Bioorganic & Medicinal Chemistry 19 (2011) 4868–4875

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

LuxR dependent quorum sensing inhibition by *N,N′*-disubstituted imidazolium salts

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ARTICLE INFO

Article history:
Received 18 April 2011
Revised 24 June 2011
Accepted 26 June 2011
Available online 29 June 2011

Keywords:
Quorum sensing
Imidazolium
Antagonist
LuxR
AHL

ABSTRACT

Thirty *N,N′*-disubstituted imidazolium salts have been synthesized and evaluated as LuxR antagonists. Substitution on one of the imidazolium nitrogen atoms includes benzhydryl, fluorenyl or cyclopentyl substituent, and alkyl chains of various lengths on the second one. Most of these compounds displayed antagonist activity, with IC_{50} reaching the micromolar range for the most active ones. The disubstituted imidazolium scaffold is thus shown to be a new pertinent pharmacophore in the field of AHL dependent QS inhibition.

1. Introduction

Bacterial quorum sensing (QS) refers to the communication system used by bacteria to adapt themselves to their environment.[1–9] This communication occurs via small molecules, known as autoinducers, which interact with their cognate proteins, referred to as transcriptional regulators, enabling the expression of genes encoding for diverse phenotypes such as virulence, bioluminescence and biofilm formation. Consequently QS inhibition has been considered as a possible strategy to fight pathogenic bacteria, encouraging in the last decades, numerous studies focusing either on the biological or the chemical points of view.[10–19]

In Gram negative bacteria, the autoinducers are mainly *N*-acyl homoserine lactones (AHLs) and the transcriptional factors are LuxR-type proteins.[19] As part of our research aimed at the discovery of potential modulators of AHLs-dependent transcriptional regulators,[20–25] we recently described the computer aided identification of several inhibitors of LuxR dependent quorum sensing, all structurally unrelated to AHL.[26] Such AHL unrelated analogues are still less investigated than more classical AHL analogues.[14,19,27–31] In this series, calmidazolium, a *N,N′*-disubstituted imidazolium salt (Fig. 1), proved to be the most active. Also, imidazolium derivatives are known to be biologically relevant molecules,[32] particularly as antibacterial agents.[33] This opened the way for designing a new family of potential AHLs QS dependent inhibitors. The results of our study on various imidazolium derivatives screened as LuxR dependent QS inhibitors are presented hereafter.

2. Results and discussion

2.1. Design and synthesis of imidazolium derived compounds

Calmidazolium is constituted of a central imidazolium ring which is substituted on both nitrogen atoms, with a dichlorobenzhydryl moiety on one nitrogen atom, and a tetrachloro bisaromatic system on the other. Based on the activity of calmidazolium as QS inhibitor, the synthesis and the biological evaluation towards QS of diversely substituted *N,N′*-disubstituted imidazolium derivatives were investigated. A first series of analogues incorporate, as substituents on one nitrogen atom of the imidazolium ring, an halogenated or non halogenated benzhydryl motif (as found in calmidazolium), and, on the other nitrogen atom, alkyl chains of various length (compounds **11a–g**, **12** and **13**, Fig. 1 and Scheme 1) unlike in the parent calmidazolium. A few compounds include an aromatic ring on this alkyl side chain (**11i–k**, Scheme 1). In a second series, a fluorenyl group was used as a conformationally constrained benzhydryl motif (compound **14**). Finally, alkylimidazolium salts with a smaller cyclic substituent, namely *N*-cyclopentyl-*N*-alkyl imidazolium, with alkyl chains of various length (compound **15**) were studied (Fig. 1 and Scheme 1).

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doi:10.1016/j.bmc.2011.06.075

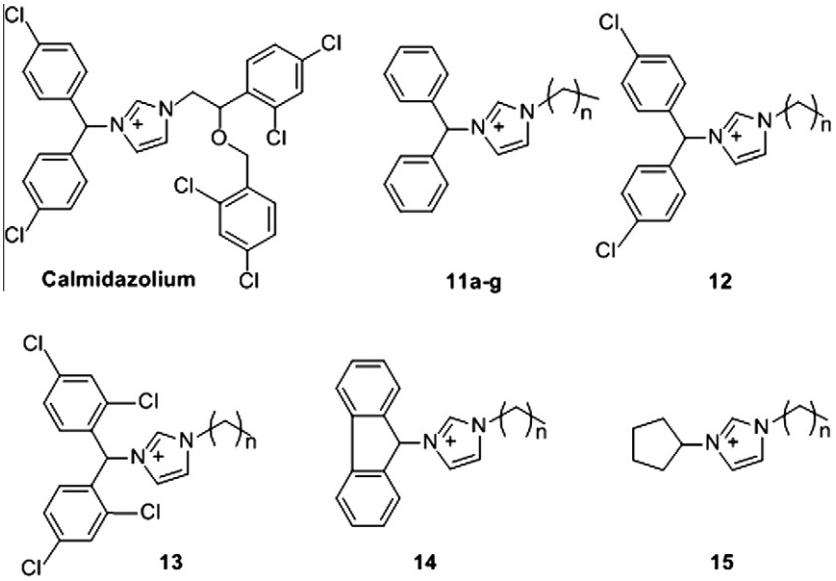
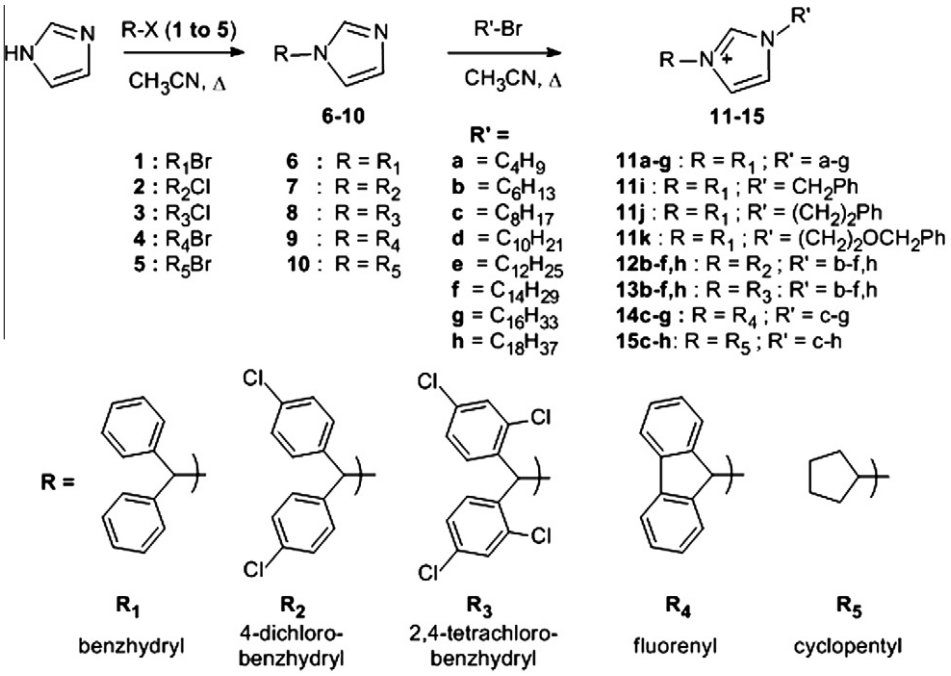


Figure 1. Structure of calmidazolium and analogues **11**–**15**.



