Hydrogen bond acceptors and additional cationic charges in methylene blue derivatives – photophysics and antimicrobial efficiency

Ariane Felgenträger1, Tim Maisch1, Daniel Dobler2 and Andreas Späth2,3*

SUPPORTING INFORMATION

Synthesis an Purification of the compounds

Materials and methods
Analytical characterization of the synthesized compounds was done by common methods. Melting Points were determined on Büchi SMP or a Lambda PhotometricsOptiMelt MPA 100 and are uncorrected. IR Spectra were recorded with a Bio-Rad FT-IR Excalibur FTS 3000 equipped with a Specac Golden Gate Diamond Single Reflection ATR-System. Absorption spectra were recorded on a Varian Cary BIO 50 UV/VIS/NIR spectrometer with temperature control using 1 cm quartz cuvettes (Hellma) and Uvasol solvents (Merck, Baker or Acros). Fluorescence measurements were performed with UV-grade solvents (Baker or Merck) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian ‘Cary Eclipse’ fluorescence spectrophotometer with temperature control. Electro spray mass spectra were performed on a Finnigan MAT TSQ 7000 ESI-spectrometer. Other Mass Spectra were recorded on Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD); Xenon serves as the ionization gas for FAB. NMR spectra were recorded on BrukerAvance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), BrukerAvance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K) or BrukerAvance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K) relative to external standards. NMR spectra were recorded in CDCl3 at 300 MHz (1H) or 75 MHz (13C) unless stated otherwise. Characterization of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, dd = double doublet, dt = double triplet, ddd = double double doublet.

1Department of Dermatology, University of Regensburg, Franz Josef Strauss Allee 11, Regensburg 93042, Germany
2Department of Organic Chemistry, University of Regensburg, Universitätsstrasse 31, Regensburg 93053
3Corresponding author: Fax: Int +49-941-944-8943
E-mail address: andreas.spaeth@chemie.uni-regensburg.de
Integration is determined as the relative number of atoms, the coupling constants are given in Hertz [Hz]. The multiplicity of the carbon atoms is given as (+) = CH₃ or CH, (-) = CH₂ and (C_quat) for quaternary carbon atoms. Error of reported values: chemical shift: 0.01 ppm for ¹H-NMR, 0.1 ppm for ¹³C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum. Analytical TLC plates (silica gel 60 F₃₄) and silica gel 60 (70-230 or 230-400 mesh) were used for chromatographic separations. Visualization of the spots was by UV light and/or staining with ninhydrin in ethanol. PE means petrol ether with a boiling range of 70 - 90°C. All other solvents and chemicals were of reagent grade and used without further purification. 1-(tert.-Butoxycarbonyl)piperazine was purchased from TCI Europe in a purity of > 98% and phenothiazine was purchased from Aldrich in a purity of > 98%. Both were used as received.

**Synthesis and Purification**

The mono-boc-protected diamines 11 and 12 were prepared by nucleophilic substitution of an appropriate alkylbromide (E1) with methylamine or by boc-protection of commercially available 1,2-dimethyl-ethylendiamine (E2).

![Scheme S-1: Preparation of the boc-protected side chain building blocks](image)

2-(N-butoxycarbonyl-2-aminoethyl)-1-(methyl)amine (11) (literature known, improved procedure)
tert-butyl [2-bromoethyl]carbamate\textsuperscript{ii} (2.23 g, 10 mmol) in methanol (100 mL) was slowly dropped in a vigorously stirring, ice-cold solution of methylamine in methanol (40 %, 50 mL) over a period of 2h, keeping the temperature between 2-5°C. After stirring over night at room temperature, the solvent and the excess amine were removed at reduced pressure. The crude material was purified by column chromatography with silica gel using chloroform/methanol 10:1 → 6:1 as the eluent, to give the bromide salt of 11 as colourless solid (1.83 g, 7.26 mmol, 73 %).

\textbf{\textsuperscript{1}H-NMR} (300 MHz, MeOD): $\delta$ [ppm] = 3.39 (2H, t, $J = 6.4$ Hz), 3.11 (2H, t, $J = 6.4$ Hz), 2.71 (3 H, s), 1.43 (9 H, s); - \textbf{MS} (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): e/z (%) = 175.1 (100, MH$^+$), 119.1 (56, MH$^+$ - C$_4$H$_9$);

A solution of this salt in dichloromethane (50 mL) was washed four times with diluted aqueous sodium hydroxide solution (4x 20 mL, 2 M). The organic layer was separated and dried over MgSO$_4$. The solvent was removed at reduced pressure to give the free base 11 as colourless oil (1.22 g, 7.06 mmol, 97 %).

