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SUPPORTING INFORMATION – I

Second generation of diazachrysenes: protection of Ebola virus infected mice and mechanism of action

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1. Chemistry

Melting points were determined on a Boetius PMHK apparatus and were not corrected. IR spectra were taken on a Thermo-Scientific Nicolet 6700 FT-IR diamond crystal. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 spectrometer (at 200 and 50 MHz, respectively), and a Bruker Ultrashield Advance III spectrometer (at 500 and 125 MHz, respectively) in the indicated solvent (*vide infra*) using TMS as the internal standard. Chemical shifts are expressed in ppm (δ) values and coupling constants (J) in Hz. ESI–MS (HRMS) spectra of the synthesized compounds were acquired on a Agilent Technologies 1200 Series instrument equipped with Zorbax Eclipse Plus C18 (100 × 2.1 mm i.d. 1.8 µm) column and DAD detector (190-450 nm) in combination with a 6210 Time-of-Flight LC/MS instrument in positive ion mode. The samples were dissolved in pure H_2O (HPLC grade). The selected values were as follows: capillary voltage 4 kV; gas temperature 350 °C; drying gas 12 L min-1; nebulizer pressure 45 psig; fragmentator voltage: 70 V. Lobar LichroPrep Si 60 (40-63 µm) or LichroPrep RP-18 columns coupled to a Waters RI 401 detector were used for preparative column chromatography. Mass spectral analyses were done using electrospray ionization in positive ion mode on a Surveyor separations module coupled to a ThermoFinnigan TSQ AM triple quadrupole mass spectrometer.

HPLC purity determination

Compounds **2-5**, **8-15** and **19-23** were analyzed for purity (HPLC) using a Waters 1525 HPLC dual pump system equipped with an Alltech Select degasser system and dual λ 2487 UV-VIS detector and using an Agilent 1200 HPLC system equipped with a Quat pump (G1311B), an injector (G1329B) 1260 ALS, TCC 1260 (G1316A) and a detector 1260 DAD VL+ (G1315C). HPLC analysis was performed using two of several different methods:

Method A: Octadecylsilica was used as the stationary phase (Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8 µ, S.N. USWKY01594). Compounds were dissolved in water. The final concentrations were ~ 1 mg/mL, and the injection volume was 3.0 µL for compounds **2** and **20** and 4.0 µL for compounds **19** and **21-23**. The eluent was made from the following solvents: 0.2% formic acid in water (A) and methanol (B). Wavelength = 254 nm.

Method B: Octadecylsilica was used as the stationary phase (Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8 µ, S.N. USWKY01594). Compounds were dissolved in water. The final concentrations were ~ 1 mg/mL, and the injection volume was 1.0 µL for compounds **2** and **20** and 4.0 µL for compounds **19** and **21-23**. The eluent was made from the following solvents: 0.2% formic acid in water (A) and acetonitrile (B). Wavelength = 254 nm.

Method C: Octadecylsilica was used as the stationary phase (Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8 µ, S.N. USWKY01594). Compounds were dissolved in methanol. The final concentrations were ~ 1 mg/mL, and the injection volume was 0.5 µL for compounds **9**, **10** and **14**, 1 µL for compounds **4** and **5**, 2 µL for compounds **8**, **12** and **13** and 3 µL for compounds **3**, **11**, **15** and **16**. The eluent was made from the following solvents: water (A) and methanol (B). Wavelength = 239 nm (**4**, **12**, **14**), 253 nm (**5**, **8-10**), 328 nm (**13**), 338 nm (**11**), 341 nm (**3**, **15** and **16**).

Method D: Octadecylsilica was used as the stationary phase (Zorbax Eclipse Plus C18 4.6 x 150mm, 1.8 µ, S.N. USWKY01594). Compounds were dissolved in methanol. The final concentrations were ~ 1 mg/mL, and the injection volume was 0.5 µL for compounds **4**, **5**, **9**, **10**, **13** and **14**, and 1 µL for compounds **3, 8, 11, 12, 15** and **16**. The eluent was made from the following solvents: water (A) and acetonitrile (B). Wavelength = 239 nm (**4, 5, 8-10** and **12-14**), 338 nm (**11**), 341 nm (**3**, **15** and **16**).

All compounds were > 95% pure.

Synthesis

General procedure for the preparation of 1,7-bis(alkylamino)-4,10-diazachrysenes
17[1] and an excess of the appropriate amine were dissolved in NMP in a MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 6 h at 180 ºC. The cooled reaction mixture was poured onto ice-water. The obtained precipitate was filtered, washed with water, and dried under reduced pressure.

General procedure for the preparation of 1,7-bis(alkylamino)-4,10-diazachrysene hydrochlorides
The appropriate base was suspended in 40% HCl in dry MeOH, and the reaction mixture was vigorously stirred for 1 h at r.t. The solvent was then removed under reduced pressure, and the remaining solid was suspended in dry EtOH. The EtOH was removed under reduced pressure, and the same procedure with EtOH was repeated two more times. Upon drying at 40 ºC under reduced pressure, the desired product was obtained.

General procedure for the preparation of alkylaminonaphthyridines.
An appropriate chloronaphthyridine and the excess of appropriate amine were dissolved in NMP in a MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 2 hours at 180 ºC. The excess of amine and NMP were removed under reduced pressure using Kugelrohr device. The crude product was purified by column chromatography (dry flash, SiO_2, eluent DCM 100%, DCM/MeOH, gradient 9:1→ 1:9, MeOH 100%, MeOH/NH_4OH gradient 99:1 → 8:2), unless specified otherwise.

***N*-(4-Morpholin-4-ylbutyl)-*N*'-(2-morpholin-4-ylethyl)quino[8,7-*h*]quinoline-1,7-diamine tetrahydrochloride 2.**
Compound **18** (24 mg, 0.06 mmol) and excess of (4-morpholin-4-ylbutyl)amine[1]
(91 mg, 0.58 mmol) were dissolved in NMP (0.7 mL) in a MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 5 h at 180 ºC. The cooled reaction mixture was poured onto ice-water. The obtained precipitate was filtered, washed with water, and dried under reduced pressure. The crude product was purified by column chromatography (dry flash, SiO_2, eluent CH_2Cl_2/MeOH gradient 8:2 → 1:9, 100% MeOH). The product was suspended in 40% HCl in dry MeOH, and the reaction mixture was vigorously stirred for 1 hour at r.t. The solvent was then removed under reduced pressure, and the remaining solid was suspended in dry EtOH. The EtOH was removed under reduced pressure, and the same procedure with EtOH was repeated two more times. Upon drying at 40 ºC under reduced pressure, the desired product was obtained. The yield was 22 mg (56 %). **2**: yellow powder, mp > 280 °C. IR (ATR): 3426s, 2926w, 2726w, 2608w, 1624s, 1571m, 1497w, 1440w, 1347w, 1266w, 1226w, 1101w, 1048w, 744w, 608w, 559w, 532w, 501w, 456w, 424w, 404w cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD + $CDCl_3$, δ): 9.15-9.00 (m, 2H), 8.53-8.65 (m, 2H), 8.20 (d, *J* = 9.2, 1H), 8.12 (d, *J* = 9.4, 1H), 6.75-6.60 (m, 2H), 3.85-3.77 (m, 4H), 3.77-3.70 (m, 4H), 3.55-3.48 (m, 2H), 3.47-3.40 (m, 2H), 2.83 (t, *J* = 6.4, 2H), 2.62 (ps, 4H), 2.51 (bs, 4H), 2.48-2.42 (m, 2H), 1.90-1.78 (m, 2H), 1.76-1.63 (m, 2H). ^{13}C NMR (125 MHz, CD_3OD + $CDCl_3$, δ): 150.74, 150.42, 148.83, 148.75, 144.73, 130.77, 120.65, 120.31, 118.40, 117.92, 116.28, 116.22, 99.77, 99.60, 66.21, 65.98, 57.96, 55.84, 52.94, 52.79, 42.27, 38.70, 25.68, 23.19. HRMS: *m/z* 515.31264 corresponds to molecular formula $C_{30}H_{38}N_6O_2H^+$ (error in ppm -0.50), 258.16023 corresponds to molecular formula $C_{30}H_{38}N_6O_2H_2^{2+}$ (error in ppm +0.55), 172.44248 corresponds to molecular formula $C_{30}H_{38}N_6O_2H_3^{3+}$ (error in ppm +1.91). HPLC purity: method A, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.231, area 97.87 %; method B, using gradient protocol 0 - 2 min

90% A, 2 - 7 min 90% A → 0% A, 7 - 9 min 0% A, 9 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.158, are 100 %.