Scheme 1. Synthesis of compounds **6**–**15**.

The synthesis of all compounds was achieved by two successive nucleophilic substitutions starting from imidazole (Scheme 1). For the benzhydryl series (compounds **11–13**), the first step involved the reaction of imidazole with commercially available benzhydryl bromide (**1**), 4-dichloro-benzhydryl choride (**2**)[34] or bis (2,4-tetrachloro-benzydryl)chloride (**3**) easily obtained from the known alcohol bis(2,4-dichlorophenyl)methanol.[35] For compounds **14** and **15,** commercially available fluorenyl bromide (**4**) or cyclopentyl bromide (**5**) were used. The second nucleophilic substitution was achieved by reaction of intermediates **6**–**10** with the appropriate bromides (Scheme 1). Targets compounds were obtained as their bromonium salts purified using reverse-phase chromatography.

2.2. Biological evaluation

First, the compounds were tested for their ability to induce bioluminescence, but none of them exhibited any agonistic activity. Then, all compounds were tested for their ability to decrease bioluminescence induced by 3-oxo-C_6-HSL (Table 1).[20] The *N*-benzhydryl derivatives bearing an alkyl chain ending with a phenyl group (**11i–j**) or a benzyl ether substituent (**11k**) were inactive, as well as the short chain (butyl) compound **11a**. Alkyl substituted benzhydryl imidazolium salts with C_6 (**11b**) to C_{16} (**11g**) chains exhibited activities with IC_{50} ranging from 153 to 5.2 μM. The maximum activity was observed for compound **11f** equipped with a C_{14} alkyl

Table 1
Inhibition of bioluminescence with compounds **11**–**15**

Chain length	Compounds				
	11 (R = R_1)	**12** (R = R_2)	**13** (R = R_3)	**14** (R = R_4)	**15** (R = R_5)
R′ = C_4H_9	**11a** Inactive				
R′ = C_6H_{13}	**11b** 153 [a] (±2) [b]	**12b** 63 (±2)	**13b** 52 (±4)		
R' = C_8H_{17}	**11c** 76 (±2)	**12c** 9.8 (±1.1)	**13c** 8.9 (±0.3)	**14c** 35 (±2)	**15c** 68 (±3)
R′ = $C_{10}H_{21}$	**11d** 18 (±2)	**12d** 5.8 (±0.4)	**13d** 4.9 (±0.2)	**14d** 4.0 (±0.6)	**15d** 33 (±2)
R′ = $C_{12}H_{25}$	**11e** 6.1 (±0.2)	**12e** 6.2 (±0.4)	**13e** 6.5 (±0.6)	**14e** 1.40 (±0.4)	**15e** 11 (±2)
R′ = $C_{14}H_{29}$	**11f** 5.2 (±0.2)	**12f** 75 (±19)	**13f** Inactive	**14f** 2.7 (±0.2)	**15f** 3.4 (±0.2)
R′ = $C_{16}H_{33}$	**11g** 7.3 (±0.3)			**14g** 14.7 (±0.8)	**15g** 5.3 (±0.1)
R′ = $C_{18}H_{37}$		**12h** Inactive	**13h** Inactive		**15h** 86 (±7)

[a] Concentration (μM) required to reduce to 50% intensity (IC_{50}) the bioluminescence induced by 200 nM of 3-oxo-C_6-HSL.
[b] Values are the means of three experiments; standard deviation is given in brackets.

chain. This sensitivity to chain length is consistent with the known importance of hydrophobic interactions within the binding site for several other families of QS modulators.[19]

In the case of dichloro- or tetrachloro-benzhydryl compounds having C_6 to C_{18} alkyl chains (**12** and **13**), activities were in the same order of magnitude (IC_{50} ranging from 75 to 4.9 μM). However, with respect to chain length of the alkyl substituent, a 'C_4 shift' was observed compared to non halogenated compounds, the C_{10} derivatives being the most active (IC_{50} 5.8 μM for **12d** and 4.9 μM for **13d**) compared to the C_{14} **11f**. In this series, compounds with longer chains **12f,h** and **13f,h** (C_{14} and C_{18}), were significantly less active (**12f**) or inactive. This 'C_4 shift' suggests that the variation of the occupied volume in the ligand binding site by the benhydryl moiety due to the presence of several bulky halogen atoms must be compensated by a smaller size of the alkyl side chain.

Fluorenyl derivatives (**14c–g**) having C_8–C_{16} alkyl chains were then evaluated and exhibited strong ability for inhibiting bioluminescence (IC_{50} ranging from 35 to 1.4 μM). For these molecules which are conformationally constrained analogues of the benzhydryl series, increasing activity was observed upon increasing the alkyl chain length up the C_{12} and C_{14} compounds (IC_{50} 1.4 μM for the fluorenyl C_{12} **14e** and 2.7 μM for the fluorenyl C_{14} **14f**), the fluorenyl C_{12} **14e** exhibiting the best activity in the whole study. The longer C_{16} analogue **14g** was less active.

Finally, the simpler cyclopentyl derivatives (**15c–h**) having C_8–C_{18} chain length were tested and exhibited IC_{50} ranging from 86 to 3.4 μM. Activities reached a maximum for the C_{14}–C_{16} compounds (**15f** and **15g**), showing that for this series, a longer chain is necessary for achieving sufficient binding, balancing a smaller substituent on the other nitrogen atom. Even the longer *N*-cyclopentyl-*N*-C_{18} **15h** remained moderately active.

It was verified that bioluminescence inhibition did not result from antimicrobial activity rather than QS inhibition. Selected compounds **11e**, **14e** and **15f** were tested using the disk diffusion assay, a common assay for antibiotic susceptibility standardized according to the Clinical and Laboratory Standards Institute guidelines.[36] A control disk diffusion assay was conducted using the relevant kanamycin antibiotic. Compounds were tested at concentrations 20 μM, 200 μM, 2 mM and 20 mM. After overnight incubation at 37 °C, the inhibition zones of each compound were measured. The minimum inhibitory concentration (MIC) was found to be ranged from 200 μM to 2 mM for each compounds, that is, several order of magnitude higher than QS inhibition.

