Journal Pre-proofs

Phosphonium-ammonium-based di-cationic ionic liquids as antibacterial over the *ESKAPE* group

Frédéric Brunel, Christelle Lautard, Frédéric Garzino, Jean-Manuel Raimundo, Jean-Michel Bolla, Michel Camplo

PII: S0960-894X(20)30500-X
DOI: https://doi.org/10.1016/j.bmcl.2020.127389
Reference: BMCL 127389

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 14 June 2020
Revised Date: 2 July 2020
Accepted Date: 3 July 2020

Please cite this article as: Brunel, F., Lautard, C., Garzino, F., Raimundo, J-M., Bolla, J-M., Camplo, M., Phosphonium-ammonium-based di-cationic ionic liquids as antibacterial over the *ESKAPE* group, *Bioorganic & Medicinal Chemistry Letters* (2020), doi: https://doi.org/10.1016/j.bmcl.2020.127389

Ph[Journal Pre-proofs]the *ESKAPE* group.

Frédéric Brunel,[a] Christelle Lautard,[b] Frédéric Garzino,[a] Jean-Manuel Raimundo,[a,] * Jean-Michel Bolla,[b,] * and Michel Camplo[a,] *

[a] *Aix Marseille Univ., CNRS, CINaM UMR 7325, Campus de Luminy, Case 913, 13288 Marseille Cedex 09, France.*

[b]*Aix-Marseille Univ.,* INSERM, SSA, MCT, *27 boulevard Jean Moulin 13385 Marseille cedex 05, France.*

Abstract

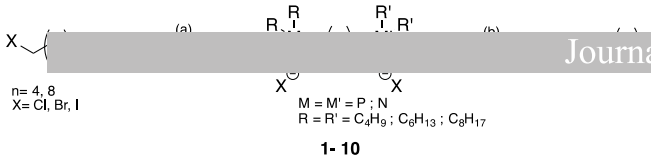
Emergence of antibioresistance is currently a major threat of public health worldwide. Hence there is an urge need of finding new antibacterial material. Herein, we report a simple and eco-friendly method to synthesize homo and heterodicationic ionic liquids based on quaternary phosphonium and ammonium salt. In order to investigate the structure activity relationship (SAR) we measured the MICs of a series of 16 derivatives with structural variations (nature of cations and counter-ions, size of linker and alkyl side chains as well as structural symmetry) over a range of Gram-positive and Gram-negative bacterial strains from the *ESKAPE* group. Some of the tested structures exhibit high antimicrobial activities (MIC = 0.5 mg/L) and are active over a wide range of bacteria from Gram-positive to Gram-negative. Overall, these results reveal the strong potential of di-cationic derivatives as antibacterial agents and the determination of activities from structural features gives decisive information for future synthesis of such di-cationic structures for biocidal purpose.