***N*,*N*'-Bis[2-(morpholin-4-yl)ethyl]-1,5-naphthyridine-4,8-diamine 3.**

General procedure given above was followed using **7** (10.00 mg, 0.05 mmol), 2-(morpholin-4-yl)ethanamine (0.27 mL, 2.05 mmol) and NMP (0.7 mL). The crude product was purified by crystalisation from methanol. The yield was 14 mg (71%). **3**: white powder, mp 174-177 °C. IR (ATR): 3379m, 2965m, 2921m, 2871w, 2844w, 2812m, 1582s, 1553s, 1476w, 1457m, 1397w, 1374w, 1337m, 1295w, 1273m, 1232m, 1144m, 1115s, 1083w, 1067w, 1031w, 978w, 947w, 918w, 859w, 815w, 767w, 665w, 629w, 559w, 499w cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.34 (d, *J* = 5.2, 2H), 6.86 (bs, 2H), 6.48 (d, *J* = 5.2, 2H), 3.76 (t, *J* = 4.6, 8H), 3.45-3.35 (m, 4H), 2.74 (t, *J* =6.4, 4H), 2,54 (bs, 8H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 150.21, 147.91, 133.69, 100.18, 66.95, 56.81, 53.50, 39.17. HRMS: *m/z* 387.24979 corresponds to molecular formula $C_{20}H_{30}N_6O_2H^+$ (error in ppm -1.32), 194.12945 corresponds to molecular formula $C_{20}H_{30}N_6O_2H_2^{2+}$ (error in ppm +3.40). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 0.755, area 95.45%; method D, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 0.722, area 100%.

***N*,*N*'-Bis[4-(diethylamino)-1-methylbutyl]-1,5-naphthyridine-4,8-diamine 4.**

General procedure given above was followed using **7** (10 mg, 0.053 mmol), *N*,*N*-diethylpentane-1,4-diamine (0.30 mL, 1.5 mmol) and NMP (0.6 mL). The yield was 12 mg (51%). **4**: light yellow film. IR (ATR): 3367m, 3055w, 2967s, 2931s, 2870m, 2800m, 1674w, 1636w, 1574s, 1545s, 1458m, 1379m, 1353m, 1293m, 1241m, 1216m, 1172m, 1154m, 1089m, 991w, 918w, 815m, 722w, 675w, 632w, 548m cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, δ): 8.23 (d, *J* = 5.5, 2H), 6.63 (d, *J* = 5.5, 2H), 3.80-3.75 (m, 2H), 2.57-2.48 (m, 12H), 1.69-1.59 (m, 8H), 1.32 (d, *J* = 6.5, 6H), 1.01 (t, *J* = 7.2, 12H). ^{13}C NMR (125 MHz, CD_3OD, δ): 149.80, 147.30, 133.32, 100.08, 52.11, 46.19, 34.13, 22.29, 19.24, 9.71. HRMS: *m/z* 443.38495 corresponds to molecular formula $C_{26}H_{46}N_6H$+ (error in ppm -1.63). HPLC purity: method C, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 3.047, area 97.30% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.926, area 98.30%.

***N*^{1},*N*^{1}-Diethyl-*N*^{4}-1,5-naphthyridin-4-ylpentane-1,4-diamine 5.**

General procedure given above was followed using **6** (10 mg, 0.060 mmol), 3-(morpholin-4-yl)propanamine (0.30 mL, 1.5 mmol) and NMP (0.5 mL). The yield was 13 mg (78%). **5**: light yellow film. IR (ATR): 3378m, 3069w, 2968s, 2933m, 2871m, 2803m, 1603s, 1578s, 1486m, 1454m, 1379m, 1369s, 1293w, 1261w, 1226m, 1176m, 1134m, 1092m, 896w, 826m, 795m, 752w, 719w, 658m, 624w, 544m cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, δ): 8.73 (dd, J_1 = 4, J_2 = 1.5, 1H), 8.40 (d, *J* = 5.5, 1H), 8.14 (dd, J_1 = 8.8, J_2 = 1.8, 1H), 7.65 (dd, J_1 = 8.5, J_2 = 4.5, 1H), 6.69 (d, *J* = 5.5, 1H), 3.85-3.82 (m, 1H), 2.55-2.47 (m, 6H), 1.73-1.59 (m, 4H), 1.34 (d, *J* = 6.5, 3H), 1.00 (t, *J* = 7.2, 6H). ^{13}C NMR (125 MHz, CD_3OD, δ): 152.58, 151.97, 148.42, 143.97, 136.86, 136.23, 126.29, 100.94, 53.70, 49.23, 47.79, 35.65, 24.07, 20.78, 11.33. HRMS: *m/z* 287.22190 corresponds to molecular formula $C_{17}H_{26}N_4H$+ (error in ppm -3.91). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 15 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.168, area 96.00% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.799, area 95.52%.

4-Chloro-1,5-naphthyridine 6.

Compound **ZS96** (162 mg, 1.11 mmol) was dissolved in $POCl_3$ (2.5 mL), and the reaction mixture was stirred at 115 °C for 15 min in a closed vessel. The solution was poured onto ice-water and K_2CO_3 was added until pH = 10. The product was extracted with CH_2Cl_2 and the combined organic layers were dried over anh. Na_2SO_4. Dichloromethane was removed under reduced pressure, and the mixture was purified by column chromatography (dry flash, SiO_2, eluent Hex/EtOAc, gradient 8:2→ 1:9, 100% EtOAc). The yield was 154 mg (84%). **6**: white powder, mp 100 °C. IR (ATR): 3068w, 3028s, 2926w, 2827w, 2658w, 1730w, 1582w, 1552w, 1485s, 1376w, 1356w, 1283m, 1250w, 1194w, 1133w, 1102w, 1072w, 1029w, 983m, 863m, 824m, 790m, 658m, 637w, 568w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 9.64-9.62 (m, 1H), 9.36 (d, J = 5.8, 1H), 9.26-9.23 (m, 1H), 8.51 (d, *J* = 5.5, 1H), 8.47 (dd, *J*1 = 8.7, *J*2 = 4.8, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 155.16, 154.04, 150.27, 140.20, 139.70, 139.46, 132.29, 129.98. HRMS: *m/z* 165.02076 corresponds to molecular formula $C_8H_5N_2ClH^+$ (error in ppm -3.88).

2,2-Dimethyl-5-[(pyridin-3-ylamino)methylidene]-1,3-dioxane-4,6-dione ZS95.
Meldrum's acid (1.50 g, 10.4 mmol) was dissolved in EtOH (24 mL), and 3-aminopyridine (0.89 g, 9.5 mmol) was added to the solution followed by triethyl orthoformate (1.40 g, 9.45 mmol). The mixture was stirred with heating at 100 ºC until ethanol evaporated. The solid residue was dissolved in a minimal amount of hot EtOH, charcoal was added, and the suspension was heated to boiling and quickly filtered through warm Cellite. The quickly formed crystals were filtered and washed with a small amount of EtOH. The yield was 1.53 g (65%). **ZS95**: off-white powder, mp 135 °C. IR (ATR): 3236w, 3206w, 3074w, 2993w, 2946w, 1726m, 1686s, 1619s, 1579m, 1480m, 1454w, 1413s, 1380m, 1325m, 1284m, 1262m, 1222m, 1144w, 1022w, 1000w, 936w, 847w, 822w, 803m, 778w, 726w, 704w, 654w, 604w, 507w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 11.26 (d, *J* = 13.4, H-N, exchangeable with D_2O), 8.64 (d, *J* = 14.2, 1H), 8.63-8.61 (m, 1H), 8.56-8.54 (m, 1H), 7.66-7.62 (m, 1H), 7.43-7.39 (m, 1H), 1.77 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 165.41, 163.04, 152.74, 147.86, 140.51, 134.55, 124.68, 124.25, 105.44, 88.71, 27.06. HRMS: *m/z* 249.08686 corresponds to molecular formula $C_{12}H_{12}N_2O_4H^+$ (error in ppm -0.48).

