gram-positive cocci *Staphylococcus aureus* (ATCC 9144), Gram-positive sporogenic rods *Bacillus subtilis* (ATCC 6051), Gram-negative rods *Escherichia coli* (ATCC 8739), and *Salmonella* Enteritidis (ATCC 13076) were grown on Tryptic soya agar (Liofilchem, Teramo, Italy) for 24 h at 37 °C. The cultures were diluted with saline to a turbidity of 0.5 McFarland units and working solutions (106 CFU/mL).

2.4.2. Determination of the Minimum Inhibitory Concentration (MIC) by the Broth Microdilution Method

Serial two-fold dilutions (volumes of 50 µL) were prepared in Mueller–Hinton broth (Liofilchem, Teramo, Italy) in 96-well plates. The prepared concentrations of the compounds were 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95, and 0.98 µg/mL. Then, these plates were inoculated with 0.05 mL of a bacterial suspension containing 5 × 104 CFU and incubated at 37 °C for 18–24 h [47,48]. The MIC values were evaluated by the lowest concentration of the compound at which bacterial growth is completely inhibited.

2.4.3. Determination of the Minimum Bactericidal Concentration (MBC)

To determine the MBC, wells showing MIC were subcultured on freshly prepared Mueller–Hinton agar plates. After being incubated at 37 °C for 18–24 h [49], the growth of relevant bacteria was observed. A decrease in colony counts by 99.9% from the original bacterial inoculum was taken as the MBC.

The antibacterial properties of synthesized compounds were evaluated according to bacteriostatic and bactericidal activities on the tested strains of bacteria, employing the determination of the MIC and the MBC and the calculation of the ratio of MBC/MIC, respectively. The ciprofloxacin for all bacteria strains was used as the standard antibiotic.

2.4.4. Agar Diffusion Methods

The agar well diffusion and disc diffusion methods were used to compare the antibacterial activity of compounds **4**, **7**, **12**, **22**, and **23** against bacteria strains.

The selected strains of bacteria were used to determine the bacterial properties and the bacterial inocula were spread over an agar plate for both methods. The bacterial inoculum was adjusted to 0.5 MacFarland, and a volume of 0.1 mL containing 1.5×10^7 bacteria was used on the entire surface of a Mueller–Hinton agar (MHA) Petri plate with a sterile cotton-tipped swab to form an even lawn.

2.4.5. The Agar Well Diffusion Method

Two volumes of 25 µL and 50 µL (25 µg and 50 µg of compounds) of the tested compounds **4**, **7**, **12**, **22**, and **23** were placed in holes of 6 mm diameter punched into the inoculated agar. The plates were incubated under aerobic conditions for 24 h at 37 °C, and the next day, the inhibition zone diameters (IZDs) were measured using a digital vernier caliper. The positive control of the ciprofloxacin (5 µg, Liofilchem, Teramo, Italy) disk was used.

2.4.6. The Disk Diffusion Method

In the disk diffusion method, filter paper disks containing compounds were placed on the surface of the agar plate. The diameter of the filter papers was 6 mm. The paper disks were impregnated with 3 µL (3 µg of the compound) and 5 µL (5 µg of the compound) of chemical compounds and placed on the surface of each MHA plate containing the inoculated tested microorganisms. Cultures were grown on agar at 37 °C for 24 h. The "zone of inhibition" was measured by a digital vernier caliper. The positive control of a ciprofloxacin (5 µg, Liofilchem, Teramo, Italy) disk was used.