\textbf{\textsuperscript{1}H-NMR} (300 MHz, CDCl$_3$): $\delta$ [ppm] = 5.43 (1 H, bs, NH), 3.09 (2 H, m), 3.52 (2 H, t, $J = 6.4$ Hz), 2.14 (3 H, s), 1.36 (1 H, s), 1.32 (9 H, s); - \textbf{\textsuperscript{13}C-NMR} (75 MHz, CDCl$_3$): $\delta$ [ppm] = 155.3 (C$_\text{quat}$), 77.9 (C$_\text{quat}$), 50.2 (-), 38.9 (-), 35.0 (+), 27.4 (+); - \textbf{MS} (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): e/z (%) = 175.1 (100, MH$^+$), 119.1 (43, MH$^+$ - C$_4$H$_9$); - \textbf{MW} = 174.24 g/mol; \textbf{MF} = C$_8$H$_{18}$N$_2$O$_2$

\textit{2-(N-butyloxycarbonyl-methylamino)ethyl-1-methylamine (12) (literature known, improved procedure)\textsuperscript{iii}}

\[
\begin{array}{c}
\text{HN} \\
\text{N} \\
\text{O} \\
\text{O} \\
\end{array}
\]

A solution of 1,2-dimethyl-ethylenediamine (8.60 g, 100 mmol) in dichloromethane (100 mL) was stirred in the ice bath under moisture protection. Bocanhydride (5.45 g, 25 mmol) in dichloromethane (300 mL) was slowly added over a period of 6 h at 0°C. After stirring at room temperature over night in a nitrogen atmosphere, the solution was washed with brine (2x 100 mL) and water (2x 100 mL), dried over MgSO$_4$ and the solvent was removed at reduced
pressure. The crude oil was purified by column chromatography with silica gel and chloroform/methanol 6:1 containing 0.5 % aqueous, conc. ammonia solution, to give 12 as pale yellow oil (2.87 g, 15.24 mmol, 61 %).

\[\text{1H-NMR (300 MHz, CDCl}_3\]: } \delta [\text{ppm}] = 3.21 (2 H, t, J = 6.4 Hz), 2.76 (3 H, s), 2.58 (2 H, t, J = 6.4 Hz), 2.32 (3 H, s), 1.36 (9 H, s), 1.12 (1 H, bs, NH); - \text{13C-NMR (150 MHz, CDCl}_3\]: } \delta [\text{ppm}] = 155.8 (C\text{quat}), 79.3 (C\text{quat}), 49.6 (+), 48.3 (-), 36.2 (+), 34.6 (-), 28.3 (+); - \text{MS (ESI-MS, CH}_2\text{Cl}_2/\text{MeOH + 10 mmol NH}_4\text{OAc): } e/z (%) = 377.2 (12, 2MH\text{+}), 230.0 (26, MH\text{+} + \text{MeCN}), 189.0 (100, MH\text{+}), 123.1 (5, MH\text{+} - \text{C}_4\text{H}_9); - \text{MW} = 188.27 \text{ g/mol}; \text{MF} = \text{C}_9\text{H}_{20}\text{N}_2\text{O}_2

3-Dimethylaminophenothiazin-5-iium triiodide (10) (literature known, improved procedure)

\[\text{To a solution of phenothiazin-5-iium tetraiodide hydrate} (9) (7.23 g, 10 mmol) in dichloromethane (500 mL) was added solution of dimethylamine in methanol (2 M, 10.0 mL, 20 mmol) dropwise over 6 h. The reaction mixture was allowed to stand overnight at room temperature and the resultant precipitate was filtered off, washed with dichloromethane and allowed to air dry. The product was recrystallised from methanol to give 10 as dark-blue solid (3.30 g, 5.30 mmol, 53 %)

