Synthesis and Antibacterial Activity of New Azole, Diazole and Triazole Derivatives Based on \( p \)-Aminobenzoic Acid

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Abstract: The \( p \)-aminobenzoic acid was applied for the synthesis of substituted 1-phenyl-5-oxopyrrolidine derivatives containing benzimidazole, azole, oxadiazole, triazole, dihydroazine, and dithiosemicarbazide moieties in the structure. All the obtained compounds were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli*, and *Pseudomonas aeruginosa* by using MIC and MBC assays. This study showed a good bactericidal activity of \( \gamma \)-amino acid and benzimidazoles derivatives. The antimicrobial activity of the most promising compounds was higher than ampicillin. Furthermore, two benzimidazoles demonstrated good antimicrobial activity against *L. monocytogenes* (MIC 15.62 \( \mu \)g/mL) that was four times more potent than ampicillin (MIC 65 \( \mu \)g/mL). Further studies are needed to better understand the mechanism of the antimicrobial activity as well as to generate antimicrobial compounds based on the 1-phenyl-5-oxopyrrolidine scaffold.

Keywords: hydrazides; 2-pyrrolidinone; azoles; benzimidazole; antimicrobial activity

1. Introduction

Rapidly growing antimicrobial resistance (AMR) has become a major source of morbidity and mortality worldwide [1]. Increasing AMR among various pathogens has led to fewer treatment options for patients suffering from severe infections caused by drug-resistant (DR) pathogens. Moreover, infections caused by DR microorganisms require more extensive treatment, therefore resulting in a longer course of illness and prolonged hospitalization duration [2,3].

The extensive use of various antimicrobials in agriculture and veterinary sectors played a pivotal role in the development of AMR and the selection of highly virulent bacterial strains [4–7]. The DR pathogens of veterinary origin can further colonize the environment and can be transferred to humans [8,9]. In addition, the genetic determinants encoding AMR phenotypes can be further disseminated via horizontal gene transfer and accumulate in various bacterial species [10,11]. These processes created a vicious cycle that gave rise to multidrug-resistant (MDR) pathogens harboring multiple resistance mechanisms, resulting in bacterial resistance to two and more antimicrobial drugs [12]. To overcome this problem, it is important to develop novel compounds targeting MDR pathogens.
The ESKAPE group of pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species complex) is the leading cause of hospital-acquired infections worldwide [13–15]. This group of Gram-positive and Gram-negative bacteria causes life-threatening infections amongst critically and chronically ill or immunocompromised individuals [15]. The growing antimicrobial resistance among ESKAPE pathogens creates a significant burden on healthcare systems and has important global economic costs. Therefore, it is important to develop novel compounds targeting clinically relevant multidrug-resistant ESKAPE group of pathogens.

\( p \)-Aminobenzoic acid (\( p \)-ABA) and its derivatives are well-known for their chemical properties and the broad spectrum of biological activity, and they have attracted considerable pharmacological and industrial interest. \( p \)-ABA is widely distributed in nature and is abundant in various plant and animal tissues. Moreover, \( p \)-ABA is found in various animal and plant-based sources such as grains, eggs, milk, and meat. Furthermore, \( p \)-ABA is frequently found as a structural moiety in drugs and plays an important role as a pharmacophore. In a voluminous database of commercial pharmaceuticals, 1.5% were found to contain the \( p \)-ABA moiety [16].

Various compounds bearing a \( p \)-ABA nucleus exert strong antimicrobial [17,18], antimitagenic [19], antioxidant, cytoprotective [20], and immunomodulatory [21,22] properties. Moreover, various \( p \)-ABA derivatives have antineoplastic, anesthetic, antiarrhythmic, anticonvulsant, antiemetic, and gastrokinetic [16,23] properties. \( p \)-ABA is also involved in the biosynthesis of coenzyme Q [24,25]; it also increases the thermotolerance [26] in plants and plays an important role as a signaling molecule in the recognition of the plant pathogens [27]. Various \( p \)-ABA derivatives were previously explored as promising immunomodulatory agents. \( p \)-ABA was shown to induce the transcriptional activation of interferons in various cell types [22]. In addition to this, compounds bearing the \( p \)-ABA nucleus were found to be a promising cyclophilin inhibitors [28]. Furthermore, \( p \)-ABA derivatives were shown to prevent steel corrosion [29] and increase dye adsorption during textile dyeing [30]. The wide spectrum of biological activity of \( p \)-ABA derivatives could be potentially exploited for the development of novel antimicrobial and immunomodulatory drugs.

The 5-oxopyrrolidine or \( \gamma \)-lactam moiety is a constituent of many natural and non-natural biologically active compounds. The broad range of biological activity displayed by functionalized 2-pyrrolidinones makes them an attractive group with profound therapeutic [31] and antioxidant [32] use. Moreover, compounds bearing \( \gamma \)-lactam moiety were previously demonstrated to bind the CCR4 chemokine receptor, making these compounds potential therapeutics in treating T-cell neoplasms [33]. Besides immunomodulatory and antiviral activity, 5-oxopyrrolidine derivatives were shown to have promising antimicrobial [34,35], antioxidant [36], antitumor [37], and anti-inflammatory [38] properties, showing high binding affinity towards carbonic anhydrase isoforms [39,40].

The benzimidazole scaffold attracts considerable attention due to its numerous biological properties. One of the most known structures containing the benzimidazole scaffold is cyanocobalamin, also known as cobalamin (vitamin \( B_{12} \)), which is involved in cellular metabolism processes.

With a great affinity displayed towards a numerous enzymes and receptors, the benzimidazoles could be potentially explored for the development of new pharmaceuticals. Numerous studies revealed that compounds derived from a benzimidazole nucleus exhibit analgesic and anti-inflammatory activity [41], as well as antiulcerative [42], anticancer [43], antiparasitic [44], antimicrobial [45–47], antioxidant [46], anticonvulsant [48], anticancer/antiestrogenic [49,50], antihypertensive [51], antiinflammatory [52], and many others [53–60] activities. Therefore, the strategies of developing compounds bearing the benzimidazole scaffold should be further explored to generate pharmacologically active molecules.

1,2,4-Triazole derivatives have been reported to possess a wide range of bioactivities such as neuroprotective [61], antifungal [62], anticancer [63], antibacterial [64], antihypertensive, and cardioprotective [65], antiviral [66], and anticonvulsant [67]. The diverse
pharmaceutical properties of triazoles induced a deep interest in discovering new entities for their broader applications. This fragment is a constituent of a variety of pharmaceuticals (etizolam, estazolam, trazodone, ribavirin, trapidil, rizatriptan, anastrozole) that are available in a clinical setting for the treatment of patients suffering from various diseases, including muscle tension, suppression of seizures, depression, viral diseases, antiplatelet action, migraine pains and breast cancer [68,69]. The introduction of the thione group, either in 3- or 5-position, leads to an enhancement of biological activities related to triazole moiety [70]. The triazolethione system is a cyclic analog of very important components available in a clinical setting for the treatment of patients suffering from various diseases, including muscle tension, suppression of seizures, depression, viral diseases, antiplatelet action, migraine pains and breast cancer [68,69]. The introduction of the thione group, including muscle tension, suppression of seizures, depression, viral diseases, antiplatelet action, migraine pains and breast cancer [68,69]. The introduction of the thione group, including muscle tension, suppression of seizures, depression, viral diseases, antiplatelet action, migraine pains and breast cancer [68,69].

The profound biological activity of pABA and various azoles makes them attractive building blocks for the development of novel antimicrobial compounds targeting clinically important pathogens. With this notion, in this paper we aim to synthesize a series of newazole, diazole, and triazole derivatives based on p-aminobenzoic acid and evaluate their in vitro antimicrobial properties against clinically important bacterial pathogens.

2. Results

2.1. Synthesis

Considering the wide structural and biological diversity of p-aminobenzoic acid and its derivatives, herein we present the synthesis and antibacterial evaluation of a series of 1-(4-carboxyphenyl)-5-oxopyrrolidine-3-carboxylic acid derivatives using p-aminobenzoic acid as the starting compound (Scheme 1). Compound 2 was prepared from the 4-aminobenzoic and itaconic acids by the method described in [72]. The esterification of compound 2 with methanol afforded methyl ester 3, which then under the action of hydrazine monohydrate in 2-propanol was converted into hydrazide 4, containing only one hydrazinocarbonyl moiety.

![Scheme 1](image-url)

Scheme 1. Synthesis of 5-oxopyrrolidine derivatives 2–13. 7a, Ar = C₆H₅; 7b, Ar = 4-MeO-C₆H₄; 7c, Ar = 4-Me₂N-C₆H₄; 11a, Ar₁ = 4-O₂N-C₆H₄; 11b, Ar₁ = 4-Cl-C₆H₄.

The hydrazide functional group can undergo various chemical transformations; using this ability, we thus performed a series of chemical reactions, applying different carbonyl compounds. The reaction of hydrazide 4 with pentane-2,4-dione (2,4-PD) in refluxing ethanol produced pyrazole derivative 5, and the Paal–Knorr pyrrole synthesis using hexane-2,5-dione (2,5-HD) afforded pyrrole 6. A catalytic amount of glacial acetic acid was used in the reaction. In the ¹H NMR spectrum of 6, the intense singlets at 2.0 and 5.65 ppm
were assigned to the protons of two methyl (2- and 5-positions) and two C=CH groups of the pyrrole ring, respectively. The resonances at 103.10 and 126.74 ppm in the $^{13}$C NMR spectrum of compound 6 finally approved the formed pyrrole cycle in the molecule. All NMR spectra of the synthesized compounds are given in the Supplementary Materials.

Hydrazones 7a–c were prepared by the condensation of acid hydrazide 4 with benzaldehyde, 4-methoxybenzaldehyde, and 4-dimethylaminobenzaldehyde in refluxing ethanol (a,c) or a mixture of ethanol and 1,4-dioxane (1:2). In the reaction with itaconic acid, the hydrazide that has the amine group can readily undergo autocatalyzed intramolecular amidation–cyclization reaction to yield a stable 5-membered N-substituted pyrrolidinone cycle. The reaction was carried out in water at reflux for 15 h. Multiplets in the ranges of 2.50–2.90 (COCH$_2$), 3.20–3.40 (CH) 3.56–3.72 (NCH$_2$), and 3.87–4.18 (NCH$_2$) ppm, integrated for 10 protons in total in the $^1$H NMR spectrum as well as double sets of the resonances of carbons of the COCH$_2$, CH, NCH$_2$ fragments in the $^{13}$C NMR spectra of compound 8 approve the presence of two pyrrolidinone rings.

