Synthesis and antimicrobial activity of novel bis-benzimidazolium salts

Amélie Dubreuil, Pierre Mas and Andreea R. Schmitzer*

SUPPORTING INFORMATION

Département de chimie, Université de Montréal, CP 6128, Succursale Centre-Ville, Montréal, Québec, H3C 3J7, Canada

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Experimental part

Materials and methods

$^1$H- and $^{13}$C-NMR spectra were recorded on 400 and 75 MHz spectrometers, respectively, in the indicated solvent. Chemical shifts are reported in ppm with internal reference to TMS, and $J$ values are given in Hertz. The purity of final compounds used in biological assays was determined by ESI/LC-MS on Quantum TSQ Ultra instruments (C18 (2.1 x 100 mm) column, water +0.1% formic acid). Chemicals were purchased from Sigma-Aldrich and used without further purification.

Synthesis and characterization

**1-Methylbenzimidazole (2):** To a solution of benzimidazole (3.00 g, 25.4 mmol) in 12.7 mL of DMF, KOH (2.85 g, 50.8 mmol) was added. The mixture was stirred for 15 min at 20 °C and iodomethane (3.96 g, 27.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organic layers were combined and washed with brine and dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-methylbenzimidazole as a brown oil in 94 % yield. $^1$H NMR (400 MHz, DMSO) $\delta$ 8.18 (s, 1H), 7.66 (dt, $J = 7.8, 1.0$ Hz, 1H), 7.58 – 7.51 (m=dt, $J = 7.8, 1.0$ Hz 1H), 7.27 (td, $J 7.6, 1.3$ Hz, 1H), 7.21 (td, $J = 7.2, 1.3$ Hz, 1H), 3.83 (s, 3H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 162.78, 145.00, 143.78, 135.04, 122.68, 121.88, 119.73, 110.61, 31.08. LCMS (ESI): calcd. for [M+H]$^+$ C$_8$H$_8$N$_2$: 133.0760, found 133.0759.
**1-Butylbenzimidazole (3):** To a solution of benzimidazole (3.00 g, 25.4 mmol) in 11.5 mL of DMSO, KOH (2.85 g, 50.8 mmol) was added. The mixture was stirred for 15 min at 20 °C and 1-bromobutane (3.83 g, 27.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organics layers were combined and washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-butylbenzimidazole as a brown oil in 83 % yield. ¹H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.65 (dt, J = 7.8, 1.0 Hz, 1H), 7.58 (dt, J = 7.8, 1.0 Hz 1H), 7.24 (td, J = 7.6, 1.3 Hz, 1H), 7.19 (td, J = 7.1, 1.3 Hz, 1H), 4.23 (t, J = 7.1 Hz, 2H), 1.82 – 1.70 (p, J = 7Hz 2H), 1.31 – 1.17 (h, J = 7Hz 2H), 0.87 (t, J = 7.4 Hz, 3H). ¹³C NMR (101 MHz, DMSO) δ 144.47, 143.95, 134.29, 122.63, 121.98, 121.80, 119.89, 110.83, 44.25, 31.92, 19.83, 13.88. LCMS (ESI): calcd. for [M+H]⁺ C₁₁H₁₄N₂: 175.1230, found 175.1227.