1,5-Naphthyridin-4-ol ZS96.
The solution of **ZS95** (100 mg, 0.403 mmol) in diphenyl ether (3.0 mL) was refluxed for 50 min on a Bunsen burner. The mixture was then cooled to room temperature and formed precipitate was then filtered and washed with hexane. The crude product was dried, and purified by sublimation at 210 °C and 20 mbar. The yield was 40 mg (68%). **ZS96**: white crystals, slowly sublimes at >200 °C. IR (ATR): 3012m, 2925s, 2855s, 2077w, 1975w, 1623s, 1583m, 1559m, 1502s, 1461m, 1421m, 1328w, 1192w, 1137w, 1073w, 977w, 885w, 818w, 779w, 724w, 682w, 590w, 546w, 481w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$ + d-TFA, δ): 9.15 (dd, J_1 = 8.8, J_2 = 1.2, 1H), 9.12-9.10 (m, 1H), 8.51 (d, *J* = 7.4), 8.41 (dd, J_1 = 8.9, J_2 = 5.4, 1H), 7.19 (d, *J* = 7.4, 1H). ^{13}C NMR (125 MHz, $CDCl_3$ + d-TFA, δ): 173.25, 147.30, 144.98, 142.03, 131.98, 131.43, 121.01, 116.06. HRMS: *m/z* 147.05496 corresponds to molecular formula $C_8H_6N_2OH^+$ (error in ppm -2.22).

4,8-Dichloro-1,5-naphthyridine 7.
The solution of **ZS153** (2.00 g, 6.814 mmol) in diphenyl ether (200 mL) was refluxed for 25 min with Bunsen burner. The mixture was then quickly cooled to room temperature and poured into hexane (300 mL). The formed precipitate was filtered, suspended in ethyl acetate, and then filtered again. Crude product was dried in vacuum desiccator, suspended in $POCl_3$ (70 mL), and the reaction mixture was stirred at 120 °C for 4 h in closed vessel. Solution was poured onto ice-water (150 mL) and K_2CO_3 was added until pH=10. The product was extracted with CH_2Cl_2 (3×50 mL) and the combined organic layers were dried over anhydrous sodium sulfate. Dichloromethane was removed under reduced pressure, and the obtained residue was crudely purified by column chromatography (dry flash, SiO_2, eluent Hex/EtOAc, gradient 1:1→ 1:9, 100% EtOAc). The crude product (446.2 mg) was dissolved in 48% hydrobromic acid

(45 mL), and the solution was stirred at 120 °C for 48 hours in a closed vessel. The solvent was then removed under reduced pressure, and the solid residue (701.4 mg) was dissolved in $POCl_3$ (28 mL). The mixture was stirred at 120 °C for 4 hours in a closed vessel. The solution was poured onto ice-water (50 mL) and K_2CO_3 was added until pH=10. The product was extracted with CH_2Cl_2 (3×30 mL) and the combined organic layers were dried over anhydrous sodium sulfate. Dichloromethane was removed under reduced pressure, and the obtained residue was crudely purified by column chromatography (dry flash, SiO_2, eluent Hex/EtOAc, gradient 1:1→ 1:9, 100% EtOAc). The yield was 312 mg (23%). **7**: white powder, mp 270 °C. IR (ATR): 3067m, 3027m, 1724w, 1579w, 1467s, 1378m, 1263m, 1190w, 1046w, 865m, 758m, 614w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$ + d-TFA, δ): 9.32 (d, *J* = 5, 2H), 8.25 (d, *J* = 5, 2H). ^{13}C NMR (125 MHz, $CDCl_3$ + d-TFA, δ): 150.43, 148.18, 137.60, 128.42. HRMS: *m/z* 198.98195 corresponds to molecular formula $C_8H_4N_2Cl_2H^+$ (error in ppm -2.42).

3-Nitropyridine-4-ol ZS107.
Synthesis according to established procedures.[2] **ZS107**: light yellow crystals, mp 234-235 °C. IR (ATR): 3158w, 3071m, 2810w, 2168w, 2110w, 1967w, 1946w, 1815w, 1643s, 1612s, 1544m, 1519w, 1497s, 1340s, 1242w, 1157w, 1096w, 1021w, 972w, 902w, 836m, 770w, 664w, 589w, 563w, 516w cm^{-1}. ^1H NMR (500 MHz, d-DMSO, δ): 12.28 (bs, H-O, exchangeable with D_2O), 8.78 (d, *J* = 1.5, 1H), 7.76 (dd, J_1 = 7.5, J_2 = 1.5, 1H), 6.47 (d, *J* = 7.5, 1H), 3.37 (bs, 1H). ^{13}C NMR (125 MHz, d-DMSO, δ): 168.36, 139.74, 138.38, 137.84, 122.39. HRMS: *m/z* 281.05184 corresponds to molecular formula $[2C_5H_4N_2O_3 + H]^+$ (error in ppm +0.62), 141.02942 corresponds to molecular formula $C_5H_4N_2O_3H^+$ (error in ppm -0.36).

4-Methoxy-3-nitropyridine ZS151.
Potassium carbonate (4.16 g, 0.030 mol) and CH_3I (1.72 mL, 0.028 mol) were added to a solution of **ZS107** (3.50 g, 0.025 mol) in acetone (130 mL) and the mixture was stirred at 40 °C. After 3 h methanol was added (200 mL), the obtained precipitate was filtered, washed with methanol, and methanol was then removed under reduced pressure. The solid residue was purified by column chromatography (dry flash, SiO_2, eluent MeOH 100%, MeOH/NH_4OH gradient 9:1 → 3:7). The yield was 3.08 g (80%). **ZS151**: yellow powder, mp 81-82 °C. IR (ATR): 3456s, 3088w, 3061w, 2920w, 2851w, 1661m, 1595m, 1504w, 1394w, 1364w, 1316m, 1205w, 930w, 833w cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 8.92 (d, *J* = 2.3, 1H), 7.79 (dd, J_1 = 7.6, J_2 = 2.3, 1H), 6.66 (d, *J* = 7.6, 1H), 3.87 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD, δ): 171.06, 165.02, 145.35, 143.84, 124.49, 45.04. HRMS: *m/z* 177.02655 corresponds to molecular formula $C_6H_6N_2O_3Na^+$ (error in ppm -2.88), 155.04484 corresponds to molecular formula $C_6H_6N_2O_3H^+$ (error in ppm -1.82).

4-Methoxypyridine-3-amine ZS152.
Synthesis according to established procedures.[3] **ZS152**: brown powder, mp 88-89 °C. IR (ATR): 3365s, 1638m, 1556s, 1534m, 1501m, 1435w, 1393w, 1364w, 1316w, 1248w, 1191w, 986w, 833w, 721w cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 7.44 (dd, J_1 = 6.9, J_2 = 2, 1H), 7.31 (d, *J* = 2.3, 1H), 6.33 (d, *J* = 6.9, 1H), 3.75 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD, δ): 171.16, 140.64, 137.79, 123.76, 112.98, 44.89. HRMS: *m/z* 249.13390 corresponds to molecular formula $2C_6H_8N_2OH^+$ (error in ppm -2.80), 125.07043 corresponds to molecular formula $C_6H_8N_2OH^+$ (error in ppm -4.07).

Diethyl {[(4-methoxypyridin-3-yl)amino]methylidene}propanedioate ZS153.
Diethyl ethoxymethylenemalonate (3.84 mL, 0.019 mol) was added dropwise to a solution of 4-methoxypyridine-3-amine (2.41 g, 0.019 mol) in ethanol (70 mL). The mixture was heated to 100 °C and stirred in an open flask until ethanol evaporated. The solid residue was purified by column chromatography (dry flash, SiO_2, eluent EtOAc/MeOH gradient 9:1 → 1:1). The yield was 4.83 g (85%). **ZS153**: light brown powder, mp 91-92 °C. IR (ATR): 3578w, 3413w, 3225w, 3053w, 3002w, 2905w,

1698s, 1666m, 1600s, 1568s, 1447w, 1426w, 1385w, 1355w, 1306w, 1264m, 1223m, 1089m, 1020w, 1003w, 926w, 843w, 797w, 615w, 553w cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, δ): 8.53-8.43 (m, 1H), 8.08 (d, *J* = 2, 1H), 7.67 (dd, J_1 = 7.2, J_2 = 1.8, 1H), 6.44 (d, *J* = 7.1, 1H), 4.35-4.13 (m, 4H), 3.86 (s, 3H), 1.42-1.23 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD, δ): 171.28, 169.50, 168.75, 167.74, 150.35, 141.70, 131.77, 127.91, 115.64, 95.71, 61.53, 45.16, 14.87. HRMS: *m/z* 589.24793 corresponds to molecular formula $[2C_{14}H_{18}N_2O_5 + H]^+$ (error in ppm -4.22), 317.11044 corresponds to molecular formula $C_{14}H_{18}N_2O_5Na^+$ (error in ppm -1.11), 295.12967 corresponds to molecular formula $C_{14}H_{18}N_2O_5H^+$ (error in ppm +2.80).

