ORGANIC CHEMISTRY FRONTIERS

RESEARCH ARTICLE

View Article Online
View Journal | View Issue

Cite this: *Org. Chem. Front.*, 2024, **11**, 4101

Novel octacationic-resorcin[4]arenes featuring quaternary ammonium groups as multivalent biocides†

Vittoria Ferrara, [a] Veronica Iuliano, *[a] Placido Neri, [a] Silvano Geremia, [b] Neal Hickey, [b] Luca Di Stasio, [a] Giovanni Vigliotta, *[a] Gaetana Paolella, [a] Ivana Caputo, [a] Paolo Della Sala, [a] Carmine Gaeta [a] and Carmen Talotta *[a]

Novel resorcin[4]arene-based octacationic quaternary ammonium compounds (**ResQAC^{R}**) were obtained by linking tetraalkylammonium groups on the macrocycle wider rim to give multivalent multi-QACs. Their structures were elucidated through 1D and 2D NMR experiments, HR-MS analysis, and crystallographic studies. Additionally, the conformational dynamics of these octacationic-resorcinarenes were investigated using ^{1}H variable-temperature (VT) NMR experiments. The antimicrobial properties of **ResQAC^{R}** derivatives were studied by *in vitro* biological investigations. We identified that the **ResQAC^{butyl}** derivative shows an impressive bacteriostatic activity against *S. aureus*. A remarkable multivalent effect was observed for this bacteriostatic activity. Interestingly, cytotoxic studies indicate that **ResQAC^{butyl}** showed no adverse impact on cell viability of a human cell line, even at concentrations 30 times greater than the MIC for *S. aureus* and approximately 3 times higher than the MIC for *E. coli*. **ResQAC^{undecyl}** exhibits greater bacteriostatic activity against *E. coli* than against *S. aureus*, but it is cytotoxic at lower concentrations (IC_{50} of 12.1 μM) than its MIC. **ResQAC^{phenyl}** shows scarce bacteriostatic activity against both *S. aureus* and *E. coli*, while no multivalent effect was observed. This is likely attributed to the conformational rigidity of the boat C_{2v} conformation of **ResQAC^{phenyl}**, which hinders optimal matching between the cationic chains and the negatively charged bacterial surface.

Received 22nd April 2024,
Accepted 26th May 2024

DOI: 10.1039/d4qo00728j

rsc.li/frontiers-organic

Introduction

Staphylococcus aureus and *Escherichia coli* represent two of the eight bacteria considered significant for public health.[1] These pathogenic microorganisms are responsible for causing a considerable number of diseases.[1] It is crucial to control the environmental transmission of these organisms to safeguard public health. In recent decades, genetic recombination, widespread antibiotic usage, and other factors have led to bacterial resistance, raising the possibility that common infections will once again become potentially fatal. Very recently, analyses have been conducted[1] to study the correlations between antimicrobial consumption (AMC) and antimicrobial resistance (AMR) for specific combinations of bacteria and antibiotics. In detail, statistically significant decreasing trends in AMC and AMR were observed in food-producing animals and humans from 2014 to 2021.[1] Nevertheless, these measures necessitate reinforcement to maintain reductions in AMC. This emphasizes the importance of initiatives promoting human and animal health, such as vaccination and improved hygiene practices, alongside the use of disinfectants.

Among disinfectants, quaternary ammonium compounds (Fig. 1, QACs)[2] are a well-known class of cationic biocides, boasting a wide spectrum of antimicrobial activity.[3,4] QACs are commonly present in cleaning products, hand sanitizers, personal care products, various types of wipes, and diverse pesticidal products. They are frequently integrated into polymeric materials (poly-QAC in Fig. 1). Interestingly, QACs serve as antimicrobial treatments for biomedical instruments and high-touch surfaces in public areas.[2–4] In addition, the use of QACs as antimicrobial agents has experienced a notable increase in response to the COVID-19 pandemic.[5]

Over recent decades, numerous studies have highlighted the role of macrocycles as useful scaffolds for obtaining antibacterial derivatives (spacer in Fig. 2).[6–11] Interestingly, the synthetic versatility of pillararenes and calixarenes enabled the incorporation of multiple functionalities onto their rims.[6–15]

[a]*Dipartimento di Chimica e Biologia "A. Zambelli". Università di Salerno, Via Giovanni Paolo II, I-84084 Fisciano (Salerno), Italy. E-mail: ctalotta@unisa.it*
[b]*Centro di Eccellenza in Biocristallografia, Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Trieste, Via L. Giorgieri 1, I-34127 Trieste, Italy*

†Electronic supplementary information (ESI) available. CCDC 2336815. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d4qo00728j

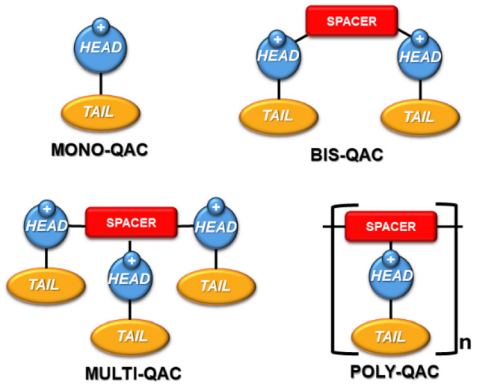


Fig. 1 Schematic representation of mono-QAC, bis-QAC, multi-QAC, and poly-QAC.[3] A mono-QAC is constituted by a single tetraalkylammonium group. In the case of bis-QAC, multi-QAC, and poly-QAC, the segment connecting the charged nitrogen atoms ("head") is named "spacer". The alkyl chains extending from the head are called "tails".