3. Results and Discussion

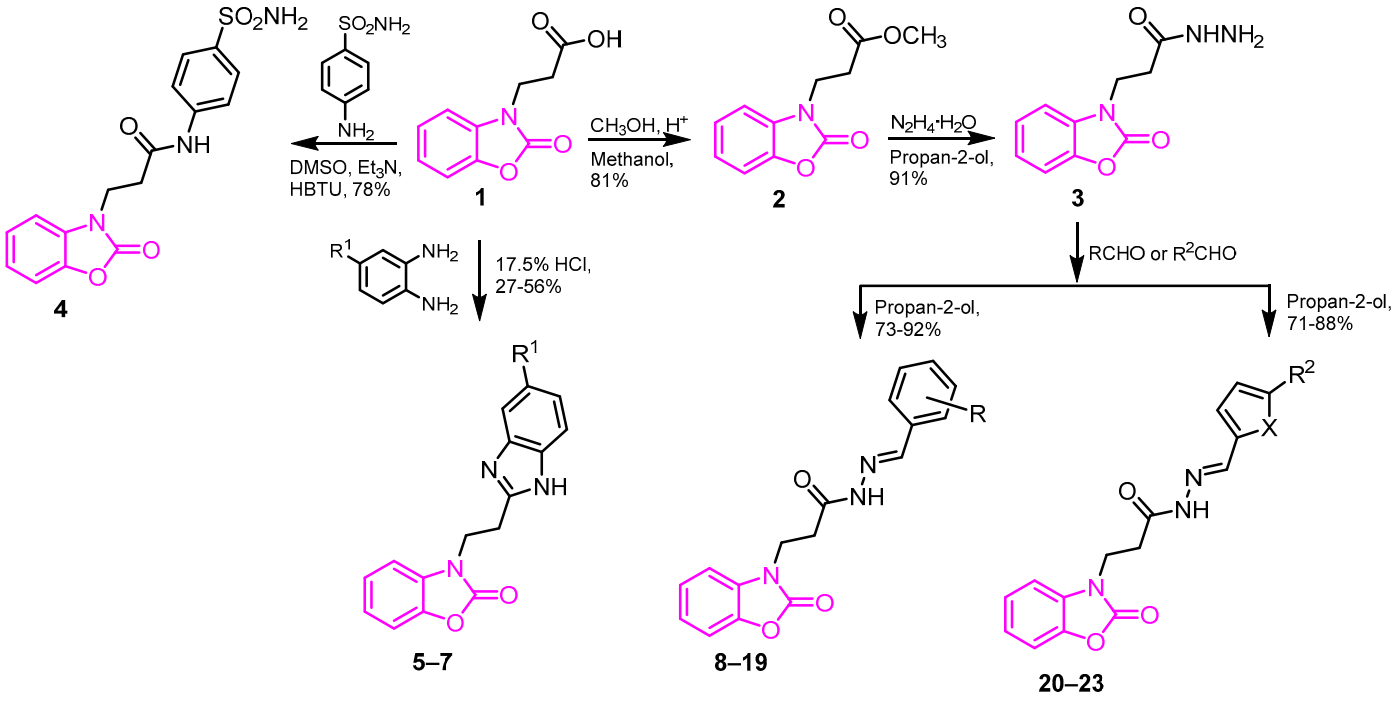
3.1. Chemistry

Since the *β*-amino acid core is a valuable building block for many biologically active compounds [50] and can be used for the synthesis of pharmacologically important hydrazones and heterocycles, it was thus decided to incorporate a carboxyalkyl moiety at the 3-position of the fused benzoxazolin-2-one structure. For this purpose, sodium 3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanoate was applied, which has shown good properties as a plant growth promoter in vitro and in vivo [46,51]. The initial acid **1** was obtained by the procedure described in [52].

To reach the target acid hydrazide **3** (Scheme 1), the standard synthesis pathway "acid → ester → hydrazide" was followed, i.e., after the esterification of the carboxylic acid **1** with methanol in the presence of sulfuric acid as a catalyst, the obtained ester **2** was subjected to hydrazinolysis to afford acid hydrazide **3**. The molecular structures of the obtained compounds **2** and **3** were confirmed by the ^{1}H, ^{13}C NMR, IR spectroscopy, and microanalysis data.

The ^{1}H NMR spectrum of **3** exhibited two characteristic proton singlets at 9.11 and 4.16 ppm due to the presence of the $CONHNH_2$ fragment. Four aromatic protons resonated in the range of 7.12–7.32 ppm. Two triplets of the alkyl chain NCH_2CH_2 were observed at 2.50 and 4.02 ppm, with a coupling constant of J = 6.8 Hz. After evaluating the peak values of the ^{13}C NMR spectrum of molecule **3**, it can be stated that the resonance lines were in good agreement with the target molecular structure.

The sulfanilamide derivative **4** was synthesized by reacting acid **1** with sulfanilamide in DMSO at room temperature for 20 h in the presence of triethylamine as a strong base and HBTU, a standard coupling agent for carboxylic acid activation. The additional spectral lines of aromatic (7.67 and 7.73 ppm (2d, J = 8.5 Hz)), NH (10.39 ppm), and NH_2 (7.24 ppm) protons of the attached sulfanilamide moiety in the ^{1}H NMR spectrum confirmed the formed structure **4**. In the ^{13}C NMR spectrum, six new resonances in the aromatic region of the spectrum indicated the presence of an additional phenyl ring, and the presence of a resonance line at 169.20 ppm confirmed the C=O of the amide moiety.



5 R^1=H; **6** R^1=F, **7** R^1=Cl;
8 R=H; **9** R=4-F; **10** R=2,4-diF; **11** R=2-Cl; **12** R=3-Cl; **13** R=4-Cl; **14** R=4-Br;
15 R=4-NO_2; **16** R=4-$N(CH_3)_2$; **17** R=2-Cl-5-NO_2; **18** R=2,3,4-triOCH_3;
19 R=3,4,5-triOCH_3; **20** X=S; R^2=H; **21** X=S; R^2=NO_2; **22** X=O; R^2=H; **23** X=O; R^2=NO_2

Scheme 1. Synthesis of benzoxazolin-2-ones **2**–**23**.