***N*-(3-Morpholin-4-ylpropyl)-1,5-naphthyridin-4-amine 8.**
General procedure given above was followed using **6** (10 mg, 0.061 mmol), 3-(morpholin-4-yl)propanamine (0.30 mL, 2.0 mmol) and NMP (0.5 mL). The yield was 15 mg (91%). **8**: light yellow film. IR (ATR): 3384m, 3237m, 3070m, 3005w, 2951m, 2856m, 2813m, 1699w, 1650w, 1603s, 1580s, 1533s, 1486m, 1459m, 1390m, 1359s, 1306m, 1270w, 1220m, 1168w, 1137m, 1116s, 1071w, 1031w, 988w, 862m, 827m, 795m, 768w, 723w, 680w, 657w, 613w, 581w, 548w cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, δ): 8.74 (dd, J_1 = 4.25, J_2 = 1.2, 1H), 8.39 (d, *J* = 5.5, 1H), 8.14 (dd, J_1 = 8.8, *J*2 = 4.2, 1H), 7.66 (dd, J_1 = 8.8, J_2 = 4.2, 1H), 6.66 (d, *J* = 5.5, 1H), 3.78 (t, *J* = 4.8, 4H), 3.48 (t, *J* = 6.5, 2H), 2.56 (t, *J* = 6.8, 2H), 2.51 (bs, 4H), 1.95 (quint, *J* = 6.5, 2H). ^{13}C NMR (125 MHz, CD_3OD, δ): 153.06, 152.18, 148.48, 143.50, 136.40, 136.34, 126.38, 100.60, 67.89, 58.36, 55.03, 42.68, 25.97. HRMS: *m/z* 273.17014 corresponds to molecular formula $C_{15}H_{20}N_4OH+$ (error in ppm -3.11). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 15 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.002, area 95.76% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.903, area 98.02%.

***N*,*N*-Diethyl-*N*'-1,5-naphthyridin-4-ylpropane-1,3-diamine 9.**
General procedure given above was followed using **6** (21 mg, 0.13 mmol), *N*,*N*-diethylpropane-1,3-diamine (0.30 mL, 1.9 mmol) and NMP (0.5 mL). The yield was 20 mg (59%). **9**: light yellow film. IR (ATR): 3664w, 3390s, 3254s, 3068m, 2968s, 2934s, 2872s, 2809s, 2570w, 2425w, 1916w, 1604s, 1579s, 1532s, 1468s, 1383s, 1360s, 1293m, 1267m, 1219s, 1172m, 1126s, 1071m, 1019w, 980w, 940w, 884w, 827s, 794s, 723m, 684w, 657m, 615m, 545m cm^{-1}. ^{1}H NMR (200 MHz, CD_3OD, δ): 8.71 (dd, J_1 = 4, J_2 = 1.2, 1H), 8.38 (d, *J* = 5.6, 1H), 8.12 (dd, J_1 = 9, J_2 = 1.7, 1H), 7.62 (dd, J_1 = 8.6, J_2 = 4.2, 1H), 6.63 (d, *J* = 5.6, 1H), 3.42 (t, *J* = 6.7, 2H), 2.66-2.48 (m, 6H), 1.89 (quint, *J* = 6.7, 2H), 1.04 (t, *J* = 7.3, 6H). ^{13}C NMR (50 MHz, CD_3OD, δ): 152.64, 152.41, 148.36, 143.72, 136.58, 126.14, 100.54, 51.66, 47.78, 42.04, 26.35, 11.40. HRMS: *m/z* 259.19099 corresponds to molecular formula $C_{15}H_{22}N_4H+$ (error in ppm -2.82). HPLC purity: method C, gradient protocol 0 - 1 min 90% A, 1 - 6 min 90% A → 0% A, 6 - 8 min 0% A, flow rate 0.5 mL/min, RT 2.222, area 98.30% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.814, area 99.49%.

1-Morpholin-4-yl-3-(1,5-naphthyridin-4-ylamino)propan-2-ol 10.
General procedure given above was followed using **6** (30 mg, 0.18 mmol), 1-amino-3-morpholin-4-ylpropan-2-ol[1] (0.30 mL, 1.9 mmol) and NMP (0.6 mL). The yield was 28 mg (54%). **10**: light yellow film. IR (ATR): 3854w, 3749w, 3673w, 3649w, 3384s, 3220m, 2953m, 2858m, 2815s, 2693m, 2572w, 1698w, 1652w, 1605s, 1581s, 1535s, 1490m, 1456m, 1361s, 1298m, 1272m, 1221m, 1116s, 1072m, 1037w, 1007m, 910w, 891w, 865m, 827m, 795m, 732m, 701w, 657m, 631m, 567m cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD, δ): 8.73-8.71 (m, 1H), 8.40 (d, *J* = 5.5, 1H), 8.15-8.11 (m, 1H), 7.64 (dd, J_1 = 8.5, J_2 = 4, 1H), 6.70 (d, *J* = 5.5, 1H), 4.10 (quint, *J* = 6, 1H), 3.74 (t, *J* = 4.8, 4H), 3.47 (ddd, J_1= 32.8, J_2 = 13.5, J_3 = 5, 2H), 2.56-2.52 (m, 6H). ^{13}C NMR (125 MHz, CD_3OD, δ): 152.84, 152.51, 148.51, 143.78, 136.72, 136.35, 126.28, 100.90, 67.98, 67.57, 64.44, 55.61, 48.46. HRMS: *m/z* 289.16586 corresponds to molecular formula $C_{15}H_{20}N_4O_2H+$ (error in ppm -0.15). HPLC purity: method C, gradient protocol 0 - 1 min 90% A, 1 - 6 min

90% A → 0% A, 6 - 8 min 0% A, flow rate 0.5 mL/min, RT 2.326, area 97.49% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.804, area 100%.

***N*-(7C-hloroquinolin-4-yl)-*N*'-(1,5-naphthyridin-4-yl)butane-1,4-diamine 11.**

General procedure given above was followed using **6** (10 mg, 0.06 mmol), *N*-(7-chloroquinolin-4-yl)butane-1,4-diamine (36 mg, 0.14 mmol) and NMP (0.7 mL). The cooled reaction mixture was poured onto ice-water. The obtained precipitate was filtered, washed with water, and dried under reduced pressure. The crude product was purified by column chromatography (dry flash, SiO_2, eluent MeOH, MeOH/NH_4OH gradient 99:1 → 8:2). The yield was 9 mg (38%). **11**: colorless film. IR (ATR): 3235m, 3065w, 2953w, 2868w, 1664w, 1607w, 1580s, 1533m, 1490w, 1448w, 1358w, 1335w, 1309w, 1275w, 1204w, 1136w, 898w, 848w, 823w, 801w, 760w, 572w, 464w cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 8.68 (dd, J_1 = 4.1, J_2 = 1.6, 1H), 8.35 (d, J = 5.5, 1H), 8.29 (d, J = 5.8, 1H), 8.11 (dd, J_1 = 8.5, J_2 = 1.6, 1H), 8.05 (d, J = 9.1, 1H), 7.73 (d, J = 2.0, 1H), 7.66-7.60 (m, 1H), 7.37 (dd, J_1 = 9, J_2 = 2, 1H), 6.65 (d, J = 5.6, 1H), 6.53 (d, J = 5.8, 1H), 3.52-3.43 (m, 4H), 1.97-1.88 (m, 4H). ^{13}C NMR (125 MHz, CD_3OD, δ): 151.26, 151.19, 150.35, 150.31, 148.30, 146.88, 142.45, 136.31, 135.11, 134.61, 127.38, 125.19, 124.74, 121.50, 117.03, 99.55, 98.60, 42.55, 41.98, 26.19, 25.92. HRMS: *m/z* 378.14721 corresponds to molecular formula $C_{21}H_{20}ClN_5H^+$ (error in ppm -2.09), 189.57781 corresponds to molecular formula $C_{21}H_{20}ClN_5H_2^{2+}$ (error in ppm +0.89). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 0.995, area 99.34%; method D, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 0.766, area 96.95%.