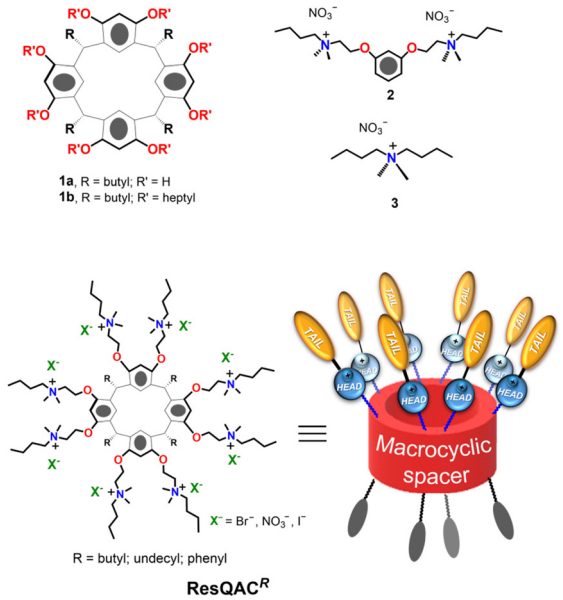


Fig. 2 Resorcin[4]arene-based multi-QACs (**ResQAC^R**) investigated in this work.

Thus, in 2016, Cohen and coworkers[12] were able to obtain pillar[*n*]arenes decorated with 10 quaternary ammonium groups (five ammonium groups on each side of the macrocycle) which inhibited the formation of biofilms by two significant Gram-positive pathogens, *Staphylococcus aureus* and *Enterococcus faecalis*.

Consoli and colleagues[13,14] focused on calixarene scaffolds, designing and synthesizing novel water-soluble polycationic calix[4]arenes. Subsequently, the authors demonstrated the ability of these novel calix[4]arene-multi-QACs to work as antibacterial agents against Gram-positive and Gram-negative bacteria, both independently and in combination with appropriate antibiotics. The same research group reported a calixarene featuring long alkyl chains at the lower rim, which exhibited micellar behavior.[14] These micelles inhibited the formation of biofilms by Gram-negative bacteria.

In the literature, a few rare examples have been reported where macrocycle-based antibacterial agents exhibited increased antibacterial efficacy by exploiting the multivalent effect.[15,16] Multivalency[17–19] refers to the phenomenon where multiple receptor/ligand interactions occur simultaneously, leading to enhanced overall affinity and selectivity of binding. Natural systems extensively utilize multivalency, such as in immunoglobulin function, virus particle adhesion to target cells, and regulation of cell–cell interactions. This concept has acquired increasing attention in the literature,[17–19] where multivalency was exploited in designing macrocyclic ligands[20–22] targeting pathologically relevant enzymes.

Resorcin[4]arene[23,24] (**1a**, Fig. 2) is a macrocycle bearing four resorcinol rings, and is obtained from resorcinol/aldehyde acid-catalysed condensation.[23,24] This macrocycle possesses eight functionalizable OH groups, all positioned on one side of the macrocycle (wider rim, see Fig. 2). Thus, the resorcin[4]arene scaffold can be considered as an ideal candidate to design multivalent quaternary ammonium compounds (multi-QACs, Fig. 1 and 2), where the valence indicates the number of ammonium units (heads in Fig. 1 and 2) linked to the macrocycle. To the best of our knowledge, no examples of polycationic ammonium resorcinarene compounds have been reported in the literature to data.

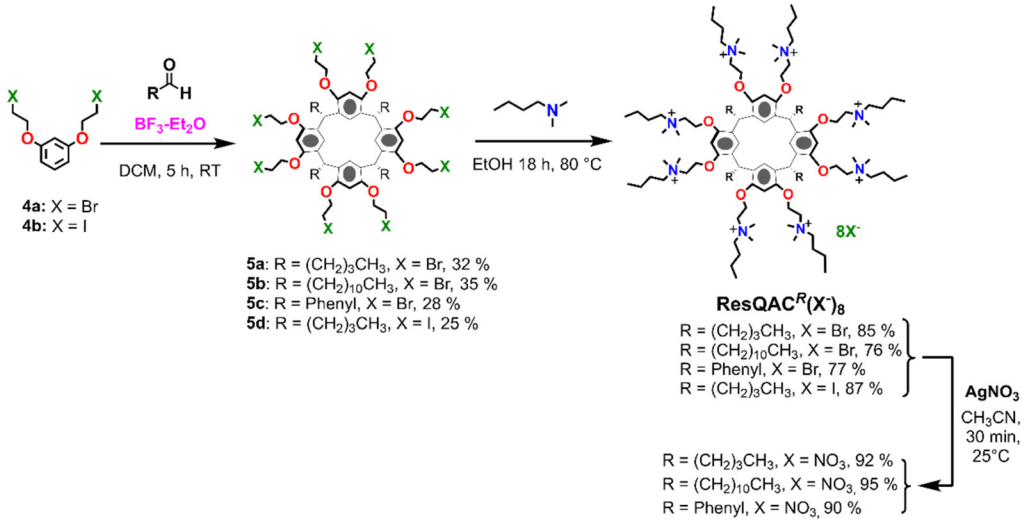
Inspired by these considerations, this study focused on the synthesis of novel multi-QAC resorcin[4]arenes (denoted as **ResQAC^R** in Fig. 2), featuring eight cationic ammonium groups on the wider rim (Fig. 2). In addition, conformational dynamics of these novel octacationic resorcinarenes will be investigated.

Finally, the antimicrobial effects of **ResQAC^R** against *Staphylococcus aureus* and *Escherichia coli* will be evaluated, alongside an assessment of their cytotoxic properties.