**1-Octylbenzimidazole (4):** To a solution of benzimidazole (6.00 g, 50.8 mmol) in 23.0 mL of DMSO, KOH (5.70 g, 102 mmol) was added. The mixture was stirred for 15 min at 20 °C and 1-bromo-octane (10.8 g, 55.9 mmol) was added dropwise under vigorous stirring. After 12 h at 80 °C, the mixture was diluted with 50 mL of water and the organic layer was extracted with ethyl acetate (3 x 25 mL). The organics layers were combined and washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by flash chromatography (100 % ethyl acetate) to obtain 1-octylbenzimidazole as a brown oil in 77 % yield. ¹H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 7.65 (dt, J = 7.5, 1.3 Hz, 1H), 7.58 (dt, 7.5, 1.3 Hz, 1H), 7.23 (td, J = 7.6, 1.3 Hz, 1H), 7.18 (td, J = 7.5, 1.3 Hz, 1H), 4.21 (t, J = 7.1 Hz, 2H), 1.76 (p, J = 7.1 Hz, 2H), 1.28 – 1.14 (m, 11H), 0.81 (t, J = 6.8 Hz, 3H). ¹³C NMR (101 MHz, DMSO) δ 144.44, 143.95, 134.27, 122.59, 121.76, 119.89, 110.76, 44.52, 31.63, 29.84, 29.05, 28.94, 26.58, 22.51, 14.34. LCMS (ESI): calcd. for [M+H]⁺ C₁₅H₂₂N₂: 231.1856, found 231.1855.
((1,3-phenylenebis(ethyne-2,1-diyl))bis(4.1-phenylene))dimethanol (6): To a solution of 4-iodobenzyl alcohol (2.56 g, 10.09 mmol) in degassed piperidine (15 mL, copper iodide (I) (16.3 mg, 85.6 μmol), bis(triphenylphosphine) palladium (II) chloride (57.8 mg, 82.3 μmol) were added. The solution was cooled down to 0 °C under nitrogen and 1.3-diethynylbenzene (600 mg, 4.76 mmol) was added dropwise over 5 min. The reaction mixture was warmed to room temperature and stirred for 3h. The crude was dissolved in acetone and precipitated in water. The product was isolated by filtration to afford the diol as white powder in 100 % yield. \[\text{^1H NMR (400 MHz, DMSO) } \delta 7.71 (t, J = 1.7 Hz, 1H), 7.60 – 7.51 (m, 6H), 7.47 (t, J=8Hz, 1H), 7.39 (d, J = 7.9 Hz, 4H), 5.31 (t, J = 5.7 Hz, 2H), 4.54 (d, J = 5.7 Hz, 4H).\][13C NMR (101 MHz, DMSO) \(\delta\) 144.23, 134.30, 131.82, 131.76, 129.84, 127.11, 123.54, 120.60, 90.80, 88.35, 62.97; LCMS (ESI): calcd. for [M+Na]^+ C_{24}H_{18}O_2: 361.1199, found 361.1197.]

1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (7): To a solution of ((1,3-phenylenebis(ethyne-2,1-diyl))bis(4.1-phenylene))dimethanol (500 mg, 1.48 mmol) in anhydrous CH\(_2\)Cl\(_2\) (5.00 mL), phosphorus tribromide (694 μL, 7.39 mmol) was added dropwise at 0 °C. The reaction was warmed to room temperature. After 2h, the solution was slowly quenched with saturated aqueous NaHCO\(_3\) and extracted with CH\(_2\)Cl\(_2\) (3x 20 mL). The organic layers were combined and dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure and the crude reaction mixture was purified on silica gel (1:99 ethyl acetate / hexane) to yield the dibromide as a yellow powder with 68 % yield. \[\text{^1H NMR (500 MHz, DMSO) } \delta 7.80 – 7.73 (m, 1H), 7.64 – 7.47 (m, 11H), 4.76 (s, 4H).\][13C NMR (126 MHz, DMSO) \(\delta\) 138.94, 134.00, 132.36, 132.17, 131.76, 129.71, 122.79, 121.81, 89.78, 88.93, 33.81. It was impossible to determine the exact mass because the compound does not ionize.
1.1″-(((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-methyl-1H-benzo[d]imidazol-3-iium) dibromide (8):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (100 mg, 215 μmol) in acetonitrile (1.15 mL), 1-methylbenzimidazole (71.2 mg, 539 μmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted in CH₂Cl₂ and precipitated in hexane. The product was dried under vacuum to obtain 95 % yield as white-grey powder. Purity 100 %. ¹H NMR (400 MHz, DMSO) δ 9.86 (s, 2H), 8.05 (dd, J = 7.7, 1.5 Hz, 2H), 7.92 (dd, J = 7.7, 1.5 Hz, 2H), 7.74 – 7.53 (m, 15H), 7.50 (t, J = 8Hz 1H), 5.83 (s, 4H), 4.11 (s, 6H). ¹³C NMR (101 MHz, DMSO) δ 143.15, 134.87, 133.93, 132.08, 131.97, 131.73, 130.69, 128.63, 126.69, 126.62, 122.70, 122.25, 113.81, 113.62, 89.53, 88.91, 49.41, 33.43.; LCMS (ESI): calcd. for [M⁺²] C₄₀H₃₂N₄: 284.1308, found 284.1297; IR (neat cm⁻¹) 3384, 2925, 1608, 1509, 1450, 1274, 1092; Melting point: 290-294 °C