\[\text{M.P. 144 – 145 °C; - 1H-NMR (300 MHz, DMSO-d6): } \delta [\text{ppm}] = 8.23 (1 H, dd, J = 8.0 & 1.6 Hz), 8.17 (1 H, dd, J = 8.0 & 1.6 Hz), 8.11 (1 H, d, J = 10 Hz), 8.04 (1 H, dd, J = 10 & 2.4 Hz), 7.99 (1 H, d, J = 2.4 Hz), 7.84 (2 H, m), 3.65 (3 H, s), 3.60 (3 H, s); - 13C-NMR (150 MHz, DMSO-d6): } \delta [\text{ppm}] = 156.0 (C\text{quat}), 144.0 (C\text{quat}), 139.7 (+), 139.5 (C\text{quat}), 137.9 (C\text{quat}), 134.5 (+), 133.2 (+), 129.7 (+), 126.2 (+), 125.9 (+), 125.8 (C\text{quat}), 109.6 (+), 43.3 (+), 42.9 (+); - \text{IR (neat): } \nu (\text{cm}^{-1}) = 2800 (\text{bs}), 1614 (s), 1585 (s), 1557 (s), 1492 (s), 1429 (m), 1404 (s), 1312 (m), 1245 (s), 1114 (s), 1073 (s), 880 (s), 829 (s), 765 (s); - \text{MS (ESI-MS, CH}_2\text{Cl}_2/\text{MeOH + 10 mmol NH}_4\text{OAc): } e/z (%) = 240.9 (100, M\text{+}); - \text{MW} = 241.34 + 381.72 \text{ g/mol}; \text{MF} = \text{C}_{14}\text{H}_{13}\text{N}_2\text{SI}_3\]
**General procedure I: Synthesis of boc-protected methyleneblue derivatives**

To a solution of 3-Dimethylaminophenothiazinium triiodide (10, 1.24 g, 2 mmol) in dichloromethane (500 mL) was added dropwise a solution of triethylamine (0.3 g, 0.4 mL, 3 mmol) in dichloromethane (50 mL). After stirring for 5 minutes the appropriate amine (6 mmol) in dichloromethane (250 mL) was added over a period of 2 h. The solution was stirred over night at room temperature and was then washed with water (3x 250 mL). The organic layer was dried over MgSO₄ and the solvent was evaporated at reduced pressure not exciding a water bath temperature of 40°C. The crude material was purified by repeated flash chromatography with silica gel using dichloromethane/ethanol 10:1 as the eluent.

3-[(2-N-butyloxycarbonyl-2-aminoethyl)(methyl)amino]-7-(dimethylamino)phenothiazin-5-i um iodide (14)i

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\text{ tert-butyl [2-(methylamino)ethyl]carbamate (11) (1.05 g, 6 mmol) was reacted to give 0.74 g bronze coloured glass (1.36 mmol, 68 %). R}_{f}(\text{DCM/EtOH 8:1} \sim 0.33)
\]

\[\text{1H-NMR (300 MHz, CDCl}_3\]: \(\delta [\text{ppm}] = 7.78 - 7.89 (2 \text{ H, m}), 7.12 - 7.41 (4 \text{ H, m}), 6.12 (1 \text{ H, bs}), 3.92 (2 \text{ H, t, } J = 6.4 \text{ Hz}), 3.52 (2 \text{ H, t, } J = 6.4 \text{ Hz}), 3.39 (3 \text{ H, s}), 3.31 (6 \text{ H, s}), 1.29 (9 \text{ H, s}); - \text{13C-NMR (75 MHz, DMSO-d6): } \delta [\text{ppm}] = 155.7 (C_{\text{quat}}), 153.5 (C_{\text{quat}}), 137.6 (+), 134.9 (C_{\text{quat}}), 134.8 (C_{\text{quat}}), 133.4 (C_{\text{quat}}), 119.0 (+), 118.9 (+), 106.5 (+), 78.1 (C_{\text{quat}}), 52.2 (-), 46.2 (-), 41.1 (+), 39.6 (+), 27.8 (+); - \text{IR (neat): } \nu (\text{cm}^{-1}) = 3330 (bs), 2976 (m), 2930 (m), 2906 (m), 2705 (m), 1699 (s), 1592 (s), 1546 (m), 1486 (s), 1438 (m), 1384 (s), 1314 (s), 1215 (s), 1161 (s), 1130 (s), 1070 (s), 1033 (s), 967 (s), 880 (s), 829 (s), 790 (s), 719 (m), 666 (m); - \text{MS (ESI-MS, CH}_2\text{Cl}_2/\text{MeOH + 10 mmol NH}_4\text{OAc): } e/z (%) = 413.0 (100, M^+); - \text{MW} = 413.57 +126.90 \text{ g/mol; MF} = C_{22}\text{H}_{29}\text{N}_4\text{SO}_2\text{I}\]
3-[methyl[2-(N-butyloxycarbonyl-methylammonio)ethyl]amino]-7-(dimethylamino)phenothiazin-5-ium iodide (15)