Results and discussion

Synthesis of ResQACs^R

Octacationic **ResQACs^R** (Fig. 2) were easily synthesized drawing inspiration from a method previously reported by Huang in 2011 for the preparation of cationic pillararenes.[25] Thus, resorcinarenes **5a–d**, bearing bromoalkane groups, were initially synthesized as suitable precursors of **ResQAC^R** derivatives (Scheme 1). To obtain resorcinarenes **5a–d**, we tested the direct macrocyclization of monomers **4a–b** with appropriate aldehydes in the presence of $BF_3{\cdot}Et_2O$ in CH_2Cl_2 as solvent (Scheme 1). Precursors **5a–d** were obtained in reasonable yields (25–35%). It is noteworthy that this represents the first example of direct macrocyclization of resorcinol monomers bearing alkyl bromide chains (**4a–b**) to yield the corresponding resorcin[4]arenes. Successively, **5a–d** were treated with an excess of dimethylbutyl amine (30 equiv.) in ethanol solvent (Scheme 1). The mixture was stirred for 18 hours at 80 °C, and



Scheme 1 Direct macrocyclization of resorcinols **4a,b** bearing bromoalkane groups with aldehydes to obtain resorcin[4]arenes **5a–d** as precursors for the synthesis of cationic resorcinarenes **ResQAC^{R}**.

after usual work-up, **ResQAC^{R}** derivatives were obtained as bromide or iodide salts. Finally, halide to nitrate exchange was carried out by treating **ResQAC^{R}(X^{-})$_8$** (X^{-} = Br^{-} or I^{-}) with $AgNO_3$, leading to **ResQAC^{R}(NO$_3^{-}$)$_8$** in 90–95% yields (Scheme 1). The structures of **ResQAC^{R}** were confirmed using HR FT ICR mass spectra, 1D–2D NMR spectra, and X-ray studies (see the ESI†).

Single crystals of **ResQAC^{butyl}** as an iodide salt, suitable for X-ray diffraction, were obtained by slow evaporation of a H_2O/MeOH solution (Fig. 3). The X-ray structure of resorcinarene **ResQAC^{butyl}** (Fig. 3) was determined using synchrotron radiation with cryogenic techniques. The structure of the macrocycle shows a boat conformation with a pseudo C_{2v} symmetry (Fig. 3). The two opposite aryl rings, which define the sides of the boat, make almost orthogonal dihedral angles (Fig. 3d) with respect to the mean plane of the carbon bridging atoms (Fig. 3d). Angles less than 90° indicate that the aryl rings lean inwards towards the twofold axis of the molecule (Fig. 3c and d) and *vice versa*. In particular, one aryl ring of **ResQAC^{butyl}** is slightly inward oriented, and the other is slightly outward oriented (Fig. 3c and d), defining two almost parallel inclined sides of the boat (3.8°). This observation of a more open conformation of **ResQAC^{butyl}** than other resorcinarenes in the boat conformation[26,27] is consistent with the presence of the electrostatic repulsion between the positively charged quaternary alkylammonium side chains. Close inspection of the ^{1}H NMR spectrum of **ResQAC^{butyl}** in CD_3CN (Fig. 4a, 400 MHz, 298 K) clearly indicated that the macrocycle adopts, also in solution, a C_{2v}-boat conformation, stable on the NMR time scale at 298 K (400 MHz). In fact, four $^{+}NCH_3$ singlets were detected at 3.25, 3.24, 3.04, and 3.01 ppm ("*f* and *g*" in Fig. 4), which correlated in the 2D HSQC spectrum with carbon signals between 52.0 and 52.2 ppm. Furthermore, in accordance with the C_{2v} symmetry of **ResQAC^{butyl}**, the aromatic region of its ^{1}H NMR spectrum revealed the presence of four singlets at 7.35, 6.99, 6.69, and 6.15 ppm ("*a* and *b*" in Fig. 4), which are attributable to the aromatic protons.

In addition, the ^{1}H NMR spectrum of **ResQAC^{butyl}** at 298 K showed the presence of two sets of $OCH_2CH_2N^{+}$ diastereotopic resonances between 3.0 and 5.0 ppm (*c*,*c*′ and *d*,*d*′ in Fig. 4). Subsequently, the conformational dynamics of **ResQAC^{butyl}** was studied by ^{1}H VT NMR.[27,28] At room temperature, this macrocycle is frozen in the C_{2v} boat conformation (Fig. 4c and d).

Upon heating, a broadening was observed for the aromatic signals "*a*,*b*" and the diastereotopic aliphatic "*c*,*d*" resonances (Fig. 4c and d), while their coalescence was observed at 373 K. This coalescence (Fig. 4d) clearly indicates conformational mobility of the C_{2v} boat conformation of **ResQAC^{butyl}** due to a boat(C_{2v})–crown(C_{4v})–boat(C_{2v}) conformational interconversion (Fig. 4c),[21,27] which becomes fast on the NMR time scale

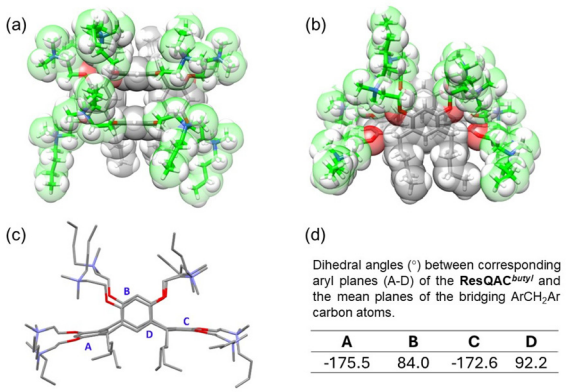


Fig. 3 (a and b) X-ray structure of **ResQAC^{butyl}** (stick representation with van der Waals surfaces). (c) Simple stick representation of **ResQAC^{butyl}**. Hydrogen atoms are omitted for clarity. (d) Dihedral angles (°) between the corresponding aryl planes (A–D in Fig. 3c) of the **ResQAC^{butyl}** structure.