1.1″-(((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-butyl-1H-benzo[d]imidazol-3-iium) dibromide (9):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (80 mg, 172 μmol) in acetonitrile (1 mL), 1-butylbenzimidazole (75.1 mg, 431 μmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH₂Cl₂ and precipitated in hexane. The product was dried under vacuum to obtain 78 % yield as white-grey powder. Purity 100 %. ¹H NMR (400 MHz, DMSO-d₆) δ: 10.01 (s, 2H), 8.11 (d, J = 8 Hz, 2H), 7.92 (d, J = 8 Hz, 2H), 7.68-7.55 (m, 16H), 5.82 (s, 4H), 4.51 (t, J = 8 Hz, 4H), 1.90 (m, 4H), 1.35 (m, 4H), 0.92 (t, J = 8 Hz, 6H); ¹³C NMR (400 MHz, DMSO-d₆) δ: 142.58. 134.87, 133.97, 132.01, 131.75, 131.37, 130.87 129.53, 128.68, 126.77, 126.71, 122.71, 122.27, 113.97, 113.84, 89.55, 88.94, 49.55, 46.64, 30.46, 19.14, 13.44; LCMS (ESI): calcd. for [M⁺²] C₄₆H₄₄N₄: 326.1777, found 326.1765; IR (neat cm⁻¹) 3400, 2956, 1607, 1557, 1445, 1272, 1112; Melting point: 253-258 °C
1.1'-((1.3-phenylenebis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-octyl-1H-benzo[d]imidazol-3-ium) dibromide (10):  

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)benzene (150 mg, 323 μmol) in acetonitrile (1.65 mL), 1-octylbenzimidazole (186 mg, 808 μmol) was added and the solution was stirred 24 h at 80 °C. The reaction mixture was diluted with CH$_2$Cl$_2$ and precipitated in hexane. The product was dried under vacuum to obtain 95 % yield as white-grey powder. Purity 99.21 %.  

$^1$H NMR (400 MHz, DMSO) δ 10.00 (s, 2H), 8.13 (dd, $J = 7.7, 1.5$ Hz, 2H), 7.95 (dd, $J = 7.7, 1.5$ Hz, 2H), 7.76 – 7.63 (m, 5H), 7.63 – 7.54 (m, 10H), 7.49 (t, $J = 6.8$ Hz, 1H), 5.83 (s, 4H), 4.52 (t, $J = 7.3$ Hz, 4H), 1.93 (q, $J = 7.1$ Hz, 4H), 1.42 – 1.11 (m, 21H), 0.84 (t, $J = 6.6$ Hz, 6H).  

$^{13}$C NMR (101 MHz, DMSO) δ 142.55, 134.89, 133.92, 131.98, 131.73, 131.33, 130.86, 129.51, 128.64, 126.77, 126.70, 122.69, 122.26, 113.95, 113.82, 89.51, 88.92, 49.52, 46.86, 31.13, 28.50, 28.39, 25.77, 22.05, 13.93. LCMS (ESI): calcd. for [M$^{+2}$] C$_{54}$H$_{60}$N$_4$: 382.2403, found 382.2420; IR (neat cm$^{-1}$) 3121, 3009, 2952, 1605, 1557, 1452, 1219, 1060; Melting point: 239-243 °C

2.6-bis((trimethylsilyl)ethynyl)pyridine (12): To a solution of 2.6-dibromopyridine (300 mg, 1.27 mmol) in degassed toluene (6.00 mL), copper (I) iodine (24.10 mg, 127 μmol), tetrakis(triphenylphosphine) palladium (0) (146 mg, 127 μmol) and piperidine (751 μL, 7.60 mmol) was added. After 5 minutes stirring, ethynyltrimethylsilane (526 μL, 3.80 mmol) was slowly added and the reaction was stirred overnight under nitrogen. The reaction was quenched with water and the organic layer was extracted 3x with CH$_2$Cl$_2$. The organic layer was washed with saturated NH$_4$Cl and dried over Na$_2$SO$_4$. The reaction mixture was concentrated on a rotavap and the product was purified by flash chromatographic. (25:75 hexane/DCM) in 100 % yield as a pink-brown powder.  

