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Research paper

Imidazolidine-4-one derivatives in the search for novel chemosensitizers of *Staphylococcus aureus* MRSA: Synthesis, biological evaluation and molecular modeling studies

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ABSTRACT

A series of amine derivatives of 5-aromatic imidazolidine-4-ones (**7**–**19**), representing three subgroups: piperazine derivatives of 5-arylideneimidazolones (**7**–**13**), piperazine derivatives of 5-arylideneimidazolidine-2,4-dione (**14**–**16**) and primary amines of 5-naphthyl-5-methylimidazolidine-2,4-diones (**17**–**19**), was evaluated for their ability to improve antibiotics effectiveness in two strains of Gram-positive *Staphylococcus aureus*: ATCC 25923 (a reference strain) and MRSA (methicillin resistant *S. aureus*) HEMSA 5 (a resistant clinical isolate). The latter compounds (**17**–**19**) were obtained by 4-step synthesis using Bucherer-Bergs condensation, two-phase bromoalkylation and Gabriel reactions. The naphthalen derivative: (Z)-5-(naphthalen-2-ylmethylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)-one (**10**) was the most potent in combination with β-lactam antibiotics and ciprofloxacin against the resistant strain. The high potency to increase efficacy of oxacillin was noted for (Z)-5-(anthracen-10-ylmethylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)one (**12**) too. In order to explain the mechanism of action of the compounds **10** and **12**, docking studies with the use of crystal structures of a penicillin binding protein (PBP2a) and MecR1 were carried out. Their outcomes suggested that the most probable mechanism of action of the active compounds is the interaction with MecR1. Molecular dynamic experiments performed for the active compounds and compound **13** (structurally similar to **12**) supported this hypothesis and provided possible explanation of activity dependencies of the tested compounds in terms of the restoration of antibiotic efficacy in *S. aureus* MRSA HEMSA 5.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram positive bacterium that can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, folliculitis, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections [1–3].

Methicillin-resistant *S. aureus* (MRSA) has caused serious problems in the use of the major classes of antibiotics in common use – cephalosporins, penicillins, carbapenems, quinolones and aminoglycosides [4] and hence it is in actuality a multi-drug resistant (MDR) bacterium. The resistance of MRSA strains to β-lactam antibiotics is well understood and is caused by the synthesis of a modified penicillin binding protein (PBP) named PBP2a. PBPs are membrane-bound enzymes that participate in the biosynthesis

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of the bacterial cell wall [5]: they catalyze the transpeptidation reaction that is necessary for cross-linkage of peptidoglycan chains [6]. Unmodified PBP is the target for β-lactam antibiotics – binding of a β-lactam antibiotic to the active center of the PBP protein inactivates the enzyme and results in bacterial growth inhibition due to the inhibition of cell wall synthesis. In turn, the modified PBP protein (PBP2a) present in MRSA strains displays much lower affinity to β-lactam antibiotics that renders the MRSA resistant to this group of antibiotics. The modified PBP2 protein of MRSA strains is due to the acquisition of a mobile genetic element from an unknown bacterial source, SCCmec (Staphylococcal Cassette Chromosome) which contains the *mecA* gene that encodes the modified PBP protein (PBP2a). Upon the acquisition of SCC*mec,* MSSA (methicillin-sensitive *S. aureus*) changes to MRSA. The SCC*mec* cassette can be ejected from the MRSA chromosome when the MRSA organism is placed under stress to another antibiotic [7] and converts the MRSA to MSSA phenotype [8]. The expression of the *mecA* gene is regulated by two proteins: MecI (a repressor protein) and MecR1 (a signal transducer protein). When MecI binds to the promoter region of *mecA*, the transcription of *mecA* is repressed. The active site centre of MecR1 penicillin binding domain is the catalytic serine residue at the beginning of helix α3, Ser391. If MecR1 detects β-lactam antibiotics in the extracellular space via MecR1 penicillin binding domain, it becomes acylated at its active-site serine residue. Thus, when β-lactam antibiotics bind to MecR1, the polypeptide having protease activity is released from MecR1 to degrade MecI, resulting in increased transcription of *mecA*, and consequently a higher level of PBP2a is reached [9].

S. aureus has also a number of efflux pumps including those of the MF family (NorA, Mef, Tet; the efflux pump Qac is plasmid mediated) and the ABC family (Msr(A)) that are able to extrude antibiotics outside the cell, thus reducing the efficacy of the drugs [10].

Since MRSA is a worldwide problem in clinical medicine, there is a need for developing new therapeutic agents against this bacterium. One of the strategies is to search for adjuvants that enhance the action of antibiotics and may thus restore the efficacy of therapeutically inactive agents. The combination of a resistance inhibitor with an antibiotic has already proven its efficacy with the clavulanic acid (inhibitor of beta-lactamase)/amoxicillin association [11]. Lines of evidence indicated several chemical families [11–16] of inhibitors of the *S. aureus* efflux pumps, mainly the NorA efflux pump (Fig 1) [11].

On the other hand, the imidazolidine-4-one pattern, that is in the area of interest of our research group, gives great opportunity for chemical modifications resulting in various biological actions, among others: GPCR-ligand properties [17,18], hypotensive, antiarrhythmic or anticonvulsant activities [19,20] as well as antibacterial, antifungal [21,22] or anticancer properties [23]. Our recent studies indicated moderate properties of chemosensitizers against multi-drug resistant *E. aerogenes* of several hydantoin derivatives (imidazolidine-2,4-dione) [24,25] and modulators of cancer efflux proteins of 5-arylideneimidazolones [26].

Therefore, the aim of this study was to test a series of imidazolidine-4-one derivatives, representing three subgroups A-C (Table 1), including the compounds that proved active in the previous assays against multi-drug resistant *E. aerogenes* [25] and the new ones, in terms of their ability to enhance the action of a β-lactam antibiotics in *S. aureus*. The synthesis of the new compounds and microbiological assays for the whole population are described herein. One of the compounds that displayed high activity against MRSA was also evaluated for cytotoxicity in preparation for further pre-clinical studies.

In order to verify the mechanism of action of the tested compounds, a molecular modeling study was carried out. The interaction with the proteins involved in MRSA β-lactam resistance (PBP2a and MecR1) was examined by docking and molecular dynamic experiments.

2. Results and discussion

2.1. Synthesis

A series of the compounds **7**–**14** were designed and synthesized in the Department of Technology and Biotechnology of Drugs JU-MC. Before synthesis, a search for their structures in the program SciFinder was performed. Syntheses of the compounds **7**–**14** are described elsewhere [25–27]. In order to obtain the compounds **15** and **16**, the 4-step synthetic path was carried out (Scheme 1). The Knoevenagel condensation was the first step in which hydantoin was condensed with aromatic aldehyde giving 5-arylideneimidazolidine-2,4-dione (**20, 21**). In the next step, the products of the Knoevenagel condensation were combined with 2,3-epoxy-1-propanol in the Mitsunobu reaction giving the N3-oxiran-5-arylideneimidazolidine-2,4-dione intermediates (**22, 23**). In the third step, which was N-alkylation, the compounds **22** and **23** were melted with 1-acetylpiperazine using microwave irradiation (**22**) and an oil bath (**23**). The last step was an N-acetyl deprotection of 3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-arylideneimidazolidine-2,4-dione (**24, 25**) producing the hydrochloride form of the final deprotected piperazine derivatives (**15**, **16**).

The compounds **17**–**19** were obtained by 4-step synthesis according to Scheme 2. In the first step, Bucherer-Bergs cyclic condensation was performed with 2-naphthylacetophenone to

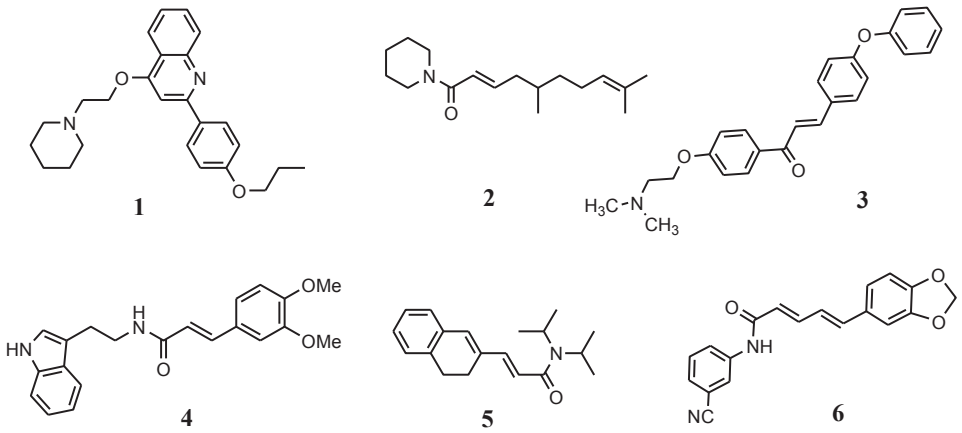
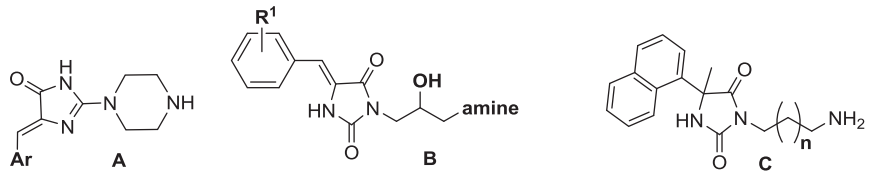
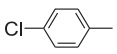
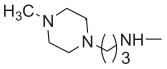
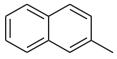
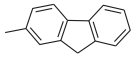
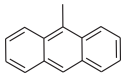
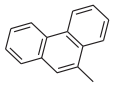


Fig. 1. Chemical families of inhibitors of MDR in S. aureus: derivatives of 2-(4-propoxyphenyl)quinoline (**1**) [10], citral amide derivatives (**2**) [11]; chalcone inhibitors (**3**) [12]; N-cinnamoylphenalkylamide derivatives (4) [9]; dihydronaphthalene inhibitors of the NorA efflux pump in S. aureus (**5**) [13]; piperine analogs (**6**) [14].

Table 1
Structure of tested derivatives of imidazolidine.



Cpd	Group	Ar	Cpd	Group	R^1	Amine	n
7	A		**14**	B	4-OCH_3		–
8	A		**15**	B	4-Cl		–
9	A		**16**	B	2,4-diCl		–
10	A		**17**	C	–	–	1
11	A		**18**	C	–	–	2
12	A		**19**	C	–	–	3
13	A						

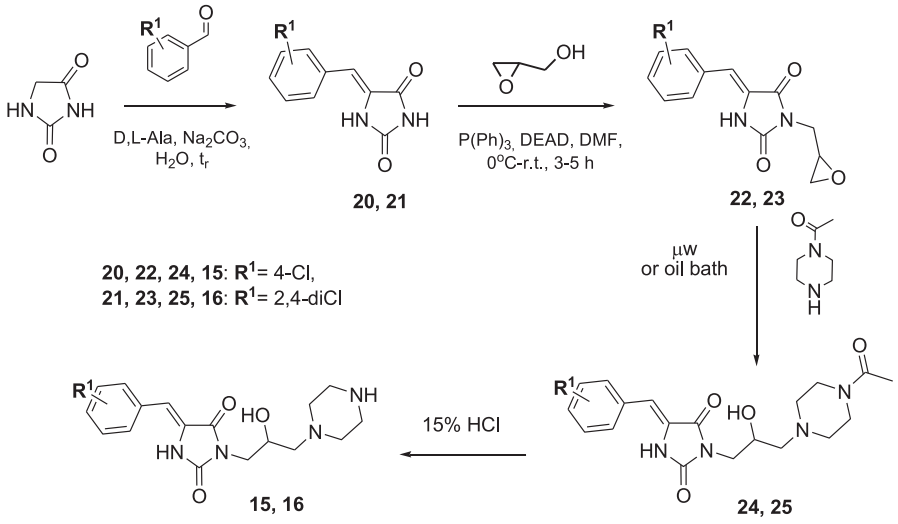
obtain 5-methyl-5-(2-naphthyl)imidazolidine-2,4-dione (**26**). The compound **26** was alkylated at position 3 using suitable dibromoalkanes to give bromoalkyl (C3–C5) derivatives **27**–**29**, which were used as alkylating agents to obtain (5-methyl-5-(naphthalene-1-yl)imidazolidin-2,4-dione-3-yl)alkyl derivatives of phthalimide (**30**–**32**). The pure compounds **17**–**19** were obtained by reaction of the phthalimide derivatives **30**–**32** with hydrazine.

2.2. Microbiological assays

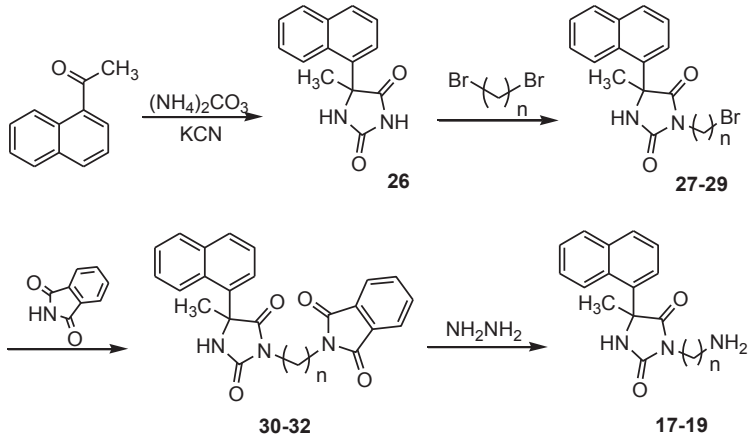
The whole series of compounds (**7**–**19**) were tested in microbiological assays [28] for their ability to increase oxacillin effectiveness in two strains of *S. aureus:* a reference strain ATCC 25923 and a clinical isolate MRSA HEMSA 5. Selected representatives of each chemical group were also tested with other β-lactam antibiotics: cloxacillin (resistant to β-lactamases) and ampicillin (susceptible to β-lactamases) in combination with sulbactam (β-lactamase inhibitor) as well as fluoroquinolones (ciprofloxacin) and aminoglycosides (neomycin) using the same strains.

2.2.1. Direct antibacterial activity

In the first step of the microbiological assays, direct antibacterial



Scheme 1. Synthesis route for compounds **15** and **16**.



Scheme 2. Synthesis route for compounds **17**–**19**, n = 3–5.

activity of the compounds was evaluated. Minimal inhibitory concentration (MIC) of the compounds **7**–**19** was determined for both strains by the broth dilution method [28] (Table 2). The tested compounds displayed varied direct antibacterial activities. Most of the compounds did not inhibit growth of both *S. aureus* strains at 1 mM. The lowest MICs were observed for piperazine derivatives of 5-arylideneimidazolones **11** and **13**. These compounds demonstrated MIC values in the range of 1.9–15.6 μM in the reference strain, whereas their MIC in MRSA HEMSA 5 was lower than those of tested β-lactam and aminoglycoside antibiotics, in particular, 23–45-fold lower than that of oxacillin (15.6–31.3 μM, Table 2).

2.2.2. Influence on MIC of antibiotics

In the next step of the microbiological assays, the compounds **7**–**19** were tested for their ability to increase the activity of oxacillin in the tested bacterial strains. The compounds were tested in the concentrations not exceeding 1/4 of their MICs. Activity gains (A) were calculated according to the equation presented in Table 3. Most of the compounds had no impact on oxacillin activity. The most active compound was **10**, which decreased the MIC of oxacillin 128-fold in the MRSA HEMSA 5 strain. Another active compound (**12**) was able to reduce the MIC of oxacillin 64-fold in the HEMSA 5 strain (Table 3). Since the corresponding reduction in the reference strain in both of these cases was negligent, this suggests that the modulation of bacterial resistance in the MRSA HEMSA 5 strain by the compounds **10** and **12** may involve proteins responsible for β-lactam resistance in MRSA strains: PBP2a and MecR1, the more so that **10** and **12** were also active with another β-lactam antibiotic cloxacillin (Table 4). This hypothesis was verified by checking the ability of these compounds to reduce the MIC of antibiotics which have a different mechanism of action: fluoroquinolones (ciprofloxacin) and aminoglycosides (neomycin) against *S. aureus* MRSA HEMSA 5 (Table 5). Here, **12** was completely inactive, and **10** showed minor activity only in case of ciprofloxacin. Apart from acting on bacterial gyrase, ciprofloxacin is also a substrate of efflux pumps so the lack of substantial activity suggests that the interaction of **10** and **12** with efflux pumps is rather unlikely. The compound **12** was inactive in case of ampicillin + sulbactam, but this does not rule out the suggested mechanism of action as ampicillin may be a too small molecule compared to oxacillin and cloxacillin to exert the desired effect. It may be also due to the fact that there are too many components in this system (ampicillin, sulbactam, compound) so they compete with each other in membrane transport.

Apart from **10** and **12**, no compound had any statistically significant impact (≥4-fold) on oxacillin activity, whereas compounds **11** and **14** improved (8-fold) the action of cloxacillin against MRSA HEMSA 5 (Table 4). The series of compounds (**7**–**19**) did not raise the efficacy of any tested antibiotics in the reference strain. Compounds **10** and **11** caused a 2-fold reduction in the MIC of oxacillin

Table 2
Direct antibacterial activity for compounds **7**–**19**.

Group	Compd	*S. aureus* MRSA HEMSA 5	*S. aureus* ATCC 25923
		MIC [mM]	MIC [mM]
A	**7**	0.5	>1
A	**8**	>1	>1
A	**9**	1	>1
A	**10**	0.5	0.25
A	**11**	0.0313	0.0156
A	**12**	0.5	0.5
A	**13**	0.0156	0.0019
B	**14**	>1	>1
B	**15**	1	>1
B	**16**	>1	>1
C	**17**	>1	>1
C	**18**	>1	>1
C	**19**	1	>1
Ref	**Oxacillin**	0.71	0.00047
	Cloxacillin	0.16	0.00042
	Ampicillin + sulbactam	0.2	0.00054
	Ciprofloxacin	0.014	0.00038
	Neomycin	0.24	0.001

Table 3
Influence of compounds **7**–**19** on minimal inhibitory concentration (MIC) of oxacillin A = $\frac{\text{MIC of antibiotic in absence of compound (7–19)}}{\text{MIC of antibiotic in presence of compound (7–19)}}$.

Group	Cpd	*S. aureus* MRSA HEMSA 5			*S. aureus* ATCC 25923		
		Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction	Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction
A	**7**	0.125	No effect	–	0.5	No effect	–
A	**8**	0.5	No effect	–	0.5	No effect	–
A	**9**	0.25	No effect	–	0.5	No effect	–
A	**10**	0.125	128-fold	From 710 μM to 5.55 μM	0.0625	2-fold	From 0.47 μM to 0.23 μM
A	**11**	0.0078	No effect	–	0.0039	2-fold	From 0.47 μM to 0.23 μM
A	**12**	0.125	64-fold	From 710 μM to 11.09 μM	0.125	2-fold	From 0.47 μM to 0.23 μM
A	**13**	0.0039	No effect	–	0.0005	No effect	–
B	**14**	0.5	2-fold	From 710 μM to 355 μM	0.5	No effect	–
B	**16**	0.5	No effect	–	0.5	No effect	–
C	**17**	0.5	No effect	–	0.5	No effect	–
C	**18**	0.5	No effect	–	0.5	No effect	–
C	**19**	0.25	No effect	–	0.5	No effect	–

Table 4
Influence of selected compounds (**8**–**14** and **17**) on minimal inhibitory concentration (MIC) of other β-lactam antibiotics: cloxacillin and ampicillin + sulbactam against *S. aureus* MRSA HEMSA 5.

Group	Cpd	*Cloxacillin*			*Ampicillin + sulbactam*		
		Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction	Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction
A	**8**	0.5	No effect	–	0.5	No effect	–
A	**9**	0.25	No effect	–	0.25	No effect	–
A	**10**	0.125	256-fold	From 315 μM to 1.23 μM	0.125	4-fold	From 202 μM to 50.5 μM
A	**11**	0.0078	8-fold	From 315 μM to 39.4 μM	0.0078	No effect	–
A	**12**	0.125	8-fold	From 315 μM to 39.4 μM	0.125	No effect	–
A	**13**	0.0039	2-fold	From 315 μM to 157.5 μM	0.0039	No effect	–
B	**14**	0.5	8-fold	From 315 μM to 39.4 μM	0.5	2-fold	From 202 μM to 101 μM
C	**17**	0.5	No effect	–	0.5	No effect	–

Compounds **8**–**14** and **17** did not have any influence on minimal inhibitory concentration (MIC) of cloxacillin and ampicillin + sulbactam against *S. aureus* ATCC 25923.

Table 5
Influence of selected compounds (**8**–**14** and **17**) on minimal inhibitory concentration (MIC) of fluoroquinolones (ciprofloxacin) and aminoglycosides (neomycin) against *S. aureus* MRSA HEMSA 5.

Group	Cpd	*Ciprofloxacin*			*Neomycin*		
		Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction	Concentration of compound [mM]	Reduction of MIC	Numerical value of reduction
A	**8**	0.5	No effect	–	0.5	No effect	–
A	**9**	0.25	No effect	–	0.25	No effect	–
A	**10**	0.125	4-fold	From 15.1 μM to 3.77 μM	0.125	No effect	–
A	**11**	0.0078	No effect	–	0.0078	No effect	–
A	**12**	0.125	No effect	–	0.125	No effect	–
A	**13**	0.0039	No effect	–	0.0039	No effect	–
B	**14**	0.5	2-fold	From 15.1 μM to 7.55 μM	0.5	No effect	–
C	**17**	0.5	No effect	–	0.5	No effect	–

Compounds **8**–**14** and **17** did not have any influence on minimal inhibitory concentration (MIC) of ciprofloxacin and neomycin against *S. aureus* ATCC 25923.

in the reference strain. However, this difference is not significant as it fits within the method's inaccuracy range. Apart from compound **10**, moderately increasing ciprofloxacin action, no compound had impact neither on fluoroquinolones nor aminoglycosides (neomycin) effectiveness against MRSA HEMSA 5 (Table 5).

2.3. Molecular modeling

At first, molecular docking experiments were performed to find out with which protein the tested compounds potentially interact – all the tested compounds were docked into the crystal structures of MecrR1 and PBP2a (Table 6). Next, with the use of molecular dynamic simulations, differences in ability to restore the antibiotic efficacy against the resistant *S. aureus* strain of the selected compounds were analyzed.

2.3.1. Docking results

Docking results revealed that all of the tested compounds were

Table 6
Number of poses obtained for each compound in the procedure of docking into crystal structures of PBP2a and MecR1 binding domain.

Cmd	Number of 3D conformations	Number of poses obtained by docking	
		MecR1	PBP2a
7	3	2	0
8	8	9	5
9	4	4	2
10	3	1	0
11	3	0	0
12	6	10	3
13	6	7	3
14	6	6	6
15	2	2	0
16	2	3	0
17	1	1	1
18	1	1	2
19	1	2	1
Oxacillin	1	1	2

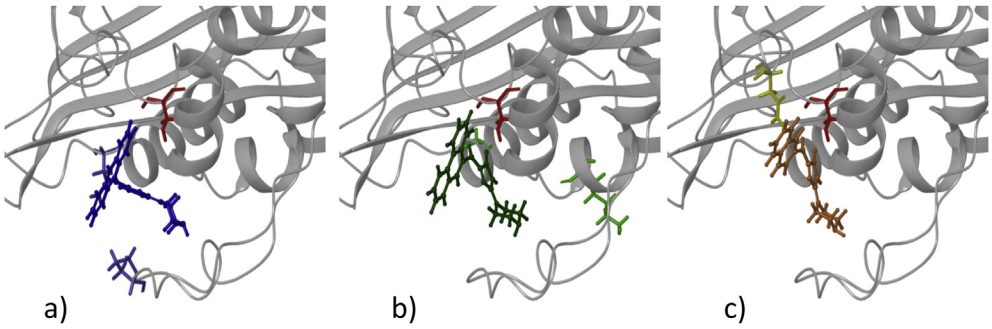


Fig. 2. Interaction mode of **12** (a), **13** (b) and **10** (c) with MecR1 binding domain (complexes with the lowest docking score were selected). a) Amino acids that interact only with **12** are marked in blue; b) amino acids that interact only with **13** are marked in green; c) amino acids that interact only with **10** are marked in yellow; Ser391 is marked with red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

much more successful in interacting with MecR1 than PBP2a as the number of the obtained ligand-protein complexes was much higher in case of MecR1 in comparison to the modified PBP (Table 6). The only compound for which the same number of ligand-protein complexes was obtained in case of MecR1 and PBP2a was **14**. Compound **14** was also the one which caused 2-fold increase in oxacillin activity. This increase is only minor and fits in the method's inaccuracy range.

As one of the active compounds (**12**) exhibits a very high structural similarity to compound **13** (inactive in *in vitro* tests), a careful analysis of the binding of **10**, **12** and **13** to MecR1 was performed. The docking studies revealed that these compounds bind in slightly different orientation modes and interact with different amino acids of MecR1. For each molecule, amino acids that interacted exclusively with one of these three compounds were identified (Fig. 2).

2.3.1.1. Docking results for oxacillin and cloxacillin. The docking studies were performed only for oxacillin and cloxacillin for two reasons. First of all, oxacillin and cloxacillin were the only antibiotics whose efficacy was substantially increased by the addition of some of the tested compounds (**10**–**14**). Secondly, similar mechanism of action of both oxacillin and cloxacillin is closely connected with the PBP protein and differs from the mechanism of action of other antibiotics used in the studies *in vitro*.

Docking results show that both oxacillin and cloxacillin fit into the binding site of MecR1 close to Ser391 (Fig. 3). The comparison of oxacillin binding mode to the binding mode of **12** (Fig. 4a) and the binding mode of cloxacillin to the binding mode of **10** (Fig. 4b)

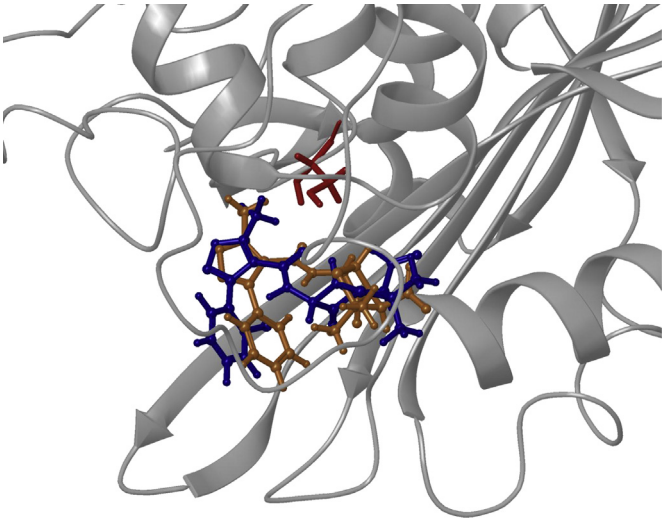


Fig. 3. Visualization of oxacillin (orange) and cloxacilin (blue) docking to MecR1 binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

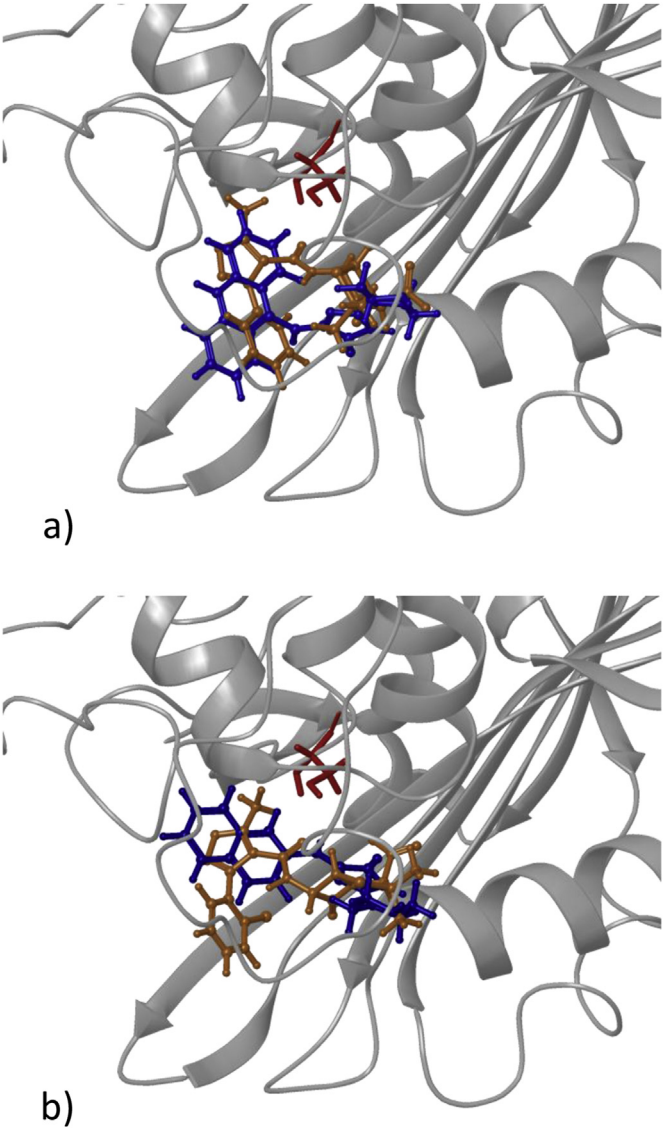


Fig. 4. Comparison of docking results of oxacillin (orange) and **12** (blue) (a) and cloxacillin (orange) and **10** (blue) (b) to MecR1. Ser391 is highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

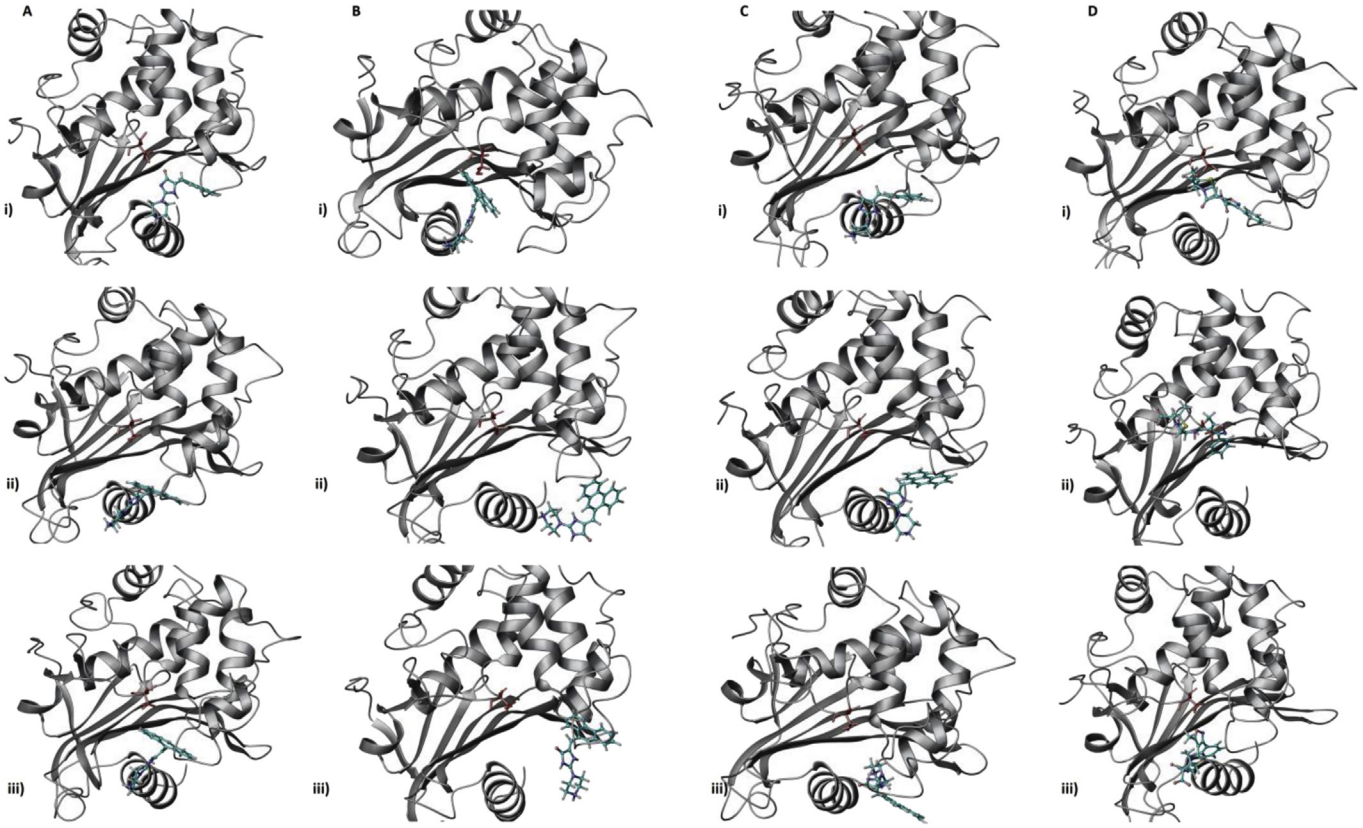


Fig. 5. Snapshots of simulations of **12** (A), **13** (B, C) and oxacillin (D) from the first (i), central (ii) and last (iii) frames of MD simulations. B refers to studies with starting pose of **13** with the lowest docking score, C – to starting pose of **13** analogous to **12**.

revealed that in case of both pair compounds, the parts of the latter molecules that come into close proximity of Ser391 are parts of aromatic rings in contrast to free methyl groups and oxygen atoms from the ketone approaching Ser391 in the case of the antibiotic molecules.

2.3.2. Molecular dynamics simulation

In vitro experiments showed that the compounds which induced the greatest overcoming of antibiotic resistance were **10** and **12**. They are structurally different from all compounds but one, and that is **13.** Therefore, these three compounds were selected for further evaluation in molecular dynamics studies. Due to extremely high similarity of the compounds **12** and **13**, they were analyzed in detail.

The compounds **12** and **13** are similar in topology. Nevertheless, only **12** enhances oxacillin antibacterial activity *in vitro.* Molecular dynamics simulation was performed for MecR1-**12**, MecR1-**13** and MecR1-oxacillin (for comparison) complexes in order to reveal differences in interaction scheme of the compounds. All simulations were conducted under the same conditions, with the starting conformations of the compounds from their complexes with MecR1 of the lowest values of docking score. As conformations with the lowest values of docking score were slightly different for compound **12** and **13**, additional simulation for compound **13** was run with the starting conformation analogous to the one of the compound **12**.

During the entire simulations (20 ns), both compounds **12** (Fig. 5) and **10** (Fig. 6) tend to stay in the entrance to the binding cavity of the MecR1 sensor domain, similar to oxacillin and cloxacillin which remain close to Ser391 (Figs. 5A and 6A). In contrast, compound **13** leaves its initial position and locates nearby the cavity in experiments with the starting pose with the lowest value

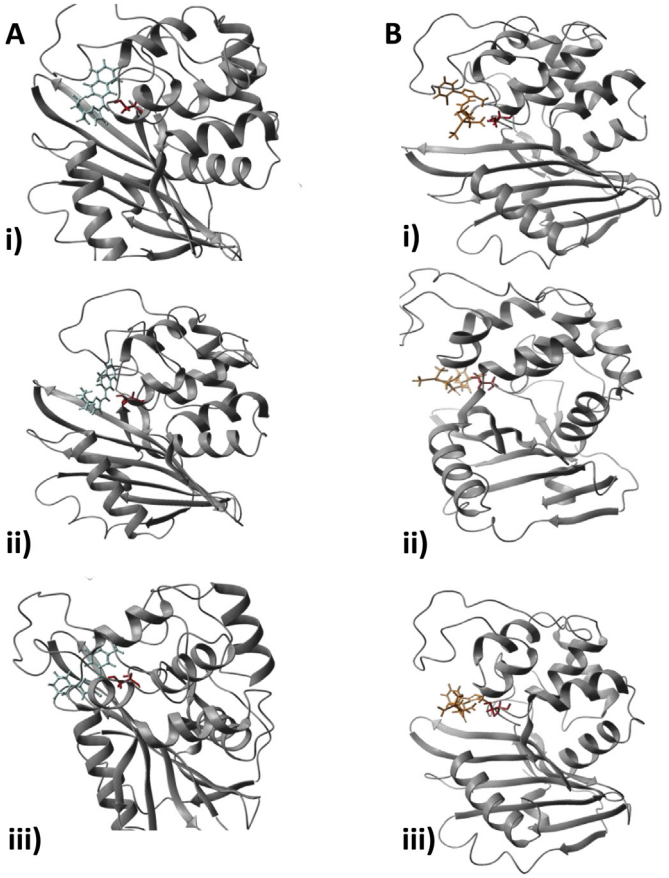


Fig. 6. Snapshots of simulations of **10** (A) and cloxacillin (B) from the first (i), central (ii) and last (iii) frames of MD simulations.

of docking score (Fig. 5B) or flips in a way that the moiety of condensed aromatic rings does not block the entrance to the binding cavity in experiments with the starting pose of **13** being analogous to **12** (Fig. 5C). Both oxacillin and cloxacillin stay in the proximity of the active site during the whole simulation (Figs. 5D and 6B) (Fig. 7).

2.4. Proliferation assay

The anti-proliferative effect of the active compound **12** against mammalian cells was tested using HEK-293 cell line and a colorimetric, commercial test EZ4U. A standard cytotoxic drug doxorubicin was used as a positive control. The results of this assay are presented in Fig. 7. Compound **12** did not exert any significant anti-proliferative effect against HEK-293 cell line: the viability of the cells exposed to its 0.125 mM concentration (active against MRSA) was 91 ± 7.67%. This makes the compound **12** a drug-like one and a good candidate for further pre-clinical tests.

2.5. SAR-discussion

The study describes an initial step in the search for new chemosensitizers of resistant Gram-positive pathogen MRSA in the new chemical family of imidazolidin-4-one derivatives, which could be helpful as "adjuvants" to increase antibiotic efficacy. Although the battle against resistant *S. aureus* is an important therapeutic question, the number of studies providing successful tools is not sufficient, and these are predominantly focused on MDR protein efflux pumps, especially NorA. Even though several families of chemical compounds that are able to modulate NorA have been described [10–16], it is difficult to find any lines of evidence which suggest pharmacophore models, lead structures or any structural requirements for chemosensitizers acting on other MDR proteins of MRSA, such as PBP2a or MecR1. In the absence of appropriate data, we selected representative imidazolidine-4-one derived compounds (**7**–**19**) based on their promising chemosensitizing- and efflux pump inhibitory properties against several strains of multidrug resistant Gram-negative bacteria [25]. The representative group contains three common structural features: the imidazolidine-4-one core, an aromatic fragment ending position 5 and an amine terminal fragment substituted at 2- (group A) or 3-position (groups B and C) of imidazolidine-4-one.

In the case of group A, the whole considered population of the compounds (**7**–**13**) has the conserved core of 1H-imidazol-5(4H)-one and free piperazine terminated fragment at position 2. The compounds differ in their size and substituents of the aromatic ring, which is placed within the 5-arylidene fragment, and this difference seems to be crucial for their "co-operative" action with the antibiotics. The members of the group A were identified as the most promising efflux pump inhibitors of tripartite efflux pumps in Gram-negative bacteria in our previous studies (data under publication elsewhere). Here, the group also seems to be the most interesting one because of the significant action against the MRSA HEMSA 5 strain that was observed for the members possessing fused rings, especially naphthalen (**10**) and anthracen (**12**) (Tables 1–5).

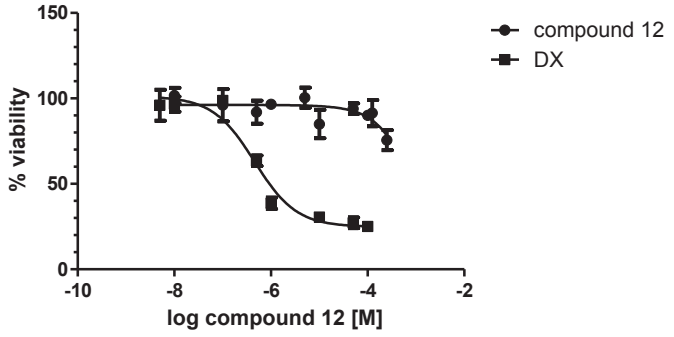


Fig. 7. Anti-proliferative effect of **12** against HEK-293 cell line. Values represent the mean of n = 4 experiments. DX stands for a positive control, doxorubicin.

In the direct antibacterial activity test for the resistant MRSA strain, the fluorenylmethylidene derivative **11** as well as the phenanthrenemethylidene one **13** displayed 23- (**11**) and 46-fold (**13**) lower MIC than that of oxacillin, whereas their MIC in the case of the reference strain was 4–32-fold higher than that of oxacillin. In general, the compounds (**11**, **13**) displayed direct antibacterial MIC against the MRSA HEMSA 5 strain, lower than that of all the tested β-lactam antibiotics and aminoglycoside neomycin (Table 2). These results suggest potential antibiotic properties of the derivatives with triple fused rings, particularly promising in the case of pathogenic MRSA strain that is resistant to β-lactam antibiotics. It is worth emphasizing that all the derivatives with fused aromatic rings (**10**–**13**), unlike the tested antibiotics, demonstrated only slight (1–8 fold) increase of their MIC values when the reference strain ATCC 25923 was replaced with the MRSA strain. In the same situation, the MIC values of β-lactam antibiotics increased from 370-fold in the case of ampicillin with sulbactam to over 1500-fold in the case of oxacillin and from 37- (ciprofloxacin) to 240-fold (neomycin) in the case of other antibiotics tested (Table 2). It confirms the chemosensitizing properties of the piperazine arylidenoimidazolones against pathogenic *S. aureus.*

The most potent antibacterial action is seen for the phenanthrene derivative **13**, but this compound did not show any ability to increase efficacy of all the tested antibiotics in both of the investigated strains. In contrast, the β-naphthylmethylidene derivative **10** and the anthracenylmethylidene one (**12**) demonstrated very weak direct antibacterial action in both of the tested strains of *S. aureus* (Table 2), but they were able to improve efficacy of oxacillin in the MRSA HEMSA 5 strain 128- and 64-fold, respectively (Table 3). This seems to be particularly interesting, especially when high structural similarities of the compounds **12** and **13**, which differ only in their triple-fused aromatic rings, are considered. The results indicate that the linear ring system of naphthalen (**10**) and a bit more branchy anthracene (**12**) favors restoration of β-lactam antibiotics activity in the resistant MRSA strain, while this property disappears with a slight twist of aromatic area in the case of the triple fused system of the phenanthrene derivative (**13**).

On the basis of molecular modeling studies, we postulate that the mechanism of action of **10** and **12** is connected with the MecR1 protein. Docking results show that **10** and **12** are likely to bind in the region of entrance to the MecR1 active site. Thus, it can be suggested that they prevent antibiotics from binding to the active site of MecR1. Moreover, it seems that both **10** and **12** do not interact with residues from the binding site of PBP2a, and therefore the mechanism of their action through this protein is less probable. In comparison, docking shows that oxacillin and cloxacillin are very likely to interact with Ser391 of MecR1, which has also been proven experimentally [29] and which in turn supports the reliability of docking results.

The comparison of the binding modes of **12** and **13** reveals differences in their interaction with MecR1. Compound **12** is more likely to bind in the region of entrance to the binding site, while **13** locates in the upper part of the cavity, leaving free space in the binding pocket and allowing oxacillin to bind. This may be the reason why **12** is more effective, as it completely blocks the entrance to binding site for oxacillin.

This hypothesis of the steric effect being the reason of activity of

10 and **12** was also supported by molecular dynamic simulations – **10** and **12** stay in the entrance to the binding cavity during the whole simulation, preventing oxacillin or cloxacillin from coming into the close proximity of Ser391 and activating MecR1. On the other hand, compound **13** moves from its initial position and leaves space for oxacillin to interact with the active site of this protein and induce the synthesis of PBP2a with lowered affinity to β-lactam antibiotics (when simulations were carried out taking the pose with the lowest docking score) or flips in a way that aromatic rings are no longer preventing oxacilin from interaction with the active site (which was proved in simulations started from pose of **13** analogous to **12**).

Despite the intriguing chemosensitizing action of the arylideneimidazolones (**10**–**13**), the rest of the investigated compounds did not demonstrate any expected pharmacological action in *S. aureus*. Groups B and C (Table 1) represent lines of modifications in which the imidazolidine-4-one core was enriched with a carbonyl fragment at position 2 forming hydantoin. The basic difference between the groups A and B is observed in the length and position of the amine terminal fragment, which has been moved from position 2 to 3. The compounds (**14**–**16**) did not display any antibacterial action in the tested concentration. However, a moderate potency to increase efficacy of cloxacillin in the MRSA strain (8-fold) was observed for the methylpiperazine-propylamine derivative **14**. Apart from this one case, the piperazine derivatives of the 5-arylidenehydantoin belonging to the group B (**15**, **16**) were inactive in all microbiological assays performed.

The results indicated that further chemical modifications (group C), that replaced the 5-arylidene moiety with 5-naphthyl-5-methyl ones and introduced primary amine at the end of 3-hydantoin substituents, did not give the expected biological results. The members of the group C (**17**–**19**) showed neither antibacterial nor chemosensitizing action against both of the tested *S. aureus* strains (Table 2, Table 3).

The results obtained from the evaluation in silico of the selected compounds indicate that further structure modifications and search for new compounds with similar mechanism of action (blocking access of antibiotic to MecR1) should be oriented at finding molecules with rigid bulky substituents. Nevertheless, the size of the molecules cannot be increased unlimitedly so as their ability to stay in the close proximity to the MecR1 binding site is not lost.

3. Conclusions

The multidisciplinary studies performed here allowed us to investigate chemosensitizing actions of three different groups of amine derivatives of 5-aromatic imidazole-4-ones in the multidrug resistant strain of *S. aureus*, MRSA HEMSA 5. According to the results obtained, the chemosensitizing properties were found for the group of piperazine derivatives of 5-arylidene-3H-imidazol-4(5H)-one (group A) whereas both primary amine derivatives of 5-arylhydantoin and piperazine-alkyl derivatives of 5-arylidenhydantoin were rather inactive. In the group A, the compounds with fused aromatic rings showed resistance against MDR mechanisms of MRSA HEMSA 5. Especially (Z)-5-(naphthalen-2-ylmethylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)-one (**10**) and (Z)-5-(anthracen-10-ylmethylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)-one (**12**) turned out to be the most promising compounds. They were deprived of their direct antibacterial activity, but were able to increase antibiotic effectiveness of β-lactam antibiotics (from 64 to 256-fold) against the MRSA strain. Furthermore, two compounds, (Z)-5-((9H-fluoren-2-yl)methylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)-one (**11**) and (Z)-5-(phenanthren-9-ylmethylene)-2-(piperazin-1-yl)-3H-imidazol-4(5H)-one (**13**), demonstrated significant antibacterial properties against the MRSA strain, but they did not improve efficacy of all the considered antibiotics (β-lactams, aminoglycosides, fluoroquinolones) against the investigated *S. aureus* pathogens.

Molecular modeling, which involved docking studies and molecular dynamics, indicated that the most probable mechanisms of chemosensitizing actions of the considered compounds are the interactions with the MecR1 protein, whereas their influence on PBP2a is rather unlikely. The computational studies allowed us to postulate some hypotheses to explain the ability of both the naphthylmethylidene (**10**) and the anthracylmethylidene (**12**) derivatives to improve efficacy of β-lactam antibiotics against the MRSA strain as well as the lack of the activity in the case of phenanthrenemethylidene derivative **13** with high structural similarity to **12**. Both docking studies and molecular dynamics indicated that the compounds **10** and **12** prevent antibiotics from binding to the active site of MecR1 while the compound **13** is able to leave free space in the binding pocket allowing oxacillin to bind. Although the hypotheses come from theoretical considerations, they seem to be of great importance for further experimental studies on the mechanism of anti-MDR actions of 5-arylideneimidazolones against the MRSA strains. In silico results suggest that the possible mechanism of action of the active compounds found in the study is connected with the inhibition of MecR1 activation through blocking of the entrance to the MecrR1 binding cavity and preventing the antibiotics from binding. Although the requirements for compounds that are supposed to act through this mechanism resulting from in silico studies are relatively simple (rigid, bulky substituents), their interaction with MecR1 will have to be carefully analyzed, as shown by the example of the two compounds with fused aromatic rings that are very similar, but only one of them is able to successfully overcome antibiotic resistance in MRSA.

The most important achievement of the work is the identification of new active chemosensitizers, compounds **10** and **12**, with high capacity to increase antibiotics efficacy of oxacillin and cloxacillin against the resistant MRSA strain. The compounds can be a good lead structure in further search for tools to combat bacterial multidrug resistance as well as promising candidates (**12**) for further pre-clinical tests considering their negligible toxic effects in the proliferation assay performed here.

4. Experimental

4.1. Chemistry

NMR spectra were recorded on a Varian Mercury VX 300 MHz PFG instrument (Varian Inc., Palo Alto, CA, USA) in DMSO-d_6 at ambient temperature using the solvent signal as an internal standard (300 MHz for ^{1}H and 75 MHz for ^{13}C). Melting points were determined using Mel-Temp II apparatus and are uncorrected. Elemental analyses were within ±0.4% of the theoretical values unless stated otherwise. LC/MS spectra were measured using the Waters ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) connected to a mass spectrometer Waters TQD (electrospray ionization (ESI), double quadrupole). Synthesis of the final compounds **7**–**14** and 5-arylidenehydantoin intermediates (**20**, **21**) has been described elsewhere [25–27,30].

4.1.1. General procedure for the preparation of the N3-oxiran-5-arylidene-imidazolidine-2,4-dione intermediates (22, 23)

5-arylideneimidazolidine-2,4-diones (**20**, **21**), obtained based on the method described previously [25], were used. 2,3-Epoxy-1-propanol (0.015 mol, 1.11 g) was solved in DMF (50 mL) and cooled down to 0 °C in an ice bath while being stirred. Then the 5-arylideneimidazolidine-2,4-dione (**20, 21**) (0.01 mol) and

triphenylphosphine (0.01 mol, 2.62 g) were added and stirred until the reactants had dissolved. The solution of diethyl azodicarboxylate DEAD (0.01 mol, 1.74 g) in dry DMF (10 mL) was added to the mixture dropwise and stirring was continued at room temperature for further 4 h. Then, the mixture was poured into 200 mL of water to precipitate and left at 0–4 °C overnight. After filtration, the precipitate was washed with water and diethyl ether. A pure product (**22** or **23**) was obtained from the precipitate by column chromatography with CH_2Cl_2/acetone (17:3).

4.1.1.1. (Z)-5-(4-chlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (22).

(Z)-5-(4-chlorobenzylidene)imidazolidine-2,4-dione (**20**) (0.01 mol, 2.23 g) was used. Yield 0.79 g, 28%; mp: 196–200 °C. LC/MS: purity: 95%, t_R = 5.64, (ESI) *m/z* [M + H^+] 279.17. ^{1}H NMR (DMSO-d_6): δ 2.69 (dd, J_1 = 4.87 Hz, J_2 = 2.57 Hz, 1H, oxiran), 2.83–2.86 (m, 1H, oxiran), 3.23–3.29 (m, 1H, oxiran), 3.76–3.89 (m, 2H, alkyl), 6.72 (s, 1H, CH=C), 7.38–7.44 (m, 4H, Ar), 8.86 (s, 1H, N_1H).

4.1.1.2. (Z)-5-(2,4-dichlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (23).

(*Z*)-5-(2,4-dichlorobenzylidene)imidazolidine-2,4-dione (**21**) (0.01 mol, 2.57 g) was used. Yield 0.53 g, 17%; mp: 185–190 °C. LC/MS: purity: 98%, t_R = 5.99, (ESI) *m/z* [M + H^+] 313.07. ^{1}H NMR (DMSO-d_6): δ 2.52–2.55 (m, 1H, oxiran), 2.71–2.74 (m, 1H, oxiran), 3.14–3.16 (m, 1H, oxiran), 3.62–3.64 (d, J = 4.7 Hz, 2H, alkyl), 6.53 (s, 1H, CH=C), 7.44–7.47 (dd, J_1 = 8.4 Hz, J_2 = 2.3 Hz, 1H, Ar), 7.69 (s, 1H, Ar), 7.78–7.81 (d, J = 8.5 Hz, 1H, Ar), 11.06 (br.s, 1H, N_1H).

4.1.2. Procedures for the preparation of the 3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-arylideneimidazolidine-2,4-diones (24, 25)

4.1.2.1. (Z)-3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-(4-chlorobenzylidene)-imidazolidine-2,4-dione (24).

(Z)-5-(4-chlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**22**) (2.3 mmol, 0.65 g), 1-acetylpiperazine (3.3 mmol, 0.42 g) were irradiated at 450 W for 3 min, then at 600 W for 4 min and then at 750 W for 26 min (2 × 5 min, 2 min, 14 min) in a standard household microwave oven. The obtained glassy residue was purified by crystallization with methanol. Yield 0.60 g, 64%; mp: 188–192 °C. LC/MS: purity: 99%, t_R = 3.46, (ESI) *m/z* [M + H^+] 407.27. ^{1}H NMR (DMSO-d_6): δ 1.93 (s, 3H, CH_3), 2.26–2.43 (m, 6H, alkyl, Pip), 3.34–3.39 (m, 4H, Pip), 3.47–3.50 (m, 2H, alkyl), 3.92–3.96 (m, 1H, C*H*OH), 4.96–4.98 (d, J = 5.38 Hz, 1H, OH), 6.49 (s, 1H, CH=C), 7.41–7.46 (m, 2H, Ar), 7.61–7.66 (m, 2H, Ar), 10.79 (s, 1H, N_1H).

4.1.2.2. (Z)-3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-(2,4-dichloro-benzylidene)imidazolidine-2,4-dione (25).

(Z)-5-(2,4-dichlorobenzylidene)-3-(oxiran-2-ylmethyl)imidazolidine-2,4-dione (**23**) (0.64 mmol, 0.2 g) was melted with 1-acetylpiperazine (1.28 mmol, 0.16 g) by using an oil bath at temperature of 160 °C for 30 min. The obtained glassy residue was purified by crystallization with methanol and activated charcoal. Yield 0.15 g, 55%; mp: 190–195 °C. LC/MS: purity: 100%, t_R = 4.03, (ESI) *m/z* [M + H^+] 441.31. ^{1}H NMR (DMSO-d_6): δ 1.93 (s, 3H, CH_3), 2.26–2.43 (m, 6H, alkyl, Pip), 3.30–3.38 (m, 4H, Pip), 3.47–3.50 (m, 2H, alkyl), 3.92–4.00 (m, 1H, C*H*OH), 4.96–4.99 (m, 1H, OH), 6.54 (s, 1H, CH=C), 7.46–7.50 (m, 1H, Ar), 7.68 (s, 1H, Ar), 7.73–7.70 (m, 1H, Ar), 10.89 (s, 1H, N_1H).

4.1.3. General procedure for the preparation of the final 5-arylidene3-(2-hydroxy-3-(piperazin-1-yl)- propyl)-imidazolidine-2,4-diones (15, 16)

The 3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-arylideneimidazolidine-2,4-dione (**24**, **25**) (0.25–1 mmol) was boiled in 15% HCl (1.5–2 mL) for 45–180 min, thus producing the hydrochloride form of the final deprotected piperazine derivatives (**15**, **16**).

4.1.3.1. (Z)-5-(4-chlorobenzylidene)-3-(2-hydroxy-3-(piperazin-1-yl)- propyl)-imidazolidine-2,4-dione hydrochloride (15).

(Z)-3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-(4-chlorobenzylidene)imidazoli-dine-2,4-dione (**24**) (1 mmol, 0.41 g) was boiled in 2 mL of 15% HCl for 180 min. Yield 0.41 g, 100%; mp: 299–301.5 °C. Anal. Calcd for $C_{17}H_{21}ClN_4O_3 \times HCl \times 2H_2O$: C, 46.69; H, 5.82; N, 12.81; found: C, 46.73; H, 544; N, 12.60. ^{1}H NMR (DMSO-d_6): δ 2.96–3.25 (m, 8H, Pip), 3.40–3.73 (m, 4H, Pip), 4.21 (m, 1H, C*H*OH), 5.95 (br.s, 1H, OH), 6.51 (s, 1H, CH=C), 7.44–7.47 (m, 2H, Ar), 7.65–7.67 (m, 2H, Ar), 9.51 (br.s, 2H, NH_2^+), 10.88 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 164.68, 155.58, 133.42, 133.29, 131.53, 129.25 127.76, 108.27, 63.45, 59.11, 42.62.

4.1.3.2. (Z)-5-(2,4-dichlorobenzylidene)-3-(2-hydroxy-3-(piperazin-1-yl) propyl)imidazolidine-2,4-dione hydrochloride (16).

(Z)-3-(3-(4-acetylpiperazin-1-yl)-2-hydroxypropyl)-5-(2,4-dichlorobenzylidene)imida-zolidine-2,4-dione (**25**) (0.25 mmol, 0.11 g) was boiled in 1.5 mL of 15% HCl for 45 min. Evaporation of water gave white crystals of **16**. Yield 0.066 g, 61%; mp: 267.8–271.2 °C. Anal. Calcd for $C_{17}H_{20}Cl_2N_4O_3 \times HCl \times 3H_2O$: C, 41.69; H, 5.56; N, 11.44; found: C, 41.3; H, 5.39; N, 11.25. ^{1}H NMR (DMSO-d_6): δ 3.20–3.56 (m, 12H, Pip, alkyl), 4.22 (br.s., 1H, C*H*OH), 5.95 (br.s, 1H, OH), 6.56 (s, 1H, CH=C), 7.46–7.50 (dd, J_1 = 8.4 Hz, J_2 = 2.2 Hz, 1H, Ar), 7.69 (s, 1H, Ar), 7.73–7.70 (m, 1H, Ar), 9.47 (br.s, 2H, NH_2^+), 11.00 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 164.40, 155.77, 134.41, 133.83, 131.61, 130.42, 130.06, 129.63, 128.16, 102.96, 64.22, 62.93, 54.04, 53.61, 49.06, 44.08.

4.1.4. Preparation of 5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (26)

The solution of ammonium carbonate (0.50 mol, 48.05 g) and potassium cyanate (0.25 mol, 16.28 g) in 200 mL of water was added to 1-acetonaphtone (0.1 mol, 15.2 mL) in 200 mL of ethanol. The mixture was stirred at 55 °C for 10 days, then densified in vacuo, cooled and acidified to pH~5 with 35% solution of HCl. The resulting solid was filtrated and crystallized from EtOH. Yield 20.14 g, 84%; mp: 213–215 °C. Anal. calcd. for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66; found C, 69.88; H, 4.99; N, 11.73. ^{1}H NMR (DMSO-d_6): δ 1.90 (s, 3H, CH_3), 7.48–7.58 (m, 3H, Ar), 7.69–7.72 (dd, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H, Ar), 7.84–7.88 (m, 1H, Ar), 7.94–8.00 (m, 2H, Ar), 8.55 (s, 1H, N_1H), 11.11 (br. s, 1H, N_3H). ^{13}C NMR (DMSO-d_6): δ 26.7, 64.7, 124.1, 125.4, 126.1, 126.7, 126.9, 129.8, 130.2, 131.1, 134.1, 134.5, 156.8, 178.6.

4.1.5. General procedure for the preparation of 3-bromoalkyl-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-diones (27–29)

A mixture of 5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione **26** (20 mmol, 4.81 g), potassium carbonate (60 mmol, 8.29 g) and triethylbenzylammonium chloride (TEBA, 3 mmol, 0.68 g) in 100 mL of acetone was stirred at room temperature for 15 min. Subsequently, the solution of an appropriate dibromoalkane (20 mmol) in acetone (20 mL) was added and the mixture was stirred for 30 min at room temperature, then refluxed for 1 h. After filtration, the solvent was removed in vacuo and the resulting crude was purified by column chromatography.

4.1.5.1. 3-(3-Bromopropyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (27). Yield 3.94 g, 55%; mp: 142–143 °C. Anal. calcd. for $C_{17}H_{17}BrN_2O_2$: C, 56.52; H, 4.74; N, 7.75; found C, 56.45; H, 4.69; N, 7.71. ^{1}H NMR (DMSO-d_6): δ 1.91 (s, 3H, CH_3), 2.13–2.23 (m, 2H, alkyl), 3.55 (t, J = 7.0 Hz, 2H, alkyl), 3.63 (t, J = 6.6 Hz, 2H, alkyl), 7.49–7.56 (m, 3H, Ar), 7.64–7.68 (m, 1H, Ar), 7.72–7.75 (dd, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H, Ar), 7.95–8.00 (m, 2H, Ar), 8.92 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 26.5, 31.3, 32.0, 37.4, 63.5, 123.9, 125.5, 126.2, 126.9, 129.9, 130.4, 130.9, 133.7, 134.5, 156.1, 176.9.

4.1.5.2. 3-(4-Bromobutyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (28). Yield 4.29 g, 57%; mp: 101–103 °C. Anal. calcd. for $C_{18}H_{19}BrN_2O_2$: C, 57.61; H, 5.10; N, 7.47; found: C, 57.77; H, 5.34; N, 7.38. ^{1}H NMR (DMSO-d_6): δ 1.73–1.88 (m, 4H, alkyl), 1.90 (s, 3H, CH_3), 3.53–3.61 (m, 4H, alkyl), 7.49–7.56 (m, 3H, Ar), 7.67–7.75 (m, 2H, Ar), 7.95–8.00 (m, 2H, Ar), 8.89 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 26.7, 26.8, 30.2, 34.9, 37.8, 63.5, 124.0, 125.5, 126.2, 126.9, 129.8, 130.3, 131.0133.7, 134.5, 156.3, 176.9.

4.1.5.3. 3-(5-Bromopentyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (29). Yield 4.29 g, 55%; mp: 102–104 °C. Anal. calcd. for $C_{19}H_{21}BrN_2O_2$: C, 58.62; H, 5.44; N, 7.20; found: C, 58.47; H, 5.62; N, 7.19. ^{1}H NMR (DMSO-d_6): δ 1.38–1.48 (m, 2H, alkyl), 1.60–1.70 (m, 2H, alkyl), 1.78–1.87 (m, 2H, alkyl), 1.90 (s, 3H, CH_3), 3.50 (t, J = 6.5 Hz, 4H, alkyl), 7.49–7.55 (m, 3H, Ar), 7.68–7.74 (m, 2H, Ar), 7.95–8.01 (m, 2H, Ar), 8.88 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 25.3, 26.7, 27.1, 32.2, 35.4, 38.3, 63.5, 124.1, 125.5, 126.2, 126.9, 129.8, 130.3, 130.9, 133.8, 134.5, 156.3, 176.9.

4.1.6. General procedure for the preparation of phthalimide derivatives (30–32)

A mixture of potassium phtalimide (5 mmol, 0.93 g), potassium carbonate (15 mmol, 2.07 g) and TEBA (0.75 mmol, 0.17 g) in 12.5 mL of acetone was stirred at room temperature for 30 min. Subsequently, the solution of an appropriate bromide **27–29** (5 mmol) in 12.5 mL of acetone was added and the mixture was refluxed for 2 h. After filtration, the solvent was removed in vacuo and the resulting crude was purified by column chromatography.

4.1.6.1. 2-(3-(5-methyl-5-(naphthalen-1-yl)-2,4-dioxoimidazolidin-1yl)propyl)isoindoline-1,3-dione (30). Yield 1.62 g, 76%; mp: 204–205 °C. Anal. calcd. for $C_{25}H_{21}N_3O_4$: C, 70.25; H, 4.95; N, 9.83; found: C, 70.46; H, 5.16; N, 9.89. ^{1}H NMR (DMSO-d_6): δ 1.93 (s, 3H, CH_3), 1.97–2.04 (m, 2H, alkyl), 3.57–3.68 (m, 4H, alkyl), 7.49–7.59 (m, 3H, Ar), 7.67 (dd, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H, Ar), 7.73 (dd, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H, Ar), 7.79–7.88 (m, 4H, Ar), 7.95–8.00 (m, 2H, Ar), 8.91 (s, 1H, N_1H); ^{13}C NMR (DMSO-d_6): δ 26.6, 27.2, 35.8, 36.5, 63.6123.5, 123.5, 124.0, 125.5, 126.2, 126.9, 127.1, 129.8, 130.4, 131.0132.1, 132.1, 133.6, 134.5, 134.8, 134.8, 156.1, 168.3, 176.8.