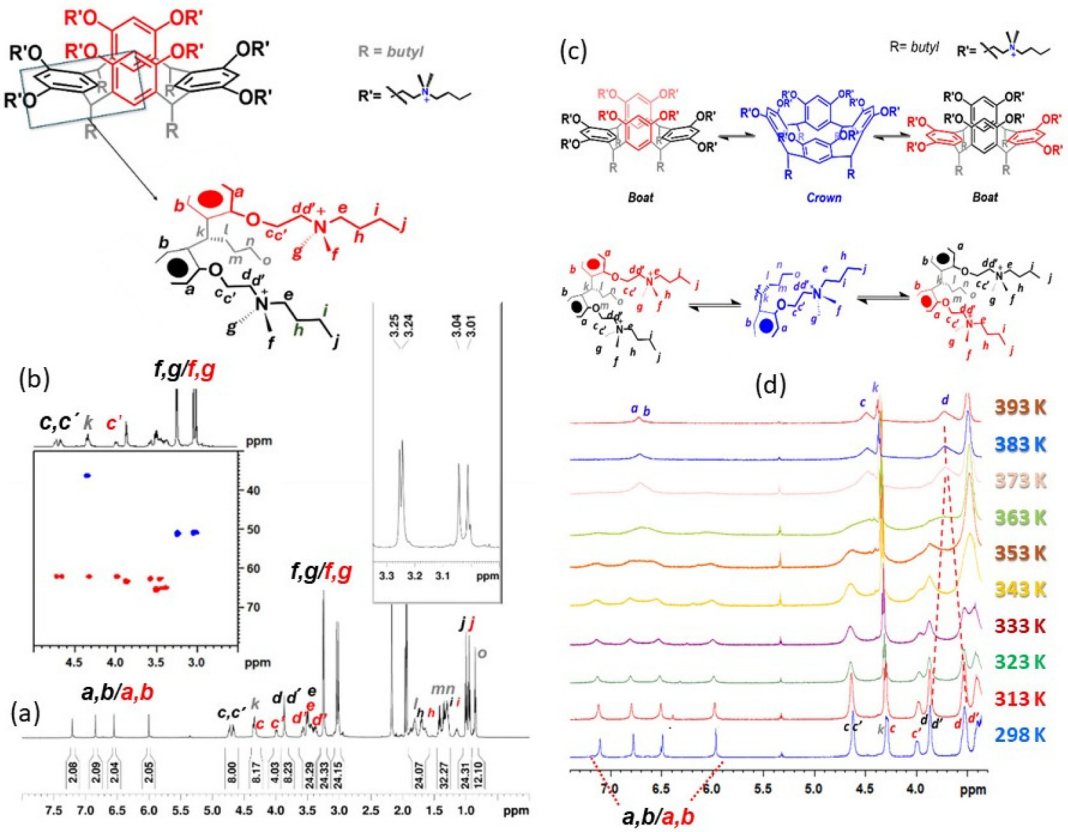


Fig. 4 (a) ^{1}H NMR spectrum and (b) 2D HSQC spectrum of **ResQAC**^{butyl} (600 MHz, CD_3CN, 298 K). (c) Chemical drawing of the boat (C_{2v}) and crown (C_{4v}) conformations of **ResQAC**^{butyl}. (d) ^{1}H VT NMR experiment (600 MHz) for **ResQAC**^{butyl} in DMSO-d_6.

at 393 K (a $\Delta G^{\ddagger}$ value of 17.3 kcal mol^{-1} was calculated for this process, see the ESI†). In fact, at 393 K, the ^{1}H NMR spectrum of **ResQAC**^{butyl} becomes simpler (Fig. 4d) and more compatible with an averaged crown-like structure with C_{4v} symmetry. In a similar way, the ^{1}H NMR spectrum at 298 K of **ResQAC**^{undecyl} reveals a stable boat C_{2v} conformation (with respect to the NMR time scale), while on heating, a broadening of its NMR signals was observed. Therefore, a boat–crown–boat conformational interconversion (Fig. 4c) occurs within the NMR time scale at higher temperature. A coalescence of NMR signals of **ResQAC**^{undecyl} was observed at 373 K, while at 393 K the ^{1}H NMR spectrum agreed with an averaged crown C_{4v} conformation. From these data, an energy barrier value of 17.2 kcal mol^{-1} was calculated for the boat–crown–boat conformational interconversion of **ResQAC**^{undecyl}, a value very similar to that found for **ResQAC**^{butyl}.

With these results in hand, we have investigated the conformational dynamics of **ResQAC**^{phenyl}. In this case, the boat conformation is blocked on the NMR time scale. In fact, on heating, the NMR signals of **ResQAC**^{phenyl} didn't show any evidence of broadening in the temperature range investigated (298–393 K, ESI†).

These results clearly indicate that the presence of phenyl groups at the methine bridges of **ResQAC**^{phenyl} increases the energy barrier to the boat–crown–boat conformational process with respect to the linear aliphatic chains (undecyl and butyl).

Antimicrobial activity of novel ResQACs^R

The antimicrobial activity of compounds **ResQAC**^{butyl}, **ResQAC**^{undecyl}, and **ResQAC**^{phenyl} was evaluated by comparative analysis of both the MIC and the MLD determined for the non-pathogenic Gram-negative strain *Escherichia coli* JM109 and Gram-positive *Staphylococcus aureus* (a pathogenic hospital isolate) (Table 1).

All **ResQAC**^{R} compounds were tested as nitrate salts to avoid bromide and iodide counter-anions, thereby enhancing the safety of these compounds for both humans and the environment. In fact, recent reports[29–31] indicate that iodide and bromide salts of QACs typically threaten water sources with pollution. During disinfection, they can produce harmful by-products such as brominated disinfection by-products (Br-DBPs) and iodinated disinfection by-products (I-DBPs), which pose significant health risks.[29–31] All **ResQAC**^{R} compounds showed antimicrobial activity against both microbial strains, but **ResQAC**^{butyl} and **ResQAC**^{undecyl} were more effective, presenting lower MIC and MLD values (Table 1). The toxic activity was variable with respect to the bacterial group. **ResQAC**^{butyl} showed high bacteriostatic and bactericidal activity with very

Table 1 Antimicrobial activity of the multivalent **ResQAC^{R}** compounds and reference compounds **1b**, **2** and **3** towards Gram-positive *Staphylococcus aureus* (a pathogenic hospital isolate) and Gram-negative strain *Escherichia coli* JM109