***N*,*N*'-Bis(3-morpholin-4-ylpropyl)-1,5-naphthyridine-4,8-diamine 12.**

General procedure given above was followed using **7** (5 mg, 0.027 mmol), 3-(morpholin-4-yl)propanamine (0.30 mL, 2.0 mmol) and NMP (0.5 mL). The yield was 4 mg (33%). **12**: light yellow film. IR (ATR): 3233m, 3072w, 3047w, 2949m, 2922m, 2890m, 2855m, 2819s, 2772m, 2692w, 2660w, 1700w, 1570s, 1545s, 1468s, 1398m, 1336m, 1304m, 1265m, 1224s, 1188w, 1146m, 1114s, 1068m, 1032w, 999w, 968w, 925w, 900w, 861m, 809m, 766m, 743m, 712m, 633m, 609w, 573w, 544m cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 8.25 (d, J = 5, 2H), 6.61 (d, J = 5.5, 2H), 3.74 (t, J = 4.5, 8H), 3.42 (t, J = 6.5, 4H), 2.54 (d, J = 7, 4H), 2.50 (bs, 8H), 1.94 (quint, J = 7, 4H). ^{13}C NMR (125 MHz, CD_3OD, δ): 150.55, 147.40, 133.35, 99.80, 66.28, 56.45, 53.41, 40.69, 24.77. HRMS: *m/z* 415.28160 corresponds to molecular formula $C_{22}H_{34}N_6O_2H+$ (error in ppm -2.35). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 15 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.227, area 95.55% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.812, area 97.48%.

***N*,*N*'-Bis[3-(diethylamino)propyl]-1,5-naphthyridine-4,8-diamine 13.**

General procedure given above was followed using **7** (6 mg, 0.031 mmol), *N*,*N*-diethylpropane-1,3-diamine (0.30 mL, 1.9 mmol) and NMP (0.5 mL). The yield was 5 mg (41%). **13**: light yellow film. IR (ATR): 3383m, 2967m, 2930m, 2871w, 2805m, 1550s, 1468m, 1348m, 1294w, 1220m, 1170w, 1138w, 1072w, 815m, 729w, 632w, 537w cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 8.25 (d, J = 5, 2H), 6.61 (d, J = 5.5, 2H), 3.38 (t, J = 6.8, 4H), 2.66-2.60 (m, 4H), 2.58 (q, J = 7.5, 8H), 1.93-1.88 (m, 4H), 1.05 (t, J = 7.2, 12H). ^{13}C NMR (125 MHz, CD_3OD, δ): 152.08, 149.03, 134.91, 101.50, 51.61, 47.93, 42.12, 26.72, 11.51. HRMS: *m/z* 387.32269 corresponds to molecular formula $C_{22}H_{38}N_6H+$ (error in ppm -0.98). HPLC purity: method C, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.977, area 99.30% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min

90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.826, area 99.81%.

1,1'-[1,5-Naphthyridine-4,8-diyldi(imino)]bis(3-morpholin-4-ylpropan-2-ol) 14.
General procedure given above was followed using **7** (11 mg, 0.056 mmol), 1-amino-3-morpholin-4-ylpropan-2-ol[1] (257 mg, 1.47 mmol) and NMP (0.8 mL). The yield was 17 mg (67%). **14**: light yellow film. IR (ATR): 3386s, 2934s, 2857s, 2814s, 2691m, 1914w, 1697w, 1665w, 1572s, 1551s, 1457s, 1395m, 1352m, 1298m, 1274m, 1222s, 1142m, 1115s, 1071m, 1038w, 1008m, 940w, 914w, 865m, 818m, 734m, 700w, 633m, 576w cm^{-1}. ^1H NMR (500 MHz, CD_3OD, δ): 8.26 (d, *J* = 5.5, 2H), 6.64 (d, *J* = 5, 2H), 4.08 (quint, *J* = 5.8, 2H), 3.72 (t, *J* = 4.5, 8H), 3.41 (ddd, J_1 = 50, J_2 = 13, *J*3 = 5.5, 4H), 2.54-2.50 (m, 12H). ^{13}C NMR (125 MHz, CD_3OD, δ): 152.19, 149.08, 135.02, 101.65, 67.99, 67.73, 64.25, 55.57, 48.41. HRMS: *m/z* 447.27104 corresponds to molecular formula $C_{22}H_{34}N_6O_4H+$ (error in ppm -0.88). HPLC purity: method C, gradient protocol 0 - 1 min 90% A, 1 - 6 min 90% A → 0% A, 6 - 7 min 0% A, flow rate 0.5 mL/min, RT 2.177, area 98.21% ; method D, gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 10 min 0% A, 10 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 6.775, area 99.17%.

***N*,*N*'-Bis[2-(diethylamino)ethyl]-1,5-naphthyridine-4,8-diamine 15.**
General procedure given above was followed using **7** (10 mg, 0.05 mmol), *N*,*N*-diethylethane-1,3-diamine (0.25 mL, 1.78 mmol) and NMP (0.7 mL). The yield was 8 mg (46%). **15**: light yellow film. IR (ATR): 3378m, 2971m, 2932w, 2869w, 2806w, 1582s, 1552s, 1462m, 1383w, 1334w, 1292w, 1255w, 1229m, 1176w, 1126w, 1068w, 974w, 808w, 746w, 593w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.31 (d, *J* = 5.2, 2H), 6.89 (bs, 2H), 6.50 (d, *J* = 5.2, 2H), 3.46 – 3.33 (m, 4H), 2.83 (t, *J* = 6.6, 4H), 2.72 – 2.62 (m, 8H), 1.09 (t, *J* = 7.1, 12H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 150.21, 147.68, 133.41, 100.18, 51.22, 47.01, 40.39, 11.48. HRMS: *m/z* 359.29188 corresponds to molecular formula $C_{20}H_{34}N_6H^+$ (error in ppm +0.31), 180.15066 corresponds to molecular formula $C_{20}H_{34}N_6H_2^{2+}$ (error in ppm +6.30). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 1.024, area 97,81%; method D, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 0.803, area 96.95%.

***N*,*N*'-Bis[4-(morpholin-4-yl)butyl]-1,5-naphthyridine-4,8-diamine 16.**
General procedure given above was followed using **7** (10 mg, 0.05 mmol), (4-morpholin-4-ylbutyl)amine[Error! Bookmark not defined.] (284.3 mg, 1.80 mmol) and NMP (0.7 mL). The yield was 9 mg (46%). **16**: light yellow film. IR (ATR): 3357s, 2940m, 2864m, 2806m, 2686w, 1660w, 1632w, 1544s, 1469m, 1396w, 1351m, 1306w , 1269w, 1249w, 1225w, 1142w, 1112m, 1066w, 1025w, 973w, 940w,911w, 869w, 845w, 821w, 781w, 741w, 633w, 611w, 554w cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.28 (d, *J* = 5.2, 2H), 6.65-6.56 (m, 2H), 6.46 (d, *J* = 5.2, 2H), 3.72 (t, *J* = 4.6, 8H), 3.37-3.28 (m, 4H), 2.53-2.34 (m, 12H), 1.85-1.74 (m, 4H), 1.70-1.60 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 150.27, 147.69, 133.53, 99.96, 66.98, 58.41, 53.73, 42.51, 26.73, 24.17. HRMS: *m/z* 443.31186 corresponds to molecular formula $C_{24}H_{38}N_6O_2H^+$ (error in ppm -2.34), 222.16077 corresponds to molecular formula $C_{24}H_{38}N_6O_2H_2^{2+}$ (error in ppm +3.06), 148.44312 corresponds to molecular formula $C_{24}H_{38}N_6O_2H_3^{3+}$ (error in ppm +4.28). HPLC purity: method C, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 1.033, area 99.92% ; method D, gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 16 min 0% A → 90% A, flow rate 0.5 mL/min, RT 1.027, area 96.27%.