2.3. Molecular modelling

The proposed binding mode of the most active compound, that is, 1-fluorenyl-3-dodecyl imidazol-3-ium derivative **14e**, was obtained as a result of flexible docking[37] in the ligand binding site of the LuxR model[38] (Fig. 2A). According to the modelling, the fluorenyl moiety occupies the space where the lactone moiety of the natural ligand 3-oxo-C_6-AHL usually binds,[24,38] and is involved in attractive interactions with Trp66 and Trp94 (Fig. 2B). The imidazolium ring, replacing the amide function of the natural ligand, is located between aromatic residues of Tyr62 and Tyr70 at distances compatible with cation-π interactions.[39–41] The proposed binding mode also suggests possible electrostatic interactions between the positively charged imidazolium ring and the carboxylate function of Asp79 (Fig. 2B). Lastly, the alkyl chain occupies the space where the alkyl chain of the natural ligand normally sits, interacting with hydrophobic residues, in particular Ile46, Ile56, Ile58, Ile76 and Pro48. Similar docking of the other most active compounds in each series, **11f**, **12e**, **13d** and **15f** showed same overall interactions and binding mode (data not shown).

3. Conclusion

In conclusion, the synthesis of new imidazolium derived compounds and their biological evaluation as QS inhibitors have been accomplished. This study showed that most of these derivatives inhibit LuxR dependent quorum sensing, some of them strongly, with IC_{50} reaching the micromolar range. It also shows that the benzhydryl motif together with an alkyl chain as substituents on both nitrogen atoms of an imidazolium ring, can be considered as a new pharmacophore which allows wide structural variability for future developments. Modelling suggests that the imidazolium ring, being located where the amide function of the natural ligands normally sits, can develop several non-covalent bonding interactions. Preliminary results on the *N*-cyclopentyl-*N*-alkyl compounds show that besides the benzhydryl moiety, but also other types of cyclic systems can be used for varying the substitution on the imidazolium ring. Alkyl chain length appears as a tuning parameter balancing the bulkiness of the cyclic or aromatic system.

4. Experimental

4.1. General

Solvents were distilled and dried before use: dichloromethane from calcium hydride, tetrahydrofuran from sodium benzophenone ketyl. Organic solutions were dried over anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen. The reactions were monitored by t.l.c. on Silica Gel 60 F254 (Merck) and detection was carried out by UV light (254 nm) and/or charring with a 5% phosphomolybdic acid solution in ethanol containing 10% of H_2SO_4, or a 1% potassium permanganate solution in water. Silica gel (Kieselgel 60, 70–230 mesh ASTM, Merck) was employed for column chromatography, and octadecyl-modified silica (C18) HPLC column for reverse-phase chromatography. Melting points were determined on a Kofler block apparatus.

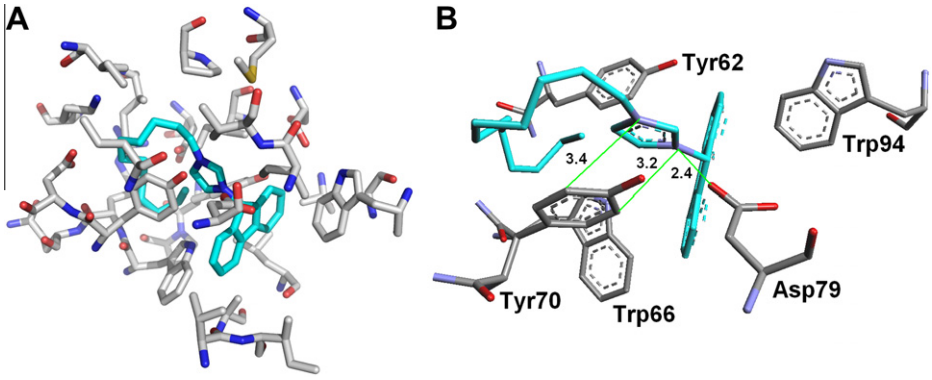


Figure 2. Proposed binding mode of 1-fluorenyl-3-dodecyl imidazol-3-ium derivative in the ligand binding site of the LuxR model. (A) Overview of 1-fluorenyl-3-dodecyl imidazol-3-ium interacting with all residues of the binding site; (B) magnification and simplification of the binding mode showing interactions with conserved residues.

The ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded with a Brucker ALS300, DRX300, and DRX400 spectrometers. Chemical shifts are given in ppm. Coupling constants are expressed in Hertz and splitting pattern abbreviations are: s, singulet; d, doublet; t, triplet; q, quartet; m, multiplet; M, massif, p, pseudo. Multiplicity (^{13}C NMR) was determined by DEPT sequences. High resolution mass spectra were obtained by electro spray technique, positive mode with a Thermo-Finnigan MAT 95 XL spectrometer.

All calculations were performed with ArgusLab[42] as software on a Dell OPTIPLEX GX 620 PC equipped with a double processor. Docking experiments were performed with the docking module of ArgusLab. The protein model of LuxR[38] was created using SWISS-MODEL[43] with ClustalW.[44]

4.2. Synthesis

4.2.1. General procedure for the preparation of compounds 6–10

These compounds were obtained from compounds **1** to **5** and imidazole according to the procedure reported by Corelli et al.[45] for the preparation of **6**. Crude products were purified by flash chromatography to provide compounds **6**–**10**. Spectroscopic data for known compounds **6** and **7** were identical to reported ones in Refs. 45 and 46, respectively.

4.2.1.1. 1-(Bis(2,4-dichlorophenyl)methyl imidazole (8). To a solution of bis(2,4-dichlorophenyl)methanol[35] (600 mg, 1.86 mmol) in dichloromethane (4 mL) was added $SOCl_2$ (146 µl, 2 mmol) and the reaction mixture was stirred at room temperature overnight. The solvent was then evaporated under vacuum to provide the crude product **3** as an oil.

From this oil **3** (450 mg) and imidazole (900 mg, 13.2 mmol), **8** (228 mg, 81% over two steps) was obtained as a white solid according to the general procedure above, after purification by column chromatography using AcOEt/pentane (90:10) as eluent. Mp = 142–143 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.66 (s, d, J = 2.1 Hz, 2H), 6.76 (s, 1H), 7.03 (s, 1H), 7.12 (s, 1H) 7.23 (dd, J = 8.4 Hz; 2.1 Hz, 2H), 7.34 (s, 1H), 7.46 (d, J = 2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 58.6, 119.27, 127.86, 129.61, 130.1, 130.33, 134.2, 134.78, 135.72, 137.42; HRMS ESI$^+$ calcd for $C_{16}H_{11}Cl_4N_2^+$: 370.9671, found: MH$^+$, 370.9677.

4.2.1.2. 1-Fluorenyl imidazole (9). From 9-bromofluorene (2 g, 8.15 mmol) and imidazole (1.66 g, 24 mmol), **9** (1.1 g, 60%) was obtained as a yellow solid after purification by column chromatography using AcOEt/pentane (80/20) as eluent. Mp = 151–153 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.7 (s, 1H), 7.06 (s, 1H), 7.26–7.4 (m, 5H), 7.46 (m, 2H), 7.69 (s, 1H), 7.77 (d, J = 7.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 61.8, 117.7, 120.4, 124.9, 128.1, 129.6, 130, 137, 140.4, 142.26; HRMS ESI$^+$ calcd for $C_{16}H_{13}N_2^+$: 233.1073, found: MH$^+$, 233.1078.

4.2.1.3. 1-Cyclopentyl imidazole (10). From cyclopentyl bromide (1.6 mL, 14.6 mmol) and imidazole (3 g, 43.8 mmol), **10** (1.55 g, 80%) was obtained as a yellow oil after purification by column chromatography using AcOEt/MeOH (80:20) as eluent. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68–1.9 (m, 6H), 2.17 (m, 2H), 4.44 (m, 1H), 6.93 (s, 1H), 7.03 (s, 1H), 7.51 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.7, 33.6, 58.3, 117.4, 129.1, 135.9; HRMS ESI$^+$ calcd for $C_8H_{13}N_2^+$: 137.1073, found: MH$^+$, 137.1075.

4.2.2. General procedure for the preparation of compounds 11–15

A mixture of compound **6**–**10** (1 equiv) and the alkyl bromide (1 equiv) in acetonitrile was refluxed at 90 °C for 72 h. The solvent was removed under vacuum and the residue was purified by reverse-phase chromatography using a stepping gradient of solvent H_2O/MeOH (9:1 to 1:9) to provide compounds **11**–**15**.

4.2.2.1. 1-Benzhydryl-3-benzyl imidazol-3-ium bromide (11i). Compound **11i** (206 mg, 99%) was obtained as an oil from **6** (150 mg, 0.64 mmol) and benzyl bromide (76 µl, 0.64 mmol). ^{1}H NMR (300 MHz, DMSO): δ 5.51 (s, 2H), 7.30 (s, 1H), 7.31–7.6 (m, 15H), 7.8 (s, 1H), 7.96 (s, 1H), 9.49 (s, 1H); ^{13}C NMR (75 MHz, DMSO): δ 52.1, 65.8, 122.7, 123.2, 127.8, 128.1, 128.4, 128.7, 129, 129.2, 134.7, 136.5, 137.1; HRMS ESI$^+$ calcd for $C_{23}H_{21}N_2^+$: 325.1705, found: M$^+$, 325.1706.

4.2.2.2. 1-Benzhydryl-3-(2-phenylethyl) imidazol-3-ium bromide (11j). Compound **11j** (180 mg, 83%) was obtained as an oil from **6** (150 mg, 0.64 mmol) and 2-phenylethyl bromide (88 µl, 0.64 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.18 (t, 2H), 4.59 (t, J = 6.6 Hz, 2H), 6.49 (s, 1H), 7–7.3 (m, 15H), 7.33 (s, 1H), 7.89 (s, 1H), 9.56 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.02, 51, 66.3, 121.2, 123, 128.4, 128.5, 128.7, 129.2, 129.5, 129.6, 135.6, 135.7, 138.9; HRMS ESI$^+$ calcd for $C_{24}H_{23}N_2^+$: 339.1861, found: M$^+$, 339.1861.

4.2.2.3. 1-Benzhydryl-3-(benzylethoxy) imidazol-3-ium bromide (11k). Compound **11k** (650 mg, 68%) was obtained as an oil from **6** (500 mg, 2.13 mmol) and 2-bromoethoxymethyl benzene (338 µl, 2.13 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (t, 2H), 4.5(s, 2H), 4.66 (t, J = 4.1 Hz, 2H), 7.11 (s, 1H), 7.05 – 7.37 (m, 15H), 7.39 (s, 1H), 7.75 (s, 1H), 9.8 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD): δ 50, 68.1, 68.5, 73.8, 123.5, 124.4, 128.7, 128.8, 129.3, 129.3, 129.4, 130.2, 130.3, 137.9, 138.8; HRMS ESI$^+$ calcd for $C_{25}H_{25}ON_2^+$: 369.1967, found: M$^+$, 369.1965.

4.2.2.4. 1-Benzhydryl-3-butyl imidazol-3-ium bromide (11a). Compound **11a** (86 mg, 36%) was obtained as an oil from **6** (150 mg, 0.64 mmol) and 1-bromobutane (69 μl, 0.64 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83 (t, J = 7.5 Hz, 3H), 1.26 (m, 2H), 1.82 (m, 2H), 4.27 (t, J = 7.5 Hz, 2H), 7.11 (s, 1H), 7.1–7.29 (m, 10H), 7.54 (s, 1H), 7.78 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 19.4, 31.9, 50, 66.3, 121.3, 122.8, 128.1, 129.1, 129.2, 136.4, 136.8; HRMS ESI$^+$ calcd for $C_{20}H_{23}N_2^+$: 291.1861, found: M$^+$, 291.1863.

4.2.2.5. 1-Benzhydryl-3-hexyl imidazol-3-ium bromide (11b). Compound **11b** (167 mg, 71%) was obtained as an oil from **6** (150 mg, 0.64 mmol) and 1-bromohexane (90 μl, 0.64 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, J = 6.7 Hz, 3H), 1.15–1.23 (m, 6H), 1.86 (m, 2H), 4.29 (t, J = 7.5 Hz, 2H), 7.13 (s, 1H), 7.15 – 7.35 (m, 10H), 7.58 (s, 1H), 7.71 (s, 1H), 10.19 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 20.1, 25.5, 29.8, 30.7, 50, 66.1, 121.2, 122.8, 127.9, 128.9, 129, 136.3, 136.5; HRMS ESI$^+$ calcd for $C_{22}H_{27}N_2^+$: 319.2174, found: M$^+$, 319.2175.

4.2.2.6. 1-Benzhydryl-3-octyl imidazol-3-ium bromide (11c). Compound **11c** (234 mg, 86%) was obtained as an oil from **6** (150 mg, 0.64 mmol) and 1-bromooctane (111 μl, 0.64 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, J = 6.7 Hz, 3H), 1.05–1.3 (m, 10H), 1.83 (m, 2H), 4.27 (t, J = 6 Hz, 2H), 7.13 (s, 1H), 7.15–7.32 (m, 10H), 7.57 (s, 1H), 7.76 (s, 1H), 10.14 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14, 22.5, 26.1, 28.8, 28.9, 30.1, 31.5, 50.3, 66.4, 121.4, 122.6, 128.2, 129.1, 129.2, 136.5, 136.8; HRMS ESI$^+$ calcd for $C_{24}H_{31}N_2^+$: 347.2487, found: M$^+$, 347.2484.

4.2.2.7. 1-Benzhydryl-3-decyl imidazol-3-ium bromide (11d). Compound **11d** (540 mg, 74%) was obtained as an oil from **6** (300 mg, 1.28 mmol) and 1-bromodecane (266 μl, 1.28 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (t, J = 6 Hz, 3H), 1.15–1.22 (m, 14H), 1.82 (m, 2H), 4.26 (t, J = 7.5 Hz, 2H), 7.14 (s, 1H), 7.15–7.31 (m, 10H), 7.55 (s, 1H), 7.75 (s, 1H), 9.98 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 22, 25.6, 28.3, 28.6, 28.7, 28.8, 29.6, 31.2, 49.7, 65.8, 121.1, 122.5, 127.6, 128.6, 128.7, 135.9, 136; HRMS ESI$^+$ calcd for $C_{26}H_{35}N_2^+$: 375.2801, found: M$^+$, 375.2800.

4.2.2.8. 1-Benzhydryl-3-dodecyl imidazol-3-ium bromide (11e). Compound **11e** (597 mg, 96%) was obtained as an oil from **6** (300 mg, 1.28 mmol) and 1-bromododecane (315 μl, 1.28 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.79 (m, 3H), 1.1–1.21 (m, 18H), 1.82 (m, 2H), 4.26 (t, J = 7.5 Hz, 2H), 7.12 (s, 1H), 7.13–7.3 (m, 10H), 7.53 (s, 1H), 7.70 (s, 1H), 9.99 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 22.1, 25.7, 28.4, 28.7, 28.8, 28.85, 28.9, 29, 31.3, 49.8, 65.9, 121.2, 122.5, 127.6, 128.7, 128.7, 135.9, 136.1; HRMS ESI$^+$ calcd for $C_{28}H_{40}N_2^+$: 403.3113, found: M$^+$, 403.3112.

4.2.2.9. 1-Benzhydryl-3-tetradecyl imidazol-3-ium bromide (11f). Compound **11f** (179 mg, 82%) was obtained as an oil from **6** (100 mg, 0.43 mmol) and 1-bromotetradecane (380 μl, 1.28 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, J = 6.7 Hz, 3H), 1.1–1.3 (m, 22H), 1.87 (m, 2H), 4.31 (t, J = 7.5 Hz, 2H), 7.12 (s, 1H), 7.15–7.4 (m, 10H), 7.54 (s, 1H), 7.6 (s, 1H), 10.3 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 22.7, 26.3, 29, 29.4, 29.45, 29.5, 29.66, 29.71, 29.75, 30.2, 32, 50.5, 66.6, 121.4, 122.2, 128.3, 129.3, 129.4, 136.5, 137.5; HRMS ESI$^+$ calcd for $C_{30}H_{43}N_2^+$: 431.3421, found: M$^+$, 431.3416.

4.2.2.10. 1-Benzhydryl-3-hexadecyl imidazol-3-ium bromide (11g). Compound **11g** (155 mg, 68%) was obtained as an oil from **6** (100 mg, 0.43 mmol) and 1-bromohexadecane (392 μl, 1.28 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, J = 6.7 Hz, 3H), 1.1–1.3 (m, 26H), 1.89 (m, 2H), 4.31 (t, J = 7.5 Hz, 2H), 7.12 (s, 1H), 7.15–7.4 (m, 10H), 7.54 (m, 2H), 10.28 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 22.7, 26.3, 29, 29.43, 29.45, 29.54, 29.67, 29.75, 30.2, 32, 50.5, 66.6, 121.4, 128.3, 129.3, 129.4, 136.5; HRMS ESI$^+$ calcd for $C_{32}H_{47}N_2^+$: 459.3734, found: M$^+$, 459.3727.

4.2.2.11. 1-(Bis(4-chlorophenyl)methyl)-3-hexyl imidazol-3-ium bromide (12b). Compound **12b** (151 mg, 98%) was obtained as an oil from **7** (100 mg, 0.33 mmol) and 1-bromohexane (139 μl, 0.99 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77 (t, J = 6.9 Hz, 3H), 1.18 (m, 6H), 1.85 (m, 2H), 4.27 (t, J = 7.5 Hz, 2H), 7.19 (d, J = 8.5 Hz, 4H), 7.25 (d, J = 8.5 Hz, 4H), 7.29 (t, J = 1.6 Hz, 1H), 7.72 (t, J = 1.6 Hz, 1H), 7.83 (s, 1H), 10.17 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.79, 22.25, 25.77, 29.95, 30.88, 50.41, 64.81, 121.47, 122.73, 129.47, 129.64, 134.68, 135.36, 136.76; HRMS ESI$^+$ calcd for $C_{22}H_{25}Cl_2N_2^+$: 387.1389, found: M$^+$, 387.1374.

4.2.2.12. 1-(Bis(4-chlorophenyl)methyl)-3-octyl imidazol-3-ium bromide (12c). Compound **12c** (191 mg, 78%) was obtained as an oil from **7** (150 mg, 0.49 mmol) and 1-bromooctane (85 μl, 0.49 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83 (t, J = 5.1 Hz, 3H), 1.15–1.3 (m, 10H), 1.88 (m, 2H), 4.3 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 4H), 7.28 (d, J = 8.5 Hz, 4H), 7.27–7.29 (m, 1H), 7.64 (t, J = 1.6 Hz, 1H), 7.83 (s, 1H), 10.21 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7. 21.1, 24.8, 27.5, 27.6, 28.7, 30.2, 49.1, 63.5, 120.3, 121.6, 128.1, 128.4, 133.5, 134, 135.2; HRMS ESI$^+$ calcd for $C_{24}H_{29}Cl_2N_2^+$: 415.1708, found: M$^+$, 415.1707.

4.2.2.13. 1-(Bis(4-chlorophenyl)methyl)-3-decyl imidazol-3-ium bromide (12d). Compound **12d** (157 mg, 91%) was obtained as an oil from **7** (100 mg, 0.33 mmol) and 1-bromodecane (206 μl, 0.99 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.8 (t, J = 6.8 Hz, 3H), 1.15–1.3 (m, 14H), 1.85 (m, 2H), 4.26 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 4H), 7.28 (d, J = 8.5 Hz, 4H), 7.27–7.29 (m, 1H), 7.61 (t, J = 1.6 Hz, 1H), 7.79 (s, 1H), 10.16 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.07, 22.61, 26.24, 28.90, 29.19, 29.33, 29.38, 30.09, 31.78, 50.51, 64.87, 121.49, 122.65, 129.54, 129.70, 134.72, 135.46, 136.93; HRMS ESI$^+$ calcd for $C_{26}H_{33}Cl_2N_2^+$: 443.2015, found: M$^+$, 443.1995.

4.2.2.14. 1-(Bis(4-chlorophenyl)methyl)-3-dodecylimidazol-3-ium bromide (12e). Compound **12e** (155 mg, 85%) was obtained as an oil from **7** (100 mg, 0.33 mmol) and 1-bromododecane (238 μl, 0.99 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.8 (t, J = 6.8 Hz, 3H), 1.14 -1.3 (m, 18 H), 1.84 (m, 2H), 4.27 (t, J = 7.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 4H), 7.27 (d, J = 8.5 Hz, 4H), 7.33 (s, 1H), 7.67 (s, 1H), 7.79 (s, 1H), 10.14 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.01, 22.55, 26.16, 28.84, 29.20, 29.26, 29.29, 29.35, 29.46, 30.03, 31.76, 50.41, 64.77, 121.50, 122.72, 129.44, 129.63, 134.67, 135.35, 136.72; HRMS ESI$^+$ calcd for $C_{28}H_{37}Cl_2N_2^+$: 471.2328, found: M$^+$, 471.2311.

4.2.2.15. 1-(Bis(4-chlorophenyl)methyl)-3-tetradecylimidazol-3-ium bromide (12f). Compound **12f** (173 mg, 90%) was obtained as an oil from **7** (100 mg, 0.33 mmol) and 1-bromotetradecane (294 μl, 0.99 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, J = 6.8 Hz, 3H), 1.19–1.3 (m, 22 H), 1.9 (m, 2H), 4.32 (t, J = 7.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 4H), 7.33 (d, J = 8.5 Hz, 4H), 7.38 (t, J = 1.6 Hz, 1H), 7.72 (t, J = 1.6 Hz, 1H), 7.86 (s, 1H), 10.22 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.05, 22.59, 26.21, 28.88, 29.26, 29.31, 29.40, 29.51, 29.55, 29.59, 30.07, 31.82, 50.45, 64.79, 76.84, 77.16, 77.48, 121.50, 122.72, 129.48, 129.66, 134.71, 135.39, 136.80; HRMS ESI$^+$ calcd for $C_{30}H_{41}Cl_2N_2^+$: 499.2641, found: M$^+$, 499.2623.

4.2.2.16. 1-(Bis(4-chlorophenyl)methyl)-3-octadecylimidazol-3-ium bromide (12h). Compound **12h** (178 mg, 85%) was obtained as a white solid from **7** (100 mg, 0.33 mmol) and 1-bromooctadecane (335 mg, 0.99 mmol). Mp = 80–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85 (t, J = 6.8 Hz, 3H), 1.2–1.3 (m, 30 H), 1.9 (m, 2H), 4.32 (t, J = 7.5 Hz, 2H), 7.27 (d, J = 8.5 Hz, 4H), 7.33 (d, J = 8.5 Hz, 4H), 7.32 - 7.34 (m, 1H), 7.66 (t, J = 1.6 Hz, 1H), 7.86 (s, 1H), 10.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.68, 26.29, 28.95, 29.35, 29.39, 29.49, 29.61, 29.65, 29.69, 30.13, 31.91, 50.55, 64.89, 121.48, 122.63, 129.58, 129.72, 134.75, 135.51, 137.02; HRMS ESI$^+$ calcd for $C_{34}H_{49}Cl_2N_2^+$: 555.3267, found: M$^+$, 555.3251.

4.2.2.17. 1-(Bis(2,4-dichlorophenyl)methyl)-3-hexyl imidazol-3-ium bromide (13b). Compound **13b** (56 mg, 49%) was obtained as an oil from **8** (80 mg, 0.21 mmol) and 1-bromohexane (32 μl, 0.21 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (m, 3H), 1.22 (m, 6H), 1.87 (m, 2H), 4.46 (t, J = 7.3 Hz, 2H), 7.1 (d, J = 2.1 Hz, 2H), 7.26 (s, 1H), 7.32 (dd, J = 2.1 Hz, 8.4 Hz, 2H), 7.35 (s, 1H), 7.44 (d, J = 2.1 Hz, 2H), 7.76 (s, 1H), 10.04 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9. 22.4, 25.8, 30.1, 31, 50.7, 61.4, 122.1, 123.4, 128.7, 130.4, 130.6, 130.9, 134.5, 136.9, 137.8. HRMS ESI$^+$ calcd for $C_{22}H_{23}Cl_4N_2^+$: 455.0610, found: M$^+$, 455.0596.

4.2.2.18. 1-(Bis(2,4-dichlorophenyl)methyl)-3-octyl imidazol-3-ium bromide (13c). Compound **13c** (71 mg, 67%) was obtained as an oil from **8** (70 mg, 0.18 mmol) and 1-bromooctane (32 μl, 0.18 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.84 (m, 3H), 1.19–1.26 (m, 10H), 1.84 (m, 2H), 4.46 (t, J = 7.3 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.23 (t, J = 1.6 Hz, 1H), 7.3–7.36 (m, 3H), 7.46 (d, J = 2 Hz, 2H), 7.64 (t, J = 1.6 Hz, 1H), 9.96 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.6, 26.1, 28.9, 29.1, 30.2, 31.6, 50.7, 61.3, 122.1, 123.4, 128.7, 130.4, 130.6, 130.9, 134.5, 136.9, 137.9; HRMS ESI$^+$ calcd for $C_{24}H_{27}Cl_4N_2^+$: 483.0923, found: M$^+$, 483.0909.

4.2.2.19. 1-(Bis(2,4-dichlorophenyl)methyl)-3-decylimidazol-3-ium bromide (13d). Compound **13d** (145 mg, 88%) was obtained as an oil from **8** (120 mg, 0.32 mmol) and 1-bromodecane (200 μl, 0.97 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, J = 6.8 Hz, 3H), 1.1–1.3 (m, 14H), 1.9 (m, 2H), 4.47 (t, J = 7.3 Hz, 2H), 7.09 (d, J = 8.4 Hz, 2H), 7.31 (m, 1H), 7.34 (dd, J = 2 Hz; 8.4 Hz, 2H), 7.39 (s, 1H), 7.46 (d, J = 2 Hz, 2H), 7.83 (t, J = 1.6 Hz, 1H), 10.11 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.08, 22.62, 26.11, 28.94, 29.21, 29.38, 30.15, 31.80, 50.68, 61.27, 122.14, 123.51, 128.59, 130.38, 130.49, 130.88, 134.44, 136.79, 137.66; HRMS ESI$^+$ calcd for $C_{26}H_{31}Cl_4N_2^+$: 511.1236, found: M$^+$, 511.1218.

4.2.2.20. 1-(Bis(2,4-dichlorophenyl)methyl)-3-dodecylimidazol-3-ium bromide (13e). Compound **13e** (173 mg, 87%) was obtained as an oil from **8** (120 mg, 0.32 mmol) and 1-bromododecane (233 μl, 0.97 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.81 (t, J = 6.8 Hz, 3H), 1.1–1.3 (m, 18H), 1.84 (m, 2H), 4.41 (t, J = 7.3 Hz, 2H), 7.02 (d, J = 8.4 Hz, 2H) 7.25–7.32 (m, 3H), 7.35 (s, 1H), 7.4 (d, J = 2 Hz, 2H), 7.83 (s, 1H), 10.1 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.03. 22.57, 26.05, 28.89, 29.23, 29.34, 29.38, 29.50, 29.51, 30.11, 31.79, 50.58, 61.17, 122.14, 123.58, 128.49, 130.32, 130.38, 130.88, 134.40, 136.68, 137.55; HRMS ESI$^+$ calcd for $C_{28}H_{35}Cl_4N_2^+$: 539.1549, found: M$^+$, 539.1525.

4.2.2.21. 1-(Bis(2,4-dichlorophenyl)methyl)-3-tetradecylimidazol-3-ium bromide (13f). Compound **13f** (152 mg, 87%) was obtained as an oil from **8** (100 mg, 0.27 mmol) and 1-bromotetradecane (240 μl, 0.81 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, J = 6.8 Hz, 3H), 1.1–1.3 (m, 22H), 1.90 (m, 2H), 4.48 (t, J = 7.3 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 7.31 (t, J = 1.6 Hz, 1H), 7.34 (dd, J = 2 Hz; 8.4 Hz, 2H), 7.47 (d, J = 2 Hz, 2H), 7.39 (s, 1H), 7.81 (s, 1H), 10.17 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.13, 22.68, 26.15, 28.99, 29.35, 29.43, 29.49, 29.62, 29.64, 29.65, 29.68, 30.18, 30.95, 31.91, 50.73, 61.33, 122.12, 123.44, 128.65, 130.41, 130.58, 130.87, 134.46, 136.85, 137.82; HRMS ESI$^+$ calcd for $C_{30}H_{39}Cl_4N_2^+$: 567.1862, found: M$^+$, 567.184.

4.2.2.22. 1-(Bis(2,4-dichlorophenyl)methyl)-3-octadecylimidazol-3-ium bromide (13h). Compound **13h** (174 mg, 92%) was obtained as an oil from **8** (100 mg, 0.27 mmol) and 1-bromotetradecane (240 μl, 0.81 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (m, 3H), 1.1–1.3 (m, 30H), 1.85 (m, 2H), 4.43 (t, J = 7.3 Hz, 2H), 7.06 (m, 2H), 7.2–7.32 (m, 3H), 7.35 (s, 1H), 7.42 (m, 2H), 7.77 (s, 1H), 10.12 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.04, 22.59, 26.07, 28.91, 29.26, 29.34, 29.37, 29.42, 29.57, 29.59, 29.62, 30.13, 31.82, 50.57, 61.18, 122.13, 123.59, 128.48, 129.43, 129.68, 130.33, 130.36, 130.89, 134.41, 136.70, 137.62; HRMS ESI$^+$ calcd for $C_{34}H_{47}Cl_4N_2^+$: 623.2488, found: M$^+$, 623.2467.

4.2.2.23. 1-Fluorenyl-3-octyl imidazol-3-ium bromide (14c). Compound **14c** (170 mg, 98%) was obtained as a white solid from **9** (100 mg, 0.4 mmol) and 1-bromooctane (219 μl, 1.2 mmol). Mp = 149–151 °C; ^{1}H NMR (300 MHz, CDCl3): δ 0.74 (t, J = 6.7 Hz, 3H), 1.1–1.25 (m, 14H), 1.8 (m, 2H), 4.21 (t, J = 7.2 Hz, 2H), 6.4 (s, 1H), 6.95 (s, 1H), 7.1 (t, J = 7.5 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 7.31 (t, J = 7.5 Hz, 2H), 7.55 (d, J = 7.5 Hz, 2H), 7.67 (s, 1H), 10.82 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 22.8, 26.5, 29.2, 29.3, 30.5, 31.9, 50.6, 63, 119.6, 120.9, 123.8, 126, 128.8, 130.7, 137.5, 139.8, 140.9; HRMS ESI$^+$ calcd for $C_{24}H_{29}N_2^+$: 345.2325, found: M+, 345.2332.

4.2.2.24. 1-Fluorenyl-3-decyl imidazol-3-ium bromide (14d). Compound **14d** (129 mg, 70%) was obtained as a white solid from **9** (100 mg, 0.4 mmol) and 1-bromodecane (267 μl, 1.2 mmol). Mp = 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.75 (t, J = 6.7 Hz, 3H), 1–1.25 (m, 14H), 1.8 (m, 2H), 4.28 (t, J = 7.2 Hz, 2H), 6.45 (s, 1H), 6.95 (s, 1H), 7.1 (t, J = 7.5 Hz, 2H), 7.3 (m, 4H), 7.54 (d, J = 7.5 Hz, 2H), 7.63 (s, 1H), 10.86 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.4, 26, 28.7, 29, 29.1, 29.2, 30, 31.6, 50.1, 62.5, 119.1, 120.4, 123.2, 125.2, 128.3, 130.2, 137.1, 139.3, 140.4; HRMS ESI$^+$ calcd for $C_{26}H_{33}N_2^+$: 373.2638, found: M$^+$, 373.2645

4.2.2.25. 1-Fluorenyl-3-dodecyl imidazol-3-ium bromide (14e). Compound **14e** (158 mg, 81%) was obtained as a white solid from **9** (100 mg, 0.4 mmol) and 1-bromodecane (310 μl, 1.2 mmol). Mp = 122–124 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.76 (t, J = 6.7 Hz, 3H), 1–1.25 (m, 18H), 1.8 (m, 2H), 4.24 (t, = 7.2 Hz, 2H), 6.46 (s, 1H), 6.96 (s, 1H), 7.14 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.5 Hz, 2H), 7.37 (d, J = 7.5 Hz, 2H), 7.57 (s, 1H), 7.58 (d, J = 7.5 Hz, 2H), 10.8 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14, 22.5, 26.1, 28.8, 29.2, 29.3, 29.4, 30.1, 31.7, 50, 63, 119.3, 120.5, 123.1, 125.4, 128.4, 130.3, 137.2, 139.5, 140.5; HRMS ESI$^+$ calcd for $C_{28}H_{37}N_2^+$: 401.2951, found: M$^+$, 401.2940

4.2.2.26. 1-Fluorenyl-3-tetradecyl imidazol-3-ium bromide (14f). Compound **14f** (201 mg, 97%) was obtained as a white solid from **9** (100 mg, 0.4 mmol) and 1-bromotetradecane (384 μl, 1.2 mmol). Mp = 127–129 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.8 (t, J = 6.7 Hz, 3H), 1–1.25 (m, 22H), 1.85 (m, 2H), 4.27 (t, J = 7.2 Hz, 2H), 6.47 (s, 1H), 7 (s, 1H), 7.16 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.5 Hz, 2H), 7.4 (d, J = 7.5 Hz, 2H), 7.56 (s, 1H), 7.6 (d, J = 7.5 Hz, 2H), 10.92 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14, 22.6, 26.2, 28.9, 29.2, 29.3, 29.4, 29.5, 29.55, 29.6, 30.1, 31.8, 50.3, 62.8, 119.3, 120.5, 123, 125.5, 128.4, 130.3, 137.4, 139.5, 140.6; HRMS ESI$^+$ calcd for $C_{30}H_{41}N_2^+$: 429.3264, found: M$^+$, 429.3264.