		Staphylococcus aureus			*Escherichia coli* JM109		
	Valency[a] (n)	MIC[b,c] μM (μg mL^{-1})	r.p.	MLD[d] μM (μg mL^{-1})	MIC[b,c] μM (μg mL^{-1})	r.p.	MLD[d] μM (μg mL^{-1})
ResQAC^{butyl}	8	0.89 (2)	182	>13 (>30)	≈13 (≈30)	4.6	>125 (>280)
ResQAC^{undecyl}	8	26 (70)	6.1	≈53 (≈140)	≈6.5 (≈17)	9.4	≈107 (≈280)
ResQAC^{phenyl}	8	≈43 (≈100)	3.8	>60 (>140)	≈43 (≈100)	1.4	>121 (>280)
1b	0	R^{e} > 187 (>280)	—	>187 (>280)	R^{e} > 187 (>280)	—	>187 (>280)
2	2	163 (80)	1	≈185 (≈140)	≈61 (≈30)	1	≈143 (≈70)
3	1	R^{e} > 954 (>280)	—	>954 (>280)	≈635 (≈140)	—	>954 (>280)

[a] n = number of ammonium units. [b] MIC (minimum inhibitory concentration). [c] r.p. = relative potency = MIC (derivative **2**)/MIC (**ResQAC^{R}**). [d] MLD (minimum lethal dose). [e] R = resistance to the derivative.

low MIC and MLD (0.89 and >13 μM, respectively) for the Gram-positive strain; on the other hand, **ResQAC^{undecyl}** was more bacteriostatic against *E. coli* than against *S. aureus*, but more bactericidal for the latter (lower MLD values). The less efficient **ResQAC^{phenyl}** showed similar bacteriostatic activity for the two bacteria (MIC = 43 μM), but a variable bactericidal effect. As reported in Table 1, resorcinarene **1b** (Fig. 2) bearing eight neutral heptyl groups on the wider rim didn't show any bacteriostatic or bactericidal effects for the doses tested. With the aim of quantifying the multivalent effect, we chose the resorcinol diammonium derivative **2** and the dibutyl–dimethylammonium derivative **3** shown in Fig. 2 as the reference divalent and monovalent compounds, respectively. The monovalent derivative **3** exhibited very low activity against both *E. coli* and *S. aureus* (MIC = 635 and >954 μM, respectively, Table 1). The resorcinol diammonium derivative **2**, which mimics a single monomeric unit of the multivalent **ResQAC^{R}** macrocycles, showed bacteriostatic activity, MIC = 163 and 61 μM, for *S. aureus* and *E. coli*, respectively (Table 1), which was significantly lower than that found for the **ResQAC^{butyl}** compound (0.89 and 13 μM).

Thus, the relative antimicrobial potencies (r.p. in Table 1) of **ResQAC^{R}** derivatives were obtained by calculating the ratio of the MIC values (μM) of the resorcinol diammonium **2** to that of the multivalent **ResQAC^{R}**.[32] In detail, a remarkable multivalent effect with an r.p. of 182 was observed for the **ResQAC^{butyl}** derivative (MIC = 0.89 μM) against *S. aureus*. According to this finding, each resorcinol diammonium monomer in **ResQAC^{butyl}** experiences an antimicrobial potency amplification of 45.5 times compared to the reference **2** (Fig. 5a and b). The data reported in Table 1 clearly show that *S. aureus* is more responsive to tetraalkylammonium multimerization than *E. coli*. In fact, multivalent **ResQAC^{R}** derivatives showed low r.p. values ranging from 1.4 to 9.4 against *E. coli*. Remarkably, the **ResQAC^{undecyl}** derivative demonstrated lower bacteriostatic activity against *S. aureus* (MIC = 26 μM) than **ResQAC^{butyl}** and exhibited six times greater efficiency than derivative **2** (r. p. = 6.1). Interestingly, **ResQAC^{phenyl}** showed a modest bacteriostatic activity towards both *S. aureus* and *E. coli* and very low multivalent effects (r.p. = 1.4 and 3.8). Thus, by close inspection of the data reported in Table 1, we can conclude that:

(a) the antibacterial activity of **ResQAC^{R}** derivatives is attributable to the interaction with the bacterial cell wall

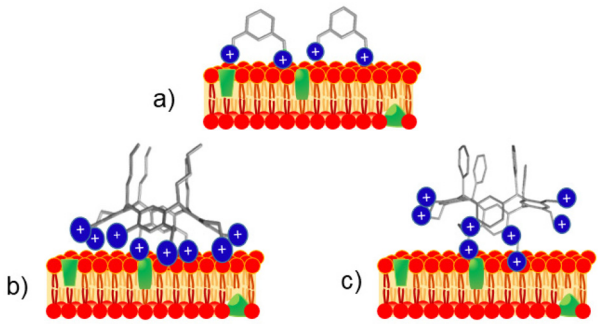


Fig. 5 Cartoon representations depicting the proposed model for the interaction between Gram-positive bacteria and (a) derivative **2**; (b) **ResQAC^{butyl}** in the crown conformation; and (c) **ResQAC^{phenyl}** in the boat conformation.

(Fig. 5a–c). As is known[33–37] for other quaternary ammonium compounds and polycationic macrocycle derivatives, **ResQAC^{R}** also can bind with the negatively charged bacterial surface by electrostatic and hydrophobic interactions (Fig. 5). Differently, no significant biocidal effect of compound **1b** was observed against both *E. coli* and *S. aureus*.

(b) A significant bacteriostatic activity was observed for the **ResQAC^{butyl}** derivative (MIC = 0.89 μM) against *S. aureus.* In addition, **ResQAC^{butyl}** shows a remarkable multivalent effect against S. *aureus*.

(c) *E. coli* is more responsive to **ResQAC^{undecyl}**.