For the synthesis of the target benzoylhydrazine derivative 9, hydrazide 4 was reacted with benzoic acid in dichloromethane at reflux for 10 min. The product 9 from the reaction mixture was isolated in 54% yield. The formation of the –CONHNHCOPh– fragment was approved by the presence of two singlets at 10.29 (NH) and 10.47 (NH) ppm, and the multiplet was integrated for nine protons of the two aromatic rings in the interval of 7.35–8.08 ppm.

To obtain a compound containing two hydrazinocarbonyl fragments, the reaction was carried out in hydrazine monohydrate using a 17 excess. The target product was obtained in a 54% yield.

The comparison of the spectra of compounds 4 and 10 demonstrated some differences that led to the easy identification of their specific structures. In the $^1$H NMR spectrum of compound 4, the singlet at 3.82 ppm ($^{13}$C, 51.99 ppm), integrated for three protons, shows the presence of the methoxy group, and the singlet at 4.35 ppm integrated for two protons proves the presence of the amino group, while in the $^1$H NMR of dihydrazide 10, the signal of methoxy group is absent, and the broad singlet at 4.39 ppm is integrated for four protons, which proves the presence of two amino groups in the molecule.

Hydrazones 11a,b were obtained by the condensation of dihydrazide 10 with aromatic aldehydes. The reaction was carried out in the mixture of 2-propanol and 1,4-dioxane (ratio of 1:1.7) at reflux for 12 (a) or 11 (b) h, and products from the reaction mixtures were separated in 77% and 88% yields, respectively.

The synthesized hydrazones 7 and 11 possess amide and azomethine groups in their structures. Based on the experimental and theoretical studies presented in literature [73], it can be stated that due to the presence of the amide fragment and the restricted rotation around the CO–NH bond, the hydrazones exist in DMSO solutions as a mixture of Z/E rotamers in which the Z rotamer predominates. The clearest proof of the existence of conformers produced due to the presence of the CONH fragment was the discovery of the two sets of resonances of the NH group in the low-field region of the $^1$H NMR spectra recorded in DMSO-$d_6$, where a stronger-field side signal was related to the resonance of the rotamer with the Z structure.

The existence of the mixtures of stereoisomers relative to the CH=N structural fragment of the molecules was also found in the $^1$H NMR spectra of the monosubstituted hydrazones 7 and 11. Based on the studies described in the academic literature [73], as well as the data of the spectra of these compounds, we can conclude that the produced mixtures of stereoisomers with the Z-isomer predominated.

The interaction of dihydrazide 10 with phenyl isothiocyanate in refluxing methanol led to the formation of thiosemicarbazide 12, which then under the action of 4% sodium hydroxide at reflux for 6 h and subsequent acidification of the mixture with dilute hydrochloric acid (1:1) to pH 2 afforded heterocyclic compound 13 with two 4-phenyl-5-thioxo-1,2,4-triazole moieties in the structure. Resonances in the interval of 9.39–9.75 (4H, 2NH/HCO) as well as 10.12 and 10.40 (2H, 2NH) ppm in the $^1$H NMR spectrum of compound 12 is clear.
evidence for the formation of the –CONHNHCSNH– moiety. Cyclodehydration of this fragment in the presence of a strong base led to the 1,2,4-triazole 13 formation, which was confirmed by the absence of thiosemicarbazide-specific spectral lines and the observation of a decrease and downfield shift of the NH resonances (1H, 13.87 ppm).

Knowing the wide range of applications of the biological properties of benzimidazole derivatives in various fields, including medicine, pharmacy, optics, and others, we decided to synthesize compound 14 to have two benzimidazole moieties in its structure (Scheme 2). Five methods to achieve this goal were used. The reaction conditions and yields of the obtained product are given in Table 1.

![Scheme 2. Synthesis of benzimidazole derivatives 14–18. 14, R = H; 15, R = CH3.](image)

### Table 1. Reaction conditions for the synthesis of benzimidazoles 14 and 15 and their corresponding product yields.

| Entry | Reagent | Solvent/Catalyst | Temperature, °C | Time, h | Yield, % |
|-------|---------|------------------|-----------------|---------|----------|
| 1     |         | -                | 170; 230        | 2; 0.5  | 51       |
| 2     |         | 15% HCl          | Reflux          | 96      | 8        |
| 3     | Benzene-1,2-diamine | -           | MW, 140 W       | 0.25    | 40       |
| 4     |         | 2-ProOH/NH₄Cl    | Reflux          | 25      | 12       |
| 5     |         | PPA              | 120             | 6       | 97 (14); 91 (15) |

The melting of carboxylic acid 2 with benzene-1,2-diamine at 170 °C and then at 230 °C gave the target compound a 51% yield. The condensation of benzene-1,2-diamine with acid 2 in 15% hydrochloric acid (by the Phillips method), at reflux yielded the desired product 14, but the process took 96 h, and the product obtained in only a 8% yield. For this reason, we tried a more modern method using microwaves, where the reaction mixture was exposed to microwave irradiation (140 W) for 15 min under solvent-free conditions. Benzimidazole 14 was obtained in a 40% yield. The reaction in 2-propanol with ammonium chloride as a catalyst did not produce the expected yield of the benzimidazole. The yield of bisbenzimidazole 14 was found to be only 12%. The most efficient method for the preparation of compound 14 appeared to be condensation of dicarboxylic acid 2 with o-phenylenediamine.
in polyphosphoric acid (PPA) at 120 °C for 6 h. The product was separated in 97% yield. The last-mentioned method was used to obtain methylbenzimidazole derivative 15.

The pyrrolidine ring of compound 14 was readily decyclized under alkaline hydrolysis conditions by refluxing it in an aqueous 20% sodium hydroxide solution for 2 h. The NMR spectra of the obtained product 16 showed chemical shifts characteristic to the open-chain structure in comparison with the initial compound 14.

The esterification of amino acid 16 with methanol in the presence of a catalytic amount of sulfuric acid was unsuccessful when the action of a strong acid led to the cyclization of the butanoic fragment to the initial pyrrolidine ring. Therefore, to prepare acid hydrazide, we had to choose another synthesis route. Butanoic acid hydrazide 17 was obtained directly from pyrrolidine derivatives 14 and its decyclized product—butanoic acid 16. The interaction of butanoic acid 16 with hydrazine monohydrate proceeded successfully under mild conditions, i.e., heating the reaction mixture in 2-propanol at reflux for 20 h afforded acid hydrazide 17. However, to obtain it from the pyrrolidine derivative 14, tightened reaction conditions were needed. Therefore, the reaction was carried out in excess hydrazine monohydrate at reflux for 6 h. The 1H and 13C NMR spectra of 17 confirmed the open-chain compound. In the NMR spectra of 17, the triplet at 6.34 ppm was ascribed to the NH proton, the singlets at 4.43 (NH2, 1H) and 9.12 (NHNH2, 1H), and the spectral line at 169.90 (C=O, 13C) approved the formed acid hydrazide moiety.

The condensation of hydrazide 17 with hexane-2,5-dione was investigated. As expected, the reaction in 2-propanol at reflux, with the presence of a catalytic amount of hydrochloric acid, afforded 2,5-dimethylpyrrole derivative 18. The singlets at 1.62 and 1.95 (CH3) and the doublets at 5.52, 5.57 (C=CH) ppm in the 1H NMR spectrum, in addition to resonances at 10.50, 11.00, and 102.76, 102.81 ppm of the corresponding groups in the 13C NMR spectrum, prove the presence of the 2,5-dimethylpyrrole fragment.

Benzimidazoles play an important role in modern medicinal chemistry by being an important pharmacophore. With this notion, it is important to develop a large amount of structurally diverse benzimidazoles with potential medicinal properties.

For this purpose, the functionalization of benzimidazole 14 was performed (Scheme 3). The functionalization at nitrogen is perhaps the most common, and therefore it was chosen for the investigation. Initially, N-substituted benzimidazole derivative 19 was synthesized by the alkylation of bisbenzimidazole 14 with ethyl chloroacetate in acetone in the presence of potassium carbonate and a catalytic amount of TBAI (tetrabutylammonium iodide). The reaction was carried out under mild conditions, i.e., heating the reaction mixture in 2-propanol at reflux for 20 h. The obtained ethyl ester 19 further was applied for the preparation of hydrazide 20. The structures of the synthesized compounds 19 and 20 were determined by spectral methods and chemical transformations.

The refluxing of 19 with hydrazine monohydrate in 1,4-dioxane for 18 h led to the formation of compound 20 containing two 2-hydrazinyl-2-oxoethyl moieties, whose presence is confirmed by the singlets at 4.49 (2NH2, 1H), 4.87, 4.95, 5.24, and 5.32 (2NCH2CO, 1H), 8.83, 8.88, 9.60, and 9.65 (2NH, 1H), as well as by the resonance lines at 166.01 and 166.34 (2CONH, 13C) ppm.

Carbonyl compounds are frequently used as derivatization agents. The condensation of acid hydrazide with diketone pentane-2,4-dione gave 3,5-dimethylpyrazole derivatives 21 a 82% yield. The reaction of 21 was performed for 5 h in refluxing 2-propanol and in the presence of a catalytic amount of hydrochloric acid. The reaction product was isolated from the reaction mixture by diluting it with water. The data of the 1H and 13C NMR spectroscopic techniques and elemental analysis confirmed the proposed structures of the synthesized pyrazole 21.

Oxadiazole derivative 22 was obtained by the ring closure reaction of the hydrazide 20 with carbon disulfide in alkaline medium obtained by using potassium hydroxide, which was dissolved in methanol, and then CS2 was added dropwise to the cooled solution. After a thorough stirring for 15 min, the required amount of hydrazide was added, and the obtained reaction mixture was refluxed for 12 h. The acidifying of the aqueous solution of the reaction mixture with hydrochloric acid to pH 1 afforded the desired derivative 22.
with two 1,3,4-oxadiazole moieties in the molecule. The signals in the NMR spectra of the compound were an exact match of the protons and carbon atoms of the obtained structure.