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\]

\textit{tert}-butylmethyl[2-(methylamino)ethyl]carbamate (12) (1.12 g, 6 mmol) was reacted to give 0.69 g bronze coloured glass (1.25 mmol, 63 %). \textbf{R}$_f$ (DCM/EtOH 8:1 ~ 0.35)

\textbf{1H-NMR} (300 MHz, DMSO-d6): \(\delta\) [ppm] = 7.76 – 7.88 (2 H, m), 7.42 – 7.56 (4 H, m), 3.81 (2 H, t, \(J = 6.4\) Hz), 3.39 (3 H, s), 3.37 (6 H, s) 3.29 (3 H, s), 3.28 (2 H, t, \(J = 6.4\) Hz), 1.27 (9 H, s); - \textbf{13C-NMR} (75 MHz, DMSO-d6): \(\delta\) [ppm] = 155.6 (C\textsubscript{quat}), 153.9 (C\textsubscript{quat}), 137.7 (+), 137.6 (+), 134.9 (C\textsubscript{quat}), 134.8 (C\textsubscript{quat}), 133.4 (C\textsubscript{quat}), 119.1 (+), 118.9 (+), 106.8 (+), 77.8 (C\textsubscript{quat}), 52.1 (-), 46.3 (-), 41.0 (+), 39.6 (+), 37.7 (+), 27.9 (+); - \textbf{IR} (neat): \(\nu\) (cm\(^{-1}\)) = 3330 (bs), 2975 (m), 2930 (m), 2908 (m), 2701 (m), 1699 (s), 1591 (s), 1546 (m), 1485 (s), 1441 (m), 1384 (s), 1312 (s), 1214 (s), 1160 (s), 1129 (s), 1066 (s), 1031 (s), 967 (m), 871 (s), 829 (s), 789 (s), 719 (m), 665 (m); - \textbf{MS} (ESI-MS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH + 10 mmol NH\textsubscript{4}OAc): e/z (%) = 427.0 (100, M\textsuperscript{+}); - \textbf{MW} = 427.59 +126.90 g/mol; \textbf{MF} = C\textsubscript{23}H\textsubscript{31}N\textsubscript{4}SO\textsubscript{2}I

3-[4-(\textit{tert}-butoxycarbonyl)piperazin-1-yl]-7-(dimethylamino)phenothiazin-5-ium iodide (16)

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\]

\textit{tert}-butyl piperazine-1-carboxylate (1.11 g, 6 mmol) was reacted to give 0.78 g purple glimmering crystals (1.41 mmol, 71 %). \textbf{R}$_f$(DCM/EtOH 8:1 ~ 0.36)

\textbf{1H-NMR} (300 MHz, DMSO-d6): \(\delta\) [ppm] = 7.68 – 7.78 (2 H, m), 7.62 (1 H, m), 7.42 (1 H, m), 7.27 (1 H, m), 7.20 (1 H, m), 3.83 (4 H, m), 3.62 (4 H, m), 3.37 (6 H, s), 1.41 (9 H, s); - \textbf{13C-NMR} (75 MHz, DMSO-d6): \(\delta\) [ppm] = 154.4 (C\textsubscript{quat}), 154.0 (C\textsubscript{quat}), 153.1 (C\textsubscript{quat}), 138.6
(+), 138.4 (+), 135.7 (C_{quat}), 135.1 (C_{quat}), 133.4 (C_{quat}), 119.4 (+), 118.9 (+), 107.5 (+), 107.2 (+), 106.6 (+), 80.6 (C_{quat}), 47.7 (-), 47.2 (-), 42.4 (+), 28.4 (+); - IR (neat): ν (cm\(^{-1}\)) = 3443 (bs), 2973 (w), 2926 (w), 2867 (w), 2704 (w), 2185 (w), 1683 (s), 1593 (s), 1487 (m), 1448 (m), 1388 (s), 1332 (s), 1219 (s), 1121 (s), 1080 (m), 1041 (m), 991 (m), 882 (s), 836 (m), 789 (m), 724 (s); - MS (ESI-MS, CH\(_2\)Cl\(_2/\)MeOH + 10 mmol NH\(_4\)OAc): e/z (%) = 425.2 (100, M\(^+\)); - MW = 425.58 +126.90 g/mol; MF = C\(_{23}\)H\(_{29}\)N\(_4\)SO\(_2\)I