$^1$H NMR (400 MHz, DMSO-d$_6$) δ: 7.83 (t, $J = 8$ Hz, 1H), 7.54 (d, $J = 8$ Hz, 2H), 0.27 (s, 18H); $^{13}$C NMR (101 MHz, DMSO) δ 142.69, 138.17, 127.51, 103.87, 95.10, 0.04. LCMS (ESI): calcd. for [M+H]$^+$ C$_{15}$H$_{21}$NSi$_2$: 272.1285, found 272.1285.
**2.6-diethynylpyridine (13):** To a solution of 2.6-bis((trimethylsilyl)ethynyl)pyridine (1.50 g, 5.52 mmol) in anhydrous CH$_2$Cl$_2$ (27.6 mL), TBAF (3.61 g, 13.8 mmol) was added. The solution was stirred at room temperature for 3-4 h. The reaction was quenched with water and the organic layer was extracted 3x with CH$_2$Cl$_2$. The organic layer was washed with brine and dried over Na$_2$SO$_4$. The solvent was removed under pressure and the crude reaction mixture was purified on silica gel (50:50 ethyl acetate / hexane) to afford 2.6-diethynylpyridine as white-brown powder in 80 % yield. $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$: 7.81 (t, $J = 10$ Hz, 1H), 7.54 (d, $J = 10$ Hz, 2H), 4.36 (s, 2H); $^{13}$C NMR (126 MHz, DMSO) $\delta$: 142.10, 137.78, 127.40, 82.31, 80.85. LCMS (ESI): calcd. for [M+H]$^+$ C$_9$H$_5$N: 128.0495, found 128.0495.

**((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))dimethanol (14):** To a solution of 4-iodobenzyl alcohol (1.24 g, 5.31 mmol) in degassed toluene (9.83 mL), triphenylphosphine (77.4 mg, 295 $\mu$mol), copper(I) iodine (37.4 mg, 197 $\mu$mol), bis(triphenylphosphine) palladium(II) chloride (138 mg, 197 $\mu$mol) and triethylamine (0.726 mL, 11.8 mmol) was added. After 5 minutes stirring, 2.6-diethynlypyridine was slowly added and the mixture was stirred 24 h under nitrogen at ambient temperature. The reaction was quenched with water and the organic layer was extracted 3x with CH$_2$Cl$_2$. The combine organic layer was washed with a 1M aqueous NH$_4$Cl solution and dried over Na$_2$SO$_4$. The reaction mixture was concentrated on a rotavap and the product was obtained by flash chromatographic (80:20 hexane/ethyl acetate and 10:35:55 methanol/hexane/ethyl acetate) in 72 % yield as a yellow-orange powder. $^1$H NMR (400 MHz, DMSO) $\delta$: 7.92 (t, $J = 7.8$ Hz, 1H), 7.68 – 7.58 (m, 6H), 7.43 (d, $J = 8.2$ Hz, 4H), 5.35 (t, $J = 5.7$ Hz, 2H), 4.56 (d, $J = 5.7$ Hz, 4H).$^{13}$C NMR (101 MHz, DMSO) $\delta$: 144.92, 143.28, 138.12, 132.07, 127.18, 127.09, 119.69, 89.62, 88.39, 62.93. LCMS (ESI): calcd. for [M+H]$^+$ C$_{23}$H$_{17}$NO$_2$: 340.1332, found 340.1344.
**2.6-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (15):** To a solution of ((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))dimethanol (330 mg, 972 μmol) in anhydrous CH₂Cl₂ (5.00 mL), phosphorus tribromide (274 μL, 2.92 mmol) was added drop wise to the solution at 0 °C. The reaction was warmed to room temperature. After 2h, the solution was slowly quenched with saturate aqueous NaHCO₃ and extracted with CH₂Cl₂ (3x 20 mL). The organic layers were combined and dried over Na₂SO₄. The solvent was removed under pressure and the crude reaction mixture was purified on silica gel (70:30 ethyl acetate / hexane) to obtain dibromide as yellow powder in 44 % yield. ¹H NMR (400 MHz, DMSO) δ 7.92 (t, J = 7.8 Hz, 1H), 7.70 – 7.59 (m, 6H), 7.54 (d, J = 8.3 Hz, 4H), 4.75 (s, 4H). ¹³C NMR (101 MHz, DMSO) δ 144.53, 142.70, 137.83, 131.81, 131.63, 126.73, 119.20, 89.37, 87.82, 62.46. LCMS (ESI): calcd. for [M+H]⁺ C₂₃H₁₅Br₂N: 465.9625, found 465.9644.