![Scheme 3](image)

Scheme 3. Synthesis of bisbenzimidazole derivatives 19–24. 24 a, Ar = C₆H₅; b, Ar = 4-O₂N-C₆H₄; c, Ar = 4-F-C₆H₄; d, Ar = 3-Cl-C₆H₄; e, Ar = 2,3-di(H₂O)-C₆H₃.

Derivative 23 with two thiosemicarbazide moieties in the molecule was obtained in the reaction of hydrazide 20 with phenyl isothiocyanate. The reaction was performed in methanol at reflux for 6 h, and the obtained hydrazinecarbothioamine 23 was then applied for the synthesis of triazolethione using a method of cyclization in basic conditions. Cyclization was performed in an aqueous 4% sodium hydroxide solution in order to prevent 5-oxopyrrolidine ring breakage with the subsequent acidification of the reaction mixture with dilute hydrochloric acid (1:1) to pH 2. However, the process failed, and efforts to separate the cyclized product were unsuccessful. The spectral data (NMR, IR, and elemental analysis) of thiosemicarbazide 23 were in full agreement with the proposed structure. The multiplet in the range of 9.48–10.04 (4NH) ppm and the singlets at 10.51 and 10.58 (2NH) ppm in the ¹H NMR spectrum for 23 as well as additional peaks in the interval of 7.08–7.95 ppm prove the presence of the CONHNHCNSNHPh fragment.

The hydrazones 24 were prepared by the condensation of hydrazide 20 with the corresponding aromatic aldehyde (benzaldehyde (a), 4-nitro- (b), 4-fluoro- (c), 3-chloro- (d) and 2,3-dimethoxybenzaldehyde (e)) in a molar ration of 1:7.5. The reactions were carried out by heating the mixtures at reflux for 5 h, except in case b when the reaction proceeded for 8 h. The products were separated in good to excellent yields in the range of 77%–94%.

When ¹H-NMR spectra of compounds 24 were observed, two sets of signals of the protons at different ppm were seen. This is because of the compounds, which have an arylidenic hydrazide structure. The restricted rotation around the CO–NH bond causes the formation of a mixture of Z/E rotamers, whereas the presence of a double bond of CH=N influences the formation of geometric isomers, which are clearly visible in the spectra of these compounds [74]. The ratio in each case was calculated by using ¹H-NMR data, and they are as follows: 0.75:0.25 for a–c, e and 0.8:0.2 for d.
2.2. The Antimicrobial Activity of the Synthesized Compounds

In this study, we aimed to synthesize a series of novel benzimidazole derivatives bearing ethyl ester, hydrazide, 3,5-dimethylpyrazole, 2,5-dimethylpyrrole, oxadiazole, thiosemicarbazide, and hydrazide moieties and to investigate their in vitro antimicrobial properties against a series of Gram-positive and Gram-negative bacterial pathogens.

The antimicrobial properties of synthesized compounds 3–24 were investigated against *Staphylococcus aureus, Listeria monocytogenes, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella enteritidis* (Supplementary Table S1). The minimal inhibitory concentration (MIC) was evaluated by the broth dilution method, while the minimal bactericidal concentration (MBC) was determined by plating.

Compounds 3–24 demonstrated acceptable antimicrobial activity against Gram-positive microorganisms, suggesting the possible existence of Gram-positive bacteria-selective targets for compounds 3, 5, 14–17, 19, and 20, which bear dimethyl ester, 3,5-dimethylpyrazole, bisbenzimidazole, bis 5(6)-methylbenzimidazole, γ-amino acid and its hydrazide, diethyl ester, and dihydrazide fragments, respectively. The antimicrobial activity was highly structure-dependent and was mostly bactericidal at near-MIC concentration (Table 2). The most promising compounds demonstrated near-MIC bactericidal activity, therefore further showing importance of the abovementioned scaffold as novel antimicrobials targeting Gram-positive bacterial pathogens.

The results obtained in this study showed that all tested methyl derivatives 3–9, dihydrazide 10, and its derivatives 12 and 13 demonstrated moderate antimicrobial activity on all tested bacteria strains (Table 2). Moreover, hydrazones 11a, b did not demonstrate antimicrobial activity on neither Gram-positive nor Gram-negative bacteria. Hydrazone 11b harboring 4-chlorobenzylidene fragment demonstrated weak but selective bactericidal activity on *S. aureus* (250 µg/mL) but not on other Gram-positive or Gram-negative organisms (Table 2).

Diester 3 and pyrazole derivative 5 exhibited good bactericidal activity (MIC and MBC of 31.25 µg/mL) against *B. cereus*. Interestingly, compounds 3 and 5 did not show a good antimicrobial activity against other Gram-positive organisms, suggesting the possible presence of *B. cereus*-specific targets of compound 5 (Table 2).

Benzimidazole 14, methylbenzimidazole 15, γ-amino acid 16, and oxadiazole 22 demonstrated broad-spectrum antimicrobial activity that targets both Gram-negative and Gram-positive microorganisms (Table 2). Compound 16 demonstrated the highest antimicrobial activity against all tested strains, suggesting the important role of γ-amino acid moiety for biological activity.

Furthermore, in this study, the bactericidal activity of hydrazide 17 and diester 19 bactericidal activity on *S. aureus* was comparable to ampicillin (62.5 µg/mL). The incorporation of γ-amino acid moiety in compound 16 and 4-(nitrobenzylidene)hydrazinyl fragment in 24b resulted in a compound with good activity against Gram-negative organisms (MIC and MBC at 31.25 µg/mL). In this study, compound 16 bearing γ-amino acid moiety demonstrated the most potent antimicrobial activity against a broad spectrum of microorganisms, demonstrating the importance of the abovementioned fragment as an antibacterial pharmacophore.

Interestingly, benzimidazoles 14 and 15 were an exception in this assay and had an exclusive activity against *L. monocytogenes*, although no activity was observed when tested against *S. aureus* or *B. cereus*. Compound 14 demonstrated slightly better, near-MIC bactericidal activity (MBC 15.62 µg/mL) (Table 2). Benzimidazole 15 bearing 5(6)-methyl moiety showed one dilution higher MBC (31.25 µg/mL), suggesting that the 5(6)-methyl moiety is important for bactericidal activity against *L. monocytogenes*.

The investigations of structure-activity based relationships revealed some evident facts that changes in the 1-phenyl-5-oxopyrrolidine backbone by an incorporation of benzimidazole moieties greatly affect the biological properties of the compounds. The data presented in Table 2 demonstrate that benzimidazole 14 shows broad-spectrum antimicrobial activity, which was most evident when tested against *L. monocytogenes*. The antimicrobial activity...
of compound bearing a nitro group (24b) in the benzene ring was confirmed in this study when stronger antibacterial properties against the *E. coli* and *P. aeruginosa* strains were seen in comparison to other hydrazones 24.

Table 2. Minimal inhibitory concentrations (MIC) as well as minimal bactericidal concentrations (MBC) of 5-oxopyrrolidine derivatives 3–24 against various bacterial strains.

| Compound | Gram-Positive Bacteria | Gram-Negative Bacteria |
|----------|------------------------|------------------------|
|          | *S. aureus* ATCC 9144   | *B. cereus* ATCC 11778  | *L. monocytogenes* ATCC 7644 | *S. enteritidis* ATCC 13076 | *E. coli* ATCC 8739 | *P. aeruginosa* NCTC 6750 |
|          | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL | MIC µg/mL | MBC µg/mL |
| 3        | 250      | 250      | 31.25    | 31.25    | 125      | 125      | 250      | 250      | 250      | 250      | 250      | 250      |
| 4        | 125      | 125      | 125      | 250      | 250      | 250      | 250      | 250      | 125      | 125      | 250      | 250      |
| 5        | 250      | 250      | 31.25    | 31.25    | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 6        | 250      | 250      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 7a       | 250      | 250      | 250      | 250      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 7b       | 250      | 250      | 250      | 250      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 7c       | 250      | 250      | 250      | 250      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 8        | 250      | 250      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 9        | 125      | 125      | 125      | 250      | 250      | 250      | 250      | 250      | 125      | 125      | 250      | 250      |
| 10       | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 11a      | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     |
| 11b      | 250      | 250      | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     | >250     |
| 12       | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 13       | 125      | 125      | 125      | 250      | 62.5     | 62.5     | 125      | 125      | 250      | 62.5     | 62.5     | 62.5     |
| 14       | 62.5     | 62.5     | 62.5     | 62.5     | 15.62    | 15.62    | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 15       | 62.5     | 125      | 62.5     | 125      | 15.62    | 31.25    | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 16       | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    | 31.25    |
| 17       | 31.25    | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 18       | 31.25    | 31.25    | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 19       | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 20       | 125      | 125      | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 21       | 125      | 125      | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 22       | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 31.25    | 31.25    | 62.5     | 62.5     | 62.5     | 62.5     |
| 24a      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      | 125      |
| 24b      | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     |
| 24c      | 125      | 125      | 125      | 125      | 62.5     | 62.5     | 62.5     | 62.5     | 125      | 125      | 125      | 125      |
| 24d      | 125      | 125      | 125      | 125      | 62.5     | 62.5     | 62.5     | 62.5     | 125      | 125      | 125      | 125      |
| 24e      | 62.5     | 125      | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 62.5     | 125      | 62.5     | 62.5     | 62.5     |

Ampicillin 62.5

The results generated during this study are expected to be a foundation for the development of novel 1-phenyl-5-oxopyrrolidine-based antimicrobials. Further studies are needed to better understand the mechanism of antimicrobial activity as well as to generate more potent antimicrobial compounds based on the 1-phenyl-5-oxopyrrolidine nucleus.

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. Chemical shifts were reported in (δ) ppm relative to tetramethylsilane (TMS) with the residual solvent as internal reference ([D₆]DMSO, δ = 2.50 ppm for H₂ 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 using KBr pellets. Mass spectra were obtained on a Bruker maXis UHRTOF mass spectrometer with ESI ionization. Elemental analyses (C, H, N) were conducted using the Elemental Analyzer CE-440; their results were found to be in good agreement (±0.3%) with the calculated values.