**General procedure II: Deprotection of boc-protected methyleneblue derivatives**

a) Deprotection

The boc-protected methyleneblue derivative (0.5 mmol) was dissolved in dichloromethane (4 mL). TFA (285 mg, 0.2 mL, 2.5 mmol) in dichloromethane (2 mL, 10% TFA) was added dropwise and the reaction mixture was stirred for 5h at room temperature. The solution was transferred to four blue caps, the product was precipitated by addition of diethylether (13.5 mL per tube) and centrifuged. The solution was decanted off the precipitate, it was resuspended in diethylether (15 mL per tube) and centrifuged again. The solvent was decanted off and the residue was dried at reduced pressure without heating.

b) Ion exchange

A small column was packed with ion exchanger (Amberlite IRA-958). The resin was rinsed with acidic sodium chloride solution (10 % aqueous NaCl cont. 0.1 % HCl, 100 mL) and conditioned with dilute hydrochloric acid (0.1 %).

The TFA salt from the former step was dissolved in double distilled water (5 mL) and was passed through the column (height 10 cm, diameter 1 cm) of anion exchanger (Amberlite IRA-958) eluting with water (40 mL). The aqueous solution was lyophilized to give the product as dark blue solid.
3-{(2-Ammoniumethyl)(methyl)amino}-7-(dimethylamino)phenothiazin-5-ium dichloride 
(literature known compound) (1)\textsuperscript{vi}

\[
\begin{array}{c}
\text{Cl}^- \\
\text{NH}_3^+ \text{Cl}^-
\end{array}
\]

Conversion of compound 14 by general procedure II gives the desired product 1 as dark blue powder (185 mg, 0.476 mmol, 95%). \(R_f\) (CHCl\textsubscript{3}/MeOH 4:1 ~ 0.1)

\(1^\text{H}-\text{NMR}\) (300 MHz, DMSO-d\textsubscript{6}): \(\delta\) [ppm] = 7.72 – 7.91 (2 H, m), 7.23 – 7.51 (4 H, m), 3.95 (2 H, m), 3.67 (6 H, s), 3.24 (3 H, s), 3.08 (2 H, m); - MS (ESI-MS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH + 10 mmol NH\textsubscript{4}OAc): e/z (%) = 313.1 (100, M\textsuperscript{+}), 157.6 (18, (M +2H\textsuperscript{+})\textsuperscript{2+}), 148.6 (51, (M-NH\textsubscript{3}+2H\textsuperscript{+})\textsuperscript{2+}); - MW = 314.46 +2x 35.45 g/mol; \textbf{MF} = C\textsubscript{17}H\textsubscript{22}N\textsubscript{4}SCl\textsubscript{2}

3-{methyl[2-(methylammonio)ethyl]amino}-7-(dimethylamino)phenothiazin-5-ium dichloride 
(2)

\[
\begin{array}{c}
\text{Cl}^- \\
\text{H}_2\text{N}^- \text{Cl}^-
\end{array}
\]

Deprotection of 15 and ion exchange after general procedure II gives product 2 as dark blue powder (185 mg, 0.481 mmol, 96%). \(R_f\) (CHCl\textsubscript{3}/MeOH 4:1 ~ 0.1)

\(1^\text{H}-\text{NMR}\) (300 MHz, DMSO-d\textsubscript{6}): \(\delta\) [ppm] = 7.83 – 7.96 (2 H, m), 7.41 – 7.63 (4 H, m), 4.03 (2 H, m), 3.65 (6 H, s), 3.31 (3 H, s), 3.19 (2 H, m), 2.63 (3 H, s); - MS (ESI-MS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH + 10 mmol NH\textsubscript{4}OAc): e/z (%) = 327.2 (100, M\textsuperscript{+}), 164.1 (43, (M +2H\textsuperscript{+})\textsuperscript{2+}), 155.6 (6, (M-NH\textsubscript{3}+2H\textsuperscript{+})\textsuperscript{2+}); - MW = 328.48 +2x 35.45 g/mol; \textbf{MF} = C\textsubscript{18}H\textsubscript{24}N\textsubscript{4}SCl\textsubscript{2}
3-(piperazin-4-ium-1-yl)-7-(dimethylamino)phenothiazin-5-ium dichloride (3)