4.1.6.2. 2-(3-(5-methyl-5-(naphthalen-1-yl)-2,4-dioxoimidazolidin-1-yl)butyl)isoindoline-1,3-dione (31). Yield 1.76 g, 80%; mp: 160–162 °C. Anal. calcd. for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52; found: C, 70.43; H, 5.35; N, 9.92. ^{1}H NMR (DMSO-d_6): δ 1.65 (m, 4H, alkyl), 1.89 (s, 3H, CH_3), 3.54 (t, J = 6.6 Hz, 2H, alkyl), 3.64 (t, J = 6.6 Hz, 2H, alkyl), 7.48–7.53 (m, 3H, Ar), 7.62–7.65 (m, 1H, Ar), 7.71 (dd, J_1 = 7.4 Hz, J_2 = 1.0 Hz, 1H, Ar), 7.81–7.89 (m, 4H, Ar), 7.94–7.99 (m, 2H, Ar), 8.85 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 25.6, 25.9, 26.6, 37.5, 38.1, 63.5, 123.5, 123.5, 123.9, 125.5, 126.1, 126.8, 126.9, 129.8, 130.3, 130.9, 132.0, 132.0, 133.6, 134.5, 134.9, 134.9, 156.3, 168.5, 176.9.

4.1.6.3. 2-(3-(5-methyl-5-(naphthalen-1-yl)-2,4-dioxoimidazolidin-1-yl)butyl)isoindoline-1,3-dione (32). Yield 1.92 g, 84%; mp: 145–146 °C; Anal. calcd. for $C_{27}H_{25}N_3O_4$: C, 71.19; H, 5.53; N, 9.22; found C, 71.36; H, 5.58; N, 9.44. ^{1}H NMR (DMSO-d_6): δ 1.30–1.38 (m, 2H, alkyl), 1.59–1.67 (m, 4H, alkyl), 1.85 (s, 3H, CH_3); 3.47–3.58 (m, 4H, alkyl), 7.48–7.54 (m, 3H, Ar), 7.64–7.72 (m, 2H, Ar), 7.79–7.87 (m, 4H, Ar), 7.94–8.00 (m, 2H, Ar), 8.83 (s, 1H, N_1H). ^{13}C NMR (DMSO-d_6): δ 24.1, 26.6, 27.7, 28.1, 37.7, 38.3, 63.5, 123.4, 123.4124.0, 125.5, 126.2, 126.8, 126.9, 129.8, 130.3130.9, 132.0, 132.1, 133.0, 133.7, 134.5, 134.8, 156.3, 168.4, 176.8.

4.1.7. General procedure for the preparation of primary amines (17–19)

A mixture of the appropriate phtalimide derivative **30–32** (2 mmol) and hydrazine monohydrate (20 mmol, 1.56 mL) in 20 mL of ethanol was refluxed for 2.5 h. After filtration and evaporation of the solvent in vacuo, the resulting crude was refluxed with 30 mL of 10% HCl for 15 min and again filtrated. The filtrate was then alkalized with 15% solution of NaOH and extracted with dichloromethane (2 × 30 mL). The combined organic layers were dried and concentrated in vacuo. The product was purified by column chromatography.

4.1.7.1. 3-(3-Aminopropyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (17). Yield 0.382 g, 64%; mp: 62–63°C. Anal. calcd. for $C_{17}H_{19}N_3O_2$: C, 68.67; H, 6.44; N, 14.13; found: C, 68.85; H, 6.69; N, 14.09. ^{1}H NMR (DMSO-d_6): δ 1.66–1.75 (m, 2H, alkyl), 1.91 (s, 3H, CH_3), 2.58 (t, J = 6.2 Hz, 2H, alkyl), 3.56 (t, J = 6.6 Hz, 2H, alkyl), 7.50–7.55 (m, 3H, Ar), 7.63–7.75 (m, 2H, Ar), 7.95–8.02 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6): δ 26.7, 31.7, 36.4, 39.1, 63.5, 124.1, 125.5, 126.2, 126.9, 126.9, 129.8, 130.3, 131.0133.7, 134.5, 156.4, 177.0.

4.1.7.2. 3-(4-Aminobutyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (18). Yield 0.376 g, 60%; mp: 191–194 °C. Anal. calcd. for $C_{18}H_{21}N_3O_2$: C, 69.43; H, 6.80; N, 13.49; found: C, 69.20; H, 6.90; N, 13.21. ^{1}H NMR (DMSO-d_6): δ 1.35–1.45 (m, 2H, alkyl), 1.59–1.69 (m, 2H, alkyl), 1.90 (s, 3H, CH_3), 2.56 (t, J = 7.0 Hz, 2H, alkyl), 3.49 (t, J = 7.0 Hz, 2H, alkyl), 7.49–7.55 (m, 3H, Ar), 7.67–7.75 (m, 2H, Ar), 7.95–8.00 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6): δ 25.5, 26.7, 29.8, 38.5, 39.1, 63.5, 124.1, 125.5, 126.2, 126.9, 126.9, 129.8, 130.3, 131.0133.7, 134.5, 156.3, 176.9.

4.1.7.3. 3-(5-Aminopentyl)-5-methyl-5-(naphthalen-1-yl)imidazolidine-2,4-dione (19). Yield 0.412 g, 63%; mp: 134–136 °C. Anal. calcd. for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91; found: C, 70.16; H, 7.39; N, 12.72. ^{1}H NMR (DMSO-d_6): δ 1.32–1.42 (m, 4H, alkyl), 1.57–1.66 (m, 2H, alkyl), 1.90 (s, 3H, CH_3), 2.47–2.53 (m, 2H, alkyl), 3.48 (t, J = 6.9 Hz, 2H, alkyl), 7.45–7.55 (m, 3H, Ar), 7.68–7.74 (m, 2H, Ar), 7.95–8.00 (m, 2H, Ar). ^{13}C NMR (DMSO-d_6): δ 24.2, 26.7, 27.9, 32.9, 38.6, 41.7, 63.5, 124.1, 125.5, 126.2, 126.8, 126.9, 129.8, 130.3, 131.0133.8, 134.5, 156.3, 176.9.

4.2. Molecular modeling methods

Crystal structures of PBP2a (PDB ID: 1VQQ) and binding domain of MecR1 (PDB ID: 2IWB) were fetched from the PDB database [31]. Three-dimensional conformations of all newly synthesized compounds, cloxacillin and oxacillin were generated using LigPrep [32] from Schrödinger Suite 2013. All molecules were generated in protonation states at pH 7 ± 2 using OPLS_2005 force field [33]. The compounds were docked into the binding sites of MecR1 and PBP2a using Schrödinger Glide in extra precision mode (maximum 5 poses for each instance from Ligprep were enabled).

For the compounds **10, 12**, **13**, cloxacillin and oxacillin (for comparison) molecular dynamic simulation studies were performed. The simulations were carried out using Schrödinger Desmond [34]. Protein structure was preprocessed and optimized in Schrödinger Protein Preparation Wizard [35–37]. The system was

composed of protein, chemical compound and solvent (TIP3P model of water). The input poses for MD were obtained from docking and complexes with the best docking scores were selected; as conformations of **12** and **13** for the ligand-protein complexes were slightly different, **13** underwent additional simulation with the analogous starting conformation as **12**. The aim of this procedure was to check whether the obtained ligand-protein complexes are stable. Each simulation lasted for 20 ns, and was performed using OPLS_2005 force field.

4.3. Microbiological assays

The assays aimed to determine the increase of antibiotic efficacy were conducted by determining if/to what extent the compounds reduce the minimum inhibitory concentrations of oxacillin, cloxacillin, ampicillin/sulbactam, ciprofloxacin and neomycin by means of a serial dilution broth microplate method according to the CLSI requirements [28].

Serial two-fold dilutions of oxacillin (Sigma–Aldrich; cat. no. 28221), cloxacillin (Sigma–Aldrich; cat. no. C9393), ampicillin/sulbactam ratio 2:1 (Sigma–Aldrich; cat. no. A9518, Sigma–Aldrich; cat. no. S9701), ciprofloxacin (Sigma–Aldrich; cat. no. 17850) or neomycin (Fagron; cat. no. 113374) were prepared in 65 μl of the Mueller-Hinton broth (Merck; cat. no. 1102930500). Suitable concentrations of the compounds (not exceeding ¼ of their MICs; total volume 10 μl) were then added. Bacterial suspensions were diluted to OD = 0.5. The resulting suspensions were then diluted 1:100 and added in the volume of 75 μl into the oxacillin serial dilutions with the compounds. The results were read after 20-h incubation at 37 °C.

4.4. Proliferation assay

In vitro proliferation assay was conducted using HEK-293 cell line and a commercial kit EZ4U (cat. no.: BI-5000, Biomedica) containing uncolored tetrazolium salt that is reduced by living cells into intensely colored formazan derivative. HEK-293 cell line was cultured in Dulbecco's Modified Eagle's complete growth Medium (DMEM) containing 10% fetal bovine serum (FBS), 100 mg/mL streptomycin and 100 U/mL penicillin. The cells were cultured at 37 °C in an atmosphere containing 5% CO_2. The cells were seeded in 96-well plates at a concentration of 1.5×10^4 cells/well in 200 μl culture medium and incubated for 24 h at 37 °C and 5% CO_2 to reach 60% confluence. Next, the compound **12** was added into the microplate at the final concentrations 0.01–250 μM. The cells were incubated with the compound for 48 h at 37 °C and 5% CO_2. Then, 20 μl of EZ4U labeling mixture was added and the cells were incubated for 5 h under the same conditions. The absorbance of the samples was measured using a microplate reader (PerkinElmer) at 492 nm. The activity of the standard drug doxorubicin (DX) was estimated at the concentrations 0.005–100 μM as we described previously [38]. All experiments were conducted in four repetitions. GraphPad Prism 5.01 software was used to calculate the experimental IC50 values.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.013.

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