7-Chloro-*N*-(2-morpholin-4-ylethyl)quino[8,7-*h*]quinolin-1-amine 18.

Compound **17** (100 mg, 0.335 mmol) and excess of 2-(morpholin-4-yl)ethanamine (420 mg, 3.22 mmol) were dissolved in NMP (3 mL) in MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 20 min at 150 ºC. The cooled reaction mixture was poured onto ice-water. The obtained precipitate was filtered, washed with water, and dried under reduced pressure. The crude product was purified by column chromatography (dry flash, SiO_2, eluent CH_2Cl_2, CH_2Cl_2/MeOH gradient 9:1 → 3:7). The yield was 83 mg (63%). **18**: off-white powder, mp = 194-196 °C. IC (ATR): 3382m, 2960w, 2927w, 2862w, 2826w, 1588s, 1538s, 1490w, 1455w, 1420w, 1376w, 1333w, 1310w, 1263w, 1234w, 1201w, 1143w, 1111m, 1070w, 1037w, 1009w, 915w, 860w, 826w, 795w, 759w cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD + $CDCl_3$): 9.18 (d, *J* = 9.4, 1H), 9.11 (d, *J* = 9.4, 1H), 8.83 (d, *J* = 4.8, 1H), 8.55 (d, *J* = 5.5, 1H), 8.29 (d, *J* = 9.4, 1H), 8.12 (d, *J* = 9.2, 1H), 7.65 (d, *J* = 4.8, 1H), 6.64 (d, *J* = 5.5, 1H), 3.82-3.70 (m, 4H), 3.52-3.42 (m, 2H), 2.85-2.75 (m, 2H), 2.58 (bs, 4H). ^{13}C NMR (125 MHz, CD_3OD + $CDCl_3$): 150.45, 149.03, 148.01, 146.01, 144.35, 142.33, 130.94, 130.67, 124.77, 127.03, 121.74, 120.82, 120.29, 118.98, 116.79, 99.98, 66.20, 55.79, 52.79, 38.70. HRMS: *m/z* 393.14686 corresponds to molecular formula $C_{22}H_{21}ClN_4OH^+$ (error in ppm -2.04), 197.07737 corresponds to molecular formula $C_{22}H_{21}ClN_4OH_2^{2+}$ (error in ppm -0.50).

_N_-[3-(Diethylamino)propyl]-N'-(2-morpholin-4-ylethyl)quino[8,7-h]quinoline-1,7-diamine tetrahydrochloride 19.

Compound **18** (10 mg, 0.026 mmol) and excess of *N*,*N*-diethylpropane-1,3-diamine (34 mg, 0.26 mmol) were dissolved in NMP (0.6 mL) in MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 5 hours at 180 ºC. The cooled reaction mixture was poured onto ice-water. The obtained precipitate was filtered, washed with water, and dried under reduced pressure. The crude product was purified by column chromatography (dry flash, SiO_2, eluent CH_2Cl_2, CH_2Cl_2/MeOH gradient 9:1 → 1:9, MeOH, MeOH/NH_3 9:1). The product was suspended in 40% HCl in dry MeOH, and the reaction mixture was vigorously stirred for 1 h at r.t. The solvent was then removed under reduced pressure, and the remaining solid was suspended in dry EtOH. The EtOH was removed under reduced pressure, and the same procedure with EtOH was repeated two more times. Upon drying at 40 ºC under reduced pressure, the desired product was obtained. The yield was 7 mg (40%). **19**: yellow powder, mp > 280 °C. IR (ATR): 3399s, 1622s, 1573s, 1504m, 1441m, 1354w, 1231w, 1097w, 748w cm^{-1}. ^{1}H NMR (500 MHz, D_2O): 9.03 – 8.99 (m, 4H), 8.92 (d, *J* = 7, 1H), 8.84 (d, *J* = 7.3, 1H), 8.79 – 8.73 (m, 2H), 7.48 (d, *J* = 7, 1H), 7.41 (d, *J* = 7.3, 1H), 4.40 (t, *J* = 6.6, 2H), 4.29 (bs, 4H), 4.07 (t, *J* = 7, 2H), 3.97 (t, *J* = 6.6, 2H), 3.79 (bs, 4H), 3.62 – 3.57 (m, 2H), 3.52 (q, *J* = 7.3, 4H), 2.56 – 2.50 (m, 2H), 1.55 (t, *J* = 7.3, 6H). ^{13}C NMR (125 MHz, D_2O): 155.95, 155.86, 142.19, 141.70, 135.00, 134.83, 124.70, 124.66, 120.99, 120.92, 120.24, 119.94, 101.10, 100.83, 63.63, 54.14, 52.13, 49.05, 47.49, 40.55, 37.42, 22.32, 8.20. HPLC purity: method A, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.254, area 99.59 %; method B, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.186, area 100 %.

1-Morpholin-4-yl-3-({7-[(2-morpholin-4-ylethyl)amino]quino[8,7-*h*]quinolin-1-yl}amino)propan-2-ol tetrahydrochloride 20.

Compound **18** (22 mg, 0.060 mmol) and excess of 1-amino-3-morpholin-4-ylpropan-2-ol[Error! Bookmark not defined.] (98 mg, 0.56 mmol) were dissolved in NMP (0.7 mL) in a MW cuvette under argon. The reaction mixture was subjected to MW irradiation using *Biotage Initiator 2.5* apparatus for 5 hours at 180 ºC. The cooled reaction mixture was poured onto ice-water and K_2CO_3 was added to pH = 13. The product was extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (dry flash, SiO_2, eluent CH_2Cl_2/MeOH gradient 9:1 → 1:9, 100% MeOH). The

product was suspended in 40% HCl in dry MeOH, and the reaction mixture was vigorously stirred for 1 hour at r.t. The solvent was then removed under reduced pressure, and the remaining solid was suspended in dry EtOH. The EtOH was removed under reduced pressure, and the same procedure with EtOH was repeated two more times. Upon drying at 40 ºC under reduced pressure, the desired product was obtained. The yield was 27 mg (73 %). **20**: light brown powder, mp > 280 °C. IR (ATR): 3419s, 2932w, 1623s, 1570m, 1499w, 1440w, 1348w, 1267w, 1229w, 1130w, 1098w, 980w, 906w, 869w, 822w, 744w, 571w cm^{-1}. ^1H NMR (500 MHz, CD_3OD + $CDCl_3$, δ): 9.10-9.05 (m, 2H), 8.61-8.55 (m, 2H), 8.19 (d, *J* = 9.4, 1H), 8.15 (d, *J* = 9.4 1H), 6.77 (d, *J* = 5.8, 1H), 6.71(d, *J* =5.8, 1H), 4.21-4.15 (m, 1H), 3.83-3.76 (m, 8H), 3.73 (bs, H-O), 3.58-3.51 (m, 3H), 3.46 (dd, J_1 = 13.0, J_2 = 6.6, 1H), 2.83 (t, *J* = 6.4, 2H), 2.67-2.57 (m, 10H). ^{13}C NMR (125 MHz, CD_3OD + $CDCl_3$, δ): 150.94, 150.59, 148.62, 148.51, 144.38, 130.62, 130.56, 120.52, 120.49, 118.25, 118.15, 116.23, 99.86, 99.81, 66.22, 66.19, 65.22, 62.57, 55.81, 53.57, 52.79, 47.30, 38.74. HRMS: *m/z* 517.29220 corresponds to molecular formula $C_{29}H_{36}N_6O_3H^+$ (error in ppm +0.06), 259.15032 corresponds to molecular formula $C_{29}H_{36}N_6O_3H_2^{2+}$ (error in ppm +2.30), 173.10265 corresponds to molecular formula $C_{29}H_{36}N_6O_3H_3^{3+}$ (error in ppm +2.37). HPLC purity: method A, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.250, area 99.17 %; method B, using gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 9 min 0% A, 9 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.161, area 100 %.