Introduction

Nosocomial infections constitute a major issue for the public health worldwide. In US alone, the number of deaths attributed to Healthcare Associated Infections (HCAIs) is about 98 000 per year while 1.7 million patients hospitalized will contract a HCAI over the same period.[1] The increasing difficulties to treat bacterial infections are directly linked to the antimicrobial resistance phenomena (AMR) caused for instance by over-used or inadequate antibiotics treatments. To overcome this resistance threat there is an urgent need to design new antibiotics or find novel strategies and new antibacterial materials. For these purposes researchers have striven several innovative alternatives based on the development of antimicrobial peptides[2], bacteriophages[3], cationic organic compounds and polymers[4] that have attracted much attention lately for their high efficiency as biocidal materials. Besides, ionic liquids have recently attracted lot of interest for biocompatible biomedical[5] and antimicrobial applications[6] due to their versatility and ability to easily fine-tune their properties in various morphologies, sizes, and surface charges. Those include imidazolium,[7] ammonium,[8] pyridinium[9] or phosphonium salts.[10] Interestingly, some studies have recently evidenced a net benefit in the bactericidal activity when the charge density of the ionic liquids increases. For example, di-cationic ionic liquids have exhibited improved efficiency[11,12] associated to broaden spectrum of activity[13] compared to their monomer counterparts. In addition, gemini-type ionic liquids constitute a new class of amphiphilic structures possessing two polar head groups (identical or not) linked either by a flexible or rigid spacer in a symmetric or asymmetric fashion. Compared to the classical monocationic structures the gemini ionic liquids have demonstrated superior physical properties[14] and enlarge the scope of applications with an exclusive way to fine-tune their biological and antimicrobial properties.[15] Although these gemini derivatives have shown unprecedented advantages in several applications, they are still underdeveloped in particular for biological and antimicrobial applications. Among them, phosphonium gemini-based molecules have been only scarcely reported and still largely underexploited opening the way to innovative structures associated with novel biological properties. In that context we will report herein the synthesis of novel phosphonium-ammonium-based di-cationic compounds as well as their gemini analogues in high yields through straightforward or two-steps reaction. The iterative process allows the possibility to vary structurally some key parameters such as the length of the linker and side-chains, the nature of the cation (ammonium or phosphonium) or the halogen counter-ion (chloride, bromide or iodide) as well as the structural and cationic symmetry (different pattern on both side of the linker). Thus, antibacterial activities were investigated by the determination of the minimum inhibition concentrations (MIC) against several bacterial strains from the *ESKAPE* group for the new synthesized compounds to study the Structure-Activity Relationship (SAR). *ESKAPE* is an acronym that stands for the major human pathogens with multi-drug resistance[16] including both Gram-positive and Gram-negative bacteria.

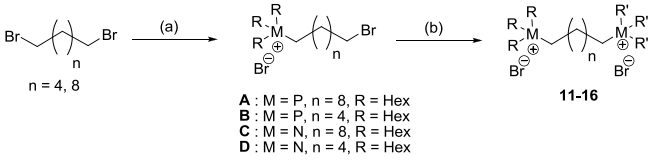
Synthesis

Homo and heterodicationic structures were designed and synthesized during the course of this study following a straightforward and eco-friendly protocol depicted in Scheme **1** and **2**. Firstly, the symmetric homodicationic compounds were obtained in one step by reacting all commercially available reactants together. Hence, the selected dihalogenated aliphatic linkers having a C_{10} or C_6 length underwent a nucleophilic substitution reaction with the appropriate trialkylphosphine or trialkylamine nucleophiles affording the desired gemini dicationic derivatives **1**-**10** in a yield from moderate to high. The reactions were performed under neat conditions using a micro-wave irradiation at high temperature for 3 hours (200 W, 130 °C) for the gemini diphosphoniums compounds (Scheme **1**, a) while for diammonium analogues conventional heating at 110°C with prolongated reaction time (48 hours) are required (Scheme **1**, b). These harsh conditions can explain the lowered yield (20-30%) obtained for the gemini diammoniums derivatives since ammonium is more susceptible to undergo undesired competitive Hofmann β-eliminations in the presence of base and/or high temperature.[17]



Scheme 1: Synthetic pathway of the homodicationic derivatives **1-10**. (a) PR_3, MW (200W), 130°C, 3h; (b) NR'_3, 110°C, 48h.

Secondly, the heterodicationic derivatives were obtained using an iterative pathway. The first step consists in a mono-substitution reaction in dilute CH_2Cl_2 under reflux conditions affording the reaction intermediaries **A**, **B**, **C** and **D** in 70, 88, 25 and 31% yield respectively after column chromatography purifications (Scheme **2** a, see SI). Subsequent nucleophilic substitution reactions with these intermediates (Scheme **2** b, see SI), using the aforementioned micro-wave irradiation protocol, led to the heterodicationic compounds **11-16** in good to excellent yields (Table **1** and see SI).



Scheme 2: Synthetic pathway of the heterodicationic derivatives **11-16**. (a) [illegible]30°C,

All synthesized dicationic derivatives have been characterized by mass spectroscopy and ^{1}H, ^{13}C and ^{31}P NMR spectroscopy (See SI).

Table 1: Structural characteristics of the homo and heterodicationic derivatives **1-16**.