**1.1’-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-methyl-1H-benzo[d]imidazol-3-ium) dibromide (16):** To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (130 mg, 279 μmol) in acetonitrile (1.40 mL), 1-methylbenzimidazole (77.6 mg, 587 μmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted in CH₂Cl₂ and crashed in hexane. The product was dried under vacuum to obtain 85 % yield as pale-brown powder. Purity 99.15 %. ¹H NMR (400 MHz, DMSO) δ 9.94 (s, 2H), 8.08 (dd, J = 8.2, 1.7 Hz 2H), 7.99 – 7.90 (m, 3H), 7.76 – 7.63 (m, 10H), 7.61 (d, J = 8.2 Hz, 4H), 5.88 (s, 4H), 4.13 (s, 6H). ¹³C NMR (101 MHz, DMSO) δ 143.17, 142.52, 137.84, 135.59, 132.28, 132.06, 130.68, 128.70, 126.69, 126.61, 121.35, 113.82, 113.62, 88.79, 88.27, 49.33, 33.46; LCMS (ESI): calcd. for [M⁺²] C₃₉H₃₁N₅: 284.6284, found 284.6287; IR (neat cm⁻¹) 3406, 2904, 2211, 1618, 1556, 1509, 1444, 1230, 1091; Melting point: 236-238 °C.
1.1’-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-butyl-1H-benzo[d]imidazol-3-iium) dibromide (17):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (210 mg, 451 μmol) in acetonitrile (1 mL), 1-butylbenzimidazole (165 mg, 948 μmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH₂Cl₂ and crashed in hexane. The product was dried under vacuum to obtain 60 % yield as pink powder. Purity 95.98 %. ¹H NMR (500 MHz, DMSO) δ 10.04 (s, 2H), 8.14 (dt, J = 8.2, 1.7 Hz 2H), 7.94 (dt, J = 8.2, 1.7 Hz 2H), 7.74 – 7.60 (m, 8H), 7.58 (m, 7H), 7.49 (t, J = 7.1 Hz, 1H), 5.84 (s, 4H), 4.53 (t, J = 7.3 Hz, 4H), 1.98 – 1.88 (p, J = 7.4 Hz 4H), 1.43 – 1.32 (h, J = 7.4 Hz 4H), 0.94 (t, J = 7.4 Hz, 6H). ¹³C NMR (126 MHz, DMSO) δ 142.58, 134.86, 133.97, 132.01, 131.75, 131.37, 130.87, 129.52, 128.68, 126.77, 126.71, 122.70, 122.27, 113.97, 113.84, 89.54, 88.94, 49.55, 46.64, 30.46, 19.14, 13.43.; LCMS (ESI): calcd. for [M⁺2] C₄₅H₄₃N₅: 326.6754, found 326.6769; IR (neat cm⁻¹) 3402, 2904, 2213, 1609, 1556, 1510, 1441, 1287, 1113; Melting point: 262-266 °C