Deprotection of 15 and ion exchange after general procedure II gives product 2 as dark blue powder (184 mg, 0.489 mmol, 98 %). \( R_f \) (CHCl₃/MeOH 4:1 ~ 0.1)

\(^1\)H-NMR (300 MHz, DMSO-d₆): \( \delta \) [ppm] = 7.84 – 7.98 (2 H, m), 7.46 – 7.67 (4 H, m), 3.64 (6 H, s), 3.36 (4 H, m), 3.24 (4 H, m); - MS (ESI-MS, CH₃Cl₂/MeOH + 10 mmol NH₄OAc): e/z (%) = 325.1 (89, M⁺), 163.1 (100, (M +2H⁺)²⁺), 154.5 (3, (M-NH₃ +2H⁺)²⁺); - MW = 326.47 +2x 35.45 g/mol; MF = C₁₈H₂₂N₄SCl₂

**General procedure III: Synthesis of asymmetric methyleneblue derivatives**

To a solution of 3-Dimethylaminophenothiazinium triiodide (10, 1.24 g, 2 mmol) in dichloromethane (360 mL) was added dropwise a solution of the appropriate amine (12 mmol) in dichloromethane (40 mL) over a period of 1 h. The solution was stirred over night at room temperature and was then washed with water (1x 200 mL). The organic layer was dried over MgSO₄ and the solvent was evaporated at reduced pressure not exciding a water bath temperature of 40°C. The crude material was dissolved in dichloromethane (10 mL) and precipitated by addition of diethylether (50 mL). The precipitate was settled by the aid of a centrifuge, the supernatant was decanted off and the dark blue residue was purified by repeated flash chromatography with silica gel.

**General procedure IV: Ion exchange protocol for methyleneblue derivatives**

The column was packed with ion exchanger (Amberlite IRA-958). The resin was rinsed with acidic sodium chloride solution (10 % aqueous NaCl cont. 0.1 % HCl, 100 mL) and conditioned with dilute hydrochloric acid (0.1 %).
The methylene blue derivative (0.5 mmol) was dissolved in hydrochloric acid (1M, 10 mL) and lyophilized. A solution of this mixed salt was dissolved in double distilled water (6 mL) and was slowly passed through a column (height 10 cm, diameter 1 cm; transferred with 4 mL dilute hydrochloric acid 0.1%) of the conditioned anion exchanger (Amberlite IRA-958) eluting with dilute hydrochloric acid (40 mL, 0.1%). The aqueous solution was lyophilized to give the product as dark blue solid.

3-{(2-Hydroxyethyl)(methyl)amino}-7-(dimethylamino)phenothiazin-5-i um chloride (4)

2-N-Methyl-aminoethanol (0.90 g, 0.96 mL, 12 mmol) was reacted and the crude material was purified with dichloromethane/ethanol 8:1 and dichloromethane/methanol 5:1 to give 0.38 g of a dark blue, purple glimmering glass (0.85 mmol, 42%). After ion exchange 0.33 g of a dark blue, purple glimmering powder is isolated. \( R_f \) (DCM/EtOH 8:1 ~ 0.16)

\begin{itemize}
  \item \textbf{\textsuperscript{1}H-NMR} (300 MHz, DMSO-d6): \( \delta \) [ppm] = 7.94 (2 H, m), 7.59 (2 H, m), 7.51 (2 H, m), 5.06 (1 H, m), 4.09 (3 H, s), 3.88 (2 H, m), 3.69 (2 H, m), 3.38 (6 H, s); - \textbf{\textsuperscript{13}C-NMR} (75 MHz, DMSO-d6): \( \delta \) [ppm] = 154.1 (C\textsubscript{quat}), 153.8 (C\textsubscript{quat}), 137.6 (+), 137.5 (+), 134.6 (C\textsubscript{quat}), 134.4 (C\textsubscript{quat}), 133.6 (C\textsubscript{quat}), 119.1 (+), 118.8 (+), 106.7 (+), 106.5 (+), 58.4 (-), 54.3 (-), 41.1 (+), 40.9 (+); - \textbf{IR} (neat): \( \nu \) (cm\(^{-1}\)) = 3384 (bs), 3045 (w), 2913 (w), 2869 (w), 2705 (w), 1592 (s), 1545 (m), 1538 (m), 1487 (m), 1443 (m), 1389 (s), 1329 (s), 1248 (m), 1224 (m), 1180 (m), 1142 (m), 1063 (s), 975 (m), 880 (m), 815 (m), 665 (m); - \textbf{MS} (ESI-MS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH + 10 mmol NH\textsubscript{4}OAc): e/z (%) = 314.1 (100, M\textsuperscript{+}); - \textbf{MW} = 314.43 + 35.45 g/mol; \textbf{MF} = C\textsubscript{17}H\textsubscript{20}N\textsubscript{3}SOCl\textsubscript{2} \end{itemize}
3-(morpholin-4-yl)-7-(dimethylamino)phenothiazin-5-ium chloride (5)\textsuperscript{vii}