Compound	M	M'	R	R'	N	X^-	Yield (%)
1	P	P	Hex	Hex	8	Cl	95
2	P	P	Hex	Hex	8	Br	85
3	P	P	Hex	Hex	8	I	96
4	N	N	Hex	Hex	8	Br	20
5	P	P	Hex	Hex	4	Br	87
6	N	N	Hex	Hex	4	Br	30
7	P	P	Oct	Oct	4	Br	91
8	P	P	Oct	Oct	8	Br	93
9	P	P	But	But	4	Br	90
10	P	P	But	But	8	Br	96
11	P	P	Hex	But	8	Br	88
12	P	P	Hex	Oct	4	Br	70
13	P	P	Hex	But	4	Br	95
14	N	P	Hex	Hex	8	Br	57
15	N	P	Hex	But	8	Br	93
16	N	P	Hex	But	4	Br	82

Table 2: Minimal Inhibition Concentration (MIC) (mg/L) of compounds **1-16** on *ESKAPE* bacteria

Bacterial strain	Compounds															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
***E. faecium* 20,477**	0.5	1	0.5	0.5	0.5	1	2	32	>64	>64	2	2	8	1	2	64
***S. aureus* CIP 7625**	0.5	0.5	0.5	0.5	0.5	1	1	16	>64	16	0.5	1	2	0.5	1	32
***K. pneumonia* CIP 82.91**	2	4	2	4	8	>64	32	>64	>64	>64	16	4	>64	2	64	>64
***A. Baumannii* ATCC 19606**	2	4	2	16	16	>64	8	>64	>64	>64	8	4	>64	8	>64	>64
***P. aeruginosa* 100720**	4	4	2	8	8	>64	>64	>64	>64	>64	64	8	>64	8	>64	>64
***E. aerogenes* ATCC 13048**	4	8	4	8	8	>64	64	>64	>64	>64	32	8	>64	8	>64	>64
***E. coli* CIP 54.8**	2	2	2	2	4	32	32	>64	>64	>64	4	8	>64	2	16	>64

Biological assays and SAR analyses

The biological assays clearly demonstrate the antibacterial effectiveness for some of the newly synthetized homo- and heterodicationic ionic liquids against Gram-positive and Gram-negative strains but associated with a certain variability (Table **2**). For example, the compound **2** exhibits a MIC of 0.5 mg/L over *S. aureus* which is comparable to the best efficiency attained with commercially available antibiotics.[18] Moreover, from a general point of view even if the effectiveness is broaden to the *ESKAPE* group the dicationic compounds display a better activity toward Gram-positive strains (*E. faecium* and *S. aureus*) than on Gram-negative but with some disparities in the tested series. At the first glance, these trends can be explained by the nature and structure of the membrane that differs in these two bacteria families.[19] However, some di-cationic structures namely **1** to **5**, **12** and **14** interestingly disclose a wider spectrum of activity for the different considered strains which can be attributed of particular structural changes on the di-cationic compounds. In that respect, we have decided to take a closer look over which kind of structural variations tend to modify those activities in order to settle a SAR study by doing a rational comparison. First of all, we have examined the influence of the halogen counter-ion as compounds **1**, **2** and **3** only differ from their halide, respectively chloride, bromide and iodide. Analogously to reported results, similar activities were obtained for compounds **1**, **2** and **3** highlighting the low impact of the halide on the efficiency whatever the strain tested. Comparison of compounds **2** (di-P^+) and **4** (di-N^+) can give some insights about the effect of pnictogen atom on the antimicrobial properties. Indeed, **2** and **4** present identical hydrocarbon scaffolds (a C_{10} linker and hexyl peripheral chains) and differ only by the nature of the pnictogen, nevertheless no clear conclusion can be done as the bactericidal effect behave similarly in both cases regardless the strain. Curiously, if the linker length is changed from C_{10} to C_6 (compounds **2→5** (di-P^+) and **4→6** (di-N^+)) clear changes appear in favor to the di-phosphonium derivative **5** that became more efficient than its nitrogen analogue **6**, particularly over Gram-negative strains while its efficiency remains similar to dicationic **2**. Furthermore, the replacement of the hexyl peripheral chains to octyl around the phosphonium core (**2→8** (di-P^+) and **5→7** (di-P^+)) have led to drastic changes associated to a clear decrease in activity in both cases. The effects are more predominant for **8** which biocidal efficiency was annihilated for all the *ESKAPE* group strains while **7** remained