1.1’-(((pyridine-2.6-diylbis(ethyne-2.1-diyl))bis(4.1-phenylene))bis(methylene))bis(3-octyl-1H-benzo[d]imidazol-3-iium) dibromide (18):

To a solution of 1.3-bis((4-(bromomethyl)phenyl)ethynyl)pyridine (109 mg, 234 μmol) in acetonitrile (1.20 mL), 1-octylbenzimidazole (124 mg, 539 μmol) was added and the solution was stirred 48 h at 80 °C. The reaction mixture was diluted with CH₂Cl₂ and precipitated in hexane. The product was dried under vacuum to obtain 30 % yield as pink powder. Purity 90.69 %. ¹H NMR (400 MHz, DMSO) δ 10.00 (s, 2H), 8.13 (dd, J = 7.1, 1.9 Hz, 2H), 7.96 (dd, J = 7.1, 1.9 Hz 2H), 7.92 (t, J = 7.8 Hz, 1H), 7.74 – 7.63 (m, 9H), 7.59 (d, J = 8.2 Hz, 4H), 5.85 (s, 4H), 4.52 (t, J = 7.3 Hz, 4H), 1.93 (p, J = 7.1 Hz, 4H), 1.35 – 1.23 (m, 18H), 0.84 (t, J = 7.1 Hz, 6H). ¹³C NMR (101 MHz, DMSO) δ 143.08, 143.01, 138.32, 136.07, 132.80, 131.82, 131.36, 129.16, 127.53, 127.27, 127.19, 121.87, 114.44, 114.28, 89.28, 88.73, 49.96, 47.35, 31.62, 28.97, 28.87, 26.26, 22.52, 14.42; LCMS (ESI): calcd. for [M⁺2]
C₅₃H₃₉N₅: 382.7380, found 382.7391; IR (neat cm⁻¹) 3375, 2921, 2212, 1608, 1554, 1509, 1439, 1165, 1113; Melting point: 239-243 °C

LogP measurement

The compounds (5 mg) suspended in 500 μL of 1-octanol, then 500 μL of PBS pH 7.4. The suspension was stirred for 24h, then centrifuged 5 min at 13 000 rpm to allow phase separation. Each phase was equally diluted and then injected in LC-UV for quantification. The peak areas were determined using OpenLab Software and Excel was used for calculations. The partitioning coefficient was calculated using the following formula:

\[ \log P = \log \frac{A_{oct \cdot Vinj \ aq}}{A_{aq \cdot Vinj \ oct}} \]

Where \(A_{oct}\) = Peak Area for Octanol phase signal

\(A_{aq}\) = Peak Area for Aqueous phase signal

\(Vinj \ aq\) = Injected volume of aqueous phase

\(Vinj \ oct\) = Injected volume of octanol phase

LC Conditions: LC System : Agilent 1200 Series, Column : Waters Symmetry C18 3.5 um, 4.6 x 75 mm. Flow Rate : 0.8 mL/min, mobile phase A: Water + 0.1% Formic Acid, mobile phase B: MeOH, gradient : 10 to 90 % B /10.5 mins.


Methods for the biological assays

Culture conditions and viability tests for bacteria and yeast

MICs were determined in 96-well cell culture plates. The tests were carried out in a growing Lauria Broth (LB) medium at 37°C in triplicate. The optical density of bacterial cells (OD 0.1 to 600 nm) was measured with a Fischer Scientific 40 cell density meter model and UV-vis spectroscopy experiments were performed on a Tecan Infinite M200 microplate reader.
Biofilm inhibition

The *S. aureus* were incubated in a 37° C LB medium for 12 h and re-diluted in the LB medium to be incubated again. After 2 h incubation, the cells were re-diluted at the final concentration (DO 600nm = 0.1-0.15). The biofilms of *S. Aureus* were marked with a Live/Dead stain after 48 h incubation with antibiotics in a growing medium (LB) in an 8-well glass plate. Negative control: DMSO (final concentration 5 % volume). Positive control: 20% Triton X.

Biofilm disruption

The *S. aureus* were incubated in a 37° C LB medium for 12 h and re-diluted in the LB medium to be incubated again. After 2 h incubation, the cells were re-diluted at the final concentration (DO 600nm = 0.1-0.15). After an incubation of 48 h in 8-well chambers, the growth media was removed and the biofilms were washed two times with PBS solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, in 200 μL of distilled water) to remove planktonic cells. The *S. aureus* biofilms were incubated with antibiotics for 24 h in PBS solution. Negative control: DMSO (final concentration 5 % volume). Positive control: 20% triton.

Biofilm staining and confocal laser scanning microscopy (LSM) analysis

Biofilms stained with FilmTracer™ LIVE / DEAD® Biofilm viability Kit (Molecular Probes, Life Technologies Ltd.). A fluorescent marker solution was prepared by adding 1 μL of SYTO® 9 dye and 1 μL of PI dye in 1 ml of filter-sterilized water. One hundred μL of staining solution were added to each well of an 8-well glass plate containing the mature biofilm. After 30 min at room temperature in the dark, the samples were washed with a saline phosphate buffer (PBS) solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, in 200 μL of distilled water). The biofilms were then observed with a confocal laser microscope (model Leica TCS SP5; Leica Microsystems CMS GmbH, Mannheim, Germany) using a 20x lens (HC PL FLUOTAR 20.0 x 0.70 DRY). A 483 nm laser was used to excite SYTO® 9, while fluorescent emission was detected from 500 to 540 nm. The PI was
excited with a laser at 535 nm and its fluorescent emission was detected from 600 to 695 nm.

Haemolytic assay

The red blood cells (RBCs) in Alserver’s solution were diluted in PBS buffer and centrifuged 10 min. at 2000 rpm. The RBCs were washed two more times with PBS buffer until the surface liquid was crystal clear and were resuspended in PBS at 2% v/v. In a 96 wells plate was added 195 μL of red blood cells solution and 5 μL of compound in DMSO. The plate was incubated with light agitation (25 rpm) for 1 h at 37 °C. The plate was then centrifuged for 10 min. at 2000 rpm and 100 μL of the supernatant was transferred to another plate to analyse the absorbance (λ = 405 nm) with a Tecan Infinite M200 microplate reader.

Figure S1: Average percent viability in triplicate for *E. Coli* (SK037).
Figure S2: Average percent viability in triplicate for *E. Coli* (SK037).

Figure S3: Average percent viability in triplicate for *MRSA = Methicillin-resistant S. aureus* (ATCC 43300).
Figure S4: Average percent viability in triplicate for MRSA = Methicillin-resistant S. aureus (ATCC 43300).

Figure S5: Average percent viability in triplicate for MRSA = Methicillin-resistant S. aureus (ATCC 43300).
Figure S6: Average percent viability in triplicate for VRE = vancomycin-resistant E. faecium (BAA-2316).

Figure S7: Average percent viability in triplicate for VRE = vancomycin-resistant E. faecium (BAA-2316).
Figure S8: Average percent viability in triplicate for VRE = vancomycin-resistant E. faecium (BAA-2316).

Figure S9: Average percent viability in triplicate for C. albicans (SC5314).
Figure S10: Average percent hemolysis in triplicate for benzimidazolium salts.

NMR spectra and LCMS of the synthesized compounds

Compound (2):
Compound (4):
Compound (6):
Compound (8):
Compound (9):

+ESI Scan (8.27 min) Frag=165.0V ARS-AD-45_C18_10-90ACN-2.d

326.17828

239.11997

651.34889

922.00980

Counts vs. Mass-to-Charge (m/z)
Compound (12):
Compound (13):
Compound (14):

[Chemical structure of Compound (14)]

[Mass spectrum of Compound (14)]

<table>
<thead>
<tr>
<th>Ion Species</th>
<th>Formula</th>
<th>Abund</th>
<th>m/z</th>
<th>m/z (Calc)</th>
<th>Diff (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(M+H)+</td>
<td>C23H17N2O2</td>
<td>3205</td>
<td>340.1344</td>
<td>340.13321</td>
<td>3.39</td>
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</tbody>
</table>
Compound (15):
Compound (17):
Compound (18):