\begin{center}
\includegraphics[width=0.2\textwidth]{structure1.png}
\end{center}

Morpholine (1.05 g, 1.05 mL, 12 mmol) was reacted and the crude material was purified with dichloromethane/ethanol 8:1 and recrystallised from ethanol to give 0.49 g bronze coloured crystal plates (1.09 mmol, 54%). After ion exchange 0.38 g of a dark blue, purple glimmering powder is isolated. R\textsubscript{f} (DCM/EtOH 8:1 ~ 0.21)

\textsuperscript{1}H-NMR (600 MHz, DMSO-d6): \(\delta\) [ppm] = 7.94 (2 H, dd, \(J = 13.2 \& 5.4\) Hz), 7.69 (1 H, d, \(J = 5.4\) Hz), 7.63 (1 H, dd, \(J = 13.2 \& 5.4\) Hz), 7.57 (1 H, d, \(J = 5.4\) Hz), 7.55 (1 H, dd, \(J = 13.2 \& 5.4\) Hz), 3.83 (4 H, t, \(J = 5.6\) Hz), 3.78 (4 H, t, \(J = 5.6\) Hz), 3.39 (6 H, s); \textsuperscript{13}C-NMR (150 MHz, DMSO-d6): \(\delta\) [ppm] = 154.1 (C\textsubscript{quat}), 153.1 (C\textsubscript{quat}), 138.1 (+), 137.8 (+), 135.9 (C\textsubscript{quat}), 134.9 (C\textsubscript{quat}), 134.2 (C\textsubscript{quat}), 133.5 (C\textsubscript{quat}), 119.9 (+), 118.6 (+), 107.1 (+), 107.0 (+), 65.9 (-), 54.9 (+), 47.5 (-), 41.3 (+); - IR (neat): \(\nu\) (cm\(^{-1}\)) = 3441 (bs), 3039 (w), 2885 (w), 2849 (w), 2700 (w), 1592 (m), 1446 (m), 1391 (s), 1351 (s), 1307 (m), 1173 (m), 1141 (m), 1112 (m), 1041 (m), 945 (m), 883 (s), 819 (m) 628 (m); - MS (ESI-MS, CH\textsubscript{2}Cl\textsubscript{2}/MeOH + 10 mmol NH\textsubscript{4}OAc): \(e/z\) (%) = 326.1 (100, M\textsuperscript{+}); - MW = 326.44 + 35.45 g/mol; MF = C\textsubscript{18}H\textsubscript{20}N\textsubscript{3}SOCl

3-(4-methylpiperazin-4-ium-1-yl)-7-(dimethylamino)phenothiazin-5-ium dichloride (6)

\begin{center}
\includegraphics[width=0.2\textwidth]{structure2.png}
\end{center}