slightly active on Gram-positive strains. On the other hand, simi
have been used (**5→9** (di-P$^+$) and **2→10** (di-P$^+$)) associated with a detrimental antimicrobial effect leading to inactive dicationic compounds in these series. Thus, for symmetric homodicationic compounds the sizes of the peripheral chains and linker appear to be interdependent factors in those series. From this study, it was also highlighted that the predominant structural parameters that dramatically affect the activity are the side chains. Indeed, comparison of the efficiency of **2**, **8** and **10**, has unequivocally evidenced that compound **2** bearing hexyl side chains exhibits the highest efficiency proving the central role of this chain length.

The heterodicationic structures **11**-**16** have been also analyzed in order to identify further structural features that could affect the antimicrobial efficiencies. During the course of the study several heterostructures have been envisioned either based on a dissymmetry at the level of the peripheral chains, or at the level of the central pnictogen or a combination of both. Moreover, the linker length has a significant impact on the activity (*vide supra*) and has been concomitantly considered. Comparison of compounds **5** (C_6H_{13}/C_6H_{13}, C_6), **12** (C_6H_{13}/C_8H_{17}, C_6) and **13** (C_6H_{13}/C_6H_9, C_6), evidenced slight effect on the antimicrobial properties that combine the effects previously observed from the homodicationic structures. Once again, the replacement of the hexyl peripheral chains, even from only one side, by longer or shorter ones led to a detrimental effect on the efficiency. The detrimental effect is more obvious in the case of shorter chains as heterodicationic compound **12** exhibits almost unaffected efficiency compared to the homodicationic parent compound **5** while **13** remains only active on Gram-positive strains. This behavior attests again that hexyl might correspond to the optimal chain length in this series. In addition, substitution of one pnictogen from **2** (di-P$^+$)→**14** (N$^+$-P$^+$) or **4** (di-N$^+$)→**14** (N$^+$-P$^+$) doesn't change the efficiency compared to the parent homodicationic derivatives **2** and **4**. However, although the activity remains almost identical it is noticeable to point out that the difference could come from the substitution of phosphonium by an ammonium, as the decrease of the antimicrobial effect is of the same order than the one observed between **2** and **4**. This behavior will suggest a greater importance of phosphonium compared to ammonium in such series. This behavior is also supported by the MICs obtained on the heterodicationic derivatives **15** and **16**. Therefore, the activity could not be strictly linked to an isolated factor such as the nature of the cation or the anion, the symmetry or the linker length, it seems that the main parameter that significantly influences the antimicrobial activity of these structures is the number of carbon, and it appears that this balance is specific for each bacterial species. To illustrate that we decided to only consider the *S. aureus* strain, and we graphically compare the MICs with the number of carbons for each dicationic structure it gives the Figure 1.

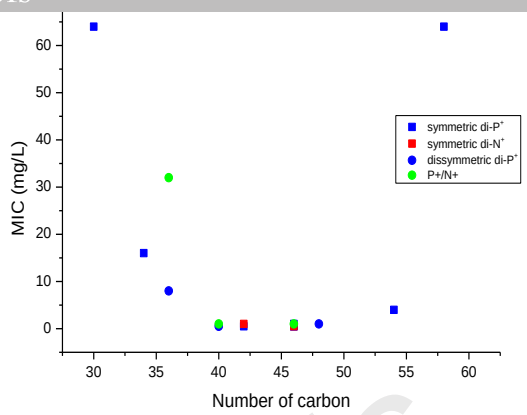


Figure 1: Effect of the total number of carbons and symmetry of compounds **1**-**16** on their bactericidal efficiency for *S. aureus*

Herein, the parabola curve obtained is representative of a square function, and we can easily release an optimal number of carbons independently of the nature and structure of the studied DCILs. In the case of *S. aureus* this optimum is located between 40 and 48 carbons. Similar results have already been demonstrated by Luczak et al.[20] In their study, they evaluate antimicrobial activity of 1-alkyl-3-imidazolium and defined a "cut-off" effect, where those ILs exhibit an optimum of activity for alkyl chain with a number of carbon between 16 and 18, above and below the activity decrease. Same phenomenon has also been observed for pyridinium[21] and quinolinium[22] ionic liquids.