(d) As demonstrated previously by some of the authors,[20,21] the effectiveness of resorcinarene-based multivalent ligands relies on achieving a delicate balance between the preorganization of the macrocycle and its conformational flexibility.[38] In fact, the effectiveness of multi-QACs against bacteria relies on their ability to align all their ammonium groups with the negatively charged bacterial surface (Fig. 5). **ResQAC^{butyl}** and **ResQAC^{undecyl}** derivatives exhibit conformational adaptability, allowing them to fit and interact optimally with the bacterial surface (Fig. 5b). In contrast, the **ResQAC^{phenyl}** derivative features a rigid boat-shaped structure, with four alkylammonium groups positioned away from the surface (Fig. 5c) and four cationic groups facing towards it. Consequently, the decreased activity of **ResQAC^{phenyl}** is likely due to its weaker interaction

with the bacterial wall, as it utilizes only four of its available eight cationic groups (Fig. 5c).[38]

Effect of ResQAC^{R} on cell viability of human HaCat cells

To evaluate the ability of **ResQAC^{R}** compounds to affect the cell viability of eukaryotic cells, a model of immortalized human keratinocytes (HaCat cells) was used. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed after 24 h of treatment with different concentrations of each compound. The percentage of cell viability measured at 100 μM and the calculated IC_{50} values are listed in Table 2. The dose–response curves in the concentration range from 1 to 150 μM for **ResQAC^{butyl}** and compound **2** are presented in Fig. 6. From all these data, we can confirm that **ResQAC^{butyl}** has no negative effect on cell viability at concentrations reaching about 30 times the MIC for *S. aureus* and about 3 times the MIC for *E. coli*. It presents only a moderate toxicity at higher concentrations (IC_{50} = 54.3 μM). On the other hand, compound **2** was less cytotoxic than **ResQAC^{butyl}**, with IC_{50} almost twice that of **ResQAC^{butyl}**; however, at 100 μM, *i.e.* a concentration below the MIC for *S. aureus* and at about 1.6 times the MIC for *E. coli*, cell viability was reduced by more than 50%.

Finally, viability assays clearly indicated that **ResQAC^{undecyl}** was cytotoxic at concentrations (IC_{50} = 12.1 μM) lower than its MIC for *S. aureus*, while **ResQAC^{phenyl}** had a moderate ability to reduce cell viability (IC_{50} = 99.8 μM), whereas compounds **1b** and **3** had no effect on the viability of HaCat cells.

Table 2 Residual cell viability measured in HaCat cells treated for 24 h with each compound at 100 μM. Calculated mean IC_{50} values are also reported

	Residual cell viability at 100 (μM)	IC_{50} (μM)
ResQAC^{butyl}	4.1%	54.3
ResQAC^{undecyl}	4.7%	12.1
ResQAC^{phenyl}	49.8%	99.8
1b	99.4%	>300
2	45.3%	97.2
3	100%	>1 mM

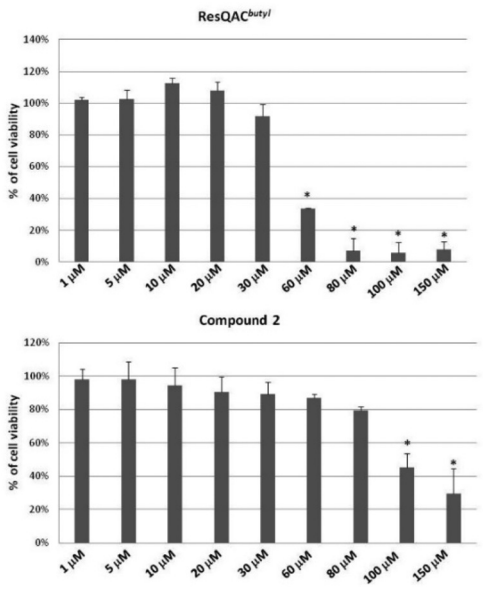


Fig. 6 Effect of **ResQAC^{butyl}** and compound **2** on the viability of human keratinocytes (HaCat) after 24 h of treatment with concentrations ranging from 1 μM to 150 μM. Mean values (± standard deviation) of biological triplicate are reported. *p < 0.0001 *vs.* vehicle-treated cells.

Conclusions

We reported the synthesis of novel resorcin[4]arene multi-QACs (**ResQAC^{R}**) bearing eight tetraalkylammonium groups on the wider rim. This study marks the first example, in the resorcin[4]arene literature, of direct macrocyclization of resorcinol monomers functionalized with bromoalkane groups. In this regard, the information described here could pave the way for the easy synthesis of new resorcin[4]arene hosts.

In the solid state, **ResQAC^{butyl}** adopts a boat conformation with a pseudo C_{2V} symmetry (Fig. 3) to minimize the electrostatic repulsion between the positively charged quaternary alkylammonium side chains. For the first time, the conformational dynamics properties of resorcin[4]arenes bearing cationic groups on the wider rim have been investigated. Dynamic NMR investigations indicate that cationic chains do not inhibit the boat-to-crown conformational process, whereas the groups on the CH-bridges play a crucial role in this process. In fact, **ResQAC^{butyl}** and **ResQAC^{undecyl}** derivatives exhibit a boat–crown–boat conformational interconversion. Conversely, for the **ResQAC^{phenyl}** derivative, conformational boat-to-crown interconversion is blocked within the NMR time scale.

Biological studies unequivocally underscore the antibacterial abilities of **ResQAC^{butyl}** and **ResQAC^{undecyl}** derivatives. **ResQAC^{butyl}** demonstrates a notable bacteriostatic activity against *S. aureus* with a remarkable multivalent effect. Interestingly, studies on the viability of HaCat cells indicate that **ResQAC^{butyl}** exhibits no detrimental effect on cell viability, even at a concentration higher than the MIC for *S. aureus* and *E. coli*. **ResQAC^{phenyl}** show a modest bacteriostatic activity against both *S. aureus* and *E. coli*. Probably, this is due to the conformational rigidity of the resorcinarene framework in **ResQAC^{phenyl}**, which does not facilitate optimal matching between the ammonium groups and the negatively charged bacterial surface. These results corroborate[21] the significance of the conformational flexibility of macrocyclic scaffolds in relation to their interaction with biological targets.[21]

Author contributions

V. F., V. I. and P. D. S. synthesized and characterized all compounds. L. D. S. and G. V. performed all antimicrobial tests. G. P. and I. C. performed all cell viability tests. S. G. and

N. H. were responsible for the X-ray crystal structure analyses. P. D. S. performed the FT ICR MS analysis. V. I., G. V. and C. T. supervised the project. C. G. secured funding and overall supervised the project. The manuscript was written by C. G., P. N., and C. T. and edited by all co-authors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

V. F. and C. T. thank the Programma Operativo Nazionale, Ricerca e Innovazione 2014–2020 (CCI 2014IT16M2OP005), Fondo Sociale Europeo, Azione I.1 "Dottorati Innovativi con caratterizzazione Industriale – CUP D43D20002240006", for the financial support. C. Gaeta thanks "NEXTGENERATIONEU (NGEU)" funded by the Ministry of University and Research (MUR) (PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022, Project code: 2022FSC2FA) for the project: New stimuli-responsive pharmacological chaperones based on prismarenes (PH-PRISM).

