



Design, synthesis and antibacterial evaluation of amphiphilic 1,2,4-oxadiazole-based pyridinium salts

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ABSTRACT

Antimicrobial resistance (AMR) is a growing global health concern that compromises the effectiveness of current antibacterial therapies and necessitates the development of new active agents. In this study, a series of 37 novel 1,2,4-oxadiazole-based pyridinium salts was designed, synthesized, and characterized. Structural variations were introduced to the positions 3- and 5- of the oxadiazole core, exploring aliphatic chains with variable length and differently substituted aromatic moieties to modulate lipophilicity and electronic properties. The compounds synthesized were evaluated in vitro against Gram-positive and Gram-negative reference strains, as well as against multidrug-resistant (MDR) clinical isolates. Overall, the derivatives showed a selective activity against Gram-positive bacteria, with several compounds exhibiting significant antibacterial activity. Notably, selected derivatives displayed minimum inhibitory concentration (MIC) values in the range of 2–16 μM (1–8 $\mu\text{g/mL}$), with compound **18b** emerging as the most potent analogue MIC = 1 μM (0.5 $\mu\text{g/mL}$) against *Staphylococcus aureus* multidrug resistant strain. Structure-activity relationship (SAR) analysis revealed that antibacterial activity is strongly influenced by the nature of the aromatic substituent. Electron-donating groups, such as methoxy ($-\text{OCH}_3$), enhanced activity, whereas electron-withdrawing substituents (i.e., $-\text{NO}_2$ and $-\text{F}$) reduced potency. In contrast, amide-containing derivatives and aliphatic analogues were inactive against resistant strains. The most active compounds exhibited low cytotoxicity in HepG2 and HDnF cell lines, together with negligible haemolytic activity at concentrations up to 25 μM . Favourable selectivity indices (SI = 1.6–33.65) were observed, with compound **21b** displaying the best safety profile. Overall, these findings identify 1,2,4-oxadiazole-based pyridinium salts as promising scaffolds and highlight the key role of amphiphilicity and electronic modulation in the development of effective antibacterial agents against resistant pathogens.

1. Introduction

Antimicrobial resistance (AMR) is an escalating worldwide health menace that undermines the efficacy of infection therapies, resulting in approximately 5 million fatalities in 2019, with projections indicating a substantial increase [1]. The growing burden of antimicrobial resistance is particularly evident in the so-called ESKAPEE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*) which are responsible for a large proportion of hospital-acquired

infections (HAIs) and are characterized by a remarkable ability to escape antimicrobial therapies and rapidly acquire resistance determinants [2]. Quaternary ammonium compounds (QACs) have historically been fundamental as disinfectants, owing to their extensive applicability and structural variety [3]. They are essential for infection control in clinical environments, acting as a fundamental measure in the prevention of HAIs and ensuring patient safety [4]. Among all QACs, cetyltrimethylammonium bromide (CTAB), benzalkonium chloride (BAC), cetylpyridinium chloride (CPC) and octenidine dihydrochloride (OCT) are the most notable examples [5].

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Their activity arises from nonspecific electrostatic interactions with cell envelopes and intracellular structures, rather than from ligand-receptor interactions [6]. This preferential interaction is generally attributed to the abundance of negatively charged membrane components in bacteria, whereas the outer leaflet of mammalian cell membranes is predominantly zwitterionic, providing the basis for the selective activity of many cationic antibacterial agents [7]. Nonetheless, bacteria resistant to quaternary ammonium compounds, including multidrug-resistant strains, have increasingly been identified. In hospital environments, significant resistant species comprised *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* [8].

Nowadays, the design of novel QACs has focused on the development of structurally optimized heteroatomic and multi-cationic systems, to improve antimicrobial performance and overcome resistance mechanisms. For instance, quinoline-based QACs have recently demonstrated high activity against multidrug-resistant strains such as MRSA (Methicillin-resistant *Staphylococcus aureus*) and VRSA (Vancomycin-resistant *Staphylococcus aureus*), with sub-micromolar MIC values and a favourable toxicity profile, for example selectivity index of 72 w.r.t. with Chinese Hamster Ovary cells (CHO-K1) and high haemolytic concentration [9]. Notably, selected bis-QACs derivatives have also shown superior efficacy compared to established antiseptics such as octenidine, highlighting the key role of aromatic scaffolds in the development of next-generation cationic antimicrobials [10].

In line with these findings, quaternary pyridinium salts based on a pyridine-3-yl chalcone framework have shown activity against *Staphylococcus aureus* and *Escherichia coli*, with MIC values in the range of 1.56–6.25 μM [11]. (Fig. 1)

Despite these advances, the development of structurally refined QACs capable of simultaneously addressing antimicrobial resistance, biofilm formation, and safety concerns remains a significant challenge [12].

In this context, the incorporation of additional heteroaromatic motifs represents a valuable strategy to further modulate the physicochemical and biological properties of cationic antimicrobial agents. Among these, the 1,2,4-oxadiazole ring has emerged as a versatile scaffold in medicinal chemistry, widely employed as a bioisosteric replacement for amide and ester functionalities. Its introduction can enhance metabolic stability, modulate lipophilicity, and improve molecular rigidity, all of which are key factors influencing membrane interactions and antimicrobial activity [13].

Moreover, the 1,2,4-oxadiazole moiety can be used as aromatic linker between the pharmacophoric units, promoting favourable spatial arrangement and electronic distribution within the molecule [14]. In antimicrobial compounds design, the incorporation of 1,2,4-oxadiazole moiety into aromatic frameworks contributes to the optimization of the

amphiphilic balance, strengthening interactions with bacterial membranes particularly in resistant and biofilm-forming pathogens [15].

In a previous study it has been demonstrated that pyridinium 1,2,4-oxadiazole based compounds exhibit significant activity against a range of resistant bacterial strains and are effective in disrupting biofilm-associated cells [16]. (Fig. 1) Due to the increasing concerns regarding resistance potentially driven by QACs, these findings highlight the potential of structurally optimized cationic scaffolds. On this basis, the present work explores a refined series of pyridinium salts to further investigate the structure-activity relationships (SAR) of 1,2,4-oxadiazole-derived antimicrobial agents (Fig. 2). The new set of compounds have been rationally designed based on previous SAR findings, highlighting a bis-aromatic architecture incorporating an oxadiazole core and optimized alkyl side chains (C10–C12) as key determinants for enhanced antimicrobial and antibiofilm activity.

In particular, mono-cationic derivatives bearing an undecyl (C11) chain displayed moderate potency (MIC = 2 $\mu\text{g/mL}$, 4.5 μM), whereas bis-cationic analogues with decyl (C10) and dodecyl (C12) chains showed significantly enhanced activity against *Staphylococcus aureus* (MIC = 0.25 $\mu\text{g/mL}$, 0.3 μM) and *Escherichia coli* (MIC = 0.5 $\mu\text{g/mL}$, 0.7 μM). These findings highlight the key role of alkyl chain length and cationic distribution in modulating antibacterial properties [16].

Considering that the antibacterial activity of amphiphilic cationic compounds is strongly influenced by the balance between hydrophobicity and positive charge, the present library has been designed to systematically investigate how these structural features affect antibacterial potency and bacterial selectivity.

The amphiphilic balance of the new derivatives was tuned by varying the length of the alkyl chain (C4–C16) attached to the pyridinium nitrogen, with the aim of optimizing lipophilicity and the interaction with bacterial cells. In parallel, the introduction of either short (methyl) or longer alkyl substituents on the oxadiazole ring enabled evaluation of the contribution of aliphatic groups directly linked to the heterocyclic core. Furthermore, a para-substituted phenyl moiety was incorporated to systematically investigate electronic effects, using both electron-withdrawing ($-\text{NO}_2$, $-\text{F}$) and electron-donating groups ($-\text{OCH}_3$, amide functionalities), thereby modulating charge distribution and physicochemical properties. This systematic molecular design also aimed to identify structural determinants associated with broad-spectrum antibacterial activity while maintaining selectivity towards bacterial cells.

Overall, the design strategy was intended to elucidate the interplay between aromaticity, lipophilicity, and electronic factors in governing the antimicrobial activity of pyridinium-based agents, providing a framework for the development of more effective compounds against resistant pathogens.

2. Results and discussion

2.1. Synthesis

The synthetic procedures for pyridinium salts are illustrated in the general Schemes 1–3.

Three synthetic protocols have been carried out to obtain 1,2,4-oxadiazoles cores.

The heterocycle can be usually obtained through the reaction between an amidoxime and an aryl or acyl chloride, using pyridine and toluene followed by heating at 110°C for the cyclization process (Scheme 1A). [17] However, xylene has been used for the synthesis of the compound 4 occurring at higher temperature (Scheme 1B). Meanwhile a stronger base such as NaOH in dimethyl sulfoxide has been used to obtain oxadiazoles at room temperature (Scheme 1C, Fig. 3) [18]. In Table S1 yields and applied protocols for derivatives 1–10 are reported.

Amide intermediates were obtained from reduction of nitro derivatives 5 and 9. The amine intermediates (11 and the regioisomer 13) directly react with nonanoyl chloride and dodecanoyl chloride to obtain the corresponding amides (12a,b and 14a,b) (Scheme 2).

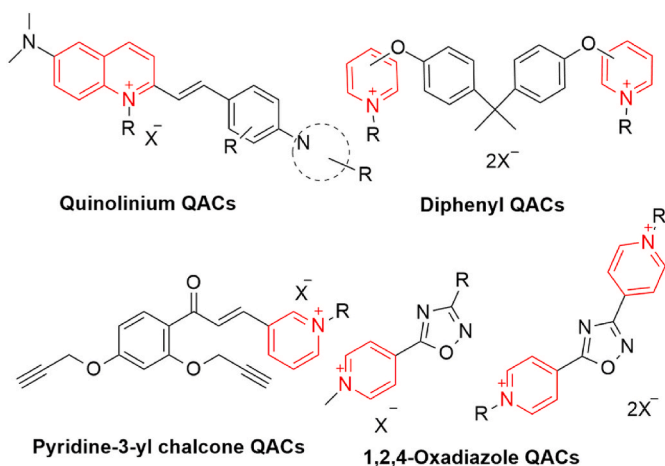


Fig. 1. Novel QACs antimicrobial compounds.

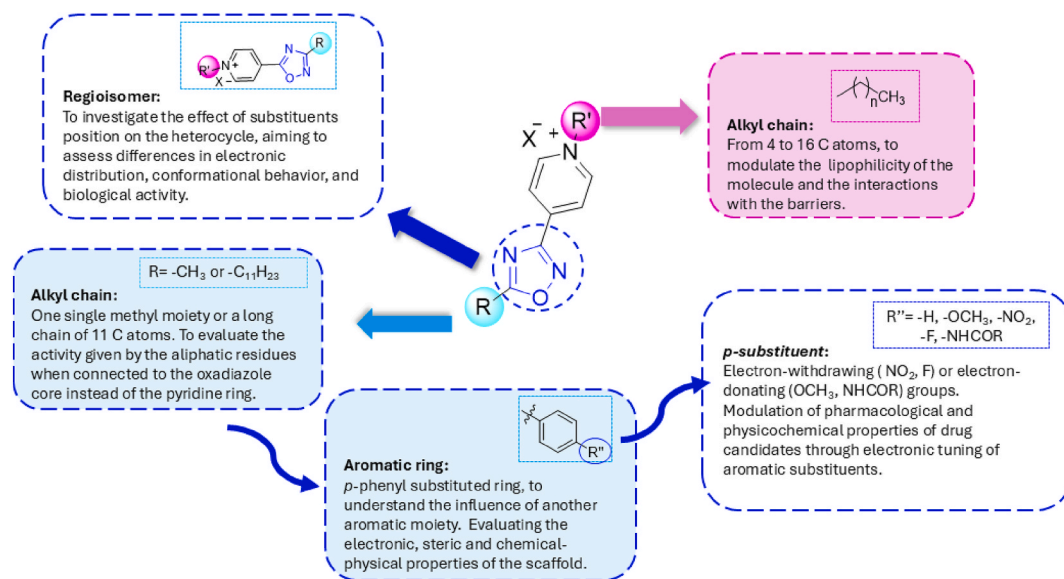
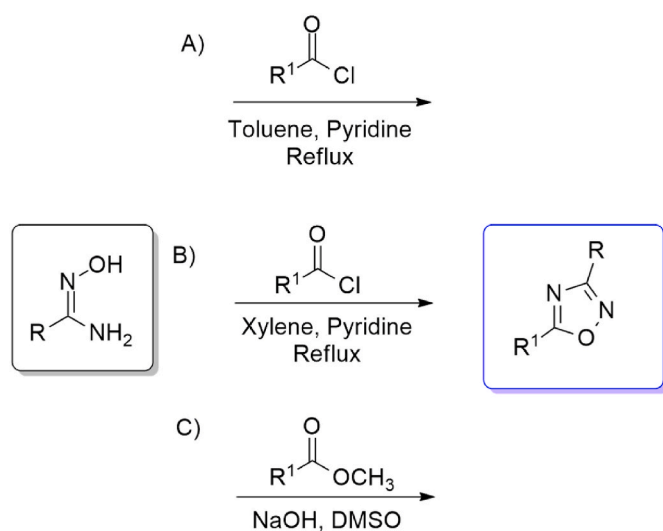


Fig. 2. Design strategy of novel 1,2,4-Oxadiazoles derivative.



Scheme 1. General schemes of synthetic procedures used to obtain 1,2,4-oxadiazole from amidoxime in three different conditions.

Subsequently, neutral intermediates described in Fig. 3 and Scheme 2 were subjected to alkylation reaction using a series of alkyl bromide or iodine (C2-C16) under different conditions, including solvent-free protocols (Scheme 3A), reactions in acetonitrile at 80°C (Scheme 3B), and microwave-assisted synthesis (Scheme 3C). These processes afforded the corresponding pyridinium salts (15-28) reported in Fig. 4 [16].