***N*,*N*'-Bis{2-[2-(dimethylamino)ethoxy]ethyl}quino[8,7-*h*]quinoline-1,7-diamine tetrahydrochloride 21.**
General procedure given above was followed using **ZS134** (10 mg, 0.020 mmol) and 40% HCl in dry MeOH (1 mL). The yield was 123 mg (98 %). **21**: off-white powder, mp > 280 °C. IR (ATR): 3380s, 3020m, 1621s, 1573m, 1502m, 1476m, 1440m, 1400w, 1345w, 1314w, 1228w, 1175w, 1122m, 990w, 929w, 826w, 748w, 568w cm^{-1}. ^1H NMR (500 MHz, D_2O): 8.46 (d, *J* = 7.1, 2H), 8.35 (d, *J* = 6.2, 2H), 8.25 (d, *J* = 9.2, 2H), 7.15 (d, *J* = 7.3, 2H), 4.01 – 3.96 (m, 4H), 3.96 – 3.90 (m, 8H), 3.43 – 3.39 (m, 4H), 2.92 (s, 12H). ^{13}C NMR (125 MHz, D_2O): 155.76, 141.45, 134.42, 124.14, 120.68, 119.72, 115.41, 100.94, 68.07, 64.26, 56.60, 43.17, 42.79. HRMS: *m/z* 491.31200 corresponds to molecular formula $C_{28}H_{38}N_6O_2H^+$ (error in ppm -1.83). HPLC purity: method A, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.273, area 98.88 %; method B, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.206, area 100 %.

***N*,*N*'-Bis(4-aminobutyl)quino[8,7-*h*]quinoline-1,7-diamine dihydrochloride 22.**
General procedure given above was followed using **ZS136** (10 mg, 0.025 mmol) and 40% HCl in dry MeOH (1 mL). The yield was 11 mg (100 %). **22**: yellow powder, mp > 280 °C. IR (ATR): 3477s, 3447s, 3208s, 3026s, 1620s, 1574s, 1503m, 1472m, 1446m, 1403w, 1363w, 1320w, 1270w, 1229m, 1159w, 1114w, 1089w, 1036w, 1007w, 929w, 905w, 827w, 779w, 749w, 700w, 572w cm^{-1}. ^1H NMR (500 MHz, D_2O): 8.41 (d, *J* = 7, 2H), 8.21 (ABq, J_{AB} = 9.3, 4H), 7.06 (d, *J* = 7.1, 2H), 3.73 – 3.97 (m, 4H), 3.16 – 3.11 (m, 4H), 1.99 – 1.85 (m, 8H). ^{13}C NMR (125 MHz, D_2O): 155.42, 141.31, 134.30, 124.00, 120.58, 119.49, 115.31, 100.67, 43.06, 39.05, 24.51, 24.27. HRMS: *m/z* 403.25983 corresponds to molecular formula $C_{24}H_{30}N_6H^+$ (error in ppm -1.59). HPLC purity: method A, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.204, area 99.70 %; method B, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.176, area 99.63 %.

***N*,*N*'-Bis(3-aminopropyl)quino[8,7-*h*]quinoline-1,7-diamine tetrahydrochloride 23.**
General procedure given above was followed using **ZS138** (10 mg, 0.027 mmol) and 40% HCl in dry MeOH (1 mL). The yield was 134 mg (99 %). **23**: yellow powder, mp > 280 °C. IR (ATR): 3394s, 3209m,

2993s, 1615s, 1571s, 1502m, 1476m, 1441m, 1404w, 1356w, 1317w, 1272w, 1231m, 1161w, 1113w, 1088w, 990w, 830w, 810w, 749w, 701w, 628w, 566w 572w cm^{-1}. ^{1}H NMR (500 MHz, D_2O): 8.41 (d, *J* = 7, 2H), 8.14 (ABq, J_{AB} = 9.3, 4H), 7.10 (d, *J* = 7.1, 2H), 3.83 – 3.77 (m, 4H), 3.29 – 3.23 (m, 4H), 2.31 – 2.23 (m, 4H). ^{13}C NMR (125 MHz, D_2O): 155.64, 141.60, 134.45, 124.18, 120.71, 119.72, 115.57, 100.78, 40.68, 37.06, 25.51. HRMS: *m/z* 375.22836 corresponds to molecular formula $C_{22}H_{26}N_6H^+$ (error in ppm -2.18). HPLC purity: method A, using gradient protocol 0 - 2 min 90% A, 2 - 7 min 90% A → 0% A, 7 - 9 min 0% A, 9 – 12 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.328, area 96.26 %; method B, using gradient protocol 0 - 3 min 90% A, 3 - 9 min 90% A → 0% A, 9 - 12 min 0% A, 12 – 14 min 0% A → 90% A, flow rate 0.5 mL/min, RT 2.231, area 98.47 %.

***N*,*N*'-Bis{2-[2-(dimethylamino)ethoxy]ethyl}quino[8,7-*h*]quinoline-1,7-diamine ZS134.**
General procedure given above was followed using **17** (50 mg, 0.17 mmol), 2-(2-aminoethoxy)-*N*,*N*-dimethylethanamine (256 mg, 1.94 mmol) and NMP (1.0 mL). The yield was 73 mg (89 %). **ZS134**: pale-brown powder, mp=122 °C. IR (ATR): 3381s, 2954s, 2864s, 2824m, 2782m, 1601s, 1544s, 1460m, 1361w, 1337m, 1251w, 1180w, 1125m, 1071w, 1036w, 959w, 831w, 810w, 760w cm^{-1}. ^{1}H NMR (500 MHz, TFA): 9.04 (d, *J* = 9.4, 2H), 8.76 (d, *J* = 7.3, 2H), 8.72 (d, *J* = 9.4, 2H), 7.35 (d, *J* = 7.4, 2H), 4.30 – 4.23 (m, 4H), 4.21 – 4.13 (m, 8H), 3.74 – 3.69 (m, 4H), 3.27 (s, 12H). ^{13}C NMR (125 MHz, TFA): 159.33, 144.20, 137.90, 127.90, 123.31, 122.79, 118.99, 103.49, 70.77, 66.41, 60.30, 46.12, 45.80. HRMS: *m/z* 491.31226 corresponds to molecular formula $C_{28}H_{38}N_6O_2H^+$ (error in ppm -1.30).

***N*,*N*'-Bis(4-aminobutyl)quino[8,7-*h*]quinoline-1,7-diamine ZS136.**
General procedure given above was followed using **17** (52 mg, 0.17 mmol), 1,4-diaminobutane (612 mg, 6.94 mmol) and NMP (1.0 mL). The yield was 50 mg (72 %). **ZS136**: pale-brown powder, mp = 224 - 226 °C. IR (ATR): 3347m, 2925m, 2854m, 1596s, 1543s, 1475m, 1433m, 1344m, 1246w, 1180w, 1119w, 1030w, 907w, 848w, 758w, 668w cm^{-1}. ^{1}H NMR (500 MHz, TFA): 8.99 (d, *J* = 9.4, 2H), 8.74 – 8.68 (m, 4H), 7.30 (d, *J* = 7.3, 2H), 3.99 (bs, 4H), 3.55 (bs, 4H), 2.25 (bs, 8H). ^{13}C NMR (125 MHz, TFA): 159.11, 144.01, 137.81, 127.80, 123.20, 122.57, 118.92, 103.21, 45.75, 43.15, 26.90, 26.77. HRMS: *m/z* 403.25996 corresponds to molecular formula $C_{24}H_{30}N_6H^+$ (error in ppm -1.27).