To date, the exact bactericidal mechanism of action remains unknown but the main hypothesis meeting consensus for such structures with cationic and hydrophobic parts involves the preferential adsorption of the cationic compound onto the negatively charged cell wall followed by the diffusion of the hydrophobic alkyl chains through the lipid bilayer causing disruption and irreversible damages to the cell membranes.[4] This two steps mechanism supposes an accurate hydrophilic/lipophilic balance (HLB), and corroborates the obtained results and the demonstrated "cut-off" effect. Another approach specifies that the improved antibacterial efficiency of di-cationic ionic liquids compared to their mono-cationic counterparts, could be conferred by their "bolaamphiphiles" structures, typically two ionic heads linked together via an alkyl linker.[12] Indeed, this specific structure is well known for its ability to auto-assemble into lipid monolayer, form stable micelles and cross cell membrane.[23] However this hypothesis is difficult to confirm with data collected herein. Additional studies currently on going such as Diffraction Light Scattering (DLS), Isothermal Titration Calorimetry (ITC), or microscopy will help us to have a better understanding of this mechanism.

From the strong antibacterial activities of some of the synthesized compounds we decided to measure the cytotoxicity to evaluate if they can be considered as new infection treatments or antibiotics. Cytotoxicity has been determined over Human hepatocytes (HepG2) for the structures **2**, **4**, **8**, **10**, **11** and **14**. As expected, all the tested DCILs show strong cytotoxicity (see SI). Indeed some alkyl-phosphonium are known to be membrane lysing agents (MLA)[24].

In summary, thanks to an innovative and eco-friendly MW based synthetic methodology, we have been able to design a series of 16 DCILs by varying several structural parameters. The measure of MICs over the bacterial strains from the *ESKAPE* group allowed us to study the SAR. Some of our compounds exhibit very low MICs along with a broad spectrum of activities (from Gram-positive to Gram-negative) but because they demonstrate high cytotoxicity they cannot be

considered as candidates for new antibiotic treatments. These phos[illegible] their ammonium counterparts, those phosphonium based DCILs have proven to be easier to synthetize and more chemically stable over time. Overall, the SAR study reveals a clear dependence to the HLB and we hope that the "cut-off" effect that we have been able to define will help to design new DCIL structures for antimicrobial purpose.

Acknowledgement

This work was supported by the Centre National de la Recherche Scientifique (CNRS) and the Ministère de l'Enseignement Supérieur et de la Recherche. F. B. thanks also the Ministère de l'Enseignement Supérieur et de la Recherche for its doctoral financial support.