1-N-Methyl-piperazin (1.2 g, 1.34 mL, 12 mmol) was reacted and the crude material was purified with dichloromethane/methanol 8:1 \(\rightarrow\) 5:1 and dichloromethane/methanol 5:1 to give 0.20 g of a dark blue, purple glimmering glass (0.43 mmol, 21%). After ion exchange 0.17 g of a dark blue, purple glimmering glass is isolated. R\textsubscript{f} (DCM/EtOH 8:1 ~ 0.02); R\textsubscript{f} (CHCl\textsubscript{3}/MeOH 4:1 ~ 0.15)
$^1$H-NMR (300 MHz, DMSO-d6): $\delta$ [ppm] = 8.03 (2 H, dd, $J = 13.2$ & $5.6$ Hz), 7.88 (1 H, m), 7.62 (3 H, m), 4.52 (2 H, m), 3.83 (2 H, m), 3.55 (2 H, m), 2.81 (6 H, s), 3.21 (2 H, m), 2.81 (3 H, s); $^{13}$C-NMR (75 MHz, DMSO-d6): $\delta$ [ppm] = 154.1 (C$_{quat}$), 153.1 (C$_{quat}$), 138.1 (+), 137.8 (+), 135.9 (C$_{quat}$), 134.9 (C$_{quat}$), 134.2 (C$_{quat}$), 133.5 (C$_{quat}$), 119.9 (+), 118.6 (+), 107.1 (+), 107.0 (+), 65.9 (-), 54.9 (+), 47.5 (-), 41.3 (+); - IR (neat): $\nu$ (cm$^{-1}$) = 3423 (bs), 3032 (w), 2941 (w), 2853 (w), 2807 (w), 2686 (w), 1593 (s), 1517 (m), 1484 (m), 1452 (m), 1394 (s), 1353 (s), 1286 (m), 1240 (s), 1221 (s), 1179 (m), 1131 (s), 1081 (m), 1042 (m), 996 (s), 947 (m), 881 (s), 823 (m), 813 (m), 631 (m); - MS (ESI-MS, CH$_2$Cl$_2$/MeOH + 10 mmol NH$_4$OAc): e/z (%) = 339.2 (100, M$^+$), 170.1 (86, (M +2H$^+$)$^{2+}$); $\text{MW} = 340.49 +2\times 35.45$ g/mol; $\text{MF} = \text{C}_{19}\text{H}_{24}\text{N}_4\text{SCl}_2$
Selected NMR spectra of prepared compounds

Figure S-2: $^1$H-NMR spectrum of compound 10

Figure S-3: $^{13}$C-NMR spectrum of compound 10
Figure S-4: $^1$H-NMR spectrum of compound 16

Figure S-5: $^{13}$C-NMR spectrum of compound 16
Figure S-6: $^1$H-NMR spectra of compound 5
Figure S-7: $^{13}$C-NMR spectrum of compound 5

Figure S-8: HSQC spectrum of compound 5
Fotographs of selected compounds

**Figure S-9:** Compound 15

**Figure S-10:** Crystalline MB derivatives, compound 16 (left) and compound 6-I (right)
Figure S-11: Absorption spectra of MB and its derivatives within a concentration range of 10 – 200 µM in H₂O; the graphs show dimerisation for MB, MB-4 and MB-5.
**Figure S-12:** Photostability measurements in a quartz cuvette with an irradiation at 600 nm with 180000 laser pulses; only MB-1 and MB-2 show a significant decrease in the absorption in the visible and in the UV range.
Figure S-13: Time- and spectrally resolved singlet oxygen luminescence of MB and its derivatives in air saturated H₂O at 25°C. Singlet oxygen is generated and detected at 1275 nm with a decay time ≈3.5 µs for all derivatives.
Antimicrobial Acitivity Data

MB-1

Figure S-14: Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-1.

Photodynamic treatment was performed using different concentrations of MB-1 with and without illumination (30 J cm\(^{-2}\)). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log\(_{10}\) steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-1 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)
Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-2.

Photodynamic treatment was performed using different concentrations of MB-2 with and without illumination (30 J cm⁻²). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log₁₀ steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-2 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)
MB-3

**Figure S-16:** Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-3.

Photodynamic treatment was performed using different concentrations of MB-3 with and without illumination (30 J cm$^{-2}$). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log$_{10}$ steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-3 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)
**Figure S-17:** Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-4.

Photodynamic treatment was performed using different concentrations of MB-4 with and without illumination (30 J cm$^{-2}$). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log$_{10}$ steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-4 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)
Figure S-18: Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-5.

Photodynamic treatment was performed using different concentrations of MB-5 with and without illumination (30 Jcm\(^{-2}\)). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log\(_{10}\) steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-5 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)
MB-6

**Figure S-19:** Photodynamic inactivation of *E. coli* and *S. aureus* by methylene blue derivative MB-6.

Photodynamic treatment was performed using different concentrations of MB-6 with and without illumination (30 J cm\(^{-2}\)). Surviving colonies were counted 24 h later. Black dashed line corresponds to a reduction of 3 log\(_{10}\) steps in bacteria numbers (99.9% killing efficacy). White and grey column: controls without illumination. Coloured columns: MB-6 + light activation, blue: *E. coli*; crosshatched: *S. aureus*. (n=3 experiments: mean values ± standard deviation)

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