References

1 European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA), and European Medicines Agency (EMA), Antimicrobial consumption and resistance in bacteria from humans and food-producing animals, *EFSA J.*, 2024, **22**, e8589.
2 D. S. Paulson, Topical Antimicrobials, in *New Biocides Development*, American Chemical Society, Washington, DC, USA, 2007, vol. 967, pp. 124–150.
3 A. N. Vereshchagin, N. A. Frolov, K. S. Egorova, M. M. Seitkalieva and V. P. Ananikov, Quaternary Ammonium Compounds (QACs) and Ionic Liquids (ILs) as Biocides: From Simple Antiseptics to Tunable Antimicrobials, *Int. J. Mol. Sci.*, 2021, **22**, 6793–6875.
4 W. A. Arnold, A. Blum, J. Branyan, T. A. Bruton, C. C. Carignan, G. Cortopassi, S. Datta, J. DeWitt, A.-C. Doherty, R. U. Halden, H. Harari, E. M. Hartmann, T. C. Hrubec, S. Iyer, C. F. Kwiatkowski, J. LaPier, D. Li, L. Li, J. G. Muñiz Ortiz, A. Salamova, T. Schettler, R. P. Seguin, A. Soehl, R. Sutton, L. Xu and G. Zheng, Quaternary Ammonium Compounds: A Chemical Class of Emerging Concern, *Environ. Sci. Technol.*, 2023, **57**, 7645–7665.
5 C.-C. Lai, S.-Y. Chen, W.-C. Ko and P.-R. Hsueh, Increased antimicrobial resistance during the COVID-19 pandemic, *Int. J. Antimicrob. Agents*, 2021, **57**, 106324.
6 D. N. Shurpik, P. L. Padnya, I. I. Stoikov and P. J. Cragg, Antimicrobial Activity of Calixarenes and Related Macrocycles, *Molecules*, 2020, **25**, 5145–5177.
7 L. Gao, H. Wang, B. Zheng and F. Huang, Combating Antibiotic Resistance: Current Strategies for the Discovery of Novel Antibacterial Materials Based on Macrocycle Supramolecular Chemistry, *Giant*, 2021, **7**, 100066.
8 R. Joseph, A. Naugolny, M. Feldman, I. M. Herzog, M. Fridman and Y. Cohen, Cationic Pillararenes Potently Inhibit Biofilm Formation without Affecting Bacterial Growth and Viability, *J. Am. Chem. Soc.*, 2016, **138**, 754–757.
9 M. Mourer, J.-B. Regnouf-de-Vains and R. E. Duval, Functionalized Calixarenes as Promising Antibacterial Drugs to Face Antimicrobial Resistance, *Molecules*, 2023, **28**, 6954–7000.
10 C. Jonkergouw, N. K. Beyeh, E. Osmekhina, K. Leskinen, S. M. Taimoory, D. Fedorov, E. Anaya-Plaza, M. A. Trant, J. F. Kostiainen, R. H. A. Ras, P. Saavalainen and M. B. Linder, Repurposing Host-Guest Chemistry to Sequester Virulence and Eradicate Biofilms in Multidrug Resistant Pseudomonas aeruginosa and Acinetobacter Baumannii, *Nat. Commun.*, 2023, **14**, 2141–2159.
11 X. Wang, L. Ma, C. Li and Y.-W. Yang, *Chem. Mater.*, 2024, **36**, 2177–2193.
12 D. Kaizerman-Kane, M. Hadar, R. Joseph, D. Logviniuk, Y. Zafrani, M. Fridman and Y. Cohen, Design Guidelines for Cationic Pillar[n]arenes that Prevent Biofilm Formation by Gram-positive Pathogens, *ACS Infect. Dis.*, 2021, **7**, 579–585.
13 G. M. L. Consoli, G. Granata, R. Picciotto, A. R. Blanco, C. Geraci, A. Marino and A. Nostro, Design, synthesis and antibacterial evaluation of a polycationic calix[4]arene derivative alone and in combination with antibiotics, *MedChemComm*, 2018, **9**, 160–164.
14 G. M. L. Consoli, G. Granata, G. Ginestra, A. Marino, G. Toscano and A. Nostro, Antibacterial Nanoassembled Calix[4]arene Exposing Choline Units Inhibits Biofilm and Motility of Gram negative Bacteria, *ACS Med. Chem. Lett.*, 2022, **13**, 916–922.
15 G. M. L. Consoli, I. Di Bari, A. R. Blanco, A. Nostro, M. D'Arrigo, V. Pistarà and S. Sortino, Design, Synthesis, and Antibacterial Activity of a Multivalent Polycationic Calix[4]arene–NO Photodonor Conjugate, *ACS Med. Chem. Lett.*, 2017, **8**, 881–885.
16 J. H. Griffin, M. S. Linsell, M. B. Nodwell, Q. Q. Chen, J. L. Pace, K. L. Quast, K. M. Krause, L. Farrington, T. X. Wu, D. L. Higgins, T. E. Jenkins, B. G. Christensen and J. K. Judice, Multivalent Drug Design. Synthesis and In Vitro Analysis of an Array of Vancomycin Dimers, *J. Am. Chem. Soc.*, 2003, **125**, 6517–6531.
17 S.-K. Choi, *Synthetic Multivalent Molecules: Concepts and Biomedical Applications*, John Wiley & Sons, Inc., Hoboken, New Jersey, 2004.
18 V. Wittmann and R. J. Pieters, Bridging Lectin Binding Sites by Multivalent Carbohydrates, *Chem. Soc. Rev.*, 2013, **42**, 4492–4503.
19 M. González-Cuesta, C. Ortiz Mellet and J. M. García Fernández, Carbohydrate supramolecular chemistry: beyond the multivalent effect, *Chem. Commun.*, 2020, **56**, 5207–5222.
20 J. P. Schneider, S. Tommasone, P. Della Sala, C. Gaeta, C. Talotta, C. Tarnus, P. Neri, A. Bodlenner and

P. Compain, Synthesis and Glycosidase Inhibition Properties of Calix[8]arene-Based Iminosugar Click Clusters, *Pharmaceuticals*, 2020, **13**, 366–386.