1-(4-Carboxyphenyl)-5-oxopyrrolidine-3-carboxylic acid (2): A mixture of itaconic acid (65.05 g, 0.5 mol) and p-aminobenzoic acid 1 (34.3 g, 0.25 mol) was refluxed in water (150 mL) for 6 h, then cooled down; the formed crystalline precipitate was filtered off, washed with water, 2-propanol, and recrystallized from 2-propanol to give the title compound 2 (white solid, yield 23.5 g, 68%, m. p. 142–143 ◦C (decomp.)).

1H-NMR (400 MHz, DMSO-d₆): δ = 2.69–2.87 (m, 2H, CH), 3.30–3.44 (m, 1H, CH), 3.96–4.11 (m, 2H, NCH) ppm. MS (APCI+, 25 V) m/z, %: 300 (100) [M + Na]+.
Calcd. for C₁₂H₁₉O₅N, %: C 57.83; H 4.45; N 5.62. Found, %: C 57.98; H 4.52; N 5.68.

Methyl 1-[4-(methoxycarbonyl)phenyl]-5-oxopyrrolidine-3-carboxylate (3): To a solution of carboxylic acid 2 (31.13 g, 0.125 mol) in methanol (350 mL), concentrated sulfuric acid (12.5 mL) was added dropwise, and the mixture was heated at reflux for 4 h. The solvent was then evaporated under reduced pressure, and the residue neutralized with 10% sodium carbonate solution to pH 6–7. After cooling, the obtained solid was filtered off, washed with plenty of water and 2-propanol, and recrystallized from 2-propanol to give the title compound 3 (white solid, yield 12.06 g, 87%, m. p. 198–199 ◦C (decomp.)).

1H-NMR (400 MHz, DMSO-d₆): δ = 2.72–2.91 (m, 2H, COCH), 3.42–3.55 (m, 1H, CH), 3.69 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.99–4.16 (m, 2H, NCH₂), 7.82 (d, J = 8.8 Hz, 2H, H₆), 7.97 (d, J = 8.8 Hz, 2H, H₆) ppm.
13C-NMR (101 MHz, DMSO-d₆): δ = 35.08 (COCH₃), 35.38 (CH), 49.85 (NCH), 112.57, 130.18, 143.25 (C = 8.4 Hz, 2H, H₆) ppm.

IR (KBr): ν max = 3121 (OH), 1706, 1671 (3C=O) cm⁻¹. MS (APCI+, 25 V) m/z, %: 272 (100) [M + Na]+.
Calcd. for C₁₂H₁₉O₅N, %: C 57.83; H 4.45; N 5.62. Found, %: C 57.98; H 4.52; N 5.68.

Methyl 4-[3-(hydrazinocarbonyl)-5-oxopyrrolidin-1-yl]benzoate (4): To a boiling mixture of methyl ester 3 (13.86 g, 0.05 mol) and 2-propanol (50 mL), hydrazine monohydrate (7.5 g, 0.15 mol) was added, and the mixture was heated at reflux for 1 h. After completion of the reaction (TLC), the mixture was cooled to room temperature; the formed precipitate was filtered off, washed with water, 2-propanol, and hexane, and recrystallized from 2-propanol to give the title compound 4 (white solid, yield 21.62 g, 87%, m. p. 198–199 ◦C (decomp.)).

1H-NMR (400 MHz, DMSO-d₆): δ = 2.61–2.82 (m, 2H, COCH), 3.09–3.26 (m, 1H, CH), 3.82 (s, 3H, OCH₃), 3.84–3.91 (m, 1H, NCH₂), 3.98–4.07 (m, 1H, NCH₂), 4.35 (s, 2H, NH₂), 7.81 (d, J = 8.4 Hz, 2H, H₆), 7.95 (d, J = 8.4 Hz, 2H, H₆) ppm.
13C-NMR (101 MHz, DMSO-d₆): δ = 33.94 (COCH₃), 50.58 (NCH), 51.99 (OCH₃), 118.41, 124.40, 130.02, 143.25 (C, 165.77, 171.44, 172.91 (3C=O) ppm.

IR (KBr): ν max = 3280, 3308 (NH, NH₂), 1724, 1685, 1637 (3C=O), 1282 (OCH₃) cm⁻¹. MS (APCI+, 25 V) m/z, %: 300 (100) [M + Na]+.
Calcd. for C_{13}H_{15}O_{4}N_{3}: %: C 56.32; H 4.52; N 15.16. Found, %: C 56.48; H 5.52; N 15.22.

Methyl 4-(4-(3,5-dimethyl-1H-pyrazole-1-carbonyl)-2-oxopyrrolidin-1-yl)benzoate (5): To a solution of hydrazide 4 (1.11 g, 4 mmol) in ethanol (8 mL), 2,4-pentanedione (1 g, 10 mmol) was added, and the reaction mixture was heated at reflux for 2 h; it then cooled down, and the mixed precipitate was filtered off, washed with ethanol, and recrystallized from methanol to give the title compound 5 (white solid, yield 0.56 g, 41%, m. p. 161–162 °C).

^1^H-NMR (400 MHz, DMSO-^d_6): δ = 2.21, 2.48 (2s, 6H, 2CH_3), 2.84–2.99 (m, 2H, COCH_3), 3.82 (s, 3H, OCH_3), 4.04–4.28 (m, 2H, HCH=N), 4.44–4.54 (m, 1H, HCH), 6.23 (s, 1H, C=CH−C), 7.26 (d, J = 8.7 Hz, 2H, H_{ar}), 7.66 (d, J = 8.7 Hz, 2H, H_{ar}) ppm.

^1^3^C-NMR (101 MHz, DMSO-^d_6): δ = 13.58, 14.03 (2CH_3), 35.27 (CH + COCH_3), 49.96 (NCH_3), 51.97 (OCH_3), 111.61, 118.58, 124.53, 130.01, 143.12, 143.91, 152.19 (C_{ar}), 165.73, 172.39, 172.43 (3C=O) ppm.

IR (KBr): ν_{max} = 3264 (NH), 1714, 1702, 1671 (3C=O), 1286 (OCH_3) cm\(^{-1}\).

MS (APCI+, 25 V) m/z: 364 (100) [M + Na]^+.

Calcd. for C_{13}H_{15}O_{4}N_{3}: %: C 63.33; H 5.61; N 12.31. Found, %: C 63.49; H 5.75; N 12.47.

Methyl 4-(4-((2,5-dimethyl-1H-pyrrol-1-yl)carbamoyl)-2-oxopyrrolidin-1-yl)benzoate (19): Calcd. for C_{18}H_{21}N_{2}O_{3}: %: C 65.74; H 5.24; N 11.50. Found, %: C 65.65; H 5.22; N 11.48.

General procedure for the preparation of hydrazones 7a–c:

To the boiling solution of hydrazide 4 (1.11 g, 4 mmol) in methanol (15 mL), 2,5-hexanedione (1.85 g, 12.5 mmol) was added, and the reaction mixture was heated at reflux for 3.5 h. Then the reaction mixture was cooled down, and the mixed precipitate was filtered off, washed with methanol and ether, and recrystallized from methanol to give the title compound 7a (white solid, yield 1.22 g, 70%, m. p. 147–148 °C).

^1^H-NMR (400 MHz, DMSO-^d_6): δ = 2.00 (s, 6H, 2CH_3), 2.74–3.01 (m, 2H, COCH_3), 3.46–3.54 (m, 1H, CH), 3.84 (s, 3H, OCH_3), 3.98–4.22 (m, 2H, NCH_2), 5.65 (s, 2H, 2C=CH), 7.85 (d, J = 8.8 Hz, 2H, H_{ar}), 7.97 (d, J = 8.8 Hz, 2H, H_{ar}), 10.94 (s, 1H, NH) ppm.

^1^3^C-NMR (101 MHz, DMSO-^d_6): δ = 10.94, 10.96 (2CH_3), 33.98 (COCH_3), 35.87 (CH), 50.21 (NCH_3), 51.99 (OCH_3), 103.10, 118.53, 124.55, 126.74, 130.05, 143.16 (C_{ar}), 165.75, 171.72, 172.41 (3C=O) ppm.

IR (KBr): ν_{max} = 3264 (NH), 1714, 1702, 1671 (3C=O), 1286 (OCH_3) cm\(^{-1}\).

MS (APCI+, 25 V) m/z: 378 (100) [M + Na]^+.

Calcd. for C_{19}H_{21}N_{3}O_{4}: %: C 64.21; H 5.96; N 11.82. Found, %: C 64.32; H 6.03; N 11.76.

To the boiling solution of hydrazide 4 (1.11 g, 4 mmol) in ethanol (50 mL) (a,c) or ethanol:1,4-dioxane (5:10 mL) (b) the corresponding aromatic aldehyde [benzaldehyde (6 mmol), 4-methoxybenzaldehyde (5 mmol), 4-dimethylaminobenzaldehyde (6 mmol)] was added, and the reaction mixture was heated at reflux for 3.5 (a,c), 2.5 (b) h. Then the mixture was cooled down; the formed solid was filtered off, washed with 2-propanol, and recrystallized from 2-propanol to give the title compound 7a (white solid, yield 0.79 g, 54%, m. p. 201–203 °C), 7b (white solid, yield 1.33 g, 84%, m. p. 230–231 °C), and 7c (yellowish solid, yield 1.22 g, 70%, m. p. 199–201 °C).

Methyl 4-(4-(2-benzylidenemethylenehydrazine-1-carbonyl)-2-oxopyrrolidin-1-yl)benzoate (7a): Calcd. for C_{20}H_{21}N_{3}O_{4}: %: C 65.74; H 5.24; N 11.50. Found, %: C 65.65; H 5.22; N 11.46.

Methyl 4-(4-(2-(4-methoxybenzylidene)hydrazine-1-carbonyl)-2-oxopyrrolidin-1-yl)benzoate (7b): Calcd. for C_{20}H_{21}N_{3}O_{4}: %: C 65.74; H 5.24; N 11.50. Found, %: C 65.65; H 5.22; N 11.46.
Molecules 2021, 26, 2597

$J = 8.5$ Hz, 2H, $H_{ar}$), 7.84 (d, $J = 8.7$ Hz, 2H, $H_{ar}$), 7.93–8.03 (m, 2H, $H_{ar} + 0.6$ H, N=CH), 8.41 (s, 0.4H, N=CH), 11.47, 11.54 (2s, 1H, NH) ppm.

IR (KBr): $\nu_{max} = 3240$ (2NH), 1717, 1680, 1653 (3C=O) cm$^{-1}$.  

MS (APCI+, 25 V) $m/z$, %: 396 (100) [M + H]$^+$.  

Calcd. for C$_{21}$H$_{21}$N$_3$O$_5$: %: C 63.79; H 5.35; N 10.63.  Found, %: C 63.69; H 5.44; N 10.67.

Methyl 4-[(4-(diethylamino)benzylidene)hydrazine-1-carbonyl]-2-oxopyrrolidin-1-yl benzoate (7): 1H-NMR (400 MHz, DMSO-d$_6$): $\delta$ = 2.50–2.90 (m, 4H, 2COCH$_3$), 3.20–3.49 (m, 2H, NCH$_3$), 3.83 (s, 3H, OCH$_3$), 5.23–5.41 (m, 4H, 2CH$_2$COH$_2$ + 0.4H, CH$_3$), 7.82 (d, $J = 8.8$ Hz, 2H, $H_{ar}$), 7.97 (d, $J = 8.8$ Hz, 2H, $H_{ar}$), 10.40 (s, 1H, NH), 12.82 (br. s, 1H, OH) ppm.

IR (KBr): $\nu_{max} = 3248$ (2NH), 1717, 1702, 1663 (3C=O) cm$^{-1}$.

MS (APCI+, 25 V) $m/z$, %: 376 (100) [M + H]$^+$.  

Calcd. for C$_{24}$H$_{28}$N$_4$O$_3$: %: C 59.18; H 4.92; N 10.79.  Found, %: C 59.18; H 4.92; N 10.79.

1-(1-(4-(Methoxycarbonyl)phenyl)-5-oxopyrrolidine-3-carboxamido)-5-oxopyrrolidine-3-carboxylic acid (8): A mixture of hydrazide (5 g, 13.85 mmol), itaconic acid (0.62 g, 4.8 mmol), and water (15 mL) was refluxed for 15 min and then cooled down; the formed precipitate was filtered off, washed with plenty of water and hexane, and recrystallized from 2-propanol to give the title compound 8 (yellowish solid, yield 1.23 g, 79%, m. p. 249–251 °C).

1H-NMR (400 MHz, DMSO-d$_6$): $\delta$ = 2.66–2.94 (m, 2H, COCH$_3$), 3.41–3.49 (m, 2H, NCH$_2$), 3.83 (s, 3H, OCH$_3$), 3.91–4.22 (m, 2H, NCH$_2$), 7.35–8.08 (m, 9H, $H_{ar}$), 10.29, 10.47 (2s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max} = 3410$ (OH), 3275 (NH), 1727, 1702, 1648, 1666 (3C=O), 1286 (OCH$_3$) cm$^{-1}$.

Calcd. for C$_{18}$H$_{19}$N$_3$O$_7$: %: C 55.53; H 4.92; N 10.79.  Found, %: C 55.47; H 4.99; N 10.92.

Methyl 4-[(4-(benzoylhydrazine-1-carbonyl)-2-oxopyrrolidin-1-yl)benzoate (9): To the boiling solution of hydrazide (5 g, 1.8 mmol) 4 in dichloromethane (100 mL), benzoyl chloride was added dropwise and the reaction mixture was heated at reflux for 10 min. It was then cooled down, and the formed precipitate was filtered off, washed with dichloromethane and ethanol, and recrystallized from methanol to give the title compound 9 (yellowish solid, yield 3.7 g, 54%, m. p. 239–241 °C).

1H-NMR (400 MHz, DMSO-d$_6$): $\delta$ = 2.66–2.94 (m, 2H, COCH$_3$), 3.41–3.49 (m, 2H, NCH$_2$), 3.83 (s, 3H, OCH$_3$), 3.91–4.22 (m, 2H, NCH$_2$), 7.35–8.08 (m, 9H, $H_{ar}$), 10.29, 10.47 (2s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max} = 3206$ (2NH), 1718, 1682, (4C=O), 1281 (OCH$_3$) cm$^{-1}$.

MS (APCI+, 25 V) $m/z$, %: 382 (100) [M + H]$^+$.  

Calcd. for C$_{26}$H$_{19}$N$_3$O$_7$: %: C 62.99; H 5.02; N 11.02.  Found, %: C 62.87; H 5.16; N 11.02.
13C-NMR (101 MHz, DMSO-d6): δ = 34.04 (COCH2), 35.90 (CH), 50.61 (NCH2), 118.40, 127.63, 128.32, 141.47 (C=N), 165.41, 171.53, 172.60 (3C=O) ppm.

IR (KBr): νmax = 3307, 3279, 3193, 3167 (2NH2, 2NH), 1687, 1635 (3C=O) cm⁻¹.

MS (APCI+, 25 V) m/z, %: 300 (100) [M + Na]⁺.

Calcd. for C12H15O3N5, %: C 51.98; H 5.45; N 25.26. Found, %: C 52.04; H 5.53; N 25.29.

General procedure for the preparation of hydrazones 11a, b:

A mixture of dihydrazide 10 (0.5 g, 1.8 mmol), the corresponding aromatic aldehyde (10.8 mmol) and 2-propanol/1,4-dioxane (1:1.7) was heated at reflux for 12 (a) or 11 (b) h. Then the mixture was cooled down, and the formed solid filtered off and washed with 2-propanol, and recrystallized from 1,4-dioxane to give the title compound 11a (yellow solid, yield 0.76 g, 77%, m. p. 295–296 °C and 11b (white solid, yield 0.83 g, 88%, m. p. 298–300 °C).

N-(4-nitrobenzylidene)-1-[4-[2-(4-chlorobenzylidenehydrazinocarbonyl)]phenyl]-5-oxopyrrolidine-3-carboxylic acid carbamide (12): A mixture of dihydrazide 10 (5 g, 18 mmol) in methanol (50 mL), phenyl isothiocyanate (9.73 g, 72 mmol) was added, and the mixture was heated at reflux for 5 h. After completion of the reaction, the mixture was cooled down, and the obtained crystalline solid was filtered off, washed with methanol and boiling water, and recrystallized from methanol to give the title compound 12 (white solid, yield 8.97 g, 97% m. p. 235–236 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 2.56–2.76 (m, 2H, COCH2), 2.89–3.41 (m, 1H, CH), 7.09–7.77 (m, 10H, Haryl), 6.28–6.74 (m, 4H, Haryl), 7.09–7.77 (m, 10H, Haryl), 6.28–6.74 (m, 4H, Haryl), 7.38–7.62 (m, 4H, Haryl), 7.63–8.20 (m, 8H, Haryl, 0.65H, CH==N), 8.23 (s, 0.35H, CH=N), 8.45 (s, 1H, CH=N), 11.66, 11.74 (2s, 1H, NH), 11.88 (2s, 1H, NH) ppm.

IR (KBr): νmax = 3431, 3235 (2NH), 1679, 1655 (3C=O) cm⁻¹.

MS (APCI+, 25 V) m/z, %: 575 (100) [M + H]⁺.

Calcd. for C26H22N2O7, %: C 57.46; H 3.89; N 18.04. Found, %: C 57.57; H 3.97; N 18.03.

N-(4-nitrobenzylidene)-1-[4-[2-(4-chlorobenzylidenehydrazinocarbonyl)]phenyl]-5-oxopyrrolidine-3-carboxylic acid carbamide (12): A mixture of dihydrazide 10 (5 g, 18 mmol) in methanol (50 mL), phenyl isothiocyanate (9.73 g, 72 mmol) was added, and the mixture was heated at reflux for 5 h. After completion of the reaction, the mixture was cooled down, and the obtained crystalline solid was filtered off, washed with methanol and boiling water, and recrystallized from methanol to give the title compound 12 (white solid, yield 8.97 g, 97% m. p. 235–236 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 3.39 (CH, COCH2), 44.37 (NCH2), 111.02, 113.98, 115.79, 116.81, 119.46, 123.68, 124.50, 124.88, 124.92, 125.12, 125.23, 125.87, 125.91, 128.10, 128.84, 129.58, 139.01, 139.34, 151.41, 166.04, 171.80, 173.00, 180.06, 180.98 (Caryl, 3C=O, 2C=S) ppm.

IR (KBr): νmax = 3312 (NH), 1611 (3C=O), 1332 (C=S) cm⁻¹.

MS (APCI+, 25 V) m/z, %: 300 (100) [M + Na]⁺.

Calcd. for C26H22N2O2S2, %: C 57.02; H 4.60; N 17.90. Found, %: C 57.10; H 4.56; N 17.93.
$^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta = 2.67$–2.88 (m, 2H, COCH$_2$), 2.89–3.25 (m, 3H, NCH$_2$ + CH), 6.81 (d, $J = 8.4$ Hz, 2H, H$_{ar}$), 7.11–7.22 (m, 4H, H$_{ar}$), 7.31–7.50 (m, 6H, H$_{ar}$), 7.58–7.62 (m, 2H, Har), 13.87 (s, 2H, 2NH) ppm.

$^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta = 26.83$ (CH), 32.48 (COCH$_2$), 45.39 (NCH$_2$), 110.84, 112.53, 127.94, 128.90, 129.33, 129.39, 129.44, 133.10, 135.16, 148.80, 150.05, 150.05, 150.93, 152.46, 167.59, 167.73, 168.16 (C$_{ar}$, C=O, C=O) ppm.

Calcd. for C$_{26}$H$_{21}$N$_7$O$_2$: %: C 61.04; H 4.14; N 19.16. Found, %: C 61.13; H 4.10; N 19.12.

General procedure for the preparation of benzimidazoles 14 and 15.

To a mixture of dicarboxylic acid 2 (5 g, 20 mmol) and benzene-1,2-diamine (14) or 4-methylbenzene-1,2-diamine (15) (52 mmol), polyphosphoric acid (15 g) was added dropwise, and the mixture was heated at reflux for 2 h. It was then cooled down and neutralized with 7% Na$_2$CO$_3$ to pH 9. The formed precipitate was filtered off, washed with plenty of water, and recrystallized from methanol to give the title compound 14 (white solid, yield 7.6 g, 97%, m. p. 215–216 °C) or compound 15 (light yellow solid, yield 7.6 g, 91%, m. p. 257–258 °C).

3-(1H-benzimidazol-2-yl)-1-[4-(1H-benzimidazol-2-yl)phenyl]pyrrolidin-5-one (14): $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta = 3.01$–3.20 (m, 2H, COCH$_2$), 4.01–4.11 (m, 1H, CH), 4.24–4.42 (m, 2H, NCH$_2$), 7.10–7.24 (m, 4H, H$_{ar}$), 7.48–7.69 (m, 4H, H$_{ar}$), 7.91 (d, $J = 8.9$ Hz, 2H, Har), 8.20 (d, $J = 8.8$ Hz, 2H, Har), 12.50, 12.87 (2s, 2H, 2NH) ppm.

$^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta = 30.61$ (COCH$_2$), 37.70 (CH), 52.08 (NCH$_2$), 111.08, 111.19, 118.52, 118.70, 119.29, 119.46, 121.19, 121.61, 122.02, 122.37, 125.54, 126.93, 128.70, 134.57, 135.00, 140.52, 142.82, 143.87 (C$_{ar}$), 150.93, 154.95 (N=C), 172.42 (C=O) ppm.

IR (KBr): $\gamma_{max} = 3197$ (2NH), 1680 (C=O) cm$^{-1}$.

MS (APCI+, 25 V): $m/z$, %: 422 (100) [M + H$^+$].

Calcd. for C$_{23}$H$_{20}$N$_4$O: %: C 73.27; H 4.87; N 17.80. Found, %: C 73.20; H 4.82; N 17.76.

3-(6-Methyl-1H-benzimidazol-2-yl)-1-[4-(6-methyl-1H-benzimidazol-2-yl)phenyl]pyrrolidin-5-one (15): $^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta = 2.39$ (s, 3H, CH$_3$), 2.42 (s, 3H, CH$_3$), 2.99–3.12 (m, 2H, COCH$_2$), 3.98–4.05 (m, 1H, CH), 4.24–4.37 (m, 2H, NCH$_2$), 6.97–7.05 (m, 2H, H$_{ar}$), 7.29–7.48 (m, 4H, H$_{ar}$), 7.88 (d, $J = 8.9$ Hz, 2H, Har), 8.17 (d, $J = 8.8$ Hz, 2H, Har), 12.51 (br. s, 2H, 2NH) ppm.

$^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta = 21.28$ (CH$_3$), 21.36 (CH$_3$), 30.63 (COCH$_2$), 37.72 (CH), 52.12 (NCH$_2$), 113.54, 117.43, 117.54, 119.26, 119.47, 119.92, 122.58, 122.97, 123.45, 125.73, 126.79, 127.62, 128.70, 130.70, 131.16, 140.37 (C$_{ar}$), 150.41, 150.64, 154.56 (N=C), 173.34 (C=O) ppm.

IR (KBr): $\gamma_{max} = 3411$, 3243 (2NH), 1684 (C=O) cm$^{-1}$.

MS (APCI+, 25 V): $m/z$, %: 422 (100) [M + H$^+$].

Calcd. for C$_{23}$H$_{20}$N$_4$O: %: C 74.06; H 5.14; N 17.02. Found, %: C 74.17; H 5.07; N 17.00.

3-(1H-benzimidazol-2-yl)-4-[4-(1H-benzimidazol-2-yl)anilino]butanoic acid (16): A mixture of benzimidazole 14 (1.97 g, 5 mmol) and aqueous 20% sodium hydroxide (30 mL) solution was heated at reflux for 2 h, and then was cooled down, diluted with water (50 mL) and filtered off. The filtrate was acidified with 10% acetic acid to pH 6. The formed solid was filtered off, washed with water, and purified by dissolving it in 5% sodium hydroxide solution, filtering and acidifying the filtrate with 10% acetic acid to pH 6 (procedure was performed twice) to give the title compound 16 (light brown solid, yield 1.03 g, 50%, m. p. 204–205 °C).

$^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta = 2.76$–2.96 (m, 2H, COCH$_2$), 3.29–3.75 (m, 3H, NHCH$_2$+CH), 6.40 (s, 1H, NHCH$_2$), 6.78 (d, $J = 8.5$ Hz, 2H, H$_{ar}$), 7.02–7.24 (m, 4H, H$_{ar}$), 7.38–7.62 (m, 4H, H$_{ar}$), 7.92 (d, $J = 8.6$ Hz, 2H, Har), 12.51 (br. s, 3H, OH + 2NH) ppm.

$^{13}$C-NMR (101 MHz, DMSO-$d_6$): $\delta = 35.64$ (COCH$_2$), 36.18 (CH), 46.49 (NCH$_2$), 111.90, 117.52, 121.24, 121.29, 127.76, 149.92 (C$_{ar}$), 152.47, 155.98 (2N=C), 173.44 (C=O) ppm.

MS (APCI+, 25 V): $m/z$, %: 412 (100) [M + H$^+$].

Calcd. for C$_{23}$H$_{21}$N$_7$O$_2$: %: C 70.06; H 5.14; N 17.00. Found, %: C 70.17; H 5.07; N 17.08.
4-((4-(1H-benzimidazol-2-yl)phenyl)amino)-3-(1H-benzimidazol-2-yl)butanoylhydrazone (17).

Method A: A mixture of butanoic acid 16 (2.06 g, 5 mmol), hydrazine monohydrate (2.10 g, 42 mmol), and 2-propanol (20 mL) was heated at reflux for 20 h and then cooled to room temperature. The obtained solid was filtered off, washed with water, and recrystallized from water to give the title compound 17 (light brown solid, yield 1.08 g, 51%, m. p. 166–167 °C).

Method B: A mixture of pyrrolidinone 14 (1.97 g, 5 mmol) and hydrazine monohydrate (20 g, 400 mmol) was heated at reflux for 6 h and then was cooled to room temperature, diluted with 2-propanol (30 mL), and filtered off. The filtrate was evaporated under reduced pressure; the residue was poured with water and stirred for 10 min. The obtained solid was filtered off, washed with plenty of water, and recrystallized from water to give the title compound 14 (light brown solid, yield 1.34 g, 63%, m. p. 166–167 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 2.56–2.75 (m, 2H, COCH2), 3.40–3.59 (m, 2H, NHCH2), 3.72–3.78 (m, 1H, CH), 4.43 (s, 2H, NH2), 6.34 (t, J = 5.9 Hz, 1H, NHCH2), 6.76 (d, J = 8.6 Hz, 2H, Hαr), 7.09–7.15 (m, 4H, Hβr), 7.42–7.58 (m, 4H, Hαr), 7.91 (d, J = 8.4 Hz, 2H, Hαr), 9.12 (s, 1H, CONH), 12.31 (s, 1H, NH), 12.47 (s, 1H, NH) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 35.54 (CH), 35.72 (COCH2), 46.46 (NHCH2), 110.88, 111.87, 117.46, 118.18, 121.26, 127.71, 134.89, 143.21, 149.95 (Cαr).

IR (KBr): νmax = 3410 (NHNH2), 1611 (C=O) cm−1.

MS (APCI+, 25 V) m/z, %: 426 (100) [M + H]+.

Calcd. for C24H23N2O, %: C 76.75; H 5.45; N 17.80. Found, %: C 76.81; H 5.52; N 17.69.

4-((4-(1H-benzimidazol-2-yl)phenyl)amino)-3-(1H-benzimidazol-2-yl)-N-(2,5-dimethyl-1H-pyrrol-2-yl)butanamide (18): To a mixture of hydrazone 17 (2.13 g, 5 mmol) and hexane-2,5-dione (3.42 g, 30 mmol) in 2-propanol (50 mL) conc. hydrochloric acid (2.5 mL) was added dropwise; the mixture was heated at reflux for 4 h, then cooled to room temperature, and the solvent was evaporated under reduced pressure. The residue was poured with water (30 mL) and stirred for 10 min. The obtained solid was filtered off, washed with plenty of water, and recrystallized from methanol to give the title compound 18 (white solid, yield 1.79 g, 71%, m. p. 227–228 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 1.56 (s, 3H, CH3), 1.95 (s, 3H, CH3), 2.93–3.09 (m, 2H, COCH2), 3.54–3.84 (m, 3H, CH + NHCH2), 5.52 (d, J = 3.1 Hz, 1H, C=CH), 5.57 (d, J = 3.1 Hz, 1H, C=CH), 6.90 (d, J = 8.7 Hz, 2H, Hαr), 7.02 (s, 1H, NHCH2), 7.19–7.24 (m, 2H, Hαr), 7.34–7.39 (m, 2H, Hαr), 7.53–7.59 (m, 2H, Hαr), 7.64–7.68 (m, 2H, Hαr), 8.06 (d, J = 8.7 Hz, 2H, Hαr), 10.86 (s, 1H, CONH), 13.68 (br. s, 2H, 2NH2) ppm.

13C-NMR (101 MHz, DMSO-d6): δ = 10.50 (CH3), 11.00 (CH3), 34.97 (COCH2), 35.55 (CH), 46.03 (NHCH2), 102.76, 102.81 (2C=CH), 112.12, 112.13, 113.52, 113.54, 122.08, 122.12, 123.87, 126.56, 126.79, 129.08, 134.17, 134.22, 139.09, 150.54 (Cαr), 151.79, 154.99 (2N=C), 169.97 (C=O) ppm.

IR (KBr): νmax = 3050 (NH), 1608 (C=O) cm−1.

Calcd. for C30H29N2O2, %: C 71.55; H 5.80; N 19.47. Found, %: C 71.78; H 5.72; N 19.33.

Ethyl 2-((4-(4-((1H-benzimidazol-2-yl)-2-oxopyrrolidin-1-yl)-1H-pyrrol-2-yl)phenyl)azo)carbonyl)acetate (19): A mixture of benzimidazole 14 (3 g, 7.6 mmol), potassium carbonate (2.1 g, 15.2 mmol), TBAI (0.02 g), and acetone (50 mL) was boiled, and ethyl chloroacetate (9.9 mL, 91.4 mmol) was slowly added dropwise. The mixture was refluxed for 20 h and filtered while hot; the filtrate was cooled to room temperature and diluted with water (100 mL). The formed solid was filtered off, washed with plenty of water, and recrystallized from methanol to give the title compound 19 (light brown solid, yield 3.78 g, 88%, m. p. 106–107 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 1.15 (t, J = 7.1 Hz, 3H, CH2CH3), 1.25 (t, J = 7.1 Hz, 3H, CH2CH3), 2.94–3.11 (m, 2H, COCH2), 4.10–4.38 (m, 7H, 2CH2CH3 + CH + NCH2), 5.23 (s, 2H, NCH2CO), 5.32 (s, 2H, NCH2CO), 7.20–7.30 (m, 4H, Hαr), 7.51–7.72 (m, 4H, Hαr), 7.75 (d, J = 8.7 Hz, 2H, Hαr), 7.90 (d, J = 8.8 Hz, 2H, Hαr).
Molecules 2021, 26, 2597

13C-NMR (101 MHz, DMSO-d6): δ = 13.94 (CH3), 14.04 (CH3), 28.41 (CH), 37.76 (COCH2),
44.41 (NCH2CO), 46.06 (NCH2CO), 51.90 (NCH2), 61.40 (CH2CH3), 61.49 (CH2CH3), 110.12,
110.59, 118.85, 119.06, 119.15, 121.92, 122.37, 125.11, 129.42, 135.76, 136.33, 140.46, 141.75,
142.40 (Car), 152.87, 155.42 (2N=C), 167.75, 167.89 (2C=O), 172.31 (NC=O) ppm.

IR (KBr): νmax = 1732, 1708, 1611 (3C=O) cm⁻¹.

MS (APCI+, 25 V) m/z, %: [M + H]+ = 566 (100).

Calcd. for C35H31N5O3, %: C 68.62; H 5.54; N 12.82.

2-(4-(4-(1-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-1H-benzimidazol-2-yl)-1-(4-(1-(3,5-dimethyl-
1H-benzimidazol-1-yl)acetohydrazide)

A mixture of diester 19 (1.98 g, 3.5 mmol), hydrazine monohydrate (1.42 g, 28.3 mmol) and 1,4-dioxane (20 mL) was refluxed for 18 h and cooled to room temperature; the formed solid was filtered off, washed with water and 2-propanol, and recrystallized from methanol to give the title compound 20 (yellowish solid, yield 1.60 g, 85%, m. p. 206–207 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 2.99–3.15 (m, 2H, COCH2), 4.00–4.74 (m, 7H, CH + NCH2 + 2NCH2), 4.87, 4.95, 5.24, 5.32 (4s, 4H, 2NCH), 7.00–7.57 (m, 6H, H-ar), 7.58–8.00 (m, 6H, H-ar), 8.83, 8.88, 9.60, 9.65 (4s, 2H, 2NH).

13C-NMR (101 MHz, DMSO-d6): δ = 28.55 (CH), 37.81 (COCH2), 44.47 (NCH2CO),
45.78 (NCH2CO), 52.09 (NCH2), 110.02, 110.54, 118.82, 119.99, 119.03, 121.75, 122.17, 125.28,
129.78, 135.79, 136.38, 140.46, 141.79, 142.49 (Car), 153.20, 155.77 (2N=C), 166.01, 166.34
(2C=O), 172.54 (NC=O) ppm.

IR (KBr): νmax = 1729, 1674 (3C=O) cm⁻¹.

MS (APCI+, 25 V) m/z, %: [M + H]+ = 566 (100).

Calcd. for C36H30N6O3, %: C 68.62; H 5.50; N 18.94. Found, %: C 68.62; H 5.25; N 18.87.

4-(1-((5-Thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-1H-benzimidazol-2-yl)-2-oxopyrrolidin-1-yl)phenyl-
1H-benzimidazol-1-yl)acetohydrazide (20): A mixture of diester 19 (1.98 g, 3.5 mmol), hydrazine monohydrate (1.42 g, 28.3 mmol) and 1,4-dioxane (20 mL) was refluxed for 18 h and cooled to room temperature; the formed solid was filtered off, washed with water and 2-propanol, and recrystallized from methanol to give the title compound 20 (yellowish solid, yield 1.60 g, 85%, m. p. 206–207 °C).

1H-NMR (400 MHz, DMSO-d6): δ = 1.14 (s, 3H, CH3), 1.16 (s, 3H, CH3), 1.24 (s, 3H, CH3), 1.25 (s, 3H, CH3), 2.91–3.11 (m, 2H, COCH2), 4.11–4.19 (m, 1H, CH), 4.25–4.37 (m, 2H, NCH2), 4.89–4.96 (m, 1H, 1H, C=CH), 4.97–5.06 (m, 1H, C=CH), 5.20, 5.30 (4s, 4H, 2NH).

13C-NMR (101 MHz, DMSO-d6): δ = 21.37 (CH), 28.55 (CH), 37.81 (CO), 7.00–7.57 (m, 6H, H-ar), 7.58–8.00 (m, 6H, H-ar), 8.83, 8.88, 9.60, 9.65 (4s, 2H, 2NH).

13C-NMR (101 MHz, DMSO-d6): δ = 21.37 (CH3), 21.53 (CH3), 28.43, 37.72, 44.60, 46.25,
51.90 (CH, COCH2, NCH2CO, NCH2), 69.19, 69.32 (C-CH-C), 110.04, 110.51, 118.86, 119.06,
119.14, 121.90, 122.25, 122.37, 125.19, 129.37, 135.79, 136.36, 140.45, 141.74, 142.39 (Car),
152.85, 155.37 (2N=C), 167.75, 167.89 (2C=O), 172.31 (NC=O) ppm.

IR (KBr): νmax = 3279, 3203 (2NH). Found, %: C 62.56; H 5.06; N 23.45. Found, %: C 62.31; H 4.99; N 23.27.

4-(1-((5-Thiao-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-1H-benzol[d]imidazol-2-yl)-1-(4-(1-
((5-thiao-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl)-1H-benzol[d]imidazol-2-yl)phenyl)pyrrolidin-2-one (22): To a cooled solution of potassium hydroxide (1 g, 18 mmol) in methanol (80 mL), carbon disulfide (0.7 mL, 11 mmol) was added dropwise and the obtained mixture was stirred at room temperature for 15 min. Then hydrazide 20 (2.15 g, 4 mmol) was added slowly, and the mixture was heated at reflux for 12 h. After completion of the reaction (TLC), the volatile fraction was evaporated under reduced pressure; the residue was dissolved in water (100 mL) and boiled for 3 min with activated carbon. The mixture was filtered off, and the filtrate was acidified with diluted hydrochloric acid to pH 1. The
formed precipitate was filtered off, washed with water, and recrystallized from methanol to give the title compound 22 (white/yellow solid, yield 2.02 g, 81%, m. p. 262–263 °C).

1H-NMR (400 MHz, DMSO-d$_6$): δ = 2.90–3.17 (m, 2H, COCH$_2$), 4.04–4.53 (m, 2H, NCH$_2$ + CH), 5.72, 5.81, 5.90, 6.01 (4s, 4H, 2NCH$_2$CO), 7.19–8.04 (m, 12H, H$_ar$), 12.41 (br. s, 2H, 2NH) ppm.

13C-NMR (101 MHz, DMSO-d$_6$): δ = 28.45, 37.82, 38.08, 44.35, 51.94 (CH, COCH$_2$, NCH$_2$CO, NCH$_2$H), 110.40, 110.87, 119.03, 119.13, 119.24, 122.29, 122.73, 124.48, 129.78, 135.35, 135.76, 140.67, 141.76, 142.42, 155.20, 164.35, 165.63, 165.93 (C$_ar$), 172.36, 172.42 (C=O, 2C=S) ppm.

IR (KBr): $\nu_{max}$ = 3425 (2NH), 1609 (C=O) cm$^{-1}$.

Calcd. for C$_{30}$H$_{26}$N$_6$O$_5$S$_2$: %: C 70.67; H 4.94; N 17.66. Found, %: C 70.62; H 4.98; N 17.58.

1H-NMR (400 MHz, DMSO-d$_6$): δ = 2.85–3.82 (m, 2H, COCH$_2$), 4.11–4.37 (m, 3H, CH + NCH$_2$), 4.89, 4.97, 5.06, 5.15 (4s, 4H, 2NCH$_2$CO), 7.08–7.95 (m, 22H, H$_ar$), 9.48–10.04 (m, 4H, 4NH), 10.51, 10.58 (2s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max}$ = 3206 (NH), 1694, 1608 (3C=O) cm$^{-1}$.

Calcd. for C$_{42}$H$_{37}$N$_{11}$O$_5$S$_2$: %: C 62.44; H 4.62; N 19.07. Found, %: C 62.57; H 4.69; N 19.16.

General procedure for the preparation of hydrazones 24a–e: To a solution of hydrazide 20 (2.15 g, 4 mmol) in DMF (100 mL), the corresponding aromatic aldehyde (30 mmol) was added, and the mixture was heated at reflux for 5 (a,c–e) or 8 (b) h. After completion of the reaction, the mixture was cooled to room temperature and diluted with water and cold methanol, and recrystallized from methanol to give the title compound 23 (light yellow solid, yield 2.91 g, 90%, m. p. 240 (decomp.) °C).

1H-NMR (400 MHz, DMSO-d$_6$): δ = 3.01–3.17 (m, 2H, COCH$_2$), 4.11–4.37 (m, 3H, CH + NCH$_2$), 4.89, 4.97, 5.06, 5.15 (4s, 4H, 2NCH$_2$CO), 7.08–7.95 (m, 22H, H$_ar$), 9.48–10.04 (m, 4H, 4NH), 10.51, 10.58 (2s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max}$ = 3206 (NH), 1694, 1608 (3C=O) cm$^{-1}$.

Calcd. for C$_{42}$H$_{37}$N$_{11}$O$_5$S$_2$: %: C 62.44; H 4.62; N 19.07. Found, %: C 62.57; H 4.69; N 19.16.

1H-NMR (400 MHz, DMSO-d$_6$): δ = 2.95–3.13 (m, 2H, COCH$_2$), 9.48–10.04 (m, 4H, 4NH), 11.82, 11.89, 12.00, 12.04 (4s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max}$ = 3200 (2NH), 1693, 1610 (3C=O) cm$^{-1}$.

Calcd. for C$_{42}$H$_{37}$N$_{11}$O$_5$S$_2$: %: C 62.76; H 4.14; N 19.17. Found, %: C 62.81; H 4.20; N 19.14.

1H-NMR (400 MHz, DMSO-d$_6$): δ = 2.93–3.13 (m, 2H, COCH$_2$), 4.08–4.20 (m, 1H, CH), 4.21–4.39 (m, 2H, NCH$_2$), 7.19–8.04 (m, 12H, H$_ar$), 12.41 (br. s, 2H, 2NH) ppm.

IR (KBr): $\nu_{max}$ = 3438 (2NH), 1694, 1610 (3C=O) cm$^{-1}$.

Calcd. for C$_{42}$H$_{37}$N$_{11}$O$_5$S$_2$: %: C 62.76; H 4.14; N 19.17. Found, %: C 62.81; H 4.20; N 19.14.
Molecules 2021, 26, 2597

5.14, 5.54, 5.61 (4s, 4H, 2NCH₂CO), 7.05–7.46 (m, 8H, H_ar), 7.47–7.96 (m, 12H, H_ar), 8.05, 8.10 (2s, 0.75(2H), 2N=CH), 8.26, 8.30 (2s, 0.25(2H), 2N=CH), 11.82, 11.88, 12.00, 12.03 (4s, 2H, 2NH) ppm.

IR (KBr): \( \nu_{\text{max}} = 3206 \) (2NH), 1694, 1608 (3C=O) cm\(^{-1}\).

Calcd. for C\(_{42}\)H\(_{33}\)F\(_2\)N\(_9\)O\(_3\), %: C 62.44; H 4.62; N 19.07. Found, %: C 62.47; H 4.69; N 19.16.

N’-3-chlorobenzylidene)-2-(2-(4-(4-(1-(2-(2-(3-chlorobenzylidene)hydrazinyl)-2-oxoethyl)-1H-benzo[d]imidazol-2-yl)-2-oxopyrrolidin-1-yl)phenyl)-1H-benzo[d]imidazol-1-yl)acetohydrazide (24d):

Light brown solid, yield 2.47 g, 79%, m. p. 256–257 ◦C.

1H-NMR (400 MHz, DMSO-d\(_6\)): \( \delta = Z/E \) 2.95–3.09 (m, 2H, COCH\(_2\)), 3.99–4.52 (m, 3H, CH + NCH\(_2\)), 5.09, 5.15, 5.56, 5.63 (4s, 4H, 2NCH\(_2\)CO), 7.08–7.96 (m, 20H, H\(_{\text{ar}}\)), 8.03, 8.08 (2s, 0.8(2H), 2N=CH), 8.23, 8.27 (2s, 0.2(2H), 2N=CH), 11.89, 11.95, 12.11, 12.14 (4s, 2H, 2NH) ppm. IR (KBr): \( \nu_{\text{max}} = 3393, 3207 \) (2NH), 1685, 1611 (3C=O) cm\(^{-1}\).

Calcd. for C\(_{42}\)H\(_{33}\)Cl\(_2\)N\(_9\)O\(_3\), %: C 64.45; H 4.25; N 16.11. Found, %: C 64.36; H 4.20; N 16.20.

N’-(2,3-dimethoxybenzylidene)-2-(2-(4-(4-(1-(2-(2-(2,3-dimethoxybenzylidene)hydrazinyl)-2-oxoethyl)-1H-benzo[d]imidazol-2-yl)-2-oxopyrrolidin-1-yl)phenyl)-1H-benzo[d]imidazol-1-yl)acetohydrazide (24e):

Light yellow solid, yield 3.14 g, 94%, m. p. 194–195 ◦C.

1H-NMR (400 MHz, DMSO-d\(_6\)): \( \delta = Z/E \) 2.94–3.11 (m, 2H, COCH\(_2\)), 3.70, 3.76, 3.79, 3.81 (4s, 12H, 4CH\(_3\)O), 4.10–4.44 (m, 1H, CH+NCH\(_2\)), 5.05, 5.11, 5.54, 5.61 (4s, 4H, 2NCH\(_2\)CO), 6.96–7.91 (m, 18H, H\(_{\text{ar}}\)), 8.36, 8.40 (2s, 0.75(2H), 2N=CH), 8.57, 8.60 (2s, 0.25(2H), 2N=CH), 11.77, 11.83, 12.00, 12.01 (4s, 2H, 2NH) ppm. IR (KBr): \( \nu_{\text{max}} = 3200, 3054 \) (2NH), 1682, 1610 (3C=O) cm\(^{-1}\). Calcd. for C\(_{46}\)H\(_{43}\)N\(_9\)O\(_7\), %: C, 66.26; H, 5.20; N, 15.12. Found, %: C 66.19; H 5.26; N 15.21.

3.2. Determination of Antimicrobial Activity

3.2.1. Preparation of Bacterial Inoculum

A panel of clinically important reference bacterial pathogens were obtained from the American Type Culture Collection (ATCC) and the National Type Culture Collection (NTCT) (Supplementary Table S1). Each test organism was subcultured on Tryptic Soy Agar (TSA) at 37 ◦C, for 24 h. After incubation, the representative colonies were suspended in 5 mL of Tryptic Soy Broth (TSB) and further incubated at 37 ◦C for 24 h to initiate the liquid culture. The bacterial cultures were normalized using a spectrophotometer (OD\(_{600\text{nm}}\)), and the final inoculum (1 × 10\(^7\) CFU/mL) was achieved by diluting the culture with fresh TSB.

3.2.2. Preparation of the Test Compounds

The test compounds 3–24 were dissolved in hybridoma grade DMSO to achieve a 50 mg/mL stock solution. The stock solution was further diluted in TSB supplemented with 1% of DMSO to produce the series of dilutions (0, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL). Ampicillin was dissolved in sterile deionized water and the series of dilutions (0, 15.62, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) were prepared as described above.

3.2.3. Evaluation of Minimal Inhibitory Concentration

The minimal inhibitory concentration (MIC) of the compounds 3–24 and ampicillin were determined by the broth dilution method as described by Balouiri et al. [75] with brief modifications. The tubes containing diluted compounds in TSB were inoculated with normalized bacterial inoculum (100 µL) to achieve a final bacterial concentration of 1 × 10\(^6\) CFU/mL. The inoculated tubes were incubated at 37 ◦C for 24 h. After incubation, the turbidity was evaluated visually and MIC was estimated. The MIC was defined as the lowest concentration of the test compound that inhibits the visual growth of the test organism.
3.2.4. Determination of Minimal Bactericidal Concentration

The minimal bactericidal concentration (MBC) was determined as described by Parvekar et al. [76]. After a MIC evaluation of the novel compounds and ampicillin, aliquots of 100 μL were taken from tubes without growth and plated on TSA. The plates were incubated at 37 °C for 48 h. After incubation, the plates were evaluated, and the minimal bactericidal concentration (MBC) was estimated. The MBC was defined as the lowest concentration of the test compound that fully suppresses the growth of the test organism.

4. Conclusions

In this study, the chemical transformations of p-aminobenzoic acid were carried out, and a series of 1-phenyl-5-oxopyrrolidine derivatives with hydrazone, pyrazole, thiosemicarbazide, triazole, oxadiazole fragments were obtained. A convenient and efficient method for the synthesis of benzimidazoles by heating reagents in polyphosphoric acid was proposed.

The synthesized compounds were evaluated for their antibacterial activity against a panel of clinically relevant Gram-positive and Gram-negative pathogens. The antimicrobial activity evaluation revealed that the γ-amino acid derivative 16 bearing two benzimidazole fragments demonstrated the strongest broad-spectrum bactericidal activity on both Gram-positive and Gram-negative organisms. The antimicrobial activity of compound 16 was notably greater than that of ampicillin. Furthermore, benzimidazoles 14 and 15 showed promising, broad-spectrum antibacterial activity against tested pathogens, with notably good bactericidal activity against L. monocytogenes. Collectively, these results demonstrated that the 5-oxopyrrolidine 14 could be further explored as a potential pharmacophore in the development of novel antimicrobials targeting clinically significant bacterial pathogens. Further studies are needed to better understand the safety, tolerability, and in vivo activity of the most promising compounds.

Supplementary Materials: The following are available online, Figure S1: 1H-NMR of compound 2, Figure S2: 13C-NMR of compound 2, Figure S3: 1H-NMR of compound 3, Figure S4: 13C-NMR of compound 3, Figure S5: 1H-NMR of compound 4, Figure S6: 13C-NMR of compound 4, Figure S7: 1H-NMR of compound 5, Figure S8: 13C-NMR of compound 5, Figure S9: 1H-NMR of compound 6, Figure S10: 13C-NMR of compound 6, Figure S11: 1H-NMR of compound 7a, Figure S12: 1H-NMR of compound 7b, Figure S13: 1H-NMR of compound 7c, Figure S14: 1H-NMR of compound 8, Figure S15: 13C-NMR of compound 8, Figure S16: 1H-NMR of compound 9, Figure S17: 13C-NMR of compound 9, Figure S18: 1H-NMR of compound 10, Figure S19: 13C-NMR of compound 10, Figure S20: 1H-NMR of compound 11a, Figure S21: 1H-NMR of compound 11b, Figure S22: 1H-NMR of compound 12, Figure S23: 13C-NMR of compound 12, Figure S24: 1H-NMR of compound 13, Figure S25: 13C-NMR of compound 13, Figure S26: 1H-NMR of compound 14, Figure S27: 13C-NMR of compound 14, Figure S28: 1H-NMR of compound 15, Figure S29: 13C-NMR of compound 15, Figure S30: 1H-NMR of compound 16, Figure S31: 13C-NMR of compound 16, Figure S32: 1H-NMR of compound 17, Figure S33: 13C-NMR of compound 17, Figure S34: 1H-NMR of compound 18, Figure S35: 13C-NMR of compound 18, Figure S36: 1H-NMR of compound 19, Figure S37: 13C-NMR of compound 19, Figure S38: 1H-NMR of compound 20, Figure S39: 13C-NMR of compound 20, Figure S40: 1H-NMR of compound 21, Figure S41: 13C-NMR of compound 21, Figure S42: 1H-NMR of compound 22, Figure S43: 13C-NMR of compound 22, Figure S44: 1H-NMR of compound 23, Figure S45: 1H-NMR of compound 24a, Figure S46: 1H-NMR of compound 24b, Figure S47: 1H-NMR of compound 24c, Figure S48: 1H-NMR of compound 24d, Figure S49: 1H-NMR of compound 24e. Table S1: Bacteria strains used in the biological evaluation.

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