4.2.2.27. 1-Fluorenyl-3-hexadecyl imidazol-3-ium bromide (14g). Compound **14g** (201 mg, 74%) was obtained as a brown solid from **9** (100 mg, 0.4 mmol) and 1-bromohexadecane (373 μl, 1.2 mmol). Mp = 118–120 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.8 (t, J = 6.7 Hz, 3H), 1–1.25 (m, 26H), 1.84 (m, 2H), 4.25 (t, J = 7.2 Hz, 2H), 6.5 (t, J = 1.6 Hz, 1H), 7.01 (s, 1H), 7.15 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.37 (d, J = 7.5 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.61 (s, 1H), 10.99 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14, 22.6, 26.2, 28.9, 29.25, 29.3, 29.4, 29.51, 29.56, 29.6, 30.1, 31.8, 50.3, 62.7, 119.3, 120.5, 123.2, 125.4, 128.4, 130.3, 137.4, 139.5, 140.6; HRMS ESI$^+$ calcd for $C_{32}H_{45}N_2^+$: 457.3577, found: M$^+$, 457.3568

4.2.2.28. 1-Cyclopentyl-3-octyl imidazol-3-ium bromide (15c). Compound **15c** (313 mg, 93%) was obtained as an oil from **10** (140 mg, 1 mmol) and 1-bromooctane (178 μl, 1 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.9 Hz, 3H), 1.25–1.36 (m, 10H), 1.71–2.1 (m, 8H), 2.35- 2.41 (m, 2H), 4.39 (t, J = 7.2 Hz, 2H), 4.94 (m, 1H), 7.27 (m, 2H), 10.71 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 22.1, 23.2, 25.8, 28.5, 28.6, 29.4, 31.2, 33, 49.5, 61, 120.7, 122.1, 135.5; HRMS ESI$^+$ calcd for $C_{16}H_{29}N_2^+$: 249.2325, found: M$^+$, 249.2335.

4.2.2.29. 1-Cyclopentyl-3-decyl imidazol-3-ium bromide (15d). Compound **15d** (235 mg, 90%) was obtained as an oil from **10** (100 mg, 0.73 mmol) and 1-bromodecane (458 μl, 2.2 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83 (t, J = 6.7 Hz, 3H), 1.15–1.3 (m, 14H), 1.6–2 (m, 8H), 2.36 (m, 2H), 4.36 (t, J = 7.2 Hz, 2H), 4.93 (m, 1H), 7.41 (s, 1H), 7.43 (s, 1H), 10.53 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.8, 22.3, 23.4, 26, 28.7, 28.9, 29.1, 29.2, 30.2, 31.5, 33.3, 49.7, 61.3, 120.7, 122.2, 135.7; HRMS ESI$^+$ calcd for $C_{18}H_{33}N_2^+$: 277.2638, found: M$^+$, 277.265.

4.2.2.30. 1-Cyclopentyl-3-dodecyl imidazol-3-ium bromide (15e). Compound **15e** (223 mg, 79%) was obtained as an oil from **10** (100 mg, 0.73 mmol) and 1-bromododecane (511 μl, 2.2 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.66 (t, J = 6.7 Hz, 3H), 0.9–1.3 (m, 18H), 1.5–1.8 (m, 8H), 2.16 (m, 2H), 4.18 (t, J = 7.2 Hz, 2H), 4.76 (m, 1H), 7.43 (s, 1H), 7.47 (s, 1H), 10.13 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.7, 22.3, 23.3, 26, 28.7, 28.9, 29, 29.1, 29.2, 30, 31.5, 33.1, 49.6, 61.1, 120.7, 122.2, 135.6; HRMS ESI$^+$ calcd for $C_{20}H_{37}N_2^+$: 305.2951, found: M$^+$, 305.296.

4.2.2.31. 1-Cyclopentyl-3-tetradecyl imidazol-3-ium bromide (15f). Compound **15f** (253 mg, 84%) was obtained as an oil from **10** (100 mg, 0.73 mmol) and 1-bromotetradecane (655 μl, 2.2 mmol). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.71 (t, J = 6.7 Hz, 3H), 0.9–1.4 (m, 22H), 1.5–1.8 (m, 8H), 2.21 (m, 2H), 4.23 (t, J = 7.2 Hz, 2H), 4.8 (m, 1H), 7.45 (s, 1H), 7.49 (s, 1H), 10.2 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.4, 23.5, 26, 28.8, 29.1, 29.2, 29.3, 29.4, 29.27, 30.2, 31.6, 33.3, 49.7, 61.3, 120.8, 122.3, 135.8; HRMS ESI$^+$ calcd for $C_{22}H_{41}N_2^+$: 333.3264, found: M$^+$, 333.3278

4.2.2.32. 1-Cyclopentyl-3-hexadecyl imidazol-3-ium bromide (15g). Compound **15g** (303 mg, 94%) was obtained as a brown solid from **10** (100 mg, 0.73 mmol) and 1-bromohexadecane (670 μl, 2.2 mmol), mp = 67–69 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (t, J = 6.7 Hz, 3H), 0.8–1.3 (m, 26H), 1.4–1.8 (m, 8H), 2.11 (m, 2H), 4.15 (t, J = 7.2 Hz, 2H), 4.7 (m, 1H), 7.43 (s, 1H), 7.47 (s, 1H), 10.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.6, 22.1, 23.2, 25.8, 28.5, 28.8, 28.9, 29, 29.1, 29.2, 29.9, 31.4, 33, 49.4, 60.9, 120.7, 122.1, 135.5; HRMS ESI$^+$ calcd for $C_{24}H_{45}N_2^+$: 361.3577, found: M$^+$, 361.3592.

4.2.2.33. 1-Cyclopentyl-3-octadecyl imidazol-3-ium bromide (15h). Compound **15h** (321 mg, 94%) was obtained as a brown solid from **10** (100 mg, 0.73 mmol) and 1-bromooctadecane (750 μl, 2.2 mmol). Mp = 79–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61 (t, J = 6.7 Hz, 3H), 0.8–1.3 (m, 30H), 1.4–1.8 (m, 8H), 2.11 (m, 2H), 4.15 (t, J = 7.2 Hz, 2H), 4.7 (m, 1H), 7.43 (s, 1H), 7.47 (s, 1H), 10.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.6, 22.2, 23.2, 25.8, 28.6, 28.9, 29.05, 29.05, 29.13, 29.15, 29.16, 29.2, 30, 31.4, 33, 49.4, 61, 120.7, 122.2, 135.5; HRMS ESI$^+$ calcd for $C_{26}H_{49}N_2^+$: 389.389, found: M$^+$, 389.3904.

4.3. Molecular modelling

In order to perform docking studies, the ligand binding site of LuxR was created by selecting residues interacting with calmidazolium, Docking experiments were performed with the following parameters: Docking box: $X = Y = Z = 15$ Å, ligand option: flexible; calculation type: Dock; Docking engine: GADock (Genetic Algorithm).[47] Genetic algorithm dock engine settings: default advanced parameters; hydrogen bonds were assigned within a distance of 3 Å.

Acknowledgments

Financial support from MESR, CNRS, and the "Cluster de recherche Chimie/infectiologie de la Région Rhône-Alpes" is gratefully acknowledged. M.S. is the recipient of a scholarship from the cluster.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.075.

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