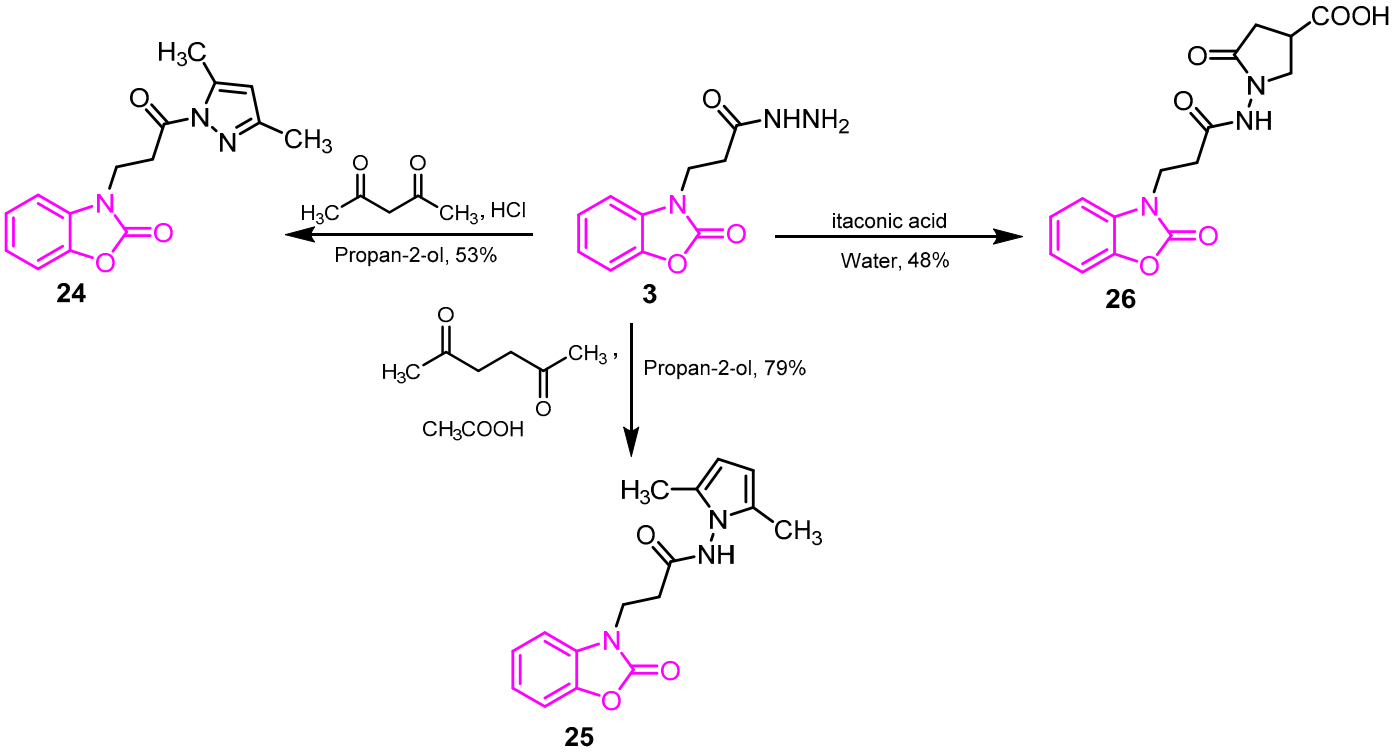
Knowing that the incorporation of the benzimidazole structure in a benzoxazole-based molecule influences an increase in its biological activity [53,54], we decided to explore it in our study. The Phillip's reaction [55] of compound **1** with a corresponding benzene-1,2-diamine in 17.5% hydrochloric acid afforded the scheduled structures **5**–**7**. The molecular structures of the benzimidazole-containing compounds **5**–**7** were easily confirmed by the data of the NMR spectra, where the presence of signals between 6.97 and 7.61 ppm integrated for 8 (compound **5**) and 7 (compounds **6** and **7**) protons reflected the presence of two aromatic rings in the synthesized derivatives. The singlets at the characteristic 12.30 (**5**), 12.46 (**6**), and 12.54 (**7**) ppm confirmed the existing NH of benzimidazole heterocycle in the molecules. In the ^{13}C NMR spectra, the resonances at 151.52 (**5**), 152.82 (**6**), and 152.94 (**7**) ppm approved the existence of the N=C-NH fragment of the benzimidazole core.

To incorporate the hydrazone moiety into the molecule, hydrazide **3** was condensed with a number of aromatic or carbaldehydes in propan-2-ol at reflux temperature. The time required for the completion of the reaction varied from 2 to 4 h, and the yields obtained were from 70.5 to 91.7%. The chemical structure of the synthesized hydrazones **8**–**23** was established based on the analysis of the ^{1}H NMR and ^{13}C NMR spectra.

Hydrazones can form four isomers owing to the presence of amide and azomethine groups in their structure. The geometrical isomers originate from the azomethine N=CH group. Hydrazone-type compounds **8**–**23** were obtained in *E* geometrical form [56]. As for rotamers, they are formed by the restricted rotation of the amide CO–NH group. The *Z* conformer is a predominating one [42,50]. From the NMR spectra, hydrazones **8**–**23** exist as mixtures of *E*/*Z* rotamers in DMSO-d_6 solutions. In all ^{1}H NMR spectra for **8**–**23**, resonances for the N=CH and CO–NH group protons were observed in double sets in the ranges of 7.80–8.57 and 11.41–11.93 ppm, respectively, and the signal intensity ratio was calculated to be 0.65:0.35, with the exception of the bulky molecules **18** and **19**, with the ratio of 0.60:0.40. In the ^{13}C NMR spectra, resonance lines for carbon atoms of the N=CH and NHC=O fragments ranged at intervals, as expected. The remaining signals in the ^{1}H NMR

and ^{13}C NMR spectra of the aliphatic and aromatic fragments were found at the expected chemical shift values. The split signals for the N=CH and NH fragments confirmed the successful preparation of the hydrazones and the correct condensation reaction.

Benzoxazolinones with attached azole structures in the molecules are documented as considerable compounds that are characterized by a number of pharmaceutical properties, namely, anticonvulsant, antihyperglycemic, antitubercular, analgesic, antimicrobial, and anticancer properties [4]. Based on it, we opted to synthesize several benzoxazolin-2-one-azole-based compounds (Scheme 2) to evaluate the antibacterial properties and structure–activity relationship.