References and notes

(1) Haque, M.; Sartelli, M.; McKimm, J.; Abu Bakar, M. *Infect. Drug. Resist.* **2018**, 11, 2321-2333.
(2) Krizsan, A.; Volke, D.; Weinert, S.; Sträter, N.; Knappe, D.; Hoffmann, R. *Angew. Chem. Int. Ed*. **2014**, 53, 12236-12239.
(3) Principi, N.; Silvestri, E.; Esposito, S. *Front. Pharmacol*. **2019**, 10, 513.
(4) Yang, Y.; Cai, Z.; Huang, Z.; Tang, X.; Zang, X. *Polym. J.* **2018**, 50, 33-44.
(5) Ergova, K. S.; Gordeev, E. G.; Ananikov, V. P. *Chem. Rev*. **2017**, 117, 10, 7132-7189.
(6) Pendelton, J. N.; Gilmore, B. F. *Int. J. Antimicrob. Agents*, **2015**, 46, 131-139.
(7) Johnson, N. A.; Southerland, M. R.; Youngs, W. J. *Molecules*, **2017**, 22, 1263/1-1263/20.
(8) Jennings, M. C.; Minbiole, K. P. C.; Wuest, W. M. *ACS Infect. Dis*., **2015**, 1, 289-303.
(9) Sowmiah, S.; Esperança, J. M. S. S.; Rebelo, L. P. N.; Afonso, C. A. M. *Org. Chem. Front.*, **2018**, 5, 453-493.
(10) Brunel, F.; Lautard, C.; Garzino, F.; Giorgio, S.; Raimundo, J. M.; Bolla, J. M.; Camplo, M. *Bioorg. Med. Chem. Lett.*, **2016**, 26, 3770-3773.
[illegible] Yan,
(12) O'Toole, G. A.; Wathier, M.; Zegans, M. E.; Shanks, R. M.; Kowalski, R.; Grinstaff, M. W. *Cornea.* **2012**, 31, 810-816.
(13) Brunel, F.; Lautard, C.; di Giorgio, C.; Garzino, F.; Raimundo, J. M.; Bolla, J. M.; Camplo, M. *Bioorg. Med. Chem. Lett*., **2018**, 28, 926-929.
(14) Payagala, T.; Huang, J.; Breitbach, Z. S.; Sharma, P. S.; Armstrong, D. W. *Chem. Mater*., **2007**, 19, 5848–5850.
(15) a) Kawai, R.; Yada, S.; Yoshimura, T. *ACS Omega*, **2019**, 4, 14242-14250; b) Yang, X.; Fang, Y. *RSC Adv*., **2018**, 8, 26255-26265; c) Al-Mohammed, N. N.; Alias, Y.; Abdullah, Z. *RSC Adv*., **2015**, 5, 92602–92617.
(16) Rice, L. B. *J. Infect. Dis.* **2008**, 197(8), 1079–1081.
(17) Sowmiah, S.; Srinivasadesikan, V.; Tseng, M. S.; Chu, Y.H. *Molecules* **2009**, 14, 3780-3813.
(18) Ling, L. L.; Schneider, T.; Peoples, A. J.; Spoering, A. L.; Engels, I.; Conlon, B. P.; Mueller, A.; Schäberle, T. F.; Hughes, D. E.; Epstein, S.; Jones, M.; Lazarides, L.; Steadman, V. A.; Cohen, D. R.; Felix, C. R.; Fetterman, K. A.; Millett, W. P.; Nitti, A. G.; Zullo, A. M.; Chen, C.; Lewis, K. *Nature.* **2015**, 517, 455-459.
(19) Silhavy, T. J.; Kahne, D.; Walker, S. *Cold Spring Harb Perspect Biol.* **2010**, 2, 5.
(20) Łuczak, J.; Jungnickel, C.; Łącka, I.; Stolte, S.; Hupka, J. *Green Chem.*, **2010**, 12, 539-601.
(21) Pernak, J.; Kalewska, J.; Ksycińska, H.; Cybulski, J. *Eur. J. Med. Chem.*, **2001**, 36, 899-907.
(22) Busetti, A.; Crawford, D.E.; Earle, M. J.; Gilea, M. A.; Gilmore, B.F.; Gorman, S.P.; Laverty, G.; Lowry, A. F.; McLaughlin, M.; Seddon, K.R. *Green Chem.*, **2010**, 12, 420-425.
(23) Fariya, M.; Jain, A.; Dhawan, V.; Shah, S.; Nagarsenker, M. S. *Adv. Pharm. Bull.*, **2014**, 4, 483-491.
(24) Bachowska, B.; Kazmierczak-Baranska, J.; Cieslak, M.; Nawrot, B.; Szczesna, D.; Skalik, J.; Balczewski, P. *ChemistryOpen*., **2012**, 1, 33-38.