All compounds were purified and characterized by ¹H NMR, ¹³C NMR and HRMS spectra (see Supporting Information). ¹H NMR spectra show the proton of the pyridinium moiety at the lowest field with a chemical shift between 9.5 and 7 ppm, and the alkyl protons of -CH₂ linked to the pyridine nitrogen giving a typical signal between 4.5 and 5 ppm, evidencing salt formation. The remaining alkyl portion appears in the higher chemical shift region from 2 to 1 ppm. Meanwhile, the ¹³C NMR spectra show the typical peak of pyridine-bound alkyl C between 50 and 65 ppm (Supporting Information). Finally, the mass spectrum confirms salt formation by identification of the positively charged molecule.

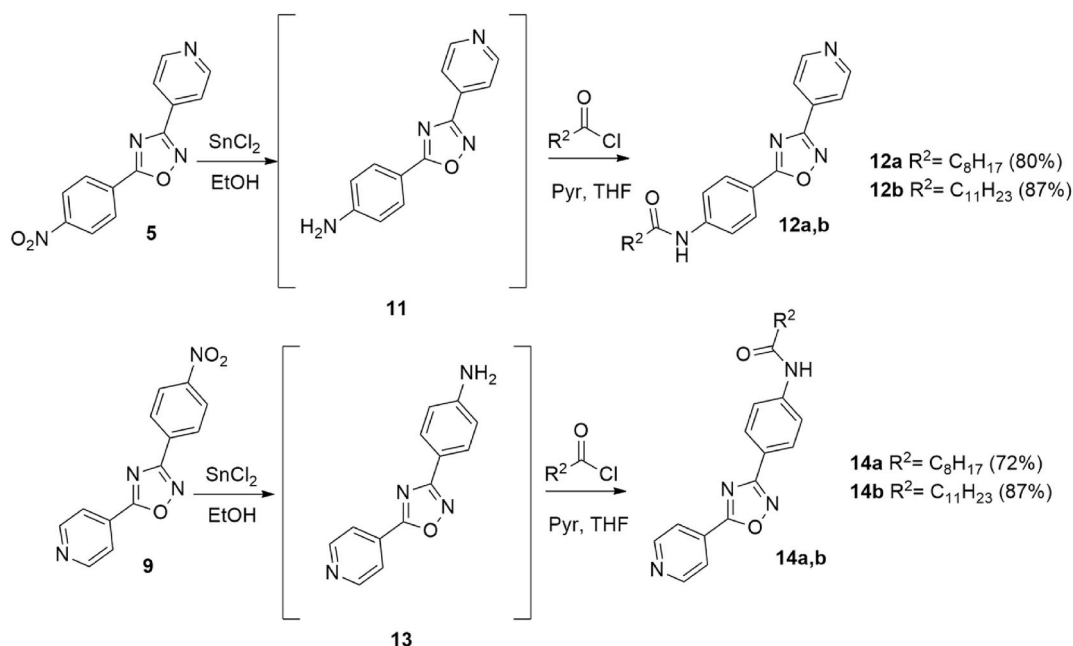
2.2. Antibacterial evaluation

The antibacterial activity of the novel pyridinium salts (Fig. 4) has been assessed in vitro against Gram-positive (*S. aureus* ATCC25923) and Gram-negative (*E. coli* ATCC25922) bacteria, since they are strictly associated with health-care-associated infections according to WHO [19]. The minimum inhibitory concentration has been determined for all the compounds. The results are reported in Table 1, except for amides (25-28), which were found to be inactive (MIC > 64 µg/mL) report in Table S2 and Table S3.

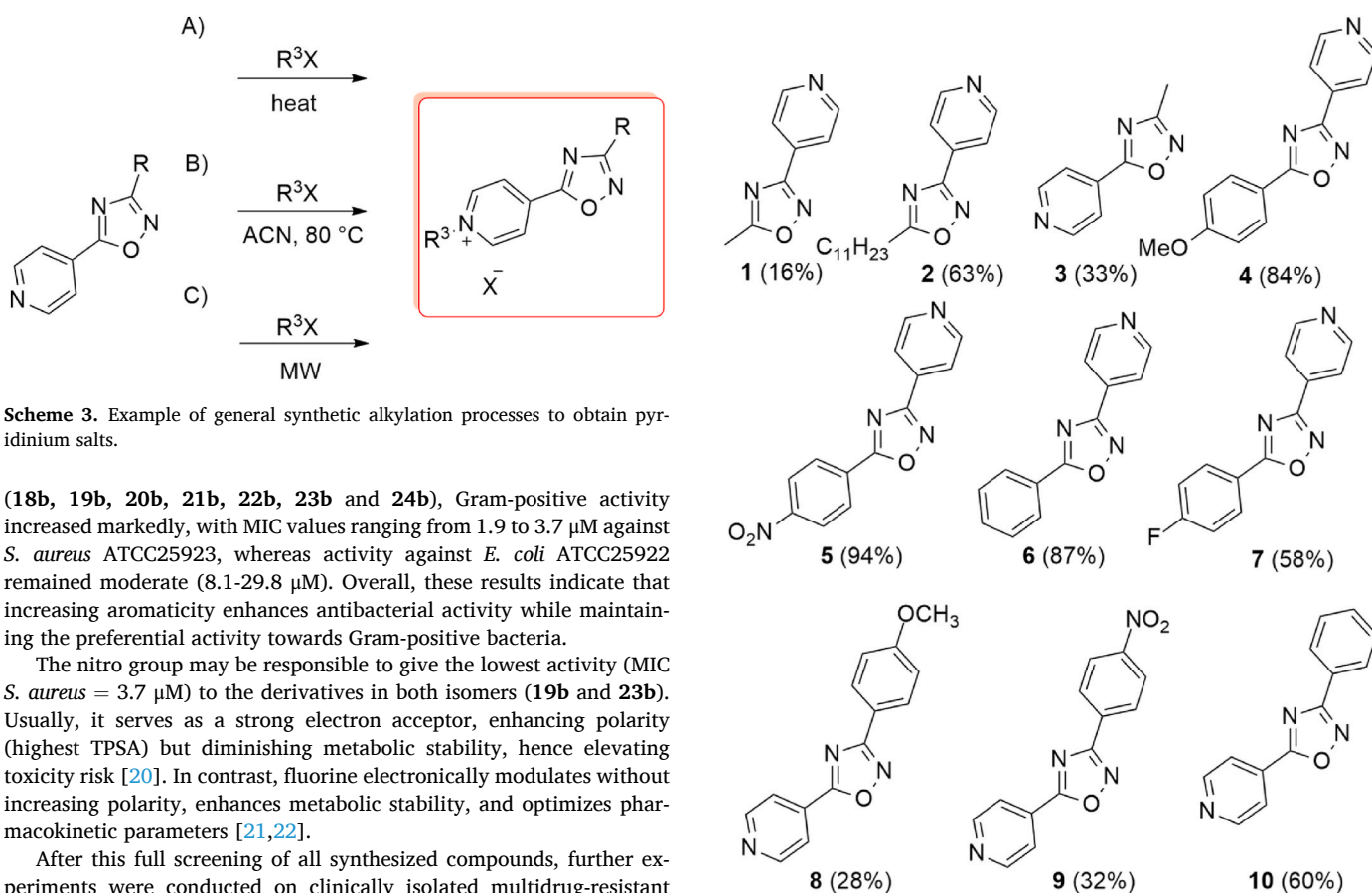
To investigate the contribution of the cationic pyridinium moiety to antibacterial activity, the corresponding neutral-bis aromatic precursors of the most active salts were evaluated against reference strains (Table S2 and Table S3). All neutral derivatives were inactive (MIC > 64 µg/mL, > 251.9 µM), indicating that quaternization of the pyridine nitrogen is an essential structural requirement for antibacterial activity.

Generally, these compounds exhibit significantly greater activity against Gram-positive bacteria compared to Gram-negative bacteria. This trend is consistent with the higher intrinsic resistance of Gram-negative bacteria, whose outer membrane represents an additional permeability barrier for many antibacterial agents. Within the methyl-substituted derivatives (15a-17b), antibacterial activity was strongly influenced by alkyl chain length. Extending the pyridinium substituent from C10 to C16 improved activity against *S. aureus* ATCC25923 (37.3 to 8.6 µM for 15a and 15b; 74.6 to 4.3 µM for 17a and 17b), suggesting that an increased hydrophobic contribution favours antibacterial potency. The presence of a longer chain (C16) in this set of pyridinium salts increases the lipophilicity of molecules and possibly their ability to interact with biological macromolecules. In contrast, to investigate the effects of oxadiazole-bound side chains, a hydrophobic segment comprising 11 carbon atoms has been employed. The antibacterial effectiveness of these compounds against Gram-positive pathogens persists when the pyridine-bound alkyl chain is short. For example, compound 16a demonstrates to have MIC of 8.7 µM (4 µg/mL). The use of long side chains on both ends may generate steric interference, obstructing interactions with targets and impairing pharmacological activity (MIC > 131.6 µM and 14 µM for 16b and 16c, respectively). These findings suggest that an appropriate balance between hydrophobicity and molecular architecture, rather than increasing lipophilicity alone, is required to achieve optimal antibacterial activity.

The introduction of a second aromatic ring further improved antibacterial potency. Among derivatives bearing a C10 pyridinium chain



Scheme 2. Reaction schemes of amides derivatives synthesis.



Scheme 3. Example of general synthetic alkylation processes to obtain pyridinium salts.

(18b, 19b, 20b, 21b, 22b, 23b and 24b), Gram-positive activity increased markedly, with MIC values ranging from 1.9 to 3.7 μM against *S. aureus* ATCC25923, whereas activity against *E. coli* ATCC25922 remained moderate (8.1–29.8 μM). Overall, these results indicate that increasing aromaticity enhances antibacterial activity while maintaining the preferential activity towards Gram-positive bacteria.

The nitro group may be responsible to give the lowest activity (MIC *S. aureus* = 3.7 μM) to the derivatives in both isomers (19b and 23b). Usually, it serves as a strong electron acceptor, enhancing polarity (highest TPSA) but diminishing metabolic stability, hence elevating toxicity risk [20]. In contrast, fluorine electronically modulates without increasing polarity, enhances metabolic stability, and optimizes pharmacokinetic parameters [21,22].

After this full screening of all synthesized compounds, further experiments were conducted on clinically isolated multidrug-resistant bacteria for the most performing ones (18b, 20b, 21b, 22b and 24b), and comparing with well-known antibiotics such as amoxicillin and clavulanic acid, ampicillin, gentamicin, fusidic acid and ciprofloxacin. A total of seven strains, comprising *K. pneumoniae*, *E. coli*, *E. faecium*, *S. aureus*, and *S. haemolyticus*, have been used (Table 2).

The results appear to be promising, particularly against the Gram-positive MDR strain, such as *S. aureus* 24023224. In particular,

Fig. 3. Structure of neutral 1,2,4-oxadiazole pyridine compounds from 1 to 10 and percentage yields in brackets.

compound 18b, presenting *p*-methoxy phenyl substituted moiety shows the lowest MIC value of 1 μM (0.5 $\mu\text{g/mL}$) against the *S. aureus* MDR and the corresponding isomer, 22b, presents MIC of 3.8 μM (2 $\mu\text{g/mL}$)

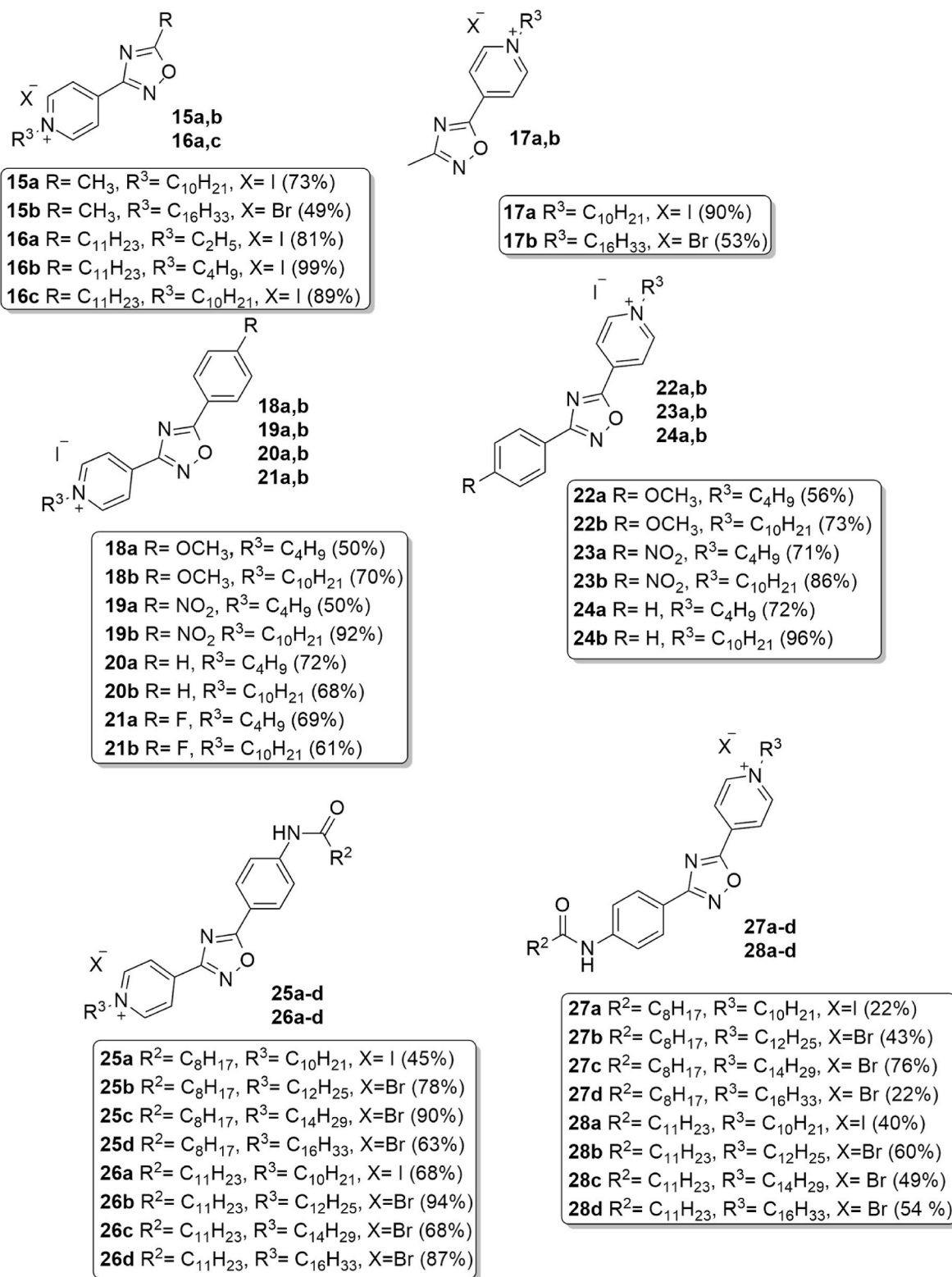


Fig. 4. Structure of 1,2,4-Oxadiazole pyridinium salts synthesized.

against two different positive strains MDR of *S. aureus* 24023224 and *S. hemolyticus* 2402453. The compounds largely retained their antibacterial activity against MDR isolates *S. aureus* and *S. haemolyticus*, whereas a moderate reduction of activity was observed against Gram-negative isolates, especially *K. pneumoniae* (**18b**: MIC = 15.4 µM; **22b**: MIC = 30.7 µM). This behaviour mirrors the trend observed for the reference strains, indicating that the antibacterial profile of the series is

maintained against clinically relevant resistant bacteria.

2.3. Selectivity and cytotoxicity

Given that an appropriate hydrophilic/lipophilic balance guarantees a favourable toxicological profile and frequently indicates the selectivity of novel antibiotics, the correlation between selective antibacterial

Table 1

MIC ($\mu\text{g/mL}$ and μM) against standard strains of *S. aureus* ATCC25923 and *E. coli* ATCC25922. The reproducibility was calculated as the Essential Agreement (EA), defined as the percentage of MIC values falling within ± 1 two-fold dilution of the modal MIC value.

Compound	R	R ³	X	<i>E. coli</i>		<i>S. aureus</i>	
				$\mu\text{g/mL}$	μM	$\mu\text{g/mL}$	μM
15a	CH ₃	C ₁₀ H ₂₁	I	>64	>149.1	16	37.3
15b	CH ₃	C ₁₆ H ₃₃	Br	64	137.3	4	8.6
16a	C ₁₁ H ₂₃	C ₂ H ₅	I	32	69.9	4	8.7
16b	C ₁₁ H ₂₃	C ₄ H ₉	I	>64	>131.6	>64	>131.6
16c	C ₁₁ H ₂₃	C ₁₀ H ₂₁	I	16	28.1	8	14.0
17a	CH ₃	C ₁₀ H ₂₁	I	>64	>149.1	32	74.6
17b	CH ₃	C ₁₆ H ₃₃	Br	>64	>137.3	2	4.3
18a	OCH ₃	C ₄ H ₉	I	>64	>146.4	>64	>146.4
18b	OCH ₃	C ₁₀ H ₂₁	I	8	15.4	1	1.9
19a	NO ₂	C ₄ H ₉	I	>64	>141.6	>64	>141.6
19b	NO ₂	C ₁₀ H ₂₁	I	8	15.0	2	3.7
20a	H	C ₄ H ₉	I	>64	>157.2	>64	>157.2
20b	H	C ₁₀ H ₂₁	I	8	16.3	1	2.0
21a	F	C ₄ H ₉	I	>64	>150.6	>64	>150.6
21b	F	C ₁₀ H ₂₁	I	8	15.7	1	2.0
22a	OCH ₃	C ₄ H ₉	I	>64	>146.4	>64	>146.4
22b	OCH ₃	C ₁₀ H ₂₁	I	8	15.4	1	1.9
23a	NO ₂	C ₄ H ₉	I	>64	>141.6	>64	>141.6
23b	NO ₂	C ₁₀ H ₂₁	I	16	29.8	2	3.7
24a	Ph	C ₄ H ₉	I	>64	>157.2	>64	>157.2
24b	Ph	C ₁₀ H ₂₁	I	4	8.1	1	2.0

activity and the lipophilicity of synthesized compounds have been explored. This initial screening enabled the selection of five viable candidates for application in antibacterial systems. The compounds **18b**, **20b**, **21b**, **22b** and **24b** exhibit MIC values against *S. aureus* of 2 μM (Table 1). The cytotoxicity, indicated through the IC₅₀ was examined using HepG2 (Human hepatocellular carcinoma G2) and HDnF (human neonatal dermal fibroblast) cells lines. The Selectivity Index (SI) is a quantitative measure of how selective an antimicrobial agent is toward microbial cells versus host (mammalian) cells. In other words, it attempts to capture the “therapeutic window” or safety margin: how much more toxic the compound is to the microbe than to mammalian cells (Table 3).

The selected compounds can be considered as selective for the bacterial cells ($1.6 \leq \text{SI} \leq 33.7$). Moreover, studies addressing haemolytic activity potentially linked to pyridinium salts demonstrate that haemolysis of erythrocytes does not take place at the doses employed for MIC of these compounds. In fact, the percentages relative to positive control are almost zero at 25 μM (Fig. 5). The pyridinium salt **21b** shows the lowest cytotoxicity and haemolytic activity than the other active compounds.

According to previous studies, bromide and iodide counterions do

not exhibit significant intrinsic cytotoxicity or haemolytic activity, while the biological properties of quaternary ammonium and pyridinium derivatives are mainly dictated by the cationic scaffold. Therefore, the IC₅₀, haemolysis and selectivity index values reported herein are expected to predominantly reflect the structure of the pyridinium cation rather than the identity of the counterion [23,24].

The observed selectivity towards bacterial cells may be explained by the different membrane compositions of bacterial and mammalian cells. Bacterial membranes are enriched in negatively charged components, including phosphatidylglycerol, cardiolipin, lipopolysaccharides (Gram-negative bacteria) and teichoic acids (Gram-positive bacteria), whereas the outer leaflet of mammalian cell membranes is predominantly composed of zwitterionic phospholipids. These compositional differences have been proposed to promote the preferential electrostatic association of cationic antibacterial agents with bacterial cell surfaces, followed by insertion of their hydrophobic moieties into the lipid bilayer [7].

2.4. Structure activity relationship (SAR)

The optimization strategy and the resulting SAR are summarized in Fig. 6. Systematic modulation of aromaticity, alkyl chain length, and aromatic substituents enabled the identification of key structural features governing antibacterial activity, selectivity and activity against multidrug-resistant bacteria.

The SAR analysis, supported by in silico evaluation using SwissADME [25], highlights the pivotal role of the pyridinium cation for antibacterial activity, as neutral precursors were inactive (MIC > 251.9 μM), suggesting that the pyridinium cation is a key structural determinant of antibacterial activity. This observation is consistent with previous reports describing cationic pyridinium derivatives as compounds that initially associate with the negatively charged bacterial surface through electrostatic interactions, facilitating subsequent insertion of their hydrophobic moieties into the membrane [12,26]. Antibacterial potency was primarily governed by the

Table 3

Selectivity Index (SI) against *S. aureus* and Half-Maximal Inhibitory Concentration (IC₅₀) in HepG2 and HDnF cells lines. IC₅₀ values obtained from triplicate runs.

Compound	SI (HepG2)	IC ₅₀ μM (HepG2)	SI (HDnF)	IC ₅₀ μM (HDnF)
18b	3.2	6.2 $\pm$ 0.2	1.6	3.2 $\pm$ 0.5
20b	5.4	11.0 $\pm$ 1.7	2.4	4.6 $\pm$ 1.2
21b	33.7	66.1 $\pm$ 0.1	>25.5	>50
22b	4.7	9 $\pm$ 0.5	3.5	7.1 $\pm$ 0.8
24b	7.3	14.9 $\pm$ 2.3	3.2	6.6 $\pm$ 0.8

Table 2

MIC values in $\mu\text{g/mL}$ and μM against clinically isolated resistant strains. Commercial antibiotics as reference. The reproducibility was calculated as the Essential Agreement (EA), defined as the percentage of MIC values falling within ± 1 two-fold dilution of the modal MIC value.

STRAIN		18b	20b	21b	22b	24b	AMX/CLV	AMP	GMC	FUA	CPX
<i>K. pneumoniae</i> 24024324	$\mu\text{g/mL}$	8	16	16	16	16	>32/2	>8	≤ 1	/	>1
	μM	15.4	32.6	31.4	30.7	32.6	>87.6/10.0	>22.9	≤ 2.1	/	>3.0
<i>K. pneumoniae</i> 24024322	$\mu\text{g/mL}$	8	16	16	16	16	>32/2	>8	>4	/	>1
	μM	15.4	32.6	31.4	30.7	32.6	>87.6/10.0	>22.9	>8.4	/	>3.0
<i>K. pneumoniae</i> 24023641	$\mu\text{g/mL}$	8	8	16	16	16	4/2	>8	≤ 1	/	0.5
	μM	15.4	16.3	31.4	30.7	32.6	11.0/10.0	>22.9	≤ 2.1	/	1.5
<i>E. coli</i> 24025100	$\mu\text{g/mL}$	8	16	16	8	16	16/2	>16	2	/	>1
	μM	15.4	32.6	31.4	15.4	32.6	43.8/10.0	>45.8	4.2	/	>3.0
<i>S. aureus</i> 24023224	$\mu\text{g/mL}$	0.5	8	4	2	8	/	/	/	4	≤ 0.5
	μM	1.0	16.3	7.9	3.8	16.3	/	/	/	7.7	≤ 1.5
<i>S. haemolyticus</i> 2402453	$\mu\text{g/mL}$	>64	8	4	2	4	/	/	/	>8	>4
	μM	>122.8	16.3	7.9	3.8	8.1	/	/	/	>15.5	>12.1
<i>E. faecium</i> 24024176	$\mu\text{g/mL}$	8	8	8	8	8	/	>16	>500	/	/
	μM	15.4	16.3	15.7	15.4	16.3	/	>45.8	>1046.9	/	/

AMX/CLV= Amoxicillin/Clavulanic Acid; AMP=Ampicillin; GMC= Gentamicin; FUA= Fusidic Acid; CPX= Ciprofloxacin.

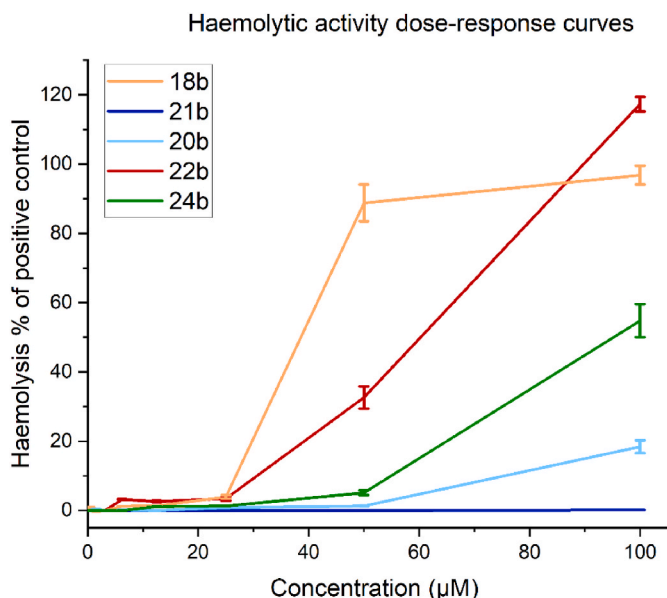


Fig. 5. Haemolytic activity (%) of **18b**, **20b**, **21b**, **22b** and **24b** at different concentrations. Data obtained from triplicate runs.

hydrophobic/hydrophilic balance, dictated by alkyl chain length, with increasing lipophilicity enhancing membrane affinity but reducing aqueous solubility, as reflected by the inverse correlation between LogP and ESOL LogS. However, excessive lipophilicity appeared detrimental, likely due to solubility limitations and steric effects. Substituents on the oxadiazole-linked aromatic ring modulated activity by tuning polarity (TPSA) and electronic properties, with strongly electron-withdrawing groups (i.e., $-\text{NO}_2$) associated with reduced activity, while moderately polar substituents (i.e., $-\text{OCH}_3$, $-\text{F}$) provided a more favourable balance. The most active derivatives (**18b–24b**) clustered within an optimal physicochemical window ($\text{LogP} \approx 4\text{--}6$; $\text{TPSA} \approx 40\text{--}60 \text{ \AA}^2$), consistent with physicochemical properties previously associated with antibacterial compounds [27,28]. Among these, **18b** emerged as the most

powerful compound, whereas cytotoxicity and selectivity were experimentally assessed only for the most active derivatives, with **21b** displaying the most favourable selectivity profile. These findings highlight a finely tuned interplay between cationic charge, lipophilicity, and electronic modulation as key determinants of activity, while supporting the use of SwissADME as a predictive tool to prioritize compounds with balanced physicochemical properties. For SwissADME results see Table S4.

3. Conclusions

Antimicrobial resistance continues to compromise the effectiveness of current antibacterial therapies, highlighting the urgent need for new active agents against resistant pathogens. In this work, a series of 1,2,4-oxadiazole-based pyridinium salts was designed, synthesized, and evaluated to investigate their potential antibacterial agents. Initial biological screening identified the pyridinium cation as an essential structural feature for antibacterial activity, since the corresponding neutral precursors were inactive ($\text{MIC} > 251.9 \text{ }\mu\text{M}$). Overall, the synthesized compounds showed a clear preference for Gram-positive bacteria, with moderate to high activity against *Staphylococcus aureus*. Longer alkyl chain on pyridinium cation increased lipophilicity and probably membrane affinity, whereas excessive hydrophobicity or the presence of long chains at both ends led to reduced activity, likely due to steric hindrance and suboptimal target interaction. Further structural optimization through the introduction of a bis-aromatic system significantly enhanced antibacterial potency. Derivatives bearing para-substituted phenyl groups and intermediate alkyl chain lengths (i.e., C10) exhibited the most favourable activity profile, with MIC values of $2 \text{ }\mu\text{M}$ against Gram-positive strains and moderate activity against Gram-negative bacteria. Among these, compound **18b**, featuring a *p*-methoxyphenyl substituent, emerged as the most potent derivative, showing an MIC value of $1 \text{ }\mu\text{M}$ against *S. aureus*. In contrast, strongly electron-withdrawing substituents such as nitro groups resulted in reduced activity, likely due to increased polarity and suboptimal amphiphilic balance.

Selected compounds retained significant activity against clinically isolated multidrug-resistant strains and displayed low haemolytic

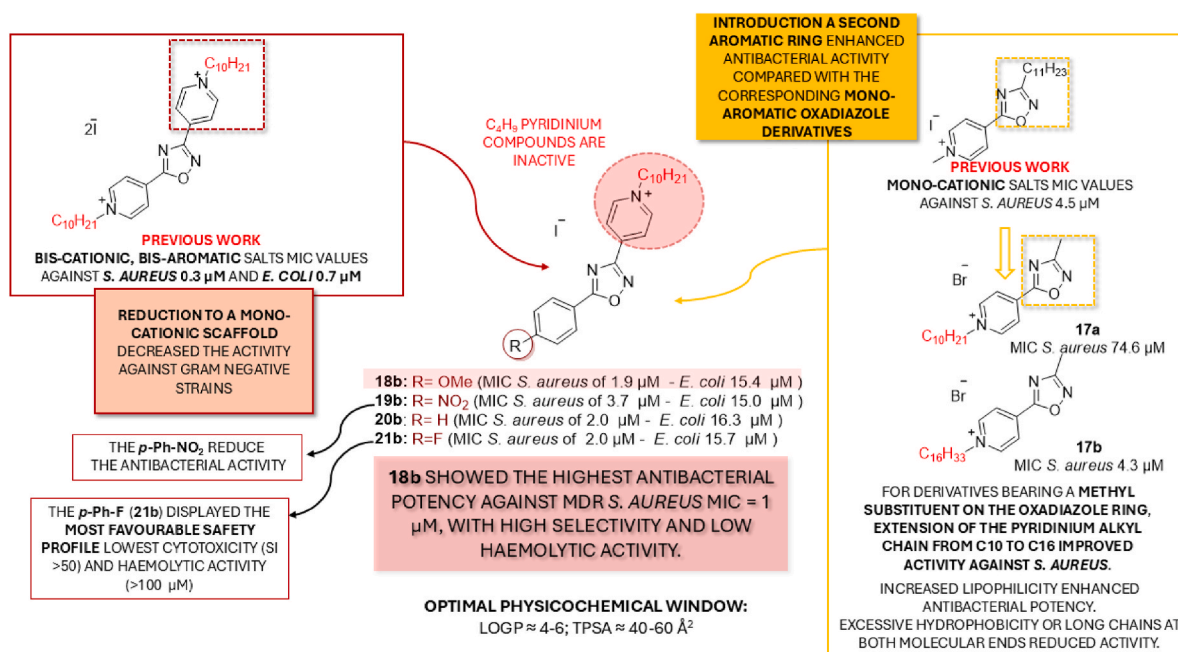


Fig. 6. Graphical summary of the SAR identified for the synthesized 1,2,4-oxadiazole-based pyridinium salts, highlighting the key structural modifications leading to improved antibacterial activity, selectivity, and activity against multidrug-resistant (MDR) bacteria.

activity together with acceptable cytotoxicity profiles, with **21b** showing the most favourable selectivity. The observed selectivity is consistent with the distinct membrane compositions of bacterial and mammalian cells generally reported for cationic antibacterial agents. Finally, SAR analysis supported by SwissADME identified an optimal physicochemical window ($\text{LogP} \approx 4\text{--}6$; $\text{TPSA} \approx 40\text{--}60 \text{ \AA}^2$), providing useful guidelines for the future design and optimization of pyridinium-based antibacterial agents.

4. Material and methods

4.1. Materials

Toluene, pyridine, xylene acetonitrile, tetrahydrofuran, dimethylsulfoxide, sodium hydroxide, sodium sulphate, acetyl chloride, undecyl chloride, benzoyl chloride, 4-methoxy benzoyl chloride, 4-fluoro benzoyl chloride, 4-nitro benzoyl chloride, methyl-4-nitro benzoate, methyl-4-methoxy benzoate, 1-iodododecane, 1-bromododecane, 1-bromohexadecane, 1-iodoethane, 1-iodobutane, 1-bromotetradecane, thionyl chloride, ethanol, isonicotinoyl chloride, nonanoyl chloride, dodecanoyl chloride, hexane, diethyl ether, ethyl acetate, petroleum ether were purchased from commercial sources and used as received. Column chromatography on silica gel, technical grade, pore size $60 \text{ \AA}$, 230–400 mesh, and particle size $40\text{--}63 \mu\text{m}$ and TLC silica gel 60 F_{254} on aluminum support were used. Amidoximes have been synthesized using the previous reported methods [29].

4.2. Methods

Chromatographic separations were carried out on silica gel (Merck, 0.040–0.063 mm), and reactions were monitored by thin-layer chromatography (TLC, from Merck KGaA, Darmstadt, Germany), using mixtures of ethyl acetate and petroleum ether (b.p. fraction 40–60°C) in different ratios as eluents. Solvents and reagents were used without further purification.

^1H NMR and ^{13}C NMR spectra were acquired using Bruker 400 MHz spectrometers, using $\text{DMSO-}d_6$ and CDCl_3 as solvent. The residual solvent peak served as the reference point. Melting points were measured using a REICHART-THERMOVAR hot-stage setup. Reversed-phase HPLC/ESI/Q-TOF HRMS investigations were conducted with HPLC/MS grade mixes of water and acetonitrile as eluents, supplemented with 0.1% (v/v) formic acid. The HPLC system utilized was an Agilent 1260 Infinity II. A reversed-phase C18 column (Luna Omega $5 \mu\text{m}$ Polar C18 $150 \times 2.1 \text{ mm}$) accompanied by a Phenomenex C18 security guard column ($4 \text{ mm} \times 3 \text{ mm}$) was employed. The flow rate was 1 mL/min, and the column temperature was maintained at 40°C. The eluent composition was modified using a linear gradient. The linear gradient went from 95% to 5% water for 10 min, then inverted to 5% to 95%. The injected volume was 10 μL . Mass spectrometry TIC and ultraviolet scanning at 250 nm were employed to analyze the eluate. An Agilent 6540 UHD accurate-mass Q-TOF spectrometer with a positive or negative Dual AJS ESI source acquired mass spectra. N_2 provided as a desolvation gas at 300°C with a flow rate of 9 L/min. The nebulizer was calibrated to 45 psi. The Sheath gas temperature was established at 350°C with a flow rate of 12 L/min. A voltage of 3.5 kV was applied to the capillary for positive ion mode, whereas 2.6 kV was utilized for negative ion mode. The fragmentor was configured to 110 V. Mass spectrometry spectra were obtained between the 100–3200 m/z range.

4.3. Synthesis and characterization

4.3.1. General procedure for the synthesis of the 1,2,4-oxadiazole - method A

1 eq. (5 mmol) of the amidoxime is weighed into a 100 mL flask and 50 mL of toluene, 1.1 eq. of pyridine and the alkyl or aryl chloride are added. The reaction mixture is stirred at reflux for 2 h. Subsequently, the

reaction solvent is eliminated using a rotary evaporator. Afterwards, the product is solubilized in ethyl acetate, and 3 extractions with distilled water and one extraction with an aqueous solution of NaOH ($\text{pH} \sim 8\text{--}9$) are performed. After collecting the organic phase was dried with Na_2SO_4 , filtered and brought to dryness with rotavapor. To allow the complete cyclization of the oxadiazole, the solid is transferred to a glass tube and heated in an oil bath at 110°C for 24 h. The reaction is monitored via TLC. The resulting reaction mixture is separated by chromatographic column into mixtures of petroleum ether and ethyl acetate.

5-methyl-3-(pyridin-4-yl)-1,2,4-oxadiazole (1) Yield 16%; m.p. = 182–185°C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) = 8.83 – 8.76 (m, 2H), 7.95 – 7.88 (m, 2H), 2.70 (s, 3H); ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ (ppm) = 178.8 (oxadiazole C), 166.7 (oxadiazole C), 151.3 (pyr-CH), 151.3 (pyr-CH), 134.0 (pyr-C), 121.3 (pyr-CH), 121.3 (pyr-CH), 12.5 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_8\text{H}_7\text{N}_3\text{O}$: 162.0662; experimental: 162.0661 [30].

3-(pyridin-4-yl)-5-undecyl-1,2,4-oxadiazole (2) Yield 63%; m.p. = 41–43°C; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 8.82 – 8.74 (m, 2H), 8.04 – 7.98 (m, 2H), 2.97 (t, $J = 7.6 \text{ Hz}$, 2H), 1.88 (m, $J = 7.5 \text{ Hz}$, 2H), 1.48 – 1.17 (m, 16H), 0.95 – 0.82 (m, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) = 181.7 (oxadiazole C), 166.6 (oxadiazole C), 151.3 (pyr-CH), 151.2 (pyr-CH), 134.0 (pyr-C), 121.3 (pyr-CH), 121.2 (pyr-CH), 31.7 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 26.3 (CH_2), 26.2 (CH_2), 22.5 (CH_2), 14.3 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{18}\text{H}_{27}\text{N}_3\text{O}$: 302.2154; experimental: 302.2158 [16].

3-methyl-5-(pyridin-4-yl)-1,2,4-oxadiazole (3) Yield 33%; m.p. = 82°C; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ (ppm) = 8.93 – 8.82 (m, 2H), 8.05 – 7.97 (m, 2H), 2.46 (s, 3H); ^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ (ppm) = 173.6 (oxadiazole C), 168.5 (oxadiazole C), 151.6 (pyr-CH), 151.6 (pyr-CH), 130.8 (pyr-C), 121.6 (pyr-CH), 121.6 (pyr-CH), 11.7 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_8\text{H}_7\text{N}_3\text{O}$: 162.0698; experimental: 162.0700 [30]. [30].

5-(4-methoxyphenyl)-3-(pyridin-4-yl)-1,2,4-oxadiazole (4) Yield 24%; m.p. = 145–146°C; ^1H NMR (300 MHz, CDCl_3) δ (ppm) = 8.72 (d, $J = 6.0 \text{ Hz}$, 2H), 8.05 (d, $J = 8.9 \text{ Hz}$, 2H), 7.67 (d, $J = 6.0 \text{ Hz}$, 2H), 6.97 (d, $J = 8.9 \text{ Hz}$, 2H), 3.89 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) = 176.3 (oxadiazole C), 167.2 (oxadiazole C), 163.4 (Ph-C), 150.6 (pyr-CH), 134.6 (pyr-C), 130.2 (pyr-CH), 121.3 (Ph-CH), 116.3 (Ph-CH), 114.6 (Ph-C), 55.6 (O-CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_2$: 254.0924; experimental: 254.0924 [31].

5-phenyl-3-(pyridin-4-yl)-1,2,4-oxadiazole (6) Yield 87%; m.p. = 95–97°C; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 8.83 (dd, $J = 4.6, 1.6 \text{ Hz}$, 2H), 8.30 – 8.04 (m, 4H), 7.60 (dd, $J = 17.2, 11.1 \text{ Hz}$, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) = 176.7 (oxadiazole C), 167.4 (oxadiazole C), 150.2 (pyr-CH), 133.3 (pyr-C), 130.0 (pyr-CH), 129.4 (Ph-CH), 128.4 (Ph-CH), 123.9 (Ph-CH), 121.7 (Ph-C); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{13}\text{H}_9\text{N}_3\text{O}$: 224.0818; experimental: 224.0832 [32].

5-(4-fluorophenyl)-3-(pyridin-4-yl)-1,2,4-oxadiazole (7) Yield 58%; m.p. = 122–123°C; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 8.84 (dd, $J = 4.5, 1.6 \text{ Hz}$, 2H), 8.31 – 8.21 (m, 2H), 8.09 (dd, $J = 4.5, 1.6 \text{ Hz}$, 2H), 7.32 – 7.23 (m, 2H); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) = 175.8 (oxadiazole C), 167.5 (oxadiazole C), 166.7 (pyr-CH), 165.0 (Ph-C), 151.1 (pyr-CH), 150.5 (pyr-C), 134.8 (pyr-CH), 130.9 (Ph-CH), 130.8 (Ph-CH), 121.6 (Ph-CH), 120.3 (Ph-CH), 116.9 (Ph-C), 116.7 (Ph-CH); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical per $\text{C}_{13}\text{H}_8\text{N}_3\text{OF}$: 242.0724; experimental: 242.0725.

3-(4-methoxyphenyl)-5-(pyridin-4-yl)-1,2,4-oxadiazole (8) Yield 27%; m.p. = 149–150°C; ^1H NMR (400 MHz, CDCl_3) δ (ppm) = 8.87 (dd, $J = 4.4, 1.7 \text{ Hz}$, 2H), 8.13 – 8.08 (m, 2H), 8.05 (dd, $J = 4.5, 1.6 \text{ Hz}$, 2H), 7.05 – 7.00 (m, 2H), 3.88 (s, 3H); ^{13}C NMR (151 MHz, CDCl_3) δ (ppm) = 173.3 (oxadiazole C), 169.3 (oxadiazole C), 162.4 (Ph-C), 150.2 (pyr-CH), 132.3 (pyr-C), 129.4 (pyr-CH), 121.9 (Ph-CH), 118.8 (Ph-CH), 114.6 (Ph-C), 55.6 (O-CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_2$: 254.0924; experimental: 254.0923.

3-phenyl-5-(pyridin-4-yl)-1,2,4-oxadiazole (10) Yield 59%; m.p. = 149–151°C; ^1H NMR (400 MHz, CDCl_3), δ (ppm) = 8.90 (dd, J = 4.5, 1.6 Hz, 2H), 8.26–8.02 (m, 4H), 7.54 (ddd, J = 5.4, 4.5, 1.4 Hz, 3H); ^{13}C NMR (151 MHz, CDCl_3), δ (ppm) = 173.6 (oxadiazole C), 169.6 (oxadiazole C), 150.3 (pyr-CH), 132.1 (pyr-C), 131.8 (pyr-CH), 129.1 (Ph-CH), 127.7 (Ph-CH), 126.4 (Ph-CH), 121.9 (Ph-C); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical for $\text{C}_{13}\text{H}_9\text{N}_3\text{O}$: 224.0818; experimental: 224.0817 [33].

4.3.2. General procedure for the synthesis of the 1,2,4-oxadiazole -method B

1 eq. (5 mmol) of the amidoxime is weighed into a 100 mL flask and 50 mL of xylene, 1.1 eq. of pyridine and chloride are added. The reaction mixture is stirred at reflux for 2 h. The reaction is monitored via TLC. Subsequently, the reaction solvent is eliminated using a rotary evaporator. Afterwards, basic water is added into the flask until pH $\approx$ 8. The obtained solid is filtered under vacuum utilizing a Buchner funnel.

(5-(4-methoxyphenyl)-3-(pyridin-4-yl)-1,2,4-oxadiazole) (4) Yield 84%.

3-phenyl-5-(pyridin-4-yl)-1,2,4-oxadiazole (10) Yield 20%.

4.3.3. General procedure for the synthesis of the 1,2,4-oxadiazole - method C

To 1 eq. of the amidoxime (5 mmol), previously solubilized in the minimum amount of dimethyl sulfoxide, 1.5 eq. of the methyl ester and 1 eq. of grounded NaOH was added. The mixture is stirred at room temperature for 30 min. The reaction is followed by TLC, using petroleum ether and ethyl acetate as eluent. Afterwards, the reaction is quenched with cold water (5°C) and the resulting solid is filtered under vacuum. Finally, the product is purified by chromatographic column into mixtures of petroleum ether and ethyl acetate.

(5-(4-nitrophenyl)-3-(pyridin-4-yl)-1,2,4-oxadiazole) (5) Yield 94%; m.p. = 169–171°C; ^1H NMR (400 MHz, CDCl_3), δ (ppm) = 8.85 (d, J = 5.6 Hz, 2H, Ar), 8.44 (s, 4H), 8.09 (d, J = 6.0 Hz, 2H); ^{13}C NMR (151 MHz, CDCl_3), δ (ppm) = 174.5 (oxadiazole C), 167.8 (oxadiazole C), 150.5 (Ph-C), 134.0 (pyr-CH), 129.3 (pyr-C), 129.0 (pyr-CH), 124.5 (Ph-C), 121.4 (Ph-CH), 77.4 (Ph-CH), 77.0 (Ph-CH), 76.6 (Ph-CH); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{13}\text{H}_8\text{N}_4\text{O}_3$: 269.0669; experimental: 269.0670.

(3-(4-methoxyphenyl)-5-(pyridin-4-yl)-1,2,4-oxadiazole) (8) Yield 20%.

3-(4-nitrophenyl)-5-(pyridin-4-yl)-1,2,4-oxadiazole (9) Yield 32%; m.p. = 168–169°C; ^1H NMR (400 MHz, CDCl_3), δ (ppm) = 8.92 (d, J = 6.1 Hz, 2H, Ar), 8.39 (s, 4H), 8.07 (d, J = 6.1 Hz, 2H); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{13}\text{H}_8\text{N}_4\text{O}_3$: 269.0669; experimental: 269.0669.

4.3.4. General procedure synthesis amides

The nitro derivatives (2.12 mmol) are solubilized in ethanol (150 mL) and 5 eq. of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ are added and placed at reflux for 5 h. Then the solvent is removed under reduced pressure, and the residue is treated with basic water (pH > 10). The organic phase was dried with Na_2SO_4 , filtered and evaporated. Without further purification, the amine (11 or 13) is solubilized in THF (40 mL), then 1.1 eq. of the acyl chloride and pyridine are added. The reaction is conducted at room temperature for 24 h under magnetic stirrer. Then the THF is removed, and the residue is treated with basic water $8 < \text{pH} < 9$ and extracted with ethyl acetate three times. Finally, the organic phase is washed with distilled water, and the solvent is removed under vacuum conditions.

N-(4-(3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl)phenyl)nonanamide (12a) Yield 80%; m.p. = 135–136°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.33 (s, 1H), 8.83 (d, J = 5.7 Hz, 2H), 8.14 (d, J = 8.8 Hz, 2H), 8.04 (d, J = 8.8 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H), 2.36 (t, J = 7.4 Hz, 2H), 1.60 (m, 2H), 1.27 (d, J = 13.2 Hz, 10H), 0.85–0.79 (m, 3H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$), δ (ppm) = 167.8 (C(O)NH), 151.3 (oxadiazole C), 144.7 (oxadiazole C), 134.0 (pyr-CH), 129.8 (pyr-C), 121.5 (pyr-CH), 119.6 (Ph-CH), 117.6 (Ph-C), 29.1 (CH_2), 25.4 (CH_2), 22.5 (CH_2),

14.4 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{22}\text{H}_{26}\text{N}_4\text{O}_2$: 379.2056; experimental: 379.2057.

N-(4-(3-(pyridin-4-yl)-1,2,4-oxadiazol-5-yl)phenyl)dodecanamide (12b) Yield 87%; m.p. = 130–131°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.33 (s, 1H), 8.83 (d, J = 5.9 Hz, 2H), 8.14 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 6.1 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H), 2.36 (t, J = 7.4 Hz, 2H), 2.08 (m, 2H), 1.67 (m, 16H), 0.84–0.80 (m, 3H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$), δ (ppm) = 174.1 (C(O)NH), 172.3 (oxadiazole C), 168.8 (oxadiazole C), 151.6 (pyr-CH), 143.0 (pyr-C), 130.9 (Ph-C), 128.5 (pyr-CH), 121.8 (Ph-CH), 120.4 (Ph-CH), 119.7 (Ph-C), 37.0 (CH_2), 34.8 (CH_2), 31.8 (CH_2), 30.9 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 25.5 (CH_2), 22.6 (CH_2), 14.4 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{25}\text{H}_{32}\text{N}_4\text{O}_2$: 421.2525; experimental: 421.2525.

N-(4-(5-(pyridin-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)nonanamide (14a) Yield 72%; m.p. = 140–141°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.20 (s, 1H), 8.91 (d, J = 6.1 Hz, 2H), 8.08 (d, J = 6.1 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.82 (d, J = 8.9 Hz, 2H), 2.34 (t, J = 7.5 Hz, 2H), 1.60 (m, 2H), 1.27 (m, 10H), 0.90–0.80 (m, 3H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$), δ (ppm) = 174.1 (C(O)NH), 172.3 (oxadiazole C), 168.7 (oxadiazole C), 151.6 (pyr-CH), 143.0 (pyr-C), 130.9 (Ph-C), 128.5 (pyr-CH), 121.8 (Ph-CH), 120.4 (Ph-CH), 119.7 (Ph-C), 37.0 (CH_2), 31.7 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 25.5 (CH_2), 22.5 (CH_2), 14.4 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{22}\text{H}_{26}\text{N}_4\text{O}_2$: 379.2129; experimental: 379.2132.

N-(4-(5-(pyridin-4-yl)-1,2,4-oxadiazol-3-yl)phenyl)dodecanamide (14b) Yield 87%; m.p. = 142–144°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.20 (s, 1H), 8.91 (d, J = 6.1 Hz, 2H), 8.09 (d, J = 6.1 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.83 (d, J = 11.2 Hz, 2H), 2.34 (t, J = 7.4 Hz, 2H), 1.60 (m, 2H), 1.26 (m, 16H), 0.88–0.80 (m, 3H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$), δ (ppm) = 174.1 (C(O)NH), 172.3 (oxadiazole C), 168.8 (oxadiazole C), 151.6 (pyr-CH), 143.0 (pyr-C), 130.9 (Ph-C), 128.5 (pyr-CH), 121.8 (Ph-CH), 120.4 (Ph-CH), 119.7 (Ph-C), 37.0 (CH_2), 34.8 (CH_2), 31.8 (CH_2), 30.9 (CH_2), 29.5 (CH_2), 29.2 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 25.5 (CH_2), 22.6 (CH_2), 14.4 (CH_3); HRMS (ESI) $[\text{M}+\text{H}]^+$: m/z theoretical $\text{C}_{25}\text{H}_{32}\text{N}_4\text{O}_2$: 421.2598; experimental: 421.2598.

4.3.5. General procedures synthesis pyridinium salts -method A

50 mg of the neutral compound (1 eq.) is placed in a closed tube, then 1 mL of halogenoalkane is added. The reaction is placed in an oil bath at a temperature of 80°C for 24 h or 5 days under continuous stirring. The solvent was removed under reduced pressure. The resulting solid was then washed three times with 20 mL of hexane and three times with 20 mL of diethyl ether. For each washing step, after solvent addition, the mixture was sonicated for 1 min and centrifuged for 5 min at 4000 rpm. The supernatant was removed after each centrifugation step, and the washing procedure was repeated as described above to afford the pure product.

1-decyl-4-(5-(4-nonanamidophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium iodide (25a) Yield 45%; m.p. = 203–204°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.39 (s, 1H), 9.30 (d, J = 6.7 Hz, 2H), 8.72 (d, J = 6.7 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.8 Hz, 2H), 4.70 (t, J = 7.4 Hz, 2H), 2.38 (t, J = 7.4 Hz, 2H), 1.96 (s, 2H), 1.66–1.54 (m, 2H), 1.27 (d, J = 19.8 Hz, 24H), 0.87–0.82 (m, 6H); ^{13}C NMR (151 MHz, $\text{DMSO}-d_6$), δ (ppm) = 177.2 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.6 (pyr-CH), 144.9 (pyr-C), 136.9 (pyr-CH), 129.9 (Ph-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 92.2 (Ph-C), 61.6 (N- CH_2), 37.0 (CH_2), 31.7 (CH_2), 31.2 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 25.9 (CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.4 (CH_3); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{32}\text{H}_{47}\text{N}_4\text{O}_2^+$: 519.3694; experimental: 519.3699.

1-dodecyl-4-(5-(4-nonanamidophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium bromide (25b) Yield 78%; m.p. = 226–227°C; ^1H NMR (400 MHz, $\text{DMSO}-d_6$), δ (ppm) = 10.42 (s, 1H), 9.32 (d, J = 6.9 Hz, 2H), 8.71 (d, J = 6.9 Hz, 2H), 8.24–8.10 (m, 2H), 7.93–7.85 (m, 2H), 4.70 (t,

$J = 7.4$ Hz, 2H), 2.43 – 2.29 (m, 2H), 1.95 (s, 2H), 1.56 (dt, $J = 45.7$, 22.8 Hz, 2H), 1.26 (dd, $J = 22.4$, 7.5 Hz, 28H), 0.84 (q, $J = 6.8$ Hz, 6H). ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.2 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (pyr-CH), 129.8 (Ph-CH), 125.9 (Ph-CH), 119.7 (Ph-C), 117.0 (Ph-C), 61.6 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.2 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\text{C}_{34}\text{H}_{51}\text{N}_4\text{O}_2^+$: 547.4007; experimental: 547.4054.

1-decyl-4-(5-(4-dodecanamidophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium iodide (26a) Yield 68%; m.p. = 236–237°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.38 (s, 1H), 9.30 (d, $J = 6.9$ Hz, 2H), 8.72 (d, $J = 6.9$ Hz, 2H), 8.18 (d, $J = 8.8$ Hz, 2H), 7.91 (d, $J = 8.8$ Hz, 2H), 4.70 (t, $J = 7.4$ Hz, 2H), 2.38 (t, $J = 7.4$ Hz, 2H), 1.96 (s, 2H), 1.66 – 1.54 (m, 2H), 1.36 – 1.18 (m, 30H), 0.85 (dd, $J = 6.9$, 5.9 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.2 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (Ph-C), 129.9 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 61.6 (N-CH₂), 37.0 (CH₂), 31.8 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.9 (CH₂), 25.9 (CH₂), 25.4 (CH₂), 22.6 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{85}{85}$: 561.4163; experimental: 561.4164.

4-(5-(4-dodecanamidophenyl)-1,2,4-oxadiazol-3-yl)-1-dodecylpyridin-1-ium bromide (26b) Yield 94%; m.p. = 237–238°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.40 (s, 1H), 9.30 (d, $J = 6.9$ Hz, 2H), 8.71 (d, $J = 6.9$ Hz, 2H), 8.18 (d, $J = 8.8$ Hz, 2H), 7.91 (d, $J = 8.8$ Hz, 2H), 4.70 (t, $J = 7.3$ Hz, 2H), 2.37 (t, $J = 7.3$ Hz, 2H), 1.95 (s, 2H), 1.64 – 1.53 (m, 2H), 1.31 – 1.18 (m, 34H), 0.84 (dd, $J = 6.9$, 6.3 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.3 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (Ph-C), 129.8 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 61.6 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\text{C}_{37}\text{H}_{57}\text{N}_4\text{O}_2^+$: 589.4476; experimental: 589.4476.

4-(5-(4-dodecanamidophenyl)-1,2,4-oxadiazol-3-yl)-1-tetradecylpyridin-1-ium bromide (26c) Yield 68%, m.p. = 242–243°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.39 (s, 1H), 9.30 (d, $J = 6.9$ Hz, 2H), 8.71 (d, $J = 6.9$ Hz, 2H), 8.17 (d, $J = 8.9$ Hz, 2H), 7.91 (d, $J = 8.9$ Hz, 2H), 4.69 (t, $J = 7.4$ Hz, 2H), 2.37 (t, $J = 7.4$ Hz, 2H), 1.95 (s, 2H), 1.67 – 1.53 (m, 2H), 1.31 – 1.18 (m, 38H), 0.84 (dd, $J = 6.9$, 5.8 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.3 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (Ph-C), 129.8 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 61.6 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{89}{89}$: 617.4789; experimental: 617.4789.

1-decyl-4-(3-(4-nonanamidophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium iodide (27a) Yield 22%; m.p. = 206–207°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.25 (s, 1H), 9.37 (d, $J = 5.6$ Hz, 2H), 8.83 (d, $J = 5.6$ Hz, 2H), 7.96 (dd, $J = 64.1$, 8.4 Hz, 4H), 4.72 (s, 2H), 2.36 (s, 2H), 1.98 (s, 2H), 1.61 (s, 2H), 1.25 (s, 24H), 0.85 (s, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.9 (oxadiazole C), 169.2 (oxadiazole C), 146.9 (pyr-CH), 143.3 (pyr-C), 137.7 (Ph-CH), 128.6 (pyr-CH), 126.5 (Ph-CH), 119.8 (Ph-CH), 119.8 (Ph-C), 61.8 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.3 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.9 (CH₂), 25.9 (CH₂), 25.5 (CH₂), 22.6 (CH₂), 21.2 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{82}{82}$: 519.3694; experimental: 519.3698.

4-(3-(4-nonanamidophenyl)-1,2,4-oxadiazol-5-yl)-1-tetradecylpyridin-1-ium bromide (27c) Yield 76%; m.p. = 170–201°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.26 (s, 1H), 9.39 (d, $J = 6.9$ Hz, 2H), 8.83 (d, $J = 6.9$ Hz, 2H), 8.07 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.8$ Hz, 2H), 4.73 (t, $J = 7.4$ Hz, 2H), 2.37 (t, $J = 7.4$ Hz, 2H), 1.98 (d, $J = 7.6$ Hz, 2H), 1.67 – 1.56 (m, 2H), 1.36 – 1.18 (m, 32H), 0.86 (q, $J = 6.8$ Hz, 6H); HRMS (ESI) $[M]^+$: m/z theoretical for $\text{C}_{36}\text{H}_{55}\text{N}_4\text{O}_2^+$: 575.432; experimental:

575.4338.

1-hexadecyl-4-(3-(4-nonanamidophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium bromide (27d) Yield 22%, m.p. = 190–220°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.39 (s, 1H), 9.40 (d, $J = 6.7$ Hz, 2H), 8.87 (dd, $J = 26.4$, 6.4 Hz, 2H), 8.22 – 7.63 (m, 4H), 4.72 (s, 2H), 2.37 (s, 2H), 1.93 (s, 2H), 1.59 (s, 2H), 1.35 – 1.10 (m, 36H), 1.00 – 0.70 (m, 6H); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{88}{88}$: 603.4633; experimental: 603.4649.

1-decyl-4-(3-(4-dodecanamidophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium iodide (28a) Yield 40%; m.p. = 199–200°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.24 (s, 1H), 9.37 (d, $J = 6.7$ Hz, 2H), 8.83 (d, $J = 6.7$ Hz, 2H), 8.07 (d, $J = 8.7$ Hz, 2H), 7.86 (d, $J = 8.7$ Hz, 2H), 4.72 (t, $J = 7.4$ Hz, 2H), 2.35 (t, $J = 7.4$ Hz, 2H), 1.96 (s, 2H), 1.60 (d, $J = 6.7$ Hz, 2H), 1.34 – 1.19 (m, 30H), 0.85 (dd, $J = 6.8$, 5.9 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.3 (oxadiazole C), 146.9 (pyr-CH), 143.3 (pyr-C), 137.7 (Ph-C), 128.6 (pyr-CH), 126.5 (Ph-CH), 119.8 (Ph-C), 61.8 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.3 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.9 (CH₂), 25.4 (CH₂), 22.6 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{85}{85}$: 561.4163; experimental: 561.4184.

4-(3-(4-dodecanamidophenyl)-1,2,4-oxadiazol-5-yl)-1-dodecylpyridin-1-ium bromide (28b) Yield 60%; m.p. = 170–210°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.23 (s, 1H), 9.36 (d, $J = 6.8$ Hz, 2H), 8.82 (d, $J = 6.8$ Hz, 2H), 8.07 (d, $J = 8.8$ Hz, 2H), 7.85 (d, $J = 8.8$ Hz, 2H), 4.71 (t, $J = 7.4$ Hz, 2H), 2.35 (t, $J = 7.4$ Hz, 2H), 1.96 (dd, $J = 9.1$, 4.3 Hz, 2H), 1.65 – 1.56 (m, 2H), 1.33 – 1.20 (m, 34H), 0.84 (t, $J = 6.7$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.2 (oxadiazole C), 146.8 (pyr-CH), 143.2 (pyr-C), 137.7 (Ph-C), 128.5 (pyr-CH), 126.5 (Ph-CH), 119.7 (Ph-CH), 61.8 (N-CH₂), 36.9 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\text{C}_{37}\text{H}_{57}\text{N}_4\text{O}_2^+$: 589.4476; experimental: 589.4487.

4-(3-(4-dodecanamidophenyl)-1,2,4-oxadiazol-5-yl)-1-tetradecylpyridin-1-ium bromide (28c) Yield 49%; m.p. = 215–227°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.26 (s, 1H), 9.38 (d, $J = 6.1$ Hz, 2H), 8.83 (d, $J = 6.1$ Hz, 2H), 8.07 (d, $J = 8.5$ Hz, 2H), 7.86 (d, $J = 8.5$ Hz, 2H), 4.72 (t, $J = 7.0$ Hz, 2H), 2.36 (t, $J = 7.0$ Hz, 2H), 1.96 (s, 2H), 1.60 (s, 2H), 1.26 (d, $J = 23.4$ Hz, 38H), 0.84 (d, $J = 6.6$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.3 (oxadiazole C), 146.9 (pyr-CH), 143.3 (pyr-C), 137.7 (Ph-C), 128.6 (pyr-CH), 126.5 (Ph-CH), 119.8 (Ph-CH), 61.8 (N-CH₂), 37.0 (CH₂), 31.8 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.5 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[M]^+$: m/z theoretical for $\frac{89}{89}$: 617.4789; experimental: 617.4789.

4.3.6. General procedures synthesis pyridinium salts - method B

100 mg of the neutral compound (**1-10**) 1 eq. is placed in a closed tube, then 2 eq. of halogenoalkane and 2 ml of acetonitrile were added. The reaction is placed in an oil bath at a temperature of 80°C for 24 h or 5 days under continuous stirring. The solvent was removed under reduced pressure. The resulting solid was then washed three times with 20 mL of hexane and three times with 20 mL of diethyl ether. For each washing step, after solvent addition, the mixture was sonicated for 1 min and centrifuged for 5 min at 4000 rpm. The supernatant was removed after each centrifugation step, and the washing procedure was repeated as described above to afford the pure product.

1-decyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium iodide (15a) Yield 73%; m.p. = 140–144°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.27 (d, $J = 6.6$ Hz, 2H), 8.65 (d, $J = 6.6$ Hz, 2H), 4.68 (t, $J = 7.4$ Hz, 2H), 2.78 (s, 3H), 1.94 (s, 2H), 1.27 (d, $J = 23.6$ Hz, 14H), 0.85 (s, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 180.0 (oxadiazole C), 165.0 (oxadiazole C), 146.6 (pyr-CH), 141.2 (pyr-C), 125.7 (pyr-CH), 61.5 (N-CH₂), 31.7 (CH₂), 31.1 (CH₂), 29.3 (CH₂), 29.2 (CH₂),

29.1 (CH₂), 28.9 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃), 12.7 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₁₈H₂₈N₃O⁺: 302.2227; experimental: 302.2227.

1-hexadecyl-4-(5-methyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium bromide (15b) Yield 49%; m.p. = 118–122°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.28 (d, *J* = 6.7 Hz, 2H), 8.64 (d, *J* = 6.5 Hz, 2H), 4.68 (t, *J* = 7.4 Hz, 2H), 2.78 (s, 3H), 1.94 (s, 2H), 1.26 (d, *J* = 26.2 Hz, 26H), 0.85 (t, *J* = 6.7 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 180.0 (oxadiazole C), 174.4 (oxadiazole C), 146.6 (pyr-CH), 141.2 (pyr-C), 132.4 (pyr-CH), 61.5 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃), 12.6 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₄H₄₀N₃O⁺: 386.3166; experimental: 386.3176.

1-ethyl-4-(5-undecyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium iodide (16a) Yield: 80.4%; m.p. = 69–72°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.28 (d, *J* = 6.8 Hz, 2H), 8.65 (d, *J* = 6.8 Hz, 2H), 4.73 (q, *J* = 7.4 Hz, 2H), 3.10 (t, *J* = 7.4 Hz, 2H), 1.88 – 1.74 (m, 2H), 1.58 (t, *J* = 7.4 Hz, 3H), 1.42 – 1.16 (m, 16H), 0.85 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 182.9 (oxadiazole C), 164.9 (oxadiazole C), 146.4 (pyr-CH), 141.2 (pyr-C), 125.7 (pyr-CH), 57.2 (N-CH₂), 31.7 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.0 (CH₂), 28.9 (CH₂), 28.7 (CH₂), 26.2 (CH₂), 22.5 (CH₂), 16.7 (CH₃), 14.4 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₀H₃₂N₃O⁺: 330.2542; experimental: 330.2542.

1-butyl-4-(5-undecyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium (16b) Yield 99%; m.p. = 45–47°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.27 (d, *J* = 6.9 Hz, 2H), 8.65 (d, *J* = 6.9 Hz, 2H), 4.70 (t, *J* = 7.5 Hz, 2H), 3.10 (t, *J* = 7.5 Hz, 2H), 1.94 (t, *J* = 7.5 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.43 – 1.19 (m, 18H), 0.93 (t, *J* = 7.4 Hz, 3H), 0.85 (t, *J* = 6.9 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 182.9 (oxadiazole C), 164.9 (oxadiazole C), 146.6 (pyr-CH), 141.3 (pyr-C), 125.8 (pyr-CH), 61.3 (N-CH₂), 33.1 (CH₂), 31.7 (CH₂), 29.3 (CH₂), 29.1 (CH₂), 28.9 (CH₂), 28.7 (CH₂), 26.3 (CH₂), 22.5 (CH₂), 19.2 (CH₂), 14.4 (CH₃), 13.8 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₂H₃₆N₃O⁺: 358.2853; experimental: 358.2854.

1-decyl-4-(5-undecyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium (16c) Yield 89%; m.p. = 60–63°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.50 (d, *J* = 6.2 Hz, 2H), 8.64 (d, *J* = 6.2 Hz, 2H), 5.04 (t, *J* = 7.5 Hz, 2H), 3.01 (t, *J* = 7.5 Hz, 2H), 2.08 (dd, *J* = 14.2, 7.5 Hz, 2H), 1.94 – 1.79 (m, 2H), 1.42 – 1.21 (m, 30H), 0.86 (d, *J* = 4.5 Hz, 6H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 182.9 (oxadiazole C), 164.9 (oxadiazole C), 146.6 (pyr-CH), 141.2 (pyr-C), 125.8 (pyr-CH), 61.5 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 28.9 (CH₂), 28.8 (CH₂), 28.7 (CH₂), 26.3 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₈H₄₈N₃O⁺: 442.3792; experimental: 442.3798.

1-decyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)pyridin-1-ium iodide (17a) Yield 90%; m.p. = 130–132°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.33 (d, *J* = 7.0 Hz, 2H), 8.75 (d, *J* = 7.0 Hz, 2H), 4.69 (d, *J* = 7.5 Hz, 2H), 2.53 (s, 3H), 1.99 – 1.89 (m, 2H), 1.32 – 1.12 (m, 14H), 0.84 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 171.3 (oxadiazole C), 169.2 (oxadiazole C), 146.8 (pyr-CH), 137.6 (pyr-C), 126.4 (pyr-CH), 61.7 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 28.9 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃), 11.7 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₁₈H₂₈N₃O⁺: 302.2227; experimental: 302.2228.

1-hexadecyl-4-(3-methyl-1,2,4-oxadiazol-5-yl)pyridin-1-ium iodide (17b) Yield 53%; m.p. = 148–150°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.33 (d, *J* = 6.6 Hz, 2H), 8.75 (d, *J* = 6.6 Hz, 2H), 4.69 (t, *J* = 7.4 Hz, 2H), 2.53 (s, 3H), 1.98 – 1.90 (m, 2H), 1.25 (d, *J* = 15.6 Hz, 26H), 0.84 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 171.3 (oxadiazole C), 169.2 (oxadiazole C), 146.8 (pyr-CH), 137.6 (pyr-C), 126.4 (pyr-CH), 61.7 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃), 11.7 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₄H₄₀N₃O⁺: 386.3166; experimental: 386.3166.

1-butyl-4-(5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium

(18a) Yield 50%; m.p. = 189–191°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.31 (d, *J* = 6.0 Hz, 2H), 8.72 (d, *J* = 6.0 Hz, 2H), 8.19 (d, *J* = 8.6 Hz, 2H), 7.25 (d, *J* = 8.6 Hz, 2H), 4.71 (t, *J* = 7.3 Hz, 2H), 3.91 (s, 3H), 2.02 – 1.87 (m, 2H), 1.35 (dd, *J* = 14.7, 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 177.3 (oxadiazole C), 165.5 (oxadiazole C), 164.1 (Ph-C), 146.6 (pyr-CH), 141.3 (pyr-C), 130.8 (pyr-CH), 125.9 (Ph-CH), 115.7 (Ph-CH), 115.3 (Ph-C), 61.3 (N-CH₂), 56.3 (O-CH₃), 33.1 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₁₈H₂₀N₃O₂⁺: 310.155, experimental: 310.1555.

1-decyl-4-(5-(4-methoxyphenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium (18b) Yield 70%; m.p. = 159–161°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.30 (d, *J* = 6.9 Hz, 2H), 8.72 (d, *J* = 6.8 Hz, 2H), 8.23 – 8.17 (m, 2H), 7.30 – 7.22 (m, 2H), 4.70 (t, *J* = 7.4 Hz, 2H), 3.91 (s, 3H), 2.01 – 1.91 (m, 2H), 1.27 (d, *J* = 27.2 Hz, 14H), 0.85 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 177.3 (oxadiazole C), 165.5 (oxadiazole C), 164.1 (Ph-C), 146.6 (pyr-CH), 141.3 (pyr-C), 130.8 (pyr-CH), 125.9 (Ph-CH), 115.7 (Ph-CH), 115.3 (Ph-C), 61.5 (N-CH₂), 56.3 (O-CH₃), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₄H₃₂N₃O₂⁺: 394.2489, experimental: 394.2491.

1-butyl-4-(5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium (19a) Yield 50%; m.p. = 150–151°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.35 (d, *J* = 6.9 Hz, 2H), 8.78 (d, *J* = 6.9 Hz, 2H), 8.55 – 8.48 (m, 4H), 4.74 (t, *J* = 7.5 Hz, 2H), 1.97 (t, *J* = 7.5 Hz, 2H), 1.36 (d, *J* = 7.5 Hz, 2H), 0.95 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 175.8 (oxadiazole C), 165.9 (oxadiazole C), 150.8 (Ph-C), 146.8 (pyr-CH), 140.9 (pyr-C), 130.3 (pyr-CH), 128.5 (Ph-C), 126.0 (Ph-CH), 125.3 (Ph-CH), 61.4 (N-CH₂), 33.1 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₁₇H₁₇N₄O₃⁺: 325.1295, experimental: 325.1298.

1-decyl-4-(5-(4-nitrophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium (19b) Yield 92%; m.p. = 136–137°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.34 (d, *J* = 7.0 Hz, 2H), 8.78 (d, *J* = 7.0 Hz, 2H), 8.55 – 8.49 (m, 4H), 4.72 (t, *J* = 7.4 Hz, 2H), 2.03 – 1.93 (m, 2H), 1.29 (d, *J* = 29.2 Hz, 14H), 0.89 – 0.82 (m, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 175.8 (oxadiazole C), 165.9 (oxadiazole C), 150.8 (Ph-C), 146.7 (pyr-CH), 140.8 (pyr-C), 130.3 (pyr-CH), 128.5 (Ph-C), 126.0 (Ph-CH), 125.3 (Ph-CH), 61.6 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₃H₂₉N₄O₃⁺: 409.2234, experimental: 409.2237.

1-butyl-4-(5-phenyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium (20a) Yield 72%; m.p. = 183–185°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.33 (d, *J* = 6.3 Hz, 2H), 8.75 (d, *J* = 6.3 Hz, 2H), 8.25 (d, *J* = 7.4 Hz, 2H), 7.75 (dt, *J* = 15.1, 7.4 Hz, 3H), 4.73 (t, *J* = 7.3 Hz, 2H), 1.96 (s, 2H), 1.35 (d, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 177.4 (oxadiazole C), 165.7 (oxadiazole C), 146.7 (pyr-CH), 141.2 (pyr-C), 134.6 (pyr-CH), 130.2 (Ph-CH), 128.6 (Ph-CH), 125.9 (Ph-CH), 123.1 (Ph-C), 61.3 (N-CH₂), 33.1 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₁₇H₁₈N₃O⁺: 280.1444; experimental: 280.1448.

1-decyl-4-(5-phenyl-1,2,4-oxadiazol-3-yl)pyridin-1-ium (20b) Yield 68%; m.p. = 190–191°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.32 (d, *J* = 6.8 Hz, 2H), 8.75 (d, *J* = 6.8 Hz, 2H), 8.31 – 8.22 (m, 2H), 7.80 (d, *J* = 7.4 Hz, 1H), 7.72 (t, *J* = 7.4 Hz, 2H), 4.71 (t, *J* = 7.4 Hz, 2H), 1.96 (s, 2H), 1.28 (d, *J* = 27.9 Hz, 14H), 0.85 (t, *J* = 6.8 Hz, 3H); ¹³C NMR (151 MHz, DMSO-*d*₆), δ (ppm) = 177.4 (oxadiazole C), 165.7 (oxadiazole C), 146.7 (pyr-CH), 141.2 (pyr-C), 134.6 (pyr-CH), 130.2 (Ph-CH), 128.6 (Ph-CH), 125.9 (Ph-CH), 123.1 (Ph-C), 61.5 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) [M]⁺: *m/z* theoretical for C₂₃H₃₀N₃O⁺: 364.2383; experimental: 364.2407.

1-butyl-4-(5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium (21a) Yield 69%; m.p. = 149–151°C; ¹H NMR (400 MHz, DMSO-*d*₆), δ (ppm) = 9.31 (d, *J* = 6.8 Hz, 2H), 8.73 (d, *J* = 6.8 Hz, 2H), 8.34 – 8.29

(m, 2H), 7.57 (dd, $J = 9.8, 7.9$ Hz, 2H), 4.71 (s, 2H), 1.95 (t, $J = 7.5$ Hz, 2H), 1.34 (dd, $J = 15.1, 7.5$ Hz, 2H), 0.93 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 176.6 (oxadiazole C), 166.6 (oxadiazole C), 165.6 (pyr-CH), 164.9 (Ph-C), 146.6 (pyr-CH), 141.1 (pyr-C), 131.7 (pyr-CH), 131.6 (Ph-CH), 125.9 (Ph-CH), 119.9 (Ph-C), 117.7 (Ph-CH), 117.5 (Ph-CH), 61.4 (N-CH₂), 33.1 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{17}\text{H}_{17}\text{FN}_3\text{O}^+$: 298.135; experimental: 298.1353.

1-decyl-4-(5-(4-fluorophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium (21b) Yield 61%; m.p. = 118–119°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.31 (d, $J = 6.9$ Hz, 2H), 8.73 (d, $J = 6.9$ Hz, 2H), 8.35–8.30 (m, 2H), 7.57 (t, $J = 8.9$ Hz, 2H), 4.70 (t, $J = 7.4$ Hz, 2H), 2.02–1.90 (m, 2H), 1.27 (d, $J = 27.9$ Hz, 14H), 0.84 (t, $J = 6.8$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 176.6 (oxadiazole C), 166.6 (oxadiazole C), 165.7 (pyr-CH), 164.9 (Ph-C), 146.7 (pyr-CH), 141.1 (pyr-C), 131.7 (pyr-CH), 131.6 (Ph-CH), 125.9 (Ph-CH), 119.9 (Ph-C), 117.7 (Ph-CH), 117.5 (Ph-CH), 61.6 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{23}\text{H}_{29}\text{FN}_3\text{O}^+$: 382.2289; experimental: 382.2289.

1-decyl-4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium (22a) Yield 56%; m.p. = 167–168°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.37 (d, $J = 6.7$ Hz, 2H), 8.82 (d, $J = 6.7$ Hz, 2H), 8.08 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 8.8$ Hz, 2H), 4.74 (t, $J = 7.4$ Hz, 2H), 3.87 (s, 3H), 2.00–1.89 (m, 2H), 1.39–1.28 (m, 2H), 0.93 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 171.7 (oxadiazole C), 169.2 (oxadiazole C), 162.7 (Ph-C), 146.8 (pyr-CH), 137.7 (pyr-C), 129.5 (pyr-CH), 126.5 (Ph-CH), 117.9 (Ph-C), 115.4 (Ph-CH), 61.6 (N-CH₂), 56.0 (O-CH₃), 33.2 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{18}\text{H}_{20}\text{N}_3\text{O}_2^+$: 310.155; experimental: 310.155.

1-butyl-4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium (22b) Yield 73%; m.p. = 165–166°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.38 (d, $J = 6.6$ Hz, 2H), 8.83 (d, $J = 6.6$ Hz, 2H), 8.08 (d, $J = 8.8$ Hz, 2H), 7.19 (d, $J = 8.8$ Hz, 2H), 4.73 (t, $J = 7.4$ Hz, 2H), 3.87 (s, 3H), 1.97 (s, 2H), 1.28 (d, $J = 26.0$ Hz, 14H), 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 171.8 (oxadiazole C), 169.2 (oxadiazole C), 162.7 (Ph-C), 146.8 (pyr-CH), 137.7 (pyr-C), 129.5 (pyr-CH), 126.5 (Ph-CH), 117.9 (Ph-C), 115.4 (Ph-CH), 61.8 (N-CH₂), 56.0 (O-CH₃), 31.7 (CH₂), 31.2 (CH₂), 29.7 (CH₂), 29.1 (CH₂), 29.0 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{24}\text{H}_{32}\text{N}_3\text{O}_2^+$: 394.2489; experimental: 394.2491.

1-butyl-4-(3-(4-nitrophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium (23a) Yield 71%; m.p. = 154–165°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.41 (d, $J = 6.9$ Hz, 2H), 8.88 (d, $J = 6.9$ Hz, 2H), 8.50 (d, $J = 9.0$ Hz, 2H), 8.41 (d, $J = 9.0$ Hz, 2H), 4.75 (t, $J = 7.4$ Hz, 2H), 2.01–1.92 (m, 2H), 1.39–1.28 (m, 2H), 0.94 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.7 (oxadiazole C), 168.2 (oxadiazole C), 150.0 (Ph-C), 147.0 (pyr-CH), 137.5 (pyr-C), 131.5 (Ph-C), 129.2 (pyr-CH), 126.6 (Ph-CH), 125.2 (Ph-CH), 61.6 (N-CH₂), 33.2 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{17}\text{H}_{17}\text{N}_4\text{O}_3^+$: 325.1295; experimental: 325.1296.

1-decyl-4-(3-(4-nitrophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium (23b) Yield 86%; m.p. = 174–176°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.40 (d, $J = 4.7$ Hz, 2H), 8.88 (d, $J = 4.7$ Hz, 2H), 8.45 (dd, $J = 37.6, 7.9$ Hz, 4H), 4.74 (s, 2H), 1.98 (s, 2H), 1.28 (d, $J = 24.7$ Hz, 14H), 0.85 (s, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.7 (oxadiazole C), 168.2 (oxadiazole C), 150.0 (Ph-C), 146.9 (pyr-CH), 137.5 (pyr-C), 131.5 (Ph-C), 129.2 (pyr-CH), 126.6 (Ph-CH), 125.2 (Ph-CH), 61.9 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{23}\text{H}_{29}\text{N}_4\text{O}_3^+$: 409.2234; experimental: 409.2235.

1-butyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)pyridin-1-ium (24a) Yield 72%; m.p. = 186–187°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.39 (d, $J = 6.7$ Hz, 2H), 8.85 (d, $J = 6.7$ Hz, 2H), 8.14 (dd, $J = 7.6, 1.7$ Hz,

2H), 7.66 (d, $J = 7.6$ Hz, 3H), 4.74 (t, $J = 7.5$ Hz, 2H), 1.95 (dd, $J = 15.0, 7.5$ Hz, 2H), 1.34 (dd, $J = 15.0, 7.5$ Hz, 2H), 0.94 (t, $J = 7.5$ Hz, 3H); ^{13}C NMR (400 MHz, DMSO- d_6), δ (ppm) = 172.1 (oxadiazole C), 169.5 (oxadiazole C), 146.9 (pyr-CH), 137.6 (pyr-C), 132.7 (pyr-CH), 130.0 (Ph-CH), 127.7 (Ph-CH), 126.6 (Ph-CH), 125.7 (Ph-C), 61.6 (N-CH₂), 33.2 (CH₂), 19.2 (CH₂), 13.8 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{17}\text{H}_{18}\text{N}_3\text{O}^+$: 280.1444; experimental: 280.1450.

1-decyl-4-(3-phenyl-1,2,4-oxadiazol-5-yl)pyridin-1-ium (24b) Yield 96%; m.p. = 188–189°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 9.38 (d, $J = 6.7$ Hz, 2H), 8.85 (d, $J = 6.7$ Hz, 2H), 8.24–8.07 (m, 2H), 7.67 (d, $J = 7.6$ Hz, 3H), 4.73 (t, $J = 7.4$ Hz, 2H), 1.97 (s, 2H), 1.28 (d, $J = 25.3$ Hz, 14H), 0.85 (t, $J = 6.7$ Hz, 3H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.1 (oxadiazole C), 169.5 (oxadiazole C), 146.9 (pyr-CH), 137.6 (pyr-C), 132.7 (pyr-CH), 130.0 (Ph-CH), 127.7 (Ph-CH), 126.6 (Ph-CH), 125.7 (Ph-C), 61.8 (N-CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{23}\text{H}_{30}\text{N}_3\text{O}^+$: 364.2383; experimental: 364.2394.