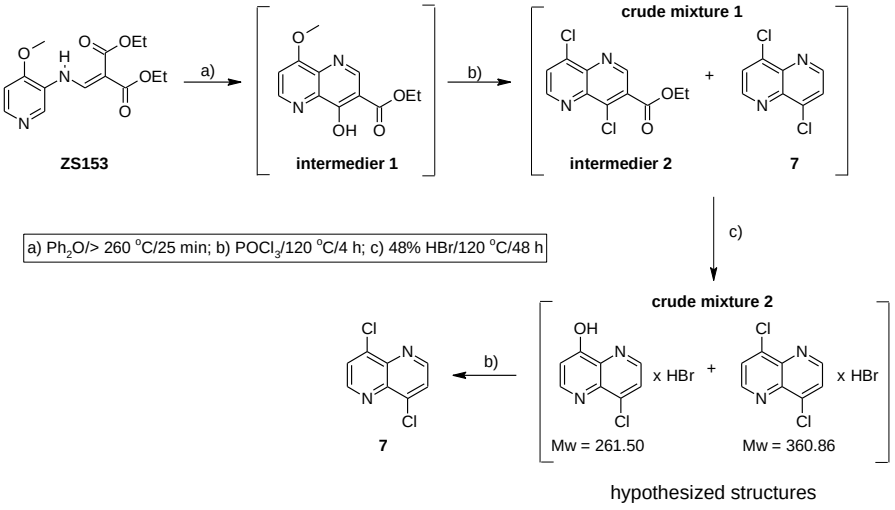
***N*,*N*'-Bis(3-aminopropyl)quino[8,7-*h*]quinoline-1,7-diamine ZS138.**
General procedure given above was followed using **17** (52 mg, 0.17 mmol), 1,3-diaminopropane (513 mg, 6.92 mmol) and NMP (1.0 mL). The yield was 64 mg (99%). **ZS138**: pale-brown powder, mp = 218 - 220 °C. IR (ATR): 3288s, 2942s, 2858m, 1596s, 1542s, 1471m, 1433m, 1393w, 1345m, 1245w, 1176w, 1125w, 956w, 920w, 856w, 801w, 754w, 547w cm^{-1}. ^{1}H NMR (500 MHz, TFA + D_2O): 7.24 (d, *J* = 9.4, 2H), 6.96 – 6.89 (m, 4H), 5.44 (d, *J* = 7.1, 2H), 2.22 – 2.15 (m, 4H), 1.69 – 1.63 (m, 4H), 0.74 – 0.66 (m, 4H). ^{13}C NMR (125 MHz, TFA + D_2O): 159.83, 145.20, 138.86, 128.82, 124.33, 123.80, 119.88, 103.92, 43.88, 40.73, 28.77. HRMS: *m/z* 375.22917 corresponds to molecular formula $C_{22}H_{26}N_6H^+$ (error in ppm -1.97).

Synthesis of compound 7

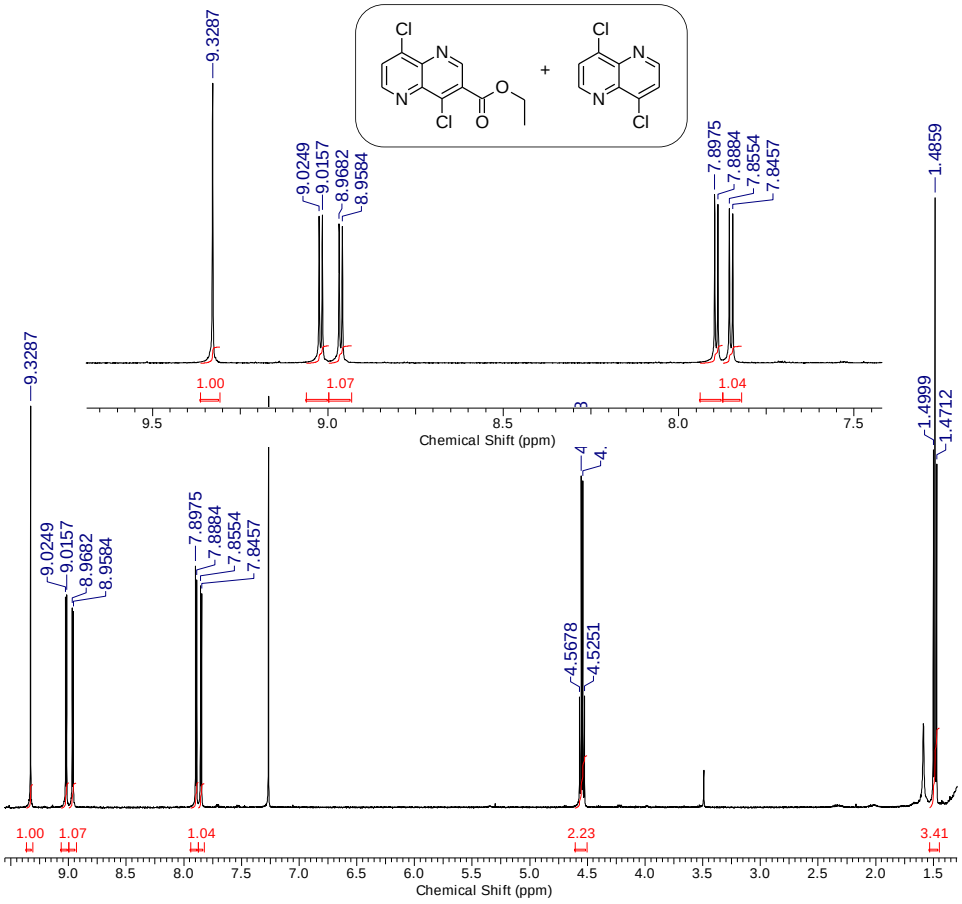
4,8-Dichloro-1,5-naphthyridine 7.

The solution of **ZS153** (2.00 g, 6.814 mmol) in diphenyl ether (200 mL) was refluxed for 25 min with Bunsen burner. The mixture was then quickly cooled to room temperature and poured into hexane (300 mL). The formed precipitate was filtered, suspended in ethyl acetate, and then filtered again. Crude product was dried in vacuum desiccator, suspended in $POCl_3$ (70 mL), and the reaction mixture was stirred at 120 °C for 4 h in closed vessel. Solution was poured onto ice-water (150 mL) and K_2CO_3 was

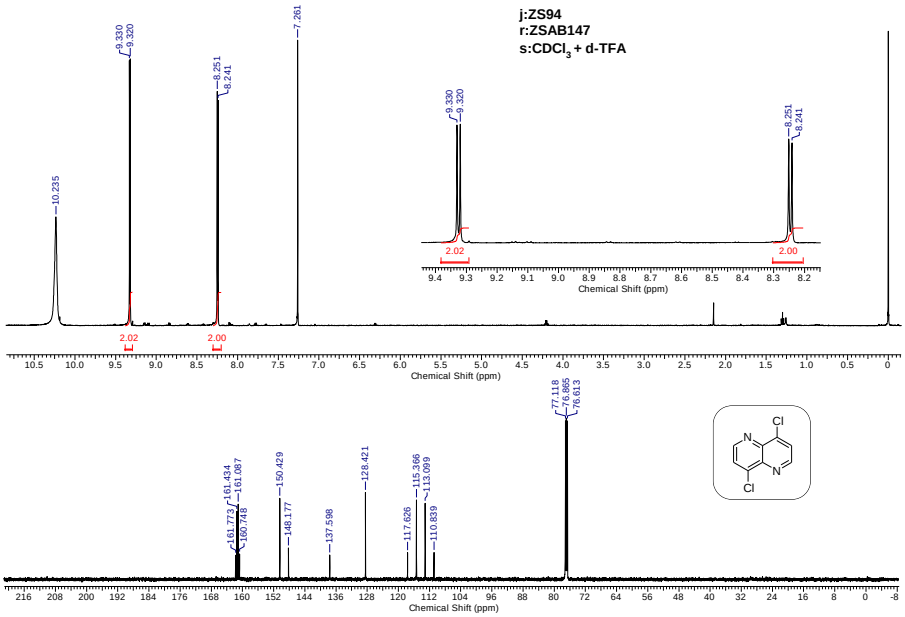
added until pH=10. The product was extracted with CH_2Cl_2 (3×50 mL) and the combined organic layers were dried over anhydrous sodium sulfate. Dichloromethane was removed under reduced pressure, and the obtained residue was crudely purified by column chromatography (dry flash, SiO_2, eluent Hex/EtOAc, gradient 1:1→ 1:9, 100% EtOAc). The crude product (446.2 mg) was dissolved in 48% hydrobromic acid (45 mL), and the solution was stirred at 120 °C for 48 hours in a closed vessel. The solvent was then removed under reduced pressure, and the solid residue (701.4 mg) was dissolved in $POCl_3$ (28 mL). The mixture was stirred at 120 °C for 4 hours in a closed vessel. The solution was poured onto ice-water (50 mL) and K_2CO_3 was added until pH=10. The product was extracted with CH_2Cl_2 (3×30 mL) and the combined organic layers were dried over anhydrous sodium sulfate. Dichloromethane was removed under reduced pressure, and the obtained residue was crudely purified by column chromatography (dry flash, SiO_2, eluent Hex/EtOAc