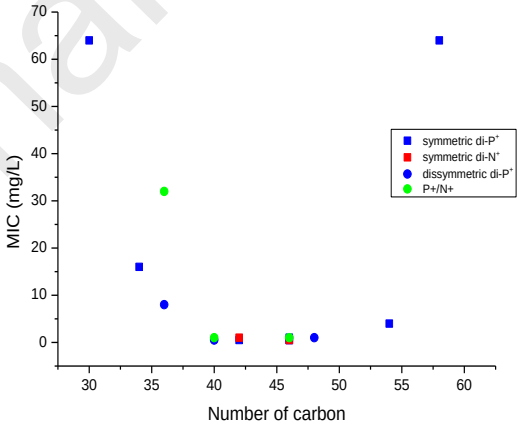
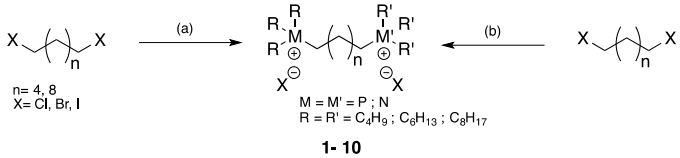
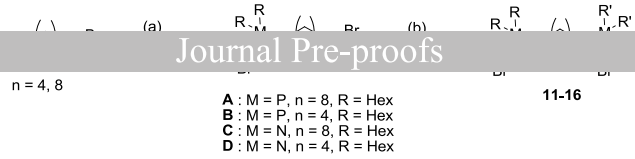


Figure 1: Effect of the total number of carbons and symmetry of compounds **1-16** on their bactericidal efficiency for *S. aureus*



1- 10

Scheme 3: Synthetic pathway of the homodicationic derivatives **1-10**. (a) PR_3, MW (200W), 130°C, 3h; (b) NR'_3, 110°C, 48h.



Scheme 4: Synthetic pathway of the heterodicationic derivatives **11-16**. (a) PR_3 or NR_3, CH_2Cl_2, reflux, 12h; (b) PR'_3 or NR'_3, MW (200W), 130°C, 3h.

Table 3: Structural characteristics of the homo and heterodicationic derivatives **1-16**.

Compound	M	M'	R	R'	N	X^-	Yield (%)
1	P	P	Hex	Hex	8	Cl	95
2	P	P	Hex	Hex	8	Br	85
3	P	P	Hex	Hex	8	I	96
4	N	N	Hex	Hex	8	Br	20
5	P	P	Hex	Hex	4	Br	87
6	N	N	Hex	Hex	4	Br	30
7	P	P	Oct	Oct	4	Br	91
8	P	P	Oct	Oct	8	Br	93
9	P	P	But	But	4	Br	90
10	P	P	But	But	8	Br	96
11	P	P	Hex	But	8	Br	88
12	P	P	Hex	Oct	4	Br	70
13	P	P	Hex	But	4	Br	95
14	N	P	Hex	Hex	8	Br	57
15	N	P	Hex	But	8	Br	93
16	N	P	Hex	But	4	Br	82

Table 2: Minimal Inhibition Concentration (MIC) (mg/L) of compounds 1-16 on *ESKAPE* bacteria

Bacterial strain	Compounds															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
***E. faecium* 20,477**	0.5	1	0.5	0.5	0.5	1	2	32	>64	>64	2	2	8	1	2	64
***S. aureus* CIP 7625**	0.5	0.5	0.5	0.5	0.5	1	1	16	>64	16	0.5	1	2	0.5	1	32
***K. pneumonia* CIP 82.91**	2	4	2	4	8	>64	32	>64	>64	>64	16	4	>64	2	64	>64
***A. Baumannii* ATCC 19606**	2	4	2	16	16	>64	8	>64	>64	>64	8	4	>64	8	>64	>64
***P. aeruginosa* 100720**	4	4	2	8	8	>64	>64	>64	>64	>64	64	8	>64	8	>64	>64
***E. aerogenes* ATCC 13048**	4	8	4	8	8	>64	64	>64	>64	>64	32	8	>64	8	>64	>64
***E. coli* CIP 54.8**	2	2	2	2	4	32	32	>64	>64	>64	4	8	>64	2	16	>64

Declaration of 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: