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In Silico and In Vitro Assessment of Antimicrobial and Antibiofilm Activity of Some 1,3-Oxazole-Based Compounds and Their Isosteric Analogues

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Abstract: In this paper, we report on the antimicrobial activity assessment of 49 compounds previously synthesized as derivatives of alanine or phenylalanine that incorporate a 4-(4-X-phenylsulfonyl)phenyl fragment (X = H, Cl, or Br), namely 21 acyclic compounds (6 × *N*-acyl-α-amino acids, 1 × *N*-acyl-α-amino acid ester, and 14 × *N*-acyl-α-amino ketones) and 28 pentatomic heterocycles from the oxazole-based compound class (6 × 4*H*-1,3-oxazol-5-ones, 16 × 5-aryl-1,3-oxazoles, and 6 × ethyl 1,3-oxazol-5-yl carbonates). Both in silico and in vitro qualitative and quantitative assays were used to investigate the antimicrobial potential of these derivatives against planktonic and biofilm-embedded microbial strains. Some of the tested compounds showed promising antimicrobial and antibiofilm activity depending on their chemical scaffold and lipophilic character.

Keywords: antimicrobial; antibiofilm; *N*-acyl-α-amino acid; 4*H*-1,3-oxazol-5-one; *N*-acyl-α-amino acid ester; *N*-acyl-α-amino ketone; 1,3-oxazole

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1. Introduction

The rise of multidrug-resistant pathogenic microorganisms is a major health concern. In response, there is an urgent need for the identification of novel antimicrobial agents.

The development of new sulfonyl-group-containing analogs is a hot research topic in medicinal chemistry [1–7]. Among these compounds, numerous diaryl sulfones have been found to exhibit a variety of biological activities, including antimicrobial, antioxidant, antimycobacterial, antimalarial, anticancer, anti-inflammatory, and anti-HIV effects [8–17]. Further, some representatives of this class selectively block the 5-HT_6 receptors being developed as therapies for Alzheimer's disease [18,19]. Recently, Alsaedi et al. synthesized a series of pyrazolo [1,5-a]pyrimidine derivatives containing the phenylsulfonyl moiety and evaluated their antimicrobial activities. The results revealed that several sulfone analogues showed effects exceeding the activity of the reference drug. Unexpectedly, it was observed that derivatives containing one sulfone group were more effective against different bacterial and fungal strains than those containing two sulfone groups [20]. Moreover, some unsaturated 4*H*-1,3-oxazol-5-ones bearing the arylsulfonylphenyl moiety in their molecules exhibited good antifungal and antibiofilm potential [21]. Very recently, the results reported by Rashdan et al. highlighted a synthetic 1,2,3-triazole-containing sulfone derivative

structurally inspired by dapsone that exhibited outstanding antimicrobial properties against various bacterial strains [22].

In addition, the 1,3-oxazole is an important heterocyclic nucleus that is present in numerous active substances and displays a wide array of biological properties. Significant research has been conducted to synthesize 1,3-oxazole derivatives and to evaluate their pharmacological profile [23,24]. Therefore, over time, a large number of natural and synthetic 1,3-oxazole–based compounds, which have been associated with a wide spectrum of pharmacological activities, such as antimicrobial, antimalarial, antidiabetic, analgesic, anti-inflammatory, and antitumoral effects, have been reported [25–32]. It has been also shown that saturated 4*H*-1,3-oxazol-5-ones—the stable 5-oxo tautomers of 1,3-oxazol-5-ols—present antimicrobial, anticancer, antiviral, and trypanocidal actions [33–36].

N-acyl amino acids, in which the acyl moiety is derived from fatty acids, are similar to endogenous cannabinoids [37]. Among these *N*-fatty acyl-α-amino acids, *N*-arachidonoylserine has been reported to display antimicrobial and antibiofilm effects against methicillin-resistant *Staphylococcus aureus* (MRSA) strains. In addition, the staphylococcal-biofilm-associated virulence determinants are altered by this agent. *N*-arachidonoylserine is able to change the bacterial membrane potential and prevent biofilm formation without killing the bacterial cells [37,38]. It has been discovered that *N*-acyltyrosine derivatives act as bacterial metabolites that exhibit antibiotic effects against the *Bacillus subtilis* and an average inhibition of the *Pseudomonas aeruginosa* biofilm formation process [39]. *N*-acyl-α-amino acids exhibit other therapeutic effects, such as antihypertensive, mucolytic, anticancer, antianemic, antiulcer, and antioxidant actions [40–46].

Some representatives of the *N*-acyl-α-amino acid ester class have antibacterial, antileishmanial, antiproliferative, antidepressant, and monoamine oxidase inhibitory activities [47–52].

Several *N*-acyl-α-amino ketone derivatives show antiviral, antihypertensive, antithrombotic, and anti-inflammatory properties [53–58].

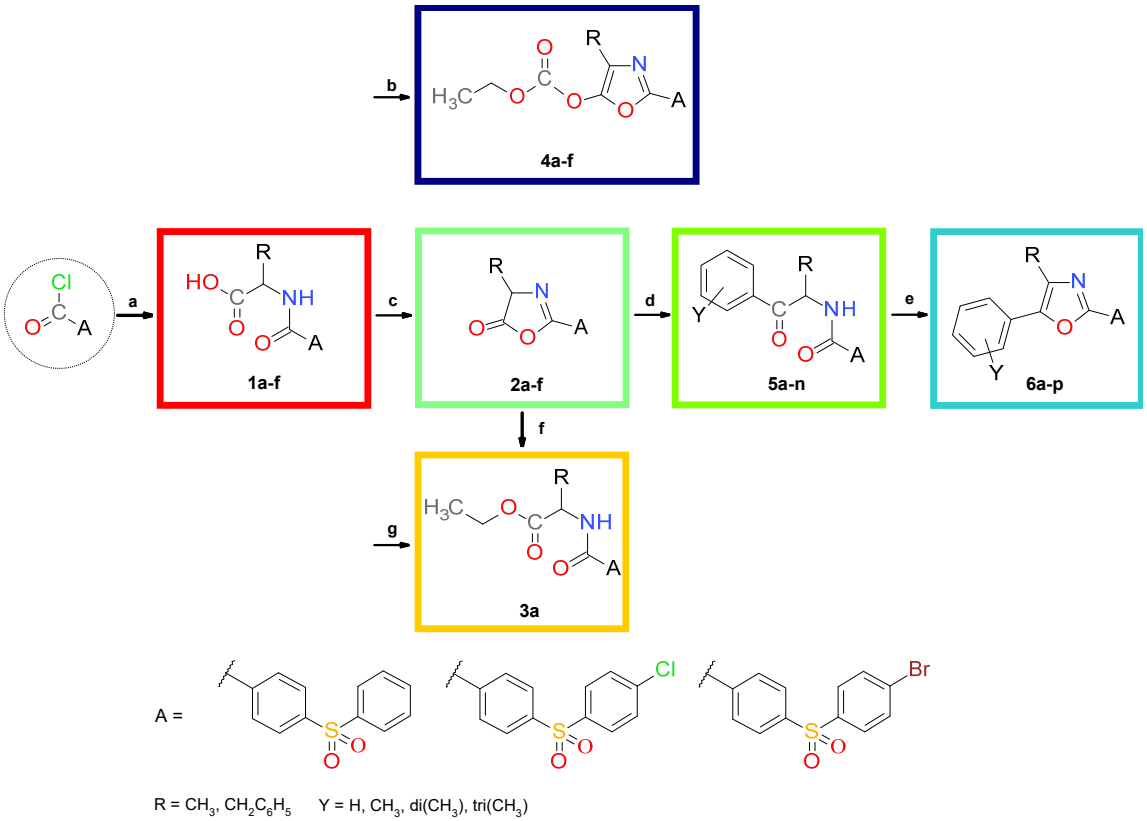
In an attempt to develop new antimicrobial agents, our group devoted considerable interest in the synthesis of compounds based on the 4-(4-X-phenylsulfonyl)phenyl fragment as the pharmacophore center [59–65]. In this study, the antimicrobial and antibiofilm effects of 49 compounds (**1a–f**, **2a–f**, **3a**, **4a–f**, **5a–n**, and **6a–p**) from the classes mentioned above were evaluated against different bacterial and fungal strains. In silico prediction of the antimicrobial, pharmacokinetic, and toxicological features of the tested compounds was also performed.

2. Results

2.1. Chemistry

Following the described synthesis procedures, a series of new *N*-acyl-α-amino acids (**1a–f**), 4*H*-1,3-oxazol-5-ones (**2a–f**), *N*-acyl-α-amino ketones (**5a–n**), 1,3-oxazoles (**4a–f**, **6a–p**), and one *N*-acyl-α-amino acid ester (**3a**) were synthesized [59–65]. The general structures of the compounds are presented in Scheme 1.

The chemical structures and purities (%) of the tested compounds **1a–f**, **2a–f**, **3a**, **4a–f**, **5a–n**, and **6a–p** are presented in Table 1.



Scheme 1. The general synthetic methodology as described in our previous papers [59–65]. Reagents and conditions: (**a**) (i) alanine or phenylalanine/NaOH, CH_2Cl_2, 0–5 °C, 30 min; (ii) room temperature (r.t.), 1 h; (iii) HCl; (**b**) $ClCO_2C_2H_5$/*N*-methylmorpholine (NMM), CH_2Cl_2, r.t., 24 h (molar ratio of **1a–f**/$ClCO_2C_2H_5$/NMM = 1:1.5:1.5); (**c**) $ClCO_2C_2H_5$/NMM, CH_2Cl_2, r.t., 30 min (molar ratio of **1a–f**/$ClCO_2C_2H_5$/NMM = 1:1:1); (**d**) benzene, toluene, *m*-xylene or mesitylene/anhyd $AlCl_3$, r.t., 20 h; (**e**) $POCl_3$, reflux, 4 h; (**f**) C_2H_5OH, reflux, 30 min; (**g**) C_2H_5OH/H_2SO_4, reflux, 12 h.

Table 1. The chemical structures and purities (%) of the tested compounds.

Compound	X	R	Purity (%)	Ref.	Compound	X	R	Y	Purity (%)	Ref.
1a	H	CH_3	99.99	[59]	**5a**	H	CH_3	H	96.55	[59]
1b	Cl	CH_3	99.99	[60]	**5b**	Cl	CH_3	H	98.02	[60]
1c	Br	CH_3	99.99	[61]	**5c**	Br	CH_3	H	97.46	[61]
1d	H	$CH_2C_6H_5$	99.99	[62]	**5d**	H	CH_3	4-CH_3	95.10	[59]
1e	Cl	$CH_2C_6H_5$	99.05	[63]	**5e**	Cl	CH_3	4-CH_3	96.65	[60]
1f	Br	$CH_2C_6H_5$	99.63	[64]	**5f**	Br	CH_3	4-CH_3	92.28	[61]
2a	H	CH_3	91.75	[59]	**5g**	H	CH_3	2,4-$(CH_3)_2$	97.16	[59]
2b	Cl	CH_3	92.92	[60]	**5h**	Cl	CH_3	2,4-$(CH_3)_2$	91.10	[60]
2c	Br	CH_3	90.78	[61]	**5i**	Br	CH_3	2,4-$(CH_3)_2$	97.55	[61]
2d	H	$CH_2C_6H_5$	92.03	[62]	**5j**	H	$CH_2C_6H_5$	4-CH_3	91.49	[62]
2e	Cl	$CH_2C_6H_5$	91.49	[63]	**5k**	Cl	$CH_2C_6H_5$	4-CH_3	98.30	[63]
2f	Br	$CH_2C_6H_5$	90.20	[64]	**5l**	Br	$CH_2C_6H_5$	4-CH_3	94.53	[64]
3a	H	CH_3	94.41	[65]	**5m**	Cl	$CH_2C_6H_5$	2,4-$(CH_3)_2$	90.83	[63]
4a	H	CH_3	99.44	[65]	**5n**	Br	$CH_2C_6H_5$	2,4-$(CH_3)_2$	92.40	[64]
4b	Cl	CH_3	93.29	[65]	**6a**	H	CH_3	H	94.90	[59]
4c	Br	CH_3	97.80	[65]	**6b**	Cl	CH_3	H	96.07	[60]
4d	H	$CH_2C_6H_5$	98.98	[65]	**6c**	Br	CH_3	H	98.79	[61]
4e	Cl	$CH_2C_6H_5$	99.36	[65]	**6d**	H	CH_3	4-CH_3	99.50	[59]
4f	Br	$CH_2C_6H_5$	98.81	[65]	**6e**	Cl	CH_3	4-CH_3	97.96	[60]
					6f	Br	CH_3	4-CH_3	97.66	[61]
					6g	H	CH_3	2,4-$(CH_3)_2$	98.68	[59]
					6h	Cl	CH_3	2,4-$(CH_3)_2$	97.51	[60]
					6i	Br	CH_3	2,4-$(CH_3)_2$	96.80	[61]
					6j	Br	CH_3	2,4,6-$(CH_3)_3$	90.58	[61]
					6k	Cl	$CH_2C_6H_5$	H	95.13	[63]
					6l	H	$CH_2C_6H_5$	4-CH_3	93.31	[62]
					6m	Cl	$CH_2C_6H_5$	4-CH_3	97.57	[63]
					6n	Br	$CH_2C_6H_5$	4-CH_3	97.70	[64]
					6o	Cl	$CH_2C_6H_5$	2,4-$(CH_3)_2$	99.15	[63]
					6p	Br	$CH_2C_6H_5$	2,4-$(CH_3)_2$	99.90	[64]

2.2. Antimicrobial Activity Assessment

2.2.1. Qualitative Screening of Antimicrobial Activity

The qualitative screening tests showed a weak growth inhibitory effect of the tested compounds against the studied microorganisms with no clear inhibition zones, the limits of the growth inhibition not exceeding the compound solution deposition area on the agar layer. This could be explained by the poor diffusion into the agar media of the tested compounds. The growth inhibition zones were detected only for compounds **1a–f**; **2a–f**; **3a**; **4a–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g–k**; **6m**; and **6o,p**, which were further evaluated by the quantitative method to determine the minimal inhibitory concentration (MIC) values.

2.2.2. Quantitative Assay of Antimicrobial Activity

The broth dilution method was used to quantitatively assess the in vitro antimicrobial profile of compounds **1a–f**; **2a–f**; **3a**; **4a–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g–k**; **6m**; and **6o,p** against two Gram-positive bacteria (*S. epidermidis* 756 and *B. subtilis* ATCC 6683), two Gram-negative bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853), and one yeast strain (*C. albicans* 128).

Compounds **1a–c**; **4b–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g,h**; **6k**; **6m**; and **6o,p** did not inhibit the growth of any of the tested microbial strains up to a concentration of 225 μg/mL. In contrast, the MIC values of compounds **1d–f**; **2a–f**; **3a**; **4a**; and **6i,j** were much lower in the case of some microbial strains with MICs in the range of 56.2 to 14 μg/mL being recorded (Table 2). Compound **1e** exhibited a wide spectrum of antimicrobial activity, being active on both Gram-positive and -negative bacterial strains as well as against the fungal strain *C. albicans* 128, for which MICs ≤56.2 μg/mL were recorded. Compounds **1d,e**; **3a**; **4a**; and **6i,j** exhibited very good antifungal activity with MIC values of 14 μg/mL.

Table 2. The MIC and MBIC values (μg/mL) measured for compounds **1d–f**; **2a–f**; **3a**; **4a**; **6i,j**; and **6p** against the tested microbial strains.

Tested Compounds	*S. epidermidis* 756		*B. subtilis* ATCC 6683		*E. coli* ATCC 25922		*P. aeruginosa* ATCC 27853		*C. albicans* 128	
	MIC	**MBIC**	**MIC**	**MBIC**	**MIC**	**MBIC**	**MIC**	**MBIC**	**MIC**	**MBIC**
1d	>225	>225	>225	>225	28.1	225	>225	>225	14	112.5
1e	56.2	56.2	>225	>225	28.1	56.2	>225	14	14	112.5
1f	56.2	56.2	>225	>225	>225	>225	>225	28.1	>225	>225
2a	>225	>225	>225	>225	28.1	225	>225	>225	>225	>225
2b	>225	>225	>225	>225	28.1	56.2	>225	>225	>225	>225
2c	>225	>225	>225	>225	28.1	56.2	>225	>225	>225	>225
2d	56.2	56.2	>225	>225	>225	>225	>225	14	>225	>225
2e	>225	112.5	>225	56.2	28.1	56.2	>225	14	>225	>225
2f	>225	225	>225	>225	28.1	56.2	>225	14	>225	>225
3a	>225	112.5	>225	>225	>225	>225	14	14	14	112.5
4a	56.2	56.2	56.2	112.5	>225	>225	>225	14	14	112.5
6i	>225	>225	>225	>225	>225	>225	>225	>225	14	112.5
6j	>225	>225	>225	225	>225	>225	>225	14	14	112.5
6p	>225	112.5	>225	>225	>225	>225	>225	>225	>225	>225
Ciprofloxacin	0.15	0.15	<0.03	<0.03	0.012	0.012	0.15	0.15	- *	-
Fluconazole	-	-	-	-	-	-	-	-	<0.12	<0.12

* -, not tested.

2.2.3. Effects of the Compounds on Biofilm Formation

To further evaluate the effects of the analyzed compounds against microbial biofilms, crystal violet microtiter assay was performed for compounds **1a–f**; **2a–f**; **3a**; **4a–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g–k**; **6m**; and **6o,p**. The analysis showed that biofilm formation was not affected by compounds **1a–c**; **4b–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g,h**; **6k**; **6m**; and **6o**. However, decreased absorbance of the stained biomass was recorded for the biofilms grown in the presence of compounds **1d–f**; **2a–f**; **3a**; **4a**; **6i,j**; and **6p**, with the minimal biofilm inhibitory assay (MBIC) ranging from 14 to 225 μg/mL (Table 2). Compounds **1e**, **2d–f**, **3a**, **4a**, and **6j** demonstrated an MBIC value of 14 μg/mL against the *P. aeruginosa* ATCC

27853 biofilm. Compound **1e** inhibited the biofilm-forming capacity of the Gram-positive *S. epidermidis* 756 strain, the Gram-negative strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, and the fungal strain *C. albicans* 128.

Regarding the antimicrobial and antibiofilm activity of the standard antibiotic (ciprofloxacin) and antifungal (fluconazole) agents used as controls, the MIC values were much lower than those obtained for the tested compounds in all of the cases. This is somehow expected given the new compounds are not yet standardized in optimal formulations and their mechanisms of action could be different from those of the control drugs.

2.3. Prediction of the Biological Properties of the Compounds

2.3.1. In Silico Evaluation of the Molecular Mechanism of Action

Based on 2D structural descriptors, the PASS application was used to calculate the probability of the target compounds **1af**, **2a–f**, **3a**, **4a–f**, **5a–n**, and **6a–p** as being active (Pa) or inactive (Pi) on a large series of targets [66]. The analysis returned Pa values higher than the corresponding Pi values for 2097 pharmacological effects, of which 14 were directly related to antibacterial effects. The maximum and minimum predicted Pa values for the 49 compounds and the compounds with Pa values over 0.5 are displayed in Table 3.

Table 3. The probability (Pa) for the compounds to produce biological effects related to antibacterial action as predicted by the PASS application.

Target	Pa Max	Pa Min	Compounds with Pa > 0.5
Anti-infective	0.702	0.218	**1a, 1b, 1c, 1d, 1e, 1f, 3a**
Antimycobacterial	0.574	0.198	**2c, 2f, 5c, 5f**
Antituberculosis	0.526	0.199	**5c, 5f**
Antibiotic glycopeptide-like	0.403	0.083	0
Peptidoglycan glycosyltransferase inhibitor	0.323	0.212	0
Antibacterial	0.312	0.168	0
UDP-*N*-acetylmuramate-*L*-alanine ligase inhibitor (MurC)	0.225	0.116	0
Antibacterial, ophthalmic	0.164	0.122	0
Bacterial efflux pump inhibitor	0.119	0.118	0
Antiseptic	0.118	0.117	0
Antibiotic	0.106	0.106	0
Peptidoglycan beta-*N*-acetylmuramidase inhibitor	0.093	0.067	0
N-acetylmuramoyl-*L*-alanine amidase inhibitor	0.083	0.054	0
UDP-*N*-acetylmuramoylalanine-*D*-glutamate ligase inhibitor (MurD)	0.079	0.059	0
Bacterial leucyl aminopeptidase inhibitor	0.064	0.051	0

A total of 33 compounds presented Pa values over 0.3 for anti-infective effect, and 37 compounds were predicted to have antimycobacterial effects with Pa value above 0.3.

Figure 1 presents the plotted Pa values for anti-infective, antimycobacterial, and antibacterial effects based on the main chemical scaffold.

The prediction results indicated a clear correlation between the chemical scaffolds of the studied compounds and the potential to have an anti-effect, with *N*-acyl-α-amino acids (scaffold 1) emerging as the most promising class (Figure 1b). In the case of antibacterial effects, the prediction indicated 4*H*-1,3-oxazol-5-one ring (scaffold 2) as the most favorable core structure (Figure 1d). The potential of the compounds to produce antimycobacterial effects was not correlated with the chemical scaffolds (Figure 1c).

The PASS application can be used to indicate a probable mechanism of action [67] of new compounds. Compounds **1a–d**, **2a**, and **2d** had significant Pa values for the inhibition of peptidoglycan glycosyltransferase, a valuable target for new antimicrobial therapies [68]. UDP-*N*-acetylmuramate-*L*-alanine ligase inhibitor (MurC) is a member of the Mur enzymes family and, similar to peptidoglycan glycosyltransferase, is involved in synthesis of peptidoglycan [69]. Compounds **1a–c**; **1e,f**; **2d,e**; and **3a** presented small but significant Pa values towards this possible mechanism.

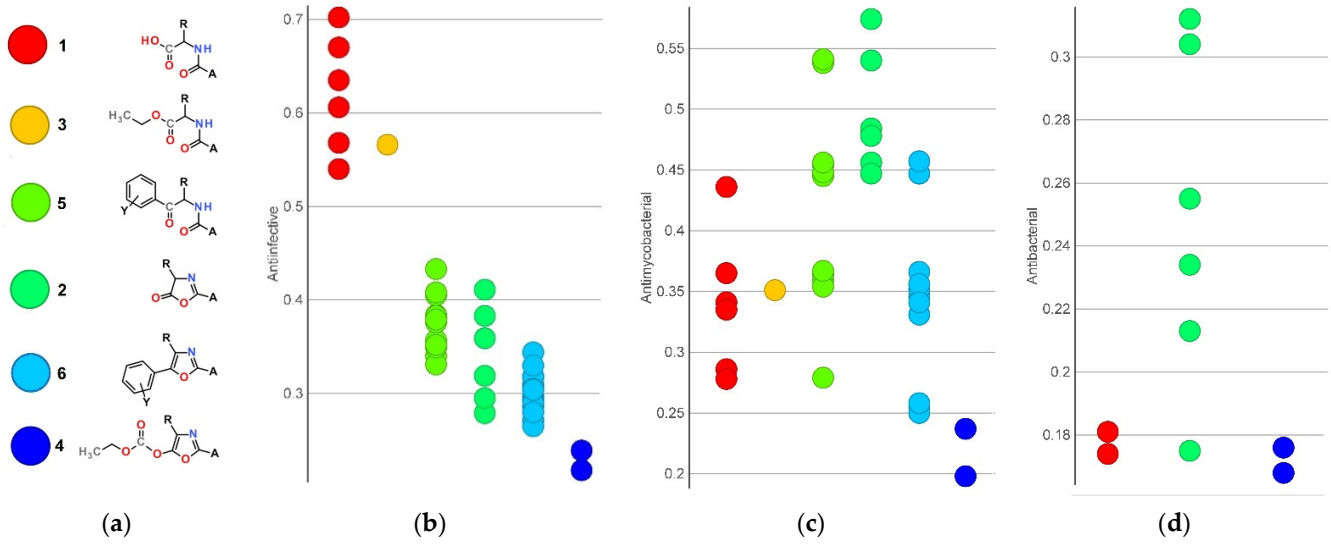


Figure 1. Pa values as predicted by the PASS application: (**a**) color codes for classification; (**b**) Pa values for anti-infective effect; (**c**) Pa values for antimycobacterial effect; (**d**) Pa values for antibacterial effect.

2.3.2. Structural Descriptors Analysis

DataWarrior v5.2.1 software [70] was used to calculate a series of structural descriptors, namely molecular weight (MW), logarithm of the octanol–water partition coefficient (cLogP), hydrogen bond donors count (HBD), hydrogen bond acceptor count (HBA), polar surface area (PSA), number of rotatable bonds (RB), and druglikeness (DLK). These descriptors are presented in Table 4 with the minimum and maximum values registered for the compounds.

Table 4. Descriptive statistics for the molecular descriptors.

Descriptor	Min	Max
MW	315.35	576.51
cLogP	1.29	7.61
HBD	0	5
HBA	0	2
RB	3	9
PSA	68.6	108.9
DLK	−19.7	4.9

In order to better understand the structure–activity relationships, each descriptor was plotted for both active and inactive compounds based on the MIC values presented in Table 2. The best difference of distribution of values was observed for cLogP (Figure 2). The results indicated that a lower lipophilic character was correlated with a higher antimicrobial effect. The chemical scaffold was also an important factor because some compounds were inactive despite a low cLogP value.

Four compounds with cLogP values in the range of 3.08–3.93 presented antimicrobial and antibiofilm effects towards *S. epidermidis*. Most of the inactive compounds had a cLogP value over 4, indicating that a high lipophilicity was detrimental. Except for the ethyl carbonate derivative **4a**, the active compounds on *S. epidermidis* were all derivatives of phenylalanine. In the case of *E. coli*, the analysis of the structure–activity relationships indicated two major factors: the presence of the *N*-acyl phenylalanine scaffold or its cyclic 4*H*-1,3-oxazol-5-one analogue and a cLogP value under 4.

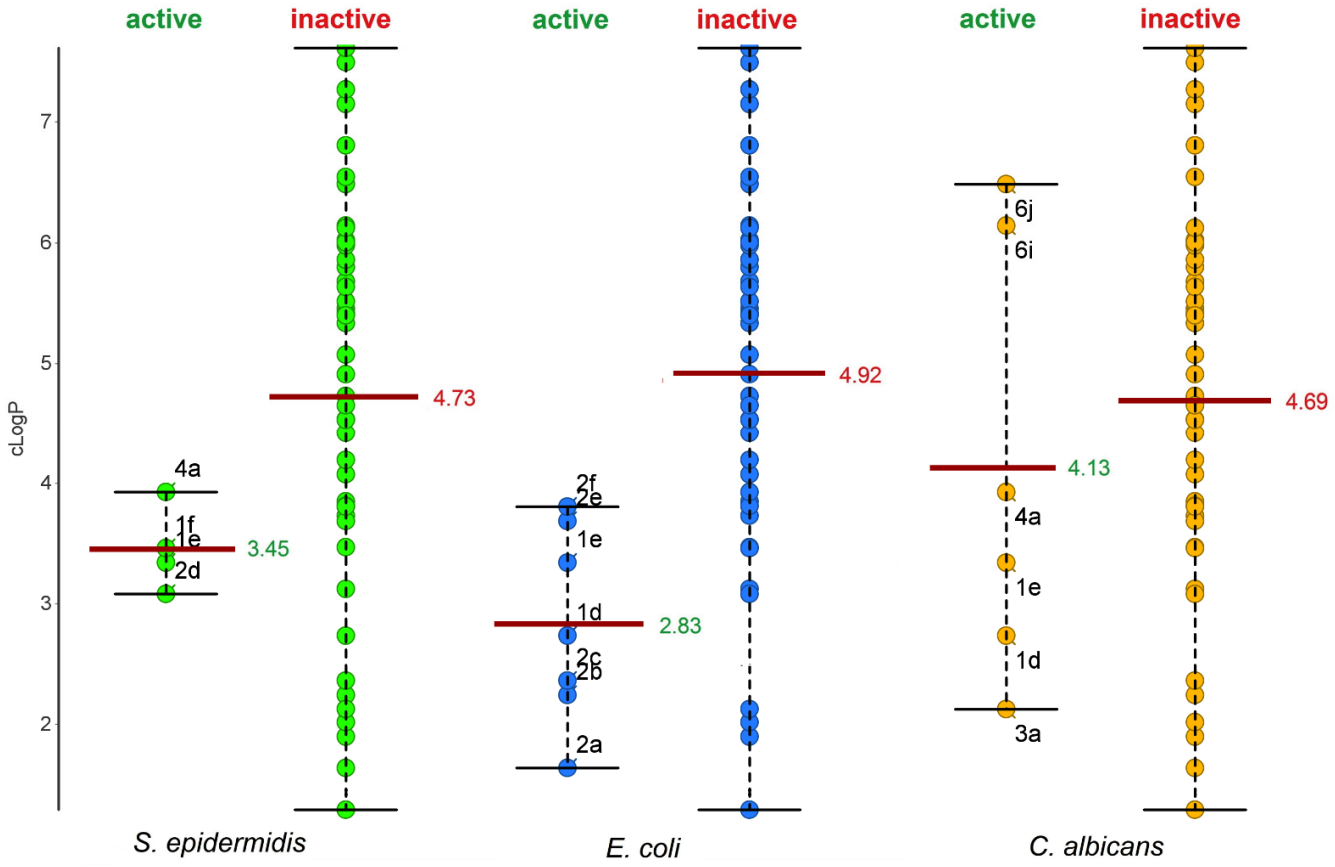


Figure 2. The cLogP values split by the active or inactive status against *S. epidermidis*, *E. coli*, and *C. albicans*. The horizontal lines represent the average values for each data column.

2.3.3. Predicted ADME-T Properties

A series of medicinal chemistry measures, ADME, and toxicity endpoints were estimated using the ADMETLab2.0 platform [71]. The data are presented in Table 5. Apart from compound **6p**, all the compounds were predicted to have good medicinal chemistry-related scores (Lipinski's rule, Pfizer rule, GSK rule, golden triangle). None of the compounds were estimated to be a pan-assay interference compound (PAINS). The new compounds were predicted to have a low therapeutic index because of their high plasma protein binding (%). For all the compounds, a significant hepatic toxicity was predicted. Except for compounds **1d–f** and **3a**, a carcinogen risk was estimated for all other analyzed compounds.

Table 5. ADMET predictions for the new compounds **1d–f**; **2a–f**; **3a**; **4a**; **6i,j**; and **6p**. yes for green, no for red.

Property	1d	1e	1f	2a	2b	2c	2d	2e	2f	3a	4a	6i	6j	6p
Lipinski's rule	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Pfizer rule	●	●	●	●	●	●	●	●	●	●	●	●	●	●
GSK rule	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Golden triangle	●	●	●	●	●	●	●	●	●	●	●	●	●	●
PAINS	no	no	no	no	no	no	no	no	no	no	no	no	no	no
Plasma protein binding (%)	99.0	98.7	98.7	98.4	99.4	98.9	100	100	100	94.3	98.2	100	100	100
Volume distribution	0.34	0.37	0.35	0.53	0.48	0.54	0.29	0.27	0.35	0.14	0.43	0.42	0.48	0.31
hERG blocker	−−	−	−	−	−−	−−−	−−−	−−	−−−	−	−−−	−−	−−−	−−−
AMES toxicity	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−
Carcinogenicity	−	−	−	++	++	++	++	++	++	−−	++	+	+	+
Hepatotoxicity	+	++	+	++	++	+	++	++	+	−−	+	++	+	+
Drug-induced liver injury	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Based on the ADMET predictions, compound **3a** had the best toxicological and pharmacokinetic profile of the new compounds. This compound was the ethyl ester of the derivative **1a**, indicating that the transformation of compounds **1d–f** could improve their toxicological profile and enhance their antibacterial properties.

3. Discussion

Over time, resistance of human pathogens to major antibiotics increases, and infectious agents that are resistant to most available antibiotics are rising globally [72]. An important strategy to help prevent and confront the resistance problem requires the discovery and development of new bioactive agents against both planktonic and adherent microorganisms. Medical devices and instruments are prone to microbial colonization and biofilm formation. Therefore, the discovery of agents that could prevent biofilm formation or adherence would be of great use. Very recently, we reported on the synthesis and antimicrobial and antibiofilm evaluation results of a series of compounds derived from valine [73,74].

In the present research, we examined the potential antimicrobial activity of some 1,3-oxazole derivatives and their isosteres sharing a 4-(4-X-phenylsulfonyl)phenyl moiety, which were synthesized using two other natural α-amino acids as raw materials, namely alanine and phenylalanine.

Preliminary qualitative antimicrobial screening revealed that compounds **1a–f**; **2a–f**; **3a**; **4a–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g–k**; **6m**; and **6o,p** exhibited inhibitory growth effects, although the growth inhibition zones were detected only in contact with the agar layer. These compounds were further evaluated in vitro to determine their effects on planktonic and adherent microbial growth. Compounds **1a–c**; **4b–f**; **5a,b**; **5d,e**; **5g,h**; **5j,k**; **5m,n**; **6g,h**; **6k**; **6m**; and **6o,p** did not exhibit growth inhibitory effects on any of the tested microbial strains up to a concentration of 225 μg/mL. Regarding the *S. epidermidis* 756 strain, among the different compounds, **1e,f**; **2d**; and **4a** were the most effective with an MIC of 56.2 μg/mL. The most active compound against *P. aeruginosa* ATCC 27853 was **3a**, the compounds **1d,e**; **3a**; **4a**; and **6i,j** were active against *C. albicans* 128 with a low MIC value of 14 μg/mL, and the compounds **1d,e**; **2a–c**; and **2e,f** proved to be active against *E. coli* ATCC 25922 with an MIC of 28.1 μg/mL. Regarding *B. subtilis* ATCC 6683, compound **4a** was found to be the best with an MIC of 56.2 μg/mL, while **3a** had an MIC of 14 μg/mL against *P. aeruginosa* ATCC 27853.

The analyzed compounds affected the adherence and biofilm formation on inert surfaces at MBIC values in the range of 14–225 μg/mL. Our data demonstrated that compounds **1e**, **2d–f**, **4a**, and **6j** had a distinctly stronger effect on *P. aeruginosa* ATCC 27853 cells embedded in the biofilm (MBIC of 14 μg/mL) than on planktonic cells. We hypothesized that the tested compounds have a specific nonbactericidal mechanism that changes the bacterial cell surface rather than destroying the bacterial cell.

From the results obtained in the quantitative screening, it was observed that 2-[4-(4-X-phenylsulfonyl)benzamido]propanoic acids **1a–c** were inactive at the concentrations used in the assay. However, by intramolecular cyclodehydration, biologically active 4*H*-1,3-oxazol-5-ones **2a–c** were obtained, which displayed growth-inhibitory action with an MIC of 28.1 μg/mL and had an antibiofilm effect on *E. coli* ATCC 25922 with MBIC values of 56.2 (**2b** and **2c**) and 225 (**2a**) μg/mL. The in silico prediction of the pharmacokinetic profile (ADME properties) indicated that compounds **2a–c** had good pharmacokinetic profiles. Compounds **1a–d**, **2a**, and **2d** were predicted to inhibit peptidoglycan glycosyltransferase.

By opening the 4*H*-1,3-oxazol-5-ones ring, the resulting *N*-acyl-α-amino ketones (**5a–i**) did not show antimicrobial properties up to a concentration of 225 μg/mL. Intramolecular cyclization of *N*-(1-aryl-1-oxopropan-2-yl)-4-(4-X-phenylsulfonyl)benzamides afforded the corresponding 1,3-oxazoles, which were inactive in the tested concentration range, with the exception of 2-{4-[(4-bromophenyl)sulfonyl]phenyl}-5-(2,4-dimethylphenyl)-4-methyl-1,3-oxazole **6i** and 2-{4-[(4-bromophenyl)sulfonyl]phenyl}-5-mesityl-4-methyl-1,3-oxazole **6j**, which showed antifungal action on *C. albicans* 128 (MIC = 14 μg/mL). Moreover, **6j** inhibited the formation of biofilm by *B. subtilis* ATCC 6683 (MBIC = 225 μg/mL) and

P. aeruginosa ATCC 27853 (MBIC = 14 μg/mL), and both 1,3-oxazoles presented antibiofilm effect against *C. albicans* 128 with an MBIC of 112.5 μg/mL. These properties were probably a consequence of the presence of a bromine atom in the *para* position of the arylsulfonylphenyl substituent linked to the C-2 and the *m*-xylyl or mesityl group grafted to the C-5 of the 1,3-oxazole ring. Esterification of 2-[4-(phenylsulfonyl)benzamido]propanoic acid **1a** or ethanolysis of 4-methyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5(4*H*)-one **2a** gave ethyl 2-[4-(phenylsulfonyl)benzamido]propanoate **3a**, which was active on *P. aeruginosa* ATCC 27853 and *C. albicans* 128 (MIC = 14 μg/mL). It exhibited an antibiofilm effect on *P. aeruginosa* ATCC 27853 (MBIC = 14 μg/mL), *S. epidermidis* 756, and *C. albicans* 128 (MBIC = 112.5 μg/mL). The conversion of *N*-acylated α-amino acid **1a** into its ester derivative **3a** led to the appearance of antimicrobial properties. Based on in silico predictions, the obtained product also had a good pharmacokinetic and toxicological profile. Derivatization of the *N*-acyl-α-amino acid **1a** also led to the ethyl {4-methyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} carbonate **4a**, which showed antimicrobial activity on *S. epidermidis* 756 and *B. subtilis* ATCC 6683 (MIC = 56.2 μg/mL) and on *C. albicans* 128 (MIC = 14 μg/mL). Compound **4a** was also capable of decreasing the biomass of *S. epidermidis* 756 (MBIC = 56.2 μg/mL), *P. aeruginosa* ATCC 27853 (MBIC = 14 μg/mL), *B. subtilis* ATCC 6683, and fungal strain *C. albicans* 128 (MBIC = 112.5 μg/mL).

All three *N*-acyl phenylalanines (**1d–f**) and all three corresponding 4*H*-1,3-oxazol-5-ones (**2d–f**) showed antimicrobial and antibiofilm activities. Thus, 3-phenyl-2-[4-(phenylsulfonyl) benzamido]propanoic acid **1d** was active on *E. coli* ATCC 25922 (MIC = 28.1 μg/mL and MBIC = 225 μg/mL) and *C. albicans* 128 (MIC = 14 μg/mL and MBIC = 112.5 μg/mL). In contrast, 4-benzyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5(4*H*)-one **2d**, which resulted from the cyclization of *N*-acyl-α-amino acid **1d**, had an inhibitory effect on Gram-positive bacterium *S. epidermidis* 756 (MIC = 56.2 μg/mL). Moreover, **2d** presented antibiofilm action against *S. epidermidis* 756 (MBIC = 56.2 μg/mL) and *P. aeruginosa* ATCC 27853 (MBIC = 14 μg/mL). The 2-{4-[(4-chlorophenyl)sulfonyl]benzamido}-3-phenylpropanoic acid **1e** showed a broad antimicrobial spectrum on Gram-positive bacterium *S. epidermidis* 756 (MIC = 56.2 μg/mL), Gram-negative bacterium *E. coli* ATCC 25922 (MIC = 28.1 μg/mL), and fungus *C. albicans* 128 (MIC = 14 μg/mL). Compound **1e** presented MBIC values of 56.2 μg/mL for *S. epidermidis* 756 and *E. coli* ATCC 25922, 14 μg/mL for *P. aeruginosa* ATCC 27853, and 112.5 μg/mL for *C. albicans* 128. These effects were probably a result of the presence of the phenylalanine fragment in the molecule and the chlorine atom in the *para* position of the arylsulfonylphenyl moiety. Intramolecular transformation of *N*-acyl-α-amino acid **1e** led to 4-benzyl-2-{4-[(4-chlorophenyl)sulfonyl]phenyl}-1,3-oxazol-5(4*H*)-one **2e**, which exhibited antimicrobial activity only on *E. coli* ATCC 25922 (MIC = 28.1 μg/mL) and antibiofilm effect on *S. epidermidis* 756 (MBIC = 112.5 μg/mL), *B. subtilis* ATCC 6683, *E. coli* ATCC 25922 (MBIC = 56.2 μg/mL), and *P. aeruginosa* ATCC 27853 (MBIC = 14 μg/mL). In addition, 2-{4-[(4-bromophenyl)sulfonyl]benzamido}-3-phenylpropanoic acid **1f** had an inhibitory action on *S. epidermidis* 756 (MIC = 56.2 μg/mL) and inhibited biofilm formation of *S. epidermidis* 756 (MBIC = 56.2 μg/mL) and *P. aeruginosa* ATCC 27853 (MBIC = 28.1 μg/mL). By intramolecular cyclization of this *N*-acyl-α-amino acid (**1f**) to the isosteric 4*H*-1,3-oxazol-5-one analogue **2f**, the antibacterial effect on *S. epidermidis* 756 disappeared, but the obtained compound inhibited the growth of *E. coli* ATCC 25922 (MIC = 28.1 μg/mL). Saturated azlactone **2f** also had antibiofilm activity against *S. epidermidis* 756 (MBIC = 225 μg/mL), *E. coli* ATCC 25922 (MBIC = 56.2 μg/mL), and *P. aeruginosa* ATCC 27853 (MBIC = 14 μg/mL). All *N*-acyl-α-amino ketones **5j–n** obtained by opening the ring of the 4-benzyl-2-[4-(4-X-phenylsulfonyl)phenyl]-1,3-oxazol-5(*4H*)-ones **2d–f** were devoid of antimicrobial action up to a concentration of 225 μg/mL. By cyclization of *N*-(1-aryl-1-oxo-3-phenylpropan-2-yl)-4-(4-X-phenylsulfonyl)benzamides, the five-membered heterocycles of the 1,3-oxazoles class (**6k–p**) were synthesized, from which only 4-benzyl-2-{4-[(4-bromophenyl)sulfonyl]phenyl}-5-(2,4-dimethylphenyl)-1,3-oxazole **6p** affected adherence and biofilm formation of *S. epidermidis* 756 on inert surfaces with an MBIC of 112.5 μg/mL. In the case of this compound, we hypothesized that the antibiofilm activity may be correlated to the substitution with

bromine in the *para* position of the C-2-linked arylsulfonylphenyl fragment, and with the presence of the benzyl substituent bonded to the C-4, and the *m*-xylyl group grafted at position 5 of the 1,3-oxazole nucleus.

In the tested concentration range, {4-(benzyl/methyl)-2-[4-(4-X-phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} ethyl carbonates **4b–f** were proved to be inactive on the studied strains. The results were confirmed by PASS analysis with small probabilities to produce anti-infective, antimycobacterial, or antibacterial effects.

Taken together, the antimicrobial activity results indicate that compounds **1d,e,f**; **2d,e,f**; **3a**; **4a**; and **6i,j** are the most promising candidates for further biological investigations and structural optimization as potential new anti-infective agents, as revealed by the lowest MIC and even MBIC values obtained.

From these compounds, the in silico assays predicted the anti-infective potential for **1e**, also exhibiting the broadest antimicrobial spectrum, and **2f**, which proved to successfully inhibit *P. aeruginosa* biofilm development. These compounds have also been predicted to have drug-like properties.

4. Materials and Methods

4.1. General Information

All solvents and reagents were purchased from commercial sources and used without further purification. The absorbance was measured on an Apollo LB 911 ELISA reader (Berthold Technologies GmbH & Co. KG, Waltham, MA, USA).

4.2. Chemistry

The tested compounds **1–6** were previously synthesized [59–65] according to the multiple-step strategy presented in Scheme 1. The *N*-acyl-α-amino acids **1a–f** were obtained by Schotten–Baumann-type *N*-acylation of α-amino acids (alanine or phenylalanine) with 4-(4-X-phenylsulfonyl)benzoyl chlorides (X = H, Cl, or Br). The intramolecular cyclodehydration of compounds **1a–f** using ethyl chloroformate in the presence of *N*-methylmorpholine (NMM) led to 4*H*-1,3-oxazol-5-ones **2a–f** when the molar ratio of **1a–f**/$ClCO_2C_2H_5$/NMM was 1:1:1 and the reaction time was 30 min and to ethyl 1,3-oxazol-5-yl carbonates **4a–f** when the molar ratio of the reactants was 1:1.5:1.5 and the reaction time was increased to 24 h. The carboxyl group of the *N*-acyl-α-amino acid **1a** was highlighted by its transformation into the corresponding ethyl ester **3a**, which was also obtained by *O*-acylation of the ethanol with 4*H*-1,3-oxazol-5-one **2a**. The Friedel–Crafts acylation, catalyzed by $AlCl_3$ of the aromatic hydrocarbons with the saturated azlactones **2a–f**, yielded *N*-acyl-α-amino ketones **5a–n**. These acyclic precursors underwent Robinson–Gabriel cyclization in the presence of phosphoryl trichloride with the formation of 5-aryl-1,3-oxazoles **6a–p**. The structures of some of the compounds were confirmed by an additional method. The compounds were purified by recrystallization from water (**1a–f**), cyclohexane (**2a–f** and **5b,c**), toluene (**3a**), ethanol (**4a–f**, **5a**, **5d–g**, **5i–n**, and **6a–p**), or ethanol–water (**5h**). Their purities were verified by RP-HPLC according to previously reported procedures [61–65], with the values ranging between 90.20 and 99.99% (Table 1). As shown in our previous works [59–65], all tested compounds were characterized using spectral methods (UV–vis, IR, MS, and ^{1}H- and ^{13}C-NMR) and elemental analyses, confirming the purity of the compounds.

4.3. Antimicrobial Activity Assessment

4.3.1. Microbial Strains

The antimicrobial activity of the synthesized compounds was tested against two Gram-positive bacteria (*Staphylococcus epidermidis* 756 and *Bacillus subtilis* ATCC 6683), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one yeast strain (*Candida albicans* 128).

4.3.2. Qualitative Screening of Antimicrobial Activity

The qualitative screening tests were performed using the agar diffusion method following the CLSI (Clinical and Laboratory Standards Institute) guidelines. The inoculums were prepared from 18–24 h microbial cultures obtained on tryptone soy broth (TSA) for bacteria and on yeast peptone glucose agar (YPGA) for yeast by direct colony suspension in sterile phosphate-buffered saline (PBS). The microbial suspensions turbidity was adjusted to 0.5 McFarland scale and used for the inoculation of the agar plates (Mueller–Hinton agar). Then, 5 µL of the solution of the tested compound at 450 µg/mL concentration, prepared in DMSO, was placed on the agar surface. The negative (DMSO) and positive (standardized antibiotic discs of ciprofloxacin 5 µg and fluconazole 25 µg) controls were prepared. The Petri dishes were incubated at 37 °C, and the diameters of inhibition growth zones were then measured.

4.3.3. Determination of the Minimal Inhibitory Concentration (MIC)

Quantitative analysis of the antimicrobial activity of the tested compounds was carried out using the broth microdilution method following the CLSI guidelines. Two-fold dilutions of the tested compounds were prepared in a liquid growth medium dispensed in a 96-well microplate. The range of final concentrations of the solutions in DMSO of all tested compounds was 1.7–225 µg/mL. Then, each well was inoculated with a microbial inoculum prepared in the same medium after dilution of the standardized microbial suspension adjusted to 0.5 McFarland scale. Binary serial dilutions for DMSO in the liquid growth medium were also prepared. The uninoculated media (MH broth or YPG) and inoculated media served as sterility controls and microbial growth controls. After mixing well, the inoculated 96-well microplates were incubated, without agitation, in aerobic conditions at 37 °C for 24 h. The MIC was measured as the lowest concentration of the tested compound showing no turbidity after 24 h, where turbidity was interpreted as visible bacterial growth. Ciprofloxacin, a broad-spectrum antibacterial agent, and antifungal fluconazole served as controls. The assays were performed in duplicate.

4.3.4. Determination of the Minimal Biofilm Inhibitory Concentration (MBIC)

The crystal violet assay was used to assess the biofilm's susceptibility to the tested compounds. After determination of the MIC values, the 96-well microplates were emptied, washed gently three times with phosphate-buffered saline (PBS) to remove the planktonic microbial cells, and then fixed with cold methanol for 5 min. The adherent cells in the plastic wells were further stained with 1% violet crystal solution for 30 min. The excess dye was removed by washing with distilled deionized water. In each well, 200 µL of 30% acetic acid was added. After 10 min of incubation to release the dye, the biofilm was assessed by measuring the absorbance at 492 nm using a plate-reading spectrophotometer. The MBIC value was determined as the lowest concentration of the tested compounds showing biofilm inhibition compared to the untreated control. The experiment was performed in duplicate.

4.4. Prediction of the Biological Properties of the Compounds

4.4.1. In Silico Evaluation of the Molecular Mechanisms of Action

The study was executed using the PASS (Prediction of Activity Spectra for Substances) software, a product that predicts the pharmacological potential of new compounds. The structures were introduced as SMILES, and the results were considered only if the Pa values were higher than the corresponding Pi values.

4.4.2. Predicted ADME-T Properties

The ADMETlab 2.0 online platform was used to evaluate the in silico ADMET profile for the 49 compounds. Several physicochemical, medicinal chemistry, and ADME properties were computed, together with toxicity endpoints and toxicophore-based assessment.

5. Conclusions

A total of 49 derivatives that incorporate a 4-(4-X-phenylsulfonyl)phenyl fragment into their structure and are designed based on the 1,3-oxazole scaffold and its isosteric analogues were investigated for their antimicrobial and antibiofilm activity. The compounds belonged to the following chemotypes: *N*-acyl-α-amino acids, 4*H*-1,3-oxazol-5-ones, *N*-acyl-α-amino acid esters, *N*-acyl-α-amino ketones, and 1,3-oxazoles classes. The assays revealed that the tested compounds **1d,e**, **3a**, and **4a** exhibited the best antimicrobial effects and could be considered as promising candidates for future biological investigations and structural optimization. Among the tested compounds, **1e** exhibited the most intense and broad spectrum of antimicrobial activity, including for the Gram-positive, Gram-negative, and fungal strains, which is probably correlated with the presence of the phenylalanine moiety in its structure and the chlorine atom in the *para* position of the arylsulfonylphenyl fragment. The predictive studies indicate the inhibition of peptidoglycan glycosyltransferase and, to a less extent, the inhibition of the UDP-*N*-acetylmuramate-*L*-alanine ligase as possible mechanisms of action.

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References

1. Regueiro-Ren, A. Cyclic sulfoxides and sulfones in drug design. In *Advances in Heterocyclic Chemistry*; Meanwell, N.A., Lolli, M.L., Eds.; Academic Press: Cambridge, CA, USA, 2021; Volume 134, pp. 1–30, ISBN 978-0-12-820181-7.
2. Kumar Verma, S.; Verma, R.; Xue, F.; Kumar Thakur, P.; Girish, Y.R.; Rakesh, K.P. Antibacterial activities of sulfonyl or sulfonamide containing heterocyclic derivatives and its structure-activity relationships (SAR) studies: A critical review. *Bioorg. Chem.* **2020**, *105*, 104400. [CrossRef] [PubMed]
3. Zhao, C.; Rakesh, K.P.; Ravidar, L.; Fang, W.-Y.; Qin, H.-L. Pharmaceutical and medicinal significance of sulfur (S^{VI})-Containing motifs for drug discovery: A critical review. *Eur. J. Med. Chem.* **2019**, *162*, 679–734. [CrossRef] [PubMed]
4. Alam, M.A.; Shimada, K.; Jahan, A.; Khan, M.W.; Bhuiyan, M.M.H.; Alam, M.S.; Matin, M.M. Synthesis, Reactions and Medicinal Importance of Cyclic Sulfone Derivatives: A Review. *Nat. Prod. Chem. Res.* **2018**, *6*, 1000350. [CrossRef]
5. Feng, M.; Tang, B.; Liang, S.H.; Jiang, X. Sulfur Containing Scaffolds in Drugs: Synthesis and Application in Medicinal Chemistry. *Curr. Top. Med. Chem.* **2016**, *16*, 1200–1216. [CrossRef] [PubMed]
6. Ahmad, I. Shagufta Sulfones: An Important Class of Organic Compounds with Diverse Biological Activities. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 19–27.
7. Kang, C.; Kim, J.; Ju, S.; Park, S.; Yoo, J.-W.; Yoon, I.-S.; Kim, M.-S.; Jung, Y. Dapsone Azo-Linked with Two Mesalazine Moieties Is a "Me-Better" Alternative to Sulfasalazine. *Pharmaceutics* **2022**, *14*, 684. [CrossRef]
8. Mady, M.F.; Awad, G.E.A.; Jørgensen, K.B. Ultrasound-assisted synthesis of novel 1,2,3-triazoles coupled diaryl sulfone moieties by the CuAAC reaction, and biological evaluation of them as antioxidant and antimicrobial agents. *Eur. J. Med. Chem.* **2014**, *84*, 433–443. [CrossRef]
9. Fernández-Villa, D.; Aguilar, M.R.; Rojo, L. Folic Acid Antagonists: Antimicrobial and Immunomodulating Mechanisms and Applications. *Int. J. Mol. Sci.* **2019**, *20*, 4996. [CrossRef]
10. Barbuceanu, S.-F.; Saramet, G.; Bancescu, G.; Draghici, C.; Apostol, T.-V.; Taran, L.; Dinu-Pirvu, C.E. Synthesis, Characterization and Antimicrobial Activity of Some Hydroxypyrazolines. *Rev. Chim.* **2013**, *64*, 355–360.
11. Guzmán-Ávila, R.; Avelar, M.; Márquez, E.A.; Rivera-Leyva, J.C.; Mora, J.R.; Flores-Morales, V.; Rivera-Islas, J. Synthesis, In Vitro, and In Silico Analysis of the Antioxidative Activity of Dapsone Imine Derivatives. *Molecules* **2021**, *26*, 5747. [CrossRef]

12. Bera, S.; Mondal, D. Insights of synthetic analogues of anti-leprosy agents. *Bioorg. Med. Chem.* **2019**, *27*, 2689–2717. [CrossRef] [PubMed]
13. Pezzella, A.T.; Fang, W. Surgical Aspects of Thoracic Tuberculosis: A Contemporary Review—Part 1. *Curr. Probl. Surg.* **2008**, *45*, 675–758. [CrossRef] [PubMed]
14. Mishra, M.; Mishra, V.K.; Kashaw, V.; Iyer, A.K.; Kashaw, S.K. Comprehensive review on various strategies for antimalarial drug discovery. *Eur. J. Med. Chem.* **2017**, *125*, 1300–1320. [CrossRef] [PubMed]
15. Al-Said, M.S.; Ghorab, M.M.; Nissan, Y.M. Dapson in heterocyclic chemistry, part VIII: Synthesis, molecular docking and anticancer activity of some novel sulfonylbiscompounds carrying biologically active 1,3-dihydropyridine, chromene and chromenopyridine moieties. *Chem. Cent. J.* **2012**, *6*, 64. [CrossRef] [PubMed]
16. Membrive Jiménez, C.; Pérez Ramírez, C.; Sánchez Martín, A.; Vieira Maroun, S.; Arias Santiago, S.; Ramírez Tortosa, M.C.; Jiménez Morales, A. Clinical Application of Pharmacogenetic Markers in the Treatment of Dermatologic Pathologies. *Pharmaceuticals* **2021**, *14*, 905. [CrossRef] [PubMed]
17. Xu, S.; Song, S.; Sun, L.; Gao, P.; Gao, S.; Ma, Y.; Kang, D.; Cheng, Y.; Zhang, X.; Cherukupalli, S.; et al. Indolylarylsulfones bearing phenylboronic acid and phenylboronate ester functionalities as potent HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorg. Med. Chem.* **2022**, *53*, 116531. [CrossRef]
18. Kucwaj-Brysz, K.; Baltrukevich, H.; Czarnota, K.; Handzlik, J. Chemical update on the potential for serotonin 5-HT_6 and 5-HT_7 receptor agents in the treatment of Alzheimer's disease. *Bioorg. Med. Chem. Lett.* **2021**, *49*, 128275. [CrossRef]
19. Millan, M.J.; Dekeyne, A.; Gobert, A.; Brocco, M.; Mannoury la Cour, C.; Ortuno, J.-C.; Watson, D.; Fone, K.C.F. Dual-acting agents for improving cognition and real-world function in Alzheimer's disease: Focus on 5-HT6 and D3 receptors as hubs. *Neuropharmacology* **2020**, *177*, 108099. [CrossRef]
20. Alsaedi, A.M.R.; Farghaly, T.A.; Shaaban, M.R. Synthesis and Antimicrobial Evaluation of Novel Pyrazolopyrimidines Incorporated with Mono- and Diphenylsulfonyl Groups. *Molecules* **2019**, *24*, 4009. [CrossRef]
21. Roșca, E.V.; Apostol, T.V.; Chifiriuc, M.C.; Grădișteanu Pîrcălăbioru, G.; Drăghici, C.; Socea, L.I.; Olaru, O.T.; Nițulescu, G.M.; Pahonțu, E.M.; Hrubaru, M.; et al. In Silico and Experimental Studies for the Development of Novel Oxazol-5(4*H*)-ones with Pharmacological Potential. *Farmacia* **2020**, *68*, 453–462. [CrossRef]
22. Rashdan, H.R.M.; Shehadi, I.A.; Abdelrahman, M.T.; Hemdan, B.A. Antibacterial Activities and Molecular Docking of Novel Sulfone Biscompound Containing Bioactive 1,2,3-Triazole Moiety. *Molecules* **2021**, *26*, 4817. [CrossRef] [PubMed]
23. Zheng, X.; Liu, W.; Zhang, D. Recent Advances in the Synthesis of Oxazole-Based Molecules via van Leusen Oxazole Synthesis. *Molecules* **2020**, *25*, 1594. [CrossRef] [PubMed]
24. Kakkar, S.; Narasimhan, B. A comprehensive review on biological activities of oxazole derivatives. *BMC Chem.* **2019**, *13*, 16. [CrossRef]
25. Chen, J.; Lv, S.; Liu, J.; Yu, Y.; Wang, H.; Zhang, H. An Overview of Bioactive 1,3-Oxazole-Containing Alkaloids from Marine Organisms. *Pharmaceuticals* **2021**, *14*, 1274. [CrossRef] [PubMed]
26. Li, Y.; Rebuffat, S. The manifold roles of microbial ribosomal peptide–based natural products in physiology and ecology. *J. Biol. Chem.* **2020**, *295*, 34–54. [CrossRef] [PubMed]
27. Mhlongo, J.T.; Brasil, E.; de la Torre, B.G.; Albericio, F. Naturally Occurring Oxazole-Containing Peptides. *Mar. Drugs* **2020**, *18*, 203. [CrossRef]
28. Zhang, H.-Z.; Zhao, Z.-L.; Zhou, C.-H. Recent advance in oxazole-based medicinal chemistry. *Eur. J. Med. Chem.* **2018**, *144*, 444–492. [CrossRef]
29. Kumar, G.; Singh, N.P. Synthesis, anti-inflammatory and analgesic evaluation of thiazole/oxazole substituted benzothiazole derivatives. *Bioorg. Chem.* **2021**, *107*, 104608. [CrossRef]
30. Sharma, V.; Bhatia, P.; Alam, O.; Javed Naim, M.; Nawaz, F.; Ahmad Sheikh, A.; Jha, M. Recent advancement in the discovery and development of COX-2 inhibitors: Insight into biological activities and SAR studies (2008–2019). *Bioorg. Chem.* **2019**, *89*, 103007. [CrossRef]
31. Guerrero-Pepinosa, N.Y.; Cardona-Trujillo, M.C.; Garzón-Castaño, S.C.; Veloza, L.A.; Sepúlveda-Arias, J.C. Antiproliferative activity of thiazole and oxazole derivatives: A systematic review of in vitro and in vivo studies. *Biomed. Pharmacother.* **2021**, *138*, 111495. [CrossRef]
32. Yan, X.; Wen, J.; Zhou, L.; Fan, L.; Wang, X.; Xu, Z. Current Scenario of 1,3-oxazole Derivatives for Anticancer Activity. *Curr. Top. Med. Chem.* **2020**, *20*, 1916–1937. [CrossRef] [PubMed]
33. de Koning, C.B.; Ngwira, K.J.; Rousseau, A.L. Biosynthesis, synthetic studies, and biological activities of the jadomycin alkaloids and related analogues. In *The Alkaloids: Chemistry and Biology*; Knölker, H.-J., Ed.; Academic Press: Cambridge, CA, USA, 2020; Volume 84, pp. 125–199, ISBN 978-0-12-820982-0.
34. Jakeman, D.L.; Bandi, S.; Graham, C.L.; Reid, T.R.; Wentzell, J.R.; Douglas, S.E. Antimicrobial Activities of Jadomycin B and Structurally Related Analogues. *Antimicrob. Agents Chemother.* **2009**, *53*, 1245–1247. [CrossRef] [PubMed]
35. Pinto, I.L.; West, A.; Debouck, C.M.; DiLella, A.G.; Gorniak, J.G.; O'Donnell, K.C.; O'Shannessy, D.J.; Patel, A.; Jarvest, R.L. Novel, selective mechanism-based inhibitors of the herpes proteases. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2467–2472. [CrossRef]
36. De Azeredo, C.M.O.; Ávila, E.P.; Pinheiro, D.L.J.; Amarante, G.W.; Soares, M.J. Biological activity of the azlactone derivative EPA-35 against *Trypanosoma cruzi*. *FEMS Microbiol. Lett.* **2017**, *364*, fnx020. [CrossRef]

37. Feldman, M.; Smoum, R.; Mechoulam, R.; Steinberg, D. Antimicrobial potential of endocannabinoid and endocannabinoid-like compounds against methicillin-resistant *Staphylococcus aureus*. *Sci. Rep.* **2018**, *8*, 17696. [CrossRef]
38. Battista, N.; Bari, M.; Bisogno, T. *N*-Acyl Amino Acids: Metabolism, Molecular Targets, and Role in Biological Processes. *Biomolecules* **2019**, *9*, 822. [CrossRef]
39. Arul Prakash, S.; Kamlekar, R.K. Function and therapeutic potential of *N*-acyl amino acids. *Chem. Phys. Lipids* **2021**, *239*, 105114. [CrossRef]
40. Li, H.-B.; Yang, T.; Richards, E.M.; Pepine, C.J.; Raizada, M.K. Maternal Treatment With Captopril Persistently Alters Gut-Brain Communication and Attenuates Hypertension of Male Offspring. *Hypertension* **2020**, *75*, 1315–1324. [CrossRef]
41. Calzetta, L.; Matera, M.G.; Rogliani, P.; Cazzola, M. Multifaceted activity of *N*-acetyl-L-cysteine in chronic obstructive pulmonary disease. *Expert Rev. Respir. Med.* **2018**, *12*, 693–708. [CrossRef]
42. Koźmiński, P.; Halik, P.K.; Chesori, R.; Gniazdowska, E. Overview of Dual-Acting Drug Methotrexate in Different Neurological Diseases, Autoimmune Pathologies and Cancers. *Int. J. Mol. Sci.* **2020**, *21*, 3483. [CrossRef]
43. Jin, X.; Cheng, Z.; Yu, X.; Tao, Q.; Huang, R.; Wang, S. Continuous supplementation of folic acid in pregnancy and the risk of perinatal depression–A meta-analysis. *J. Affect. Disord.* **2022**, *302*, 258–272. [CrossRef]
44. Menezo, Y.; Elder, K.; Clement, A.; Clement, P. Folic Acid, Folinic Acid, 5 Methyl TetraHydroFolate Supplementation for Mutations That Affect Epigenesis through the Folate and One-Carbon Cycles. *Biomolecules* **2022**, *12*, 197. [CrossRef] [PubMed]
45. Sharma, P.; Singh, S.; Siddiqui, T.I.; Singh, V.S.; Kundu, B.; Prathipati, P.; Saxena, A.K.; Dikshit, D.K.; Rastogi, L.; Dixit, C.; et al. α-Amino acid derivatives as proton pump inhibitors and potent anti-ulcer agents. *Eur. J. Med. Chem.* **2007**, *42*, 386–393. [CrossRef] [PubMed]
46. Guerini, M.; Condrò, G.; Friuli, V.; Maggi, L.; Perugini, P. N-acetylcysteine (NAC) and Its Role in Clinical Practice Management of Cystic Fibrosis (CF): A Review. *Pharmaceuticals* **2022**, *15*, 217. [CrossRef]
47. Bruns, H.; Herrmann, J.; Müller, R.; Wang, H.; Wagner Döbler, I.; Schulz, S. Oxygenated *N*-Acyl Alanine Methyl Esters (NAMEs) from the Marine Bacterium *Roseovarius tolerans* EL-164. *J. Nat. Prod.* **2018**, *81*, 131–139. [CrossRef] [PubMed]
48. Singh, I.P.; Jain, S.K.; Kaur, A.; Singh, S.; Kumar, R.; Garg, P.; Sharma, S.S.; Arora, S.K. Synthesis and Antileishmanial activity of Piperoyl-Amino Acid Conjugates. *Eur. J. Med. Chem.* **2010**, *45*, 3439–3445. [CrossRef]
49. Aboul-Fadl, T.; Al-Hamad, S.S.; Fouad, E.A. Pharmacokinetic studies of naproxen amides of some amino acid esters with promising colorectal cancer chemopreventive activity. *Bioorg. Chem.* **2018**, *76*, 370–379. [CrossRef]
50. Antoszczak, M.; Sobusiak, M.; Maj, E.; Wietrzyk, J.; Huczyński, A. Synthesis and antiproliferative activity of new bioconjugates of Salinomycin with amino acid esters. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3511–3514. [CrossRef]
51. Xiong, J.; Zhu, H.-F.; Zhao, Y.-J.; Lan, Y.-J.; Jiang, J.-W.; Yang, J.-J.; Zhang, S.-F. Synthesis and Antitumor Activity of Amino Acid Ester Derivatives Containing 5-Fluorouracil. *Molecules* **2009**, *14*, 3142. [CrossRef]
52. Sathi, G.; Gujrati, V.R.; Nath, C.; Agarwal, J.C.; Bhargava, K.P.; Shanker, K. Synthesis and Pharmacological Evaluation of New Ethyl Esters of N-Acyl Amino Acids as CNS Agents. *Arch. Pharm.* **1982**, *315*, 603–609. [CrossRef]
53. Stille, J.K.; Tjutrins, J.; Wang, G.; Venegas, F.A.; Hennecker, C.; Rueda, A.M.; Sharon, I.; Blaine, N.; Miron, C.E.; Pinus, S.; et al. Design, synthesis and in vitro evaluation of novel SARS-CoV-2 $3CL^{pro}$ covalent inhibitors. *Eur. J. Med. Chem.* **2022**, *229*, 114046. [CrossRef] [PubMed]
54. Lockbaum, G.J.; Henes, M.; Lee, J.M.; Timm, J.; Nalivaika, E.A.; Thompson, P.R.; Kurt Yilmaz, N.; Schiffer, C.A. Pan-3C Protease Inhibitor Rupintrivir Binds SARS-CoV-2 Main Protease in a Unique Binding Mode. *Biochemistry* **2021**, *60*, 2925–2931. [CrossRef] [PubMed]
55. Allen, L.A.T.; Raclea, R.-C.; Natho, P.; Parsons, P.J. Recent advances in the synthesis of α-amino ketones. *Org. Biomol. Chem.* **2021**, *19*, 498–513. [CrossRef]
56. Deng, H.; Bannister, T.D.; Jin, L.; Babine, R.E.; Quinn, J.; Nagafuji, P.; Celatka, C.A.; Lin, J.; Lazarova, T.I.; Rynkiewicz, M.J.; et al. Synthesis, SAR exploration, and X-ray crystal structures of factor XIa inhibitors containing an α-ketothiazole arginine. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3049–3054. [CrossRef] [PubMed]
57. Semple, G.; Ashworth, D.M.; Batt, A.R.; Baxter, A.J.; Benzies, D.W.M.; Elliot, L.H.; Evans, D.M.M.; Franklin, R.J.; Hudson, P.; Jenkins, P.D.; et al. Peptidomimetic aminomethylene ketone inhibitors of interleukin-1β-converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 959–964. [CrossRef]
58. Tice, C.M.; Hormann, R.E.; Thompson, C.S.; Friz, J.L.; Cavanaugh, C.K.; Michelotti, E.L.; Garcia, J.; Nicolas, E.; Albericio, F. Synthesis and SAR of α-Acylaminoketone ligands for control of gene expression. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 475–478. [CrossRef]
59. Apostol, T.-V.; Draghici, C.; Dinu, M.; Barbuceanu, S.-F.; Socea, L.I.; Saramet, I. Synthesis, Characterization and Biological Evaluation of New 5-aryl-4-methyl-2-[*para*-(phenylsulfonyl)phenyl]oxazoles. *Rev. Chim.* **2011**, *62*, 142–148.
60. Apostol, T.-V.; Saramet, I.; Draghici, C.; Barbuceanu, S.-F.; Socea, L.I.; Almajan, G.L. Synthesis and Characterization of New 5-Aryl-2-[*para*-(4-chlorophenylsulfonyl)phenyl]-4-methyloxazoles. *Rev. Chim.* **2011**, *62*, 486–492.
61. Apostol, T.-V.; Barbuceanu, S.-F.; Olaru, O.T.; Draghici, C.; Saramet, G.; Socea, B.; Enache, C.; Socea, L.-I. Synthesis, Characterization and Cytotoxicity Evaluation of New Compounds from Oxazol-5(4*H*)-ones and Oxazoles Class Containing 4-(4-Bromophenylsulfonyl)phenyl Moiety. *Rev. Chim.* **2019**, *70*, 1099–1107. [CrossRef]

62. Apostol, T.V.; Barbuceanu, S.F.; Socea, L.I.; Draghici, C.; Saramet, G.; Iscrulescu, L.; Olaru, O.T. Synthesis, Characterization and Cytotoxicity Evaluation of New Heterocyclic Compounds with Oxazole Ring Containing 4-(Phenylsulfonyl)phenyl Moiety. *Rev. Chim.* **2019**, *70*, 3793–3801. [CrossRef]
63. Apostol, T.-V.; Socea, L.-I.; Drăghici, C.; Olaru, O.T.; Șaramet, G.; Enache-Preoteasa, C.; Bărbuceanu, Ș.-F. Design, Synthesis, Characterization, and Cytotoxicity Evaluation of New 4-Benzyl-1,3-oxazole Derivatives Bearing 4-(4-Chlorophenylsulfonyl)phenyl Moiety. *Farmacia* **2021**, *69*, 314–324. [CrossRef]
64. Apostol, T.V.; Drăghici, C.; Socea, L.I.; Olaru, O.T.; Șaramet, G.; Hrubaru, M.; Bărbuceanu, Ș.F. Synthesis, Characterization and Cytotoxicity Assessment of New 4-Benzyl-1,3-oxazole Derivatives Incorporating 4-[(4-Bromophenyl)sulfonyl]phenyl Fragment. *Farmacia* **2021**, *69*, 521–529. [CrossRef]
65. Apostol, T.-V.; Drăghici, C.; Socea, L.-I.; Olaru, O.T.; Șaramet, G.; Enache-Preoteasa, C.; Bărbuceanu, Ș.-F. Synthesis, Characterization and Cytotoxicity Evaluation of New Diphenyl Sulfone Derivatives. *Farmacia* **2021**, *69*, 657–669. [CrossRef]
66. Filimonov, D.A.; Lagunin, A.A.; Gloriozova, T.A.; Rudik, A.V.; Druzhilovskii, D.S.; Pogodin, P.V.; Poroikov, V.V. Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource. *Chem. Heterocycl. Compd.* **2014**, *50*, 444–457. [CrossRef]
67. Nitulescu, G.M.; Iancu, G.; Nitulescu, G.; Iancu, R.C.; Bogdanici, C.; Vasile, D. Brave New Hope for Breast Cancer: Aminopyrazole derivates between rational design and clinical efficacy. *Rev. Chim.* **2017**, *68*, 754–757. [CrossRef]
68. Belete, T.M. Novel targets to develop new antibacterial agents and novel alternatives to antibacterial agents. *Hum. Microbiome J.* **2019**, *11*, 100052. [CrossRef]
69. Naqvi, K.F.; Patin, D.; Wheatley, M.S.; Savka, M.A.; Dobson, R.C.J.; Gan, H.M.; Barreteau, H.; Blanot, D.; Mengin-Lecreulx, D.; Hudson, A.O. Identification and Partial Characterization of a Novel UDP-*N*-Acetylenolpyruvoylglucosamine Reductase/UDP-*N*-Acetylmuramate:L-Alanine Ligase Fusion Enzyme from *Verrucomicrobium spinosum* DSM 4136^{T}. *Front. Microbiol.* **2016**, *7*, 362. [CrossRef]
70. Sander, T.; Freyss, J.; von Korff, M.; Rufener, C. DataWarrior: An Open-Source Program for Chemistry Aware Data Visualization and Analysis. *J. Chem. Inf. Model.* **2015**, *55*, 460–473. [CrossRef]
71. Xiong, G.; Wu, Z.; Yi, J.; Fu, L.; Yang, Z.; Hsieh, C.; Yin, M.; Zeng, X.; Wu, C.; Lu, A.; et al. ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Res.* **2021**, *49*, W5–W14. [CrossRef]
72. Hutchings, M.I.; Truman, A.W.; Wilkinson, B. Antibiotics: Past, present and future. *Curr. Opin. Microbiol.* **2019**, *51*, 72–80. [CrossRef]
73. Apostol, T.-V.; Marutescu, L.G.; Draghici, C.; Socea, L.-I.; Olaru, O.T.; Nitulescu, G.M.; Pahontu, E.M.; Saramet, G.; Enache-Preoteasa, C.; Barbuceanu, S.-F. Synthesis and Biological Evaluation of New *N*-Acyl-α-amino Ketones and 1,3-Oxazoles Derivatives. *Molecules* **2021**, *26*, 5019. [CrossRef] [PubMed]
74. Apostol, T.-V.; Chifiriuc, M.C.; Draghici, C.; Socea, L.-I.; Marutescu, L.G.; Olaru, O.T.; Nitulescu, G.M.; Pahontu, E.M.; Saramet, G.; Barbuceanu, S.-F. Synthesis, In Silico and In Vitro Evaluation of Antimicrobial and Toxicity Features of New 4-[(4-Chlorophenyl)sulfonyl]benzoic Acid Derivatives. *Molecules* **2021**, *26*, 5107. [CrossRef] [PubMed]