21 P. Della Sala, C. Vanni, C. Talotta, L. Di Marino, C. Matassini, A. Goti, P. Neri, S. Šesták, F. Cardona and C. Gaeta, *Org. Chem. Front.*, 2021, **8**, 6648–6656.

22 M. D. Cominetti, D. L. Hughes and S. E. Matthews, Open-resorcinarenes, a new family of multivalent scaffolds, *Org. Biomol. Chem.*, 2016, **14**, 10161–10164.

23 A. G. S. Hoegberg, Cyclooligomeric phenol-aldehyde condensation products. 2. Stereoselective synthesis and DNMR study of two 1,8,15,22-tetraphenyl[14]metacyclophan-3,5,10,12,17,19,24,26-octols, *J. Am. Chem. Soc.*, 1980, **102**, 6046–6050.

24 L. M. Tunstad, J. A. Tucker, E. Dalcanale, J. Weiser, J. A. Bryant, J. C. Sherman, R. C. Helgeson, C. B. Knobler and D. J. Cram, Host-guest complexation. 48. Octol building blocks for cavitands and carcerands, *J. Org. Chem.*, 1989, **54**, 1305–1312.

25 Y. Ma, X. Ji, F. Xiang, X. Chi, C. Han, J. He, Z. Abliz, W. Chen and F. Huang, A cationic water-soluble pillar[5]arene: synthesis and host–guest complexation with sodium 1-octanesulfonate, *Chem. Commun.*, 2011, **47**, 12340–12342.

26 M. Pamuła, M. Nissinen and K. Helttunen, *Chem. – Eur. J.*, 2020, **26**, 7374–7383.

27 P. Timmerman, W. Verboom and D. N. Reinhoudt, Resorcinarenes, *Tetrahedron*, 1996, **52**, 2663–2704.

28 R. J. Kurland, M. B. Rubin and M. B. Wise, Inversion Barrier in Singly Bridged Biphenyls, *J. Chem. Phys.*, 1964, **40**, 2426–2427.

29 Therefore, developing methods to prevent water contamination by bromine and iodide pollutants is crucial for maintaining ecological balance and protecting human health. Furthermore, recent research has shown that bromide intoxication can lead to central nervous system dysfunction. Y. Yang, Y. Komaki, S. Y. Kimura, H.-Y. Hu, E. D. Wagner, B. J. Mariñas and M. J. Plewa, Toxic Impact of Bromide and Iodide on Drinking Water Disinfected with Chlorine or Chloramines, *Environ. Sci. Technol.*, 2014, **48**, 12362–12369.

30 K. Xue, C. Yang and Y. He, A review of technologies for bromide and iodide removal from water, *Environ. Technol. Rev.*, 2023, **12**, 129–148.

31 S. Rayamajhi, S. Sharma and H. Iftikhar, Unexplained Bromide Toxicity Presenting as Hyperchloremia and a Negative Anion Gap, *Cureus*, 2023, **15**, e36218.

32 The use of the factor r.p. to highlight the multivalent effect in antimicrobial activity has previously been reported in the literature by Reymond and coworkers, see: R. U. Kadam, M. Bergmann, M. Hurley, D. Garg, M. Cacciarini, M. A. Swiderska, C. Nativi, M. Sattler, A. R. Smyth, P. Williams, M. Camara, A. Stocker, T. Darbre and J.-L. Reymond, A Glycopeptide Dendrimer Inhibitor of the Galactose-Specific Lectin LecA and of Pseudomonas aeruginosa Biofilms, *Angew. Chem., Int. Ed.*, 2011, **50**, 10631–10635.

33 G. Sautrey, M. Orlof, B. Korchowiec, J.-B. Regnouf de Vains and E. Rogalska, Membrane activity of tetra-*p* guanidinoethylcalix[4]arene as a possible reason for its antibacterial properties, *J. Phys. Chem. B*, 2011, **115**, 15002–15012.

34 B. Korchowiec, M. Gorczyca, E. Rogalska, J.-B. Regnouf-de-Vains, M. Mourer and J. Korchowiec, The selective interactions of cationic tetra-p-guanidinoethylcalix[4]arene with lipid membranes: theoretical and experimental model studies, *Soft Matter*, 2016, **12**, 181–190.

35 M. Grare, M. Mourer, S. Fontanay, J.-B. Regnouf-de-Vains, C. Finance and R. E. Duval, In vitro activity of paraguanidinoethylcalix[4]arene against susceptible and antibiotic-resistant Gram-negative and Gram-positive bacteria, *J. Antimicrob. Chemother.*, 2007, **60**, 575–581.

36 R. Lamartine, M. Tsukada, D. Wilson and A. Shirata, Antimicrobial activity of calixarenes, *C. R. Chim.*, 2002, **5**, 163–169.

37 R. Bragg, A. Jansen, M. Coetzee, W. van der Westhuizen and C. Boucher, Bacterial resistance to Quaternary Ammonium Compounds (QAC) disinfectants, *Adv. Exp. Med. Biol.*, 2014, **808**, 1–13.

38 As reported by Wagner and colleagues, flexibility is a crucial factor in achieving optimal interaction between polycationic species and bacteria: R. Ahmed, A. Vaishampayan, J. L. Cuellar-Camacho, D. J. Wight, I. Donskyi, W. Unger, E. Grohmann, R. Haag and O. Wagner, Multivalent Bacteria Binding by Flexible Polycationic Microsheets Matching Their Surface Charge Density, *Adv. Mater. Interfaces*, 2020, **7**, 1902066.