Scheme 2. Synthesis of azoles **24**–**26**.

As a result of the heterocyclization of 3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanehydrazide (**3**) with diketones, the compounds with a combination of bicyclic benzoxazolinone with pyrazole (comp. **24**) and pyrrole (comp. **25**) rings in the structure were formed.

Upon the condensation of acid hydrazide **3** with pentane-2,4-dione in refluxing propan-2-ol containing a catalytic amount of hydrochloric acid, the corresponding derivative **24** was prepared in a 52.5% yield. Compound **25** was obtained accordingly, applying hexane-2,5-dione instead of the pentane analog and using a catalytic amount of acetic acid. The pyrrole derivative was prepared in a 78.6% yield. The characteristic signals of 3,5-dimethylpyrazole and 2,5-dimethylpyrrole were clearly visible in the NMR spectra of the compounds. Furthermore, all spectral and microanalysis data were in good agreement with the predicted structures.

The chemical modification of hydrazide **3** was attempted in an aqueous itaconic acid solution to obtain compound **26**, which was resynthesized according to the procedure described in [46].

3.2. Biology

3.2.1. Evaluation of the Antibacterial Activity by the Determination of MIC and MBC Values

The synthesized benzoxazolinone derivatives **1**–**26** were evaluated for their antibacterial activity. The bacteria strains of the Gram-positive cocci *Staphylococcus aureus* (ATCC 9144), Gram-positive sporogenic rods *Bacillus subtilis* (ATCC 6051), Gram-negative rods

Escherichia coli (ATCC 8739), and *Salmonella* Enteritidis (ATCC 13076) were used for the study of antibacterial properties, employing the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) and the calculation of the ratio of MBC/MIC, respectively. MIC was evaluated by the broth dilution method [47,48], and MBC was evaluated by plating [49]. Ciprofloxacin (CFN) was used as the standard antibiotic for all bacteria strains. The tests were carried out twice. The antibacterial activity of the selected derivates **4**, **7**, **12**, **22**, and **23** was evaluated and compared via the disc diffusion and agar well diffusion methods.

The results of the antibacterial activity of the prepared compounds are shown in Table 1.

Table 1. The MIC and MBC values of compounds **1**–**26**.

Compounds	Gram-Positive Bacteria Strains				Gram-Negative Bacteria Strains			
	S. aureus		*B. subtilis*		*E. coli*		*S. enteritidis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
	μg/mL							
1	125	500	250	500	125	500	125	500
2	125	500	250	500	125	250	125	500
3	125	500	125	250	125	500	125	500
4	62.5	500	62.5	125	62.5	500	125	500
5	125	500	250	500	125	500	125	500
6	125	500	500	500	125	500	125	500
7	125	500	62.5	125	125	500	125	500
8	125	500	125	250	125	500	125	500
9	125	500	62.5	125	125	500	125	500
10	125	500	125	250	125	500	125	500
11	125	500	62.5	125	125	500	125	500
12	62.5	500	62.5	125	62.5	500	125	500
13	125	500	62.5	125	125	500	125	500
14	125	500	250	500	125	500	125	500
15	125	500	62.5	125	125	500	125	500
16	125	500	62.5	250	125	500	125	500
17	125	500	250	500	125	500	125	500
18	125	500	125	250	125	500	125	500
19	125	500	250	500	125	500	125	500
20	125	500	125	250	125	500	125	500
21	125	500	250	500	125	500	125	500
22	125	500	62.5	125	62.5	500	125	500
23	62.5	500	62.5	125	15.6	500	62.5	500
24	62.5	500	125	250	62.5	125	125	500
25	62.5	500	125	250	125	500	125	500
26	62.5	500	125	500	125	500	125	500
CFN	15.6	15.6	62.5	62.5	15.6	15.6	15.6	15.6

The evaluation of the antibacterial activity of the synthesized derivates was performed according to the synthesis scheme and by comparing the bacteriostatic and bactericidal effects of the compounds and their derivatives, employing the determination of MIC and MB values and inhibition zones. The MIC and MBC values of the synthesized derivates are shown in Table 1.

At the beginning, according to Scheme 1, "Synthesis of benzoxazolin-2-ones **2**–**23**", *the* MIC and MBC values of compound 1 and its derivates **2**, **4**, and **5**–**7** were determined and compared. The inhibitory effect of the synthesized derivates **2** and **4**–**7** of carboxylic acid **1** for the tested bacteria was distinguished by its diversity.

The same 125 μg/mL concentrations of carboxylic acid **1** and its methyl ester **2** and benzimidazoles **5**–**7** inhibited the growth of *S. aureus*, while only its amide **4** showed a two-fold lower bacteriostatic concentration of 62.5 μg/mL. The comparison of the susceptibility

of *S. aureus* and *B. subtilis* to different compounds showed that the tested bacillus strain was inhibited by higher and lower, but not the same, minimum concentrations. The minimum bacteriostatic concentrations for the Bacillus subtilis strain were as follows: 250 μg/mL of carboxylic acid **1**, its methyl ester **2,** and unsubstituted benzimidazole **5**; 5-fluorobenzimidazole **6** required 500 μg/mL, while amide **4** and 5-chlorobenzimidazole **7** inhibited at 62.5 μg/mL.

The inhibition of the growth of Gram-negative bacteria was achieved by minimum concentrations of 125 μg/mL of acid **1** and its derivatives **2**, **5**–**7**, while amide **4** showed the same inhibition only for *S. enteritidis*. Notably, amide **4** showed the highest inhibition efficiency against the E. coli strain, with the lowest MIC value of 62.5 μg/mL among acid **1** and its derivatives.

Structure–activity analysis (SAR) for acid **1** and its derivatives **2**, **4**, and **5**–**7** demonstrated that the introduction of electron-withdrawing chlorine into the benzimidazole molecule (compound **7**), as well as the incorporation of a 4-aminobenzenesulfonamide moiety in the structure of the compound (amide **4**), selectively increased the bacteriostatic effect of the compounds. Interestingly, the formed unsubstituted benzimidazole moiety did not affect the inhibition activity in comparison with the initial acid **1**, while the introduction of a highly electronegative fluorine at the fifth position of benzimidazole strongly diminished the inhibition of the *B. subtilis* but did not affect other strains tested in comparison with the action of acid **1**.

The bactericidal activity of acid **1** and its derivatives **2**, **4**, and **5**–**7** did not change for *S. aureus* and *S. enteritidis* and was found to be 500 μg/mL for the tested *Staphylococcus* sp. and *Salmonella* sp. Most of these chemicals were bactericidal against both *B. subtilis* and *E. coli* strains at a concentration of 500 μg/mL and exhibited a selective bactericidal effect. However, some compounds were selectively the best regarding the killing of bacteria strains. 5-Chlorobenzimidazole **7** and amide **4** appeared to be bactericidal for the tested Gram-positive *Bacillus subtilis* at a concentration of 125 μg/mL, with a difference of 2 $\log_2$ dilution, and methyl ester **2** killed ≥99.9% (≥3 $\log_{10}$ reduction) of bacteria *E. coli* at a concentration of 250 μg/mL, with a difference of 1 $\log_2$ dilution. Thus, as in the MIC case, the chlorine in the benzimidazole (comp. **7**) and the 4-aminobenzenesulfonamide moiety in the molecule (comp. **4**) selectively improved the bactericidal potency of these compounds. Interestingly, SAR analysis showed that replacing the carboxyl group with an ester group did not affect the inhibitory effect of the compounds, while the bactericidal effect of the ester against *E. coli* was doubled.

The best results of the MBC evaluation among compounds **1**, **2**, **4**, and **5**–**7** were obtained against the *B. subtilis* strain, where 5-chlorobenzimidazole **7** and amide **4** showed the lowest MIC of 125 μg/mL.

The comparison of the antibacterial activity of ethyl ester **2** and its hydrazide **3** is shown in Table 1. So, ester **2** and hydrazide **3** identically inhibited the growth (MICs, 125 μg/mL) and killed (MBCs, 500 μg/mL) the tested *Staphylococcus* and *Salmonella* strains. However, it is worth noting that hydrazide **3** showed antibacterial activity against *B. subtilis*, with 1 $\log_2$ lower dilution compared with ester **2**. As for the *E. coli* strain, methyl ester **2** killed ≥99.9% (≥3 $\log_{10}$ reduction) of *E. coli* after treatment with a two-fold lower concentration of 250 μg/mL when compared to the action of hydrazide **3**.

Subsequently, the antibacterial activity of three series of hydrazide derivatives **8**–**19**, **20**–**23**, and **24**–**26** was determined and compared.

The comparative evaluation of the antibacterial activity of hydrazide **3** and the first series of hydrazones **8**–**19** showed identical or close (the difference ±1 $\log_2$ dilution) MIC and MBC values for all of the tested bacteria strains.

The MIC values of hydrazide **3** and its hydrazones **8**–**19** for *S. enteritidis* were identical and equal to 125 μg/mL. Similar results were achieved with *S. aureus* and *E. coli*, except for hydrazone **12**, with the incorporated chlorine at the third position of the additional phenyl ring, which showed a two-fold higher inhibition efficacy. The higher range (62.5–250 μg/mL) of MIC values was revealed when treating the *B. subtilis* strain. Hydra-

zones **9** and **11**–**13** with halophenyl substitutions inhibited the growth of the bacillus at a concentration of 62.5 μg/mL. The incorporation of the 4-bromophenyl (**14**), the bulkier 2-chloro-5-nitrophenyl (**17**), and the very bulky 3,4,5-trimethoxyphenyl (**19**) moieties reduced the growth inhibition properties of these compounds, resulting in an MIC of 250 μg/mL.

The MBC values of hydrazide **3** and its first-series hydrazones for *S. aureus*, *E. coli*, and *S. enteritidis* were identical and equal to 500 μg/mL. The higher range (125–500 μg/mL) of MBC values was when treating *B. subtilis*. Hydrazones **9**, **11**–**13,** and **15**, bearing an electron-withdrawing halo and nitro substituents on the phenyl rings in the molecules, killed ≥99.9% of the bacillus at a concentration of 125 μg/mL, and in this case, it was found to be the best bactericidal result in this part of the bactericidal evaluation.

Hydrazones **8**, **10**, **16**, and **18**, with unsubstituted phenyl, 2,4-difluoro, 4-dimethylamino, and 2,3,4-trimethoxy substitutions on the additional phenyl rings, respectively, retained the bactericidal effect of the initial hydrazide **3** and killed ≥99.9% of the *B. subtilis* strain at a two-fold higher concentration with an MIC of 250 μg/mL.

The comparative evaluation of the antibacterial activity of hydrazide **3** and its second series of hydrazones **20**–**23** showed a higher range of results. Identical or close (the difference ±1 $\log_2$ dilution) MIC and MBC values were determined for hydrazones **20**–**22** for all tested bacteria strains.

The MIC values of compound **3** and its hydrazones **20**–**22** for *S. enteritidis* and *S. aureus* were identical and equal to 125 μg/mL, while 2-furyl substituted hydrazone **22** was effective against *B. subtilis* and *E. coli* strains after the treatment with the 1–2 $\log_2$ lower concentration of 62.5 μg/mL. The hydrazone **23** with the incorporated 5-nitro-2-thienyl fragment was more effective when compared with hydrazide **3** against all of the tested bacteria strains, and the MIC values were 62.5 μg/mL for *S. aureus*, *B. subtilis*, and *S. enteritidis*, while 15.6 μg/mL was found for *E. coli*. Compared to structure **22**, the attached electron-withdrawing nitro group in molecule **23** highly increased the inhibition properties of the compound and led to improved action against *S. aureus*, *E. coli*, and *S. enteritidis*.

The MBC values of hydrazide **3** and its second-series hydrazones for *S. aureus*, *E. coli*, and *S. enteritidis* were identical and equal to 500 μg/mL. The higher range (125–500 μg/mL) of MBC values was found when treating the *B. subtilis* strain. The 2-furyl- and 5-nitro-2-furylhydrazones **22** and **23** killed ≥99.9% of the bacillus at a two-fold lower concentration of 125 μg/mL, whereas 5-nitro-2-thienylhydrazone **21** required 500 μg/mL to produce the same bactericidal effect in comparison with the MBC of hydrazide **3**.

The comparative evaluation of the antibacterial activity of compound 3 and its azoles **24**–**26** shows that *B. subtilis* and *S. enteritidis* were the most resistant to the action of these compounds. Identical 125 μg/mL MIC values were determined for compound **3** and its azoles **24**, **25**, and **26** for the tested bacteria strains of *B. subtilis* and *S. enteritidis*. MIC values that were lower by 1 $\log_2$ dilution (62.5 μg/mL) in comparison with hydrazide **3** were achieved by the azole derivatives **24**, **25**, and **26** for coccus, while only pyrazole **24** repeated the same antibacterial activity against *E. coli*. Pyrrole **25** and 5-oxopyrrolidine **26** had identical MIC values equal to 125 μg/mL against Gram-positive *B. subtilis* and Gram-negative *E. coli* and *S. enteritidis*.

Analyzing the relationship between the structure and activity, it can be stated that the incorporation of 3,5-dimethylpyrazole, 2,5-dimethylpyrrole, and 5-oxopyrrolidine fragments in the molecules improved the growth inhibition activity of *S. aureus*, and 3,5-dimethylpyrazole (comp. **24**) also selectively inhibited the Gram-negative strain of *E. coli*.

The MBC values of pyrazole **24**, pyrrole **25**, and 5-oxopyrrolidine **26** for *S. aureus*, *E. coli*, and *S. enteritidis* ranged from 125 μg/mL to 500 μg/mL. Compound **26** showed the lowest bactericidal activity, and its MBC value of 500 μg/mL was found for all of the tested strains. Pyrrole **25** did not show higher bactericidal activity against the tested strains of bacteria. Compared to compound **26**, hydrazide **3** and pyrrole derivative **25** showed higher efficacy only for the *B. subtilis* strain. Compared to hydrazide **3** and pyrrole **25**, pyrazole **24**

had a higher bactericidal activity, with a difference of 1 $\log_2$ dilution only against *E. coli* when compared with hydrazide **3**.

SAR analysis showed that the formation of 3,5-dimethylpyrazole and 2,5-dimethylpyrrole structures in molecules **24** and **25** maintains the bactericidal effect of hydrazide **3** against *B. subtilis*, and only 3,5-dimethylpyrazole **24** improves the bactericidal effect against *E. coli*.

The comparison of MBC and MIC values for *B. subtilis* showed that the ratios of MBC/MIC were ≤4 for all compounds, and such effects were considered the bactericidal ones. Meanwhile, the ratios of MBC/MIC values for *S. aureus* and *S. enteritidis* ranged from 4 to 8, whereas for *E. coli*, they ranged from 2 to 32. When the MBC/MIC ratio is >4, the effect of the compound is bacteriostatic (Figure 2).

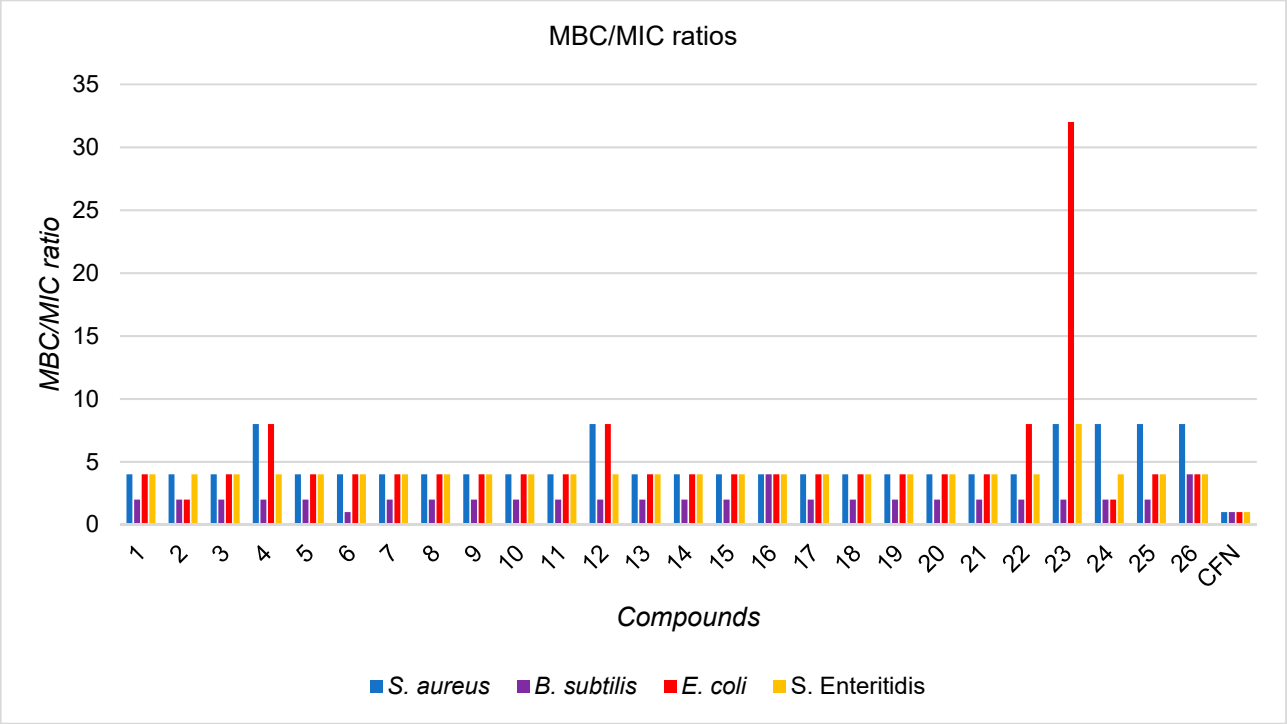


Figure 2. MBC and MIC ratios for compounds **1**–**26**.

Eighteen derivates had bactericidal effects for all of the tested bacteria, whereas derivates **4**, **12**, **22**, **23**, **24**, **25**, and **26** had bacteriostatic ones for some bacteria strains. The effect of derivates **24**, **25**, and **26** was bacteriostatic only for *S. aureus*, while **22** showed such effect for *E. coli*. The derivates **4** and **12** were bacteriostatic for two tested bacteria, *S. aureus* and *E. coli*, while **23** showed such an effect for three tested bacteria strains, *S. aureus*, *E. coli*, and *S. enteritidis*.

3.2.2. Evaluation of the Antibacterial Activity by the Determination of IZDs

The results of the in vitro antibacterial screening of the test compounds are listed in Tables 2 and 3.

The inhibitory effects of all of the selected compounds **4**, **7**, **12**, **22**, and **23** were not detected against Gram-negative and Gram-positive bacteria by both methods. Hydrazone **23**, with a 5-nitro-2-furyl substitution, showed the inhibitory effect only against Gram-positive bacteria (*S. aureus* and *B. subtilis*) strains.

Table 2. Zone of inhibition of the synthesized compounds **4**, **7**, **12**, **22**, and **23** against Gram-positive and Gram-negative bacteria by the agar well diffusion method.

Compounds		Diameter of the Zone of Inhibition (mm)			
		Gram-Positive Bacteria		Gram-Negative Bacteria	
		S. aureus	*B. subtilis*	*E. coli*	*S. enteritidis*
4	25 µg	NI	NI	NI	NI
	50 µg	NI	NI	NI	NI
7	25 µg	NI	NI	NI	NI
	50 µg	NI	NI	NI	NI
12	25 µg	NI	NI	NI	NI
	50 µg	NI	NI	NI	NI
22	25 µg	NI	NI	NI	NI
	50 µg	NI	NI	NI	NI
23	25 µg	13.11 ± 0.01	18.16 ± 0.03	NI	NI
	50 µg	18.15 ± 0.21	21.67 ± 0.08	NI	NI
Ciprofloxacin, 5 µg		26.00 ± 1.41	26.50 ± 0.71	31.50 ± 0.71	29.50 ± 2.12

Table 3. Zone of inhibition of the synthesized compounds **4**, **7**, **12**, **22**, and **23** against Gram-positive and Gram-negative bacteria by the disk diffusion method.

Compounds		Diameter of the Zone of Inhibition (mm)			
		Gram-Positive Bacteria		Gram-Negative Bacteria	
		S. aureus	*B. subtilis*	*E. coli*	*S. enteritidis*
4	3 µg	NI	NI	NI	NI
	5 µg	NI	NI	NI	NI
7	3 µg	NI	NI	NI	NI
	5 µg	NI	NI	NI	NI
12	3 µg	NI	NI	NI	NI
	5 µg	NI	NI	NI	NI
22	3 µg	NI	NI	NI	NI
	5 µg	NI	NI	NI	NI
23	3 µg	6.73 ± 0.01	10.38 ± 0.04	NI	NI
	5 µg	14.03 ± 0.01	16.07 ± 014	NI	NI
Ciprofloxacin, 5 µg		26.00 ± 1.41	26.50 ± 0.71	31.50 ± 0.71	29.50 ± 2.12

Data are presented as the mean ± SD. Values are presented for duplicate experiments. NI = No inhibition, (+ve) = Gram-positive, (−ve) = Gram-negative.

The antibacterial activity test results for the Gram-positive bacteria *S. aureus* and *B. subtilis* indicated that the size of the IZDs for both methods correlated with the amount of hydrazone **23**. It was determined by the agar well diffusion method that the IZDs for *S. aureus* were 13.11 ± 0.01 mm after using 25 µg of a compound and increased to 18.15 ± 0.21 mm when 50 µg was used. The increase in IZDs was demonstrated after the treatment of *B. subtilis* with different quantities of the compound as well. However, the size of the IZDs was greater for bacilli and formed 18.16 ± 0.03 mm and 21.67 ± 0.08 mm after using volumes containing 25 µg and 50 µg of compounds, respectively. The same was observed using the disk agar diffusion method with volumes containing 3 µg and 5 µg of the compounds. The IZDs were 6.73 ± 0.01 mm and 14.03 ± 0.01 mm for *S. aureus*

after using volumes containing 3 µg and 5 µg of compounds, respectively. The size of the IZDs correlated with the amount of the compound and were 10.38 ± 0.04 mm and 16.07 ± 0.14 mm for *B. subtilis* as well.

4. Conclusions

In this study, the antibacterial activity against *S. aureus* ATCC 9144, *B. subtilis* ATCC 6051, *E. coli* ATCC 8739, and *S. enteritidis* ATCC 13,076 pathogens of a series of derivatives based on 3-(2-oxobenzo[d]oxazol-3(2*H*)-yl) propanoic acid was investigated as a potential core for the search of antibacterials. The synthesized compounds were confirmed by the spectroscopic and microanalysis data.

The estimation of MIC and MBC showed the different potential of the antibacterial effect of the compounds on both Gram-positive and Gram-negative strains. Meanwhile, agar diffusion methods showed that the most effective was the 23-chemical compound against *Staphylococcus aureus* and *Bacillus subtilis*.

The study provides foundational synthetic procedures for subsequent compound development, opening the way for hit-to-lead optimization and structure-activity relationship data. Further studies are mandatory for a better understanding of the in vitro and in vivo safety, bioavailability, and tolerability of 3-(2-oxobenzo[d]oxazol-3(2*H*)-yl)propanoic acid derivatives, and new compounds based on this pharmacophore, which may help in the discovery of new selective antibiotics against various infectious diseases.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14114783/s1. Figure S1: ^{1}H NMR of compound **2**, Figure S2: ^{13}C NMR of compound **2**, Figure S3: ^{1}H NMR of compound **3,** Figure S4: ^{13}C NMR of compound **3,** Figure S5: ^{1}H NMR of compound **4,** Figure S6: ^{13}C NMR of compound **4**, Figure S7: ^{1}H NMR of compound **5,** Figure S8: ^{13}C NMR of compound **5**, Figure S9: ^{1}H NMR of compound **6,** Figure S10: ^{13}C NMR of compound **6**, Figure S11: ^{1}H NMR of compound **7,** Figure S12: ^{13}C NMR of compound **7**, Figure S13: ^{1}H NMR of compound **8,** Figure S14: ^{13}C NMR of compound **8**, Figure S15: ^{1}H NMR of compound **9,** Figure S16: ^{13}C NMR of compound **9**, Figure S17: ^{1}H NMR of compound **10,** Figure S18: ^{13}C NMR of compound **10**, Figure S19: ^{1}H NMR of compound **11,** Figure S20: ^{13}C NMR of compound **11,** Figure S21: ^{1}H NMR of compound **12,** Figure S22: ^{13}C NMR of compound **12,** Figure S23: ^{1}H NMR of compound **13,** Figure S24: ^{13}C NMR of compound **13,** Figure S25: ^{1}H NMR of compound **14,** Figure S26: ^{13}C NMR of compound **14,** Figure S27: ^{1}H NMR of compound **15,** Figure S28: ^{13}C NMR of compound **15,** Figure S29: ^{1}H NMR of compound **16,** Figure S30: ^{13}C NMR of compound **16,** Figure S31: ^{1}H NMR of compound **17,** Figure S32: ^{13}C NMR of compound **17,** Figure S33: ^{1}H NMR of compound **18,** Figure S34: ^{13}C NMR of compound **18,** Figure S35: ^{1}H NMR of compound **19,** Figure S36: ^{13}C NMR of compound **19,** Figure S37: ^{1}H NMR of compound **20,** Figure S38: ^{13}C NMR of compound **20,** Figure S39: ^{1}H NMR of compound **21,** Figure S40: ^{13}C NMR of compound **21,** Figure S41: ^{1}H NMR of compound **22,** Figure S42: ^{13}C NMR of compound **22**, Figure S43: ^{1}H NMR of compound **23,** Figure S44: ^{13}C NMR of compound **23,** Figure S45: ^{1}H NMR of compound **24,** Figure S46: ^{13}C NMR of compound **24,** Figure S47: ^{1}H NMR of compound **25,** Figure S48: ^{13}C NMR of compound **25,** Figure S49: ^{1}H NMR of compound **26**, Figure S50: ^{13}C NMR of compound **26**.

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