4.3.7. General procedures synthesis pyridinium salts - method C

The reaction can be also performed applying microwave irradiation, under solvent-free conditions. In fact, 50 mg of the neutral compound and 2 mL of halogenoalkane. Usually, 10 or 20 cycles of 30 min are conducted. Each cycle is heated for the initial 5 min from 25 to 140°C, followed by stabilization at 140°C for a duration of up to 30 min at a power of 200W. The reaction is observed using TLC. Subsequently, the residue was treated as reported above.

4-(5-(4-nonanamidophenyl)-1,2,4-oxadiazol-3-yl)-1-tetradecylpyridin-1-ium bromide (25c) Yield 90%; m.p. = 239–240°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.41 (s, 1H), 9.32 (d, $J = 5.7$ Hz, 2H), 8.72 (d, $J = 6.8$ Hz, 2H), 8.18 (d, $J = 8.7$ Hz, 2H), 7.93–7.91 (m, 2H), 4.71 (t, $J = 7.3$ Hz, 2H), 2.40–2.36 (m, 2H), 1.96 (s, 2H), 1.62–1.59 (m, 2H), 1.30–1.22 (m, $J = 12.9$ Hz, 32H), 0.87–0.82 (q, $J = 6.8$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.3 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (Ph-C), 129.8 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 61.6 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.5 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.0 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{36}\text{H}_{55}\text{N}_4\text{O}_2^+$: 575.4320; experimental: 575.4320.

1-hexadecyl-4-(5-(4-nonanamidophenyl)-1,2,4-oxadiazol-3-yl)pyridin-1-ium bromide (25d) Yield 63%; m.p. = 202–203°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.40 (s, 1H), 9.31 (d, $J = 6.8$ Hz, 2H), 8.72 (d, $J = 6.8$ Hz, 2H), 8.18 (d, $J = 8.8$ Hz, 2H), 7.92 (d, $J = 8.8$ Hz, 2H), 4.70 (t, $J = 7.4$ Hz, 2H), 2.38 (t, $J = 7.4$ Hz, 2H), 1.95 (s, 2H), 1.66–1.56 (m, 2H), 1.33–1.21 (m, 36H), 0.85 (q, $J = 6.7$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 177.2 (C(O)NH), 172.7 (oxadiazole C), 165.6 (oxadiazole C), 146.7 (pyr-CH), 144.9 (pyr-C), 141.4 (Ph-C), 129.8 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 117.0 (Ph-C), 61.6 (N-CH₂), 37.0 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 29.5 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 29.1 (CH₂), 29.0 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.4 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for C_{38} : 603.4633; experimental: 603.4633.

4-(5-(4-dodecanamidophenyl)-1,2,4-oxadiazol-3-yl)-1-hexadecylpyridin-1-ium bromide (26d) Yield 87%; m.p. = 242–243°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.39 (s, 1H), 9.29 (d, $J = 6.9$ Hz, 2H), 8.72 (d, $J = 6.8$ Hz, 2H), 8.18 (d, $J = 8.8$ Hz, 2H), 7.91 (d, $J = 8.8$ Hz, 2H), 4.69 (t, $J = 7.3$ Hz, 2H), 2.37 (s, 2H), 1.96 (d, $J = 12.8$ Hz, 2H), 1.67–1.53 (m, 2H), 1.30–1.20 (m, 42H), 0.84 (t, $J = 6.8$ Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.3 (oxadiazole C), 146.6 (pyr-CH), 144.9 (pyr-C), 137.7 (Ph-C), 129.8 (pyr-CH), 125.9 (Ph-CH), 119.7 (Ph-CH), 61.8 (N-CH₂), 37.0 (CH₂), 31.6 (CH₂), 31.2 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.4 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 25.8 (CH₂), 25.5 (CH₂), 22.5 (CH₂), 14.4 (CH₃); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for C_{45} : 645.5102;

experimental: 645.5108.

1-dodecyl-4-(3-(4-nonanamidophenyl)-1,2,4-oxadiazol-5-yl)pyridin-1-ium bromide (27b) Yield 43%; m.p. = 210–225°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.25 (s, 1H), 9.38 (d, J = 6.6 Hz, 2H), 8.82 (d, J = 6.6 Hz, 2H), 8.06 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 8.7 Hz, 2H), 4.73 (t, J = 7.4 Hz, 2H), 2.36 (t, J = 7.4 Hz, 2H), 1.96 (s, 2H), 1.60 (dd, J = 14.0, 6.9 Hz, 2H), 1.36–1.17 (m, 28H), 0.85 (q, J = 6.7 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.2 (oxadiazole C), 146.9 (pyr-CH), 143.3 (pyr-C), 137.7 (Ph-C), 128.5 (pyr-CH), 126.5 (Ph-CH), 119.8 (Ph-CH), 119.7 (Ph-C), 61.7 (N-CH $_2$), 36.9 (CH $_2$), 31.7 (CH $_2$), 31.7 (CH $_2$), 31.2 (CH $_2$), 29.4 (CH $_2$), 29.3 (CH $_2$), 29.2 (CH $_2$), 29.1 (CH $_2$), 29.1 (CH $_2$), 29.0 (CH $_2$), 28.8 (CH $_2$), 25.8 (CH $_2$), 25.4 (CH $_2$), 22.5 (CH $_2$), 14.4 (CH $_3$); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{34}\text{H}_{51}\text{N}_4\text{O}_2^+$: 547.4007; experimental: 547.4040.

4-(3-(4-dodecanamidophenyl)-1,2,4-oxadiazol-5-yl)-1-hexadecylpyridin-1-ium bromide (28d) Yield 54%; m.p. = 194–196°C; ^1H NMR (400 MHz, DMSO- d_6), δ (ppm) = 10.25 (s, 1H), 9.37 (d, J = 6.7 Hz, 2H), 8.83 (d, J = 6.7 Hz, 2H), 8.07 (d, J = 8.7 Hz, 2H), 7.86 (d, J = 8.7 Hz, 2H), 4.72 (t, J = 7.3 Hz, 2H), 2.36 (t, J = 7.3 Hz, 2H), 2.01–1.90 (m, 2H), 1.65–1.55 (m, 2H), 1.26 (d, J = 28.9 Hz, 42H), 0.85 (t, J = 6.6 Hz, 6H); ^{13}C NMR (151 MHz, DMSO- d_6), δ (ppm) = 172.4 (C(O)NH), 171.8 (oxadiazole C), 169.3 (oxadiazole C), 146.9 (pyr-CH), 143.3 (pyr-C), 137.7 (Ph-C), 128.6 (Pyr-CH), 126.5 (Ph-CH), 119.8 (Ph-CH), 61.8 (N-CH $_2$), 37.0 (CH $_2$), 31.7 (CH $_2$), 31.2 (CH $_2$), 29.5 (CH $_2$), 29.4 (CH $_2$), 29.4 (CH $_2$), 29.2 (CH $_2$), 29.1 (CH $_2$), 29.1 (CH $_2$), 28.8 (CH $_2$), 25.8 (CH $_2$), 25.4 (CH $_2$), 22.5 (CH $_2$), 14.4 (CH $_3$); HRMS (ESI) $[\text{M}]^+$: m/z theoretical for $\text{C}_{48}\text{H}_{77}\text{N}_4\text{O}_2^+$: 645.5102; experimental: 645.5106.

4.4. Antimicrobial activity - minimum inhibitory concentrations (MICs) determination

The antibacterial activities of compounds **4**, **6**, **7**, **8**, **10** and **15** to **28** were evaluated in vitro against reference strains *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) and toward clinically isolated strains (*K. pneumoniae*, *E. coli*, *E. faecium*, *S. aureus*, and *S. haemolyticus*).

The previously mentioned strains were tested with different classes of antibiotics according to the EUCAST guidelines using the microdilution method. All the Gram-negative clinical strains were carbapenemase-producing, extended spectrum beta-lactamase (ESBL), and multidrug-resistant (MDR), with the exception of *E. coli* ATCC 25922. Penicillins, fluoroquinolones, aminoglycosides, glycopeptides, macrolides, tetracyclines, and oxazolidinones were among the antibiotics investigated for *Staphylococcus* strains. Except for *S. aureus* ATCC 25923, every strain exhibited varying degrees of resistance to oxacillina and other compounds. Strains of *S. haemolyticus* that were resistant to several drug classes were identified.

MICs were determined through the broth microdilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [34].

Individual colonies of each strain were taken from cultivated plates and resuspended in sterile saline to produce bacterial inocula with turbidity equal to a 0.5 McFarland standard (1.5×10^8 CFU). The suspensions were plated on a sterile 96-well microtiter plate after being diluted in Mueller-Hinton broth (Sigma-Aldrich, St. Louis, MO, USA) to a final bacterial cell density of 5×10^5 CFU/mL. Each antimicrobial experiment was carried out in triplicate, and the chemical dilution range selected was 0.125–64 $\mu\text{g/mL}$ based on a 1:2 dilution series on a final well volume of 100 μL .

The minimum inhibitory concentration (MIC) was established as the lowest concentration of a chemical at which no discernible growth occurred after 18–20 h of incubation at 35–37°C.

The MIC determination showed excellent reproducibility, achieving an Essential Agreement (EA) of 100% across all the tested antimicrobial molecules, confirming the high robustness and reliability of the screening assay.

4.5. MTT assays

MTT assay was performed as described by Mossman with minor modifications [35]. Briefly, HDnF (human dermal neonatal fibroblasts) were obtained from ATCC (LCG Standards, Milan, Italy) and cultured in DMEM high glucose (Euroclone, Milan, Italy) supplemented with 10% FCS (Euroclone, Milan, Italy), 1% GlutaMAX (Gibco, Thermofisher, Milan Italy) 1% penicillin–streptomycin solution (Euroclone, Milan Italy), 2 mM sodium pyruvate (Euroclone, Milan Italy), and 1% non-essential amino acids (Euroclone, Milan, Italy). HepG2 cells were acquired from Cytion (Heidelberg, Germany) and cultured in Ham's F12 medium supplemented with 10% FBS, 1% GlutaMAX, 2 mM sodium pyruvate, and 1% penicillin–streptomycin solution.

Both cell lines were maintained at 37°C in a humidified atmosphere and subcultured once per week.

For the cytotoxicity assay, HDnF cells were seeded at a density of 3.5×10^3 cells per well in 90 μL of medium in a 96-well plate, while HepG2 cells were seeded at a density of 4×10^3 cells per well. After 24 h, 10 μL of each test compound ($10\times$ stock dilution) was added to each well to achieve a final $1\times$ concentration.

After 72 h, 10 μL of a 5 mg/mL solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, Milan, Italy) was added, and plates were incubated for an additional 4 h. The medium was then discarded, and 100 μL of DMSO along with 10 μL of Sorenson buffer (pH 10.5) was added.

Absorbance was measured at 570 nm with a 620 nm reference filter using a microplate reader (Clariostar, BMG Labtech, Germany). Absorbance values were normalized to the no-drug control (set at 100%). IC $_{50}$ values were calculated using a four-parameter logistic regression model with Prism v6.0 software (GraphPad, Boston, USA).

4.6. Selectivity index

Selectivity indexes (SI) were calculated for the analyzed compounds by relating the IC $_{50}$ values obtained from the MTT assay on HDnF and HepG2 cell lines to the corresponding MIC values determined against the tested bacterial *S. aureus*. Since MIC values were experimentally determined in $\mu\text{g/mL}$, they were converted into micromolar units (μM) using the molecular weight of each corresponding salt, including both the cationic species and the counterion, according to equation:

$$\text{MIC } (\mu\text{M}) = \frac{\text{MIC } \left(\frac{\mu\text{g}}{\text{mL}} \right) \times 1000}{\text{MW } \left(\frac{\text{g}}{\text{mol}} \right)} \quad \text{Eq. 1}$$

The SI values were subsequently calculated according to equation:

$$\text{SI} = \frac{\text{IC}_{50} (\mu\text{M})}{\text{MIC } (\mu\text{M})} \quad \text{Eq. 2}$$

4.7. Haemolysis assays

The haemolysis assay was performed as previously described by Ingwill Pedersen Sæbø et al. [36]. Briefly, blood sample was collected from a healthy donor after informed consent in lithium heparin tubes. The sample was immediately centrifuged at $1700 \times g$ for 5 min. The supernatant was removed, and PBS (pH 7.4) was added before centrifugation. This washing step was repeated three times. After thorough washing, the erythrocyte pellet was diluted in PBS at a ratio of 1:100 to obtain a 1% erythrocyte suspension.

The assay was performed in a U-shaped 96-well plate by mixing 50 μL of each test compound dilution (2x concentrated), PBS (negative control), or 10% Triton X-100 (Sigma-Aldrich, Milan, Italy) (positive control) with 50 μL of the 1% erythrocyte suspension. The mixtures were incubated at 37°C for 60 min.

After incubation, plates were centrifuged at $1700 \times g$ for 5 min to separate intact cells from released haemoglobin. Subsequently, 50 μL of

the supernatant was carefully transferred to a flat-bottom 96-well plate, and the absorbance was measured at 405 nm using a microplate reader (Clariostar, BMG Labtech, Germany).

The haemolysis ratio (%) was calculated using the following equation:

$$HR(\%) = \frac{OD_{test} - OD_{neg}}{OD_{pos} - OD_{neg}} \times 100 \quad \text{Eq. 3}$$

CRedit authorship contribution statement

Sara Amata: Data curation, Formal analysis, Investigation, Writing – original draft. **Virginia Talarico:** Formal analysis, Investigation. **Gaspare Crisà:** Formal analysis, Investigation. **Giuseppe Pizzolanti:** Formal analysis, Investigation. **Andrea Pace:** Data curation, Validation, Writing – review & editing. **Antonio Palumbo Piccionello:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. **Cinzia Calà:** Methodology, Resources, Supervision, Validation, Writing – review & editing. **Carla Rizzo:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmc.2026.100365>.

Data availability

Data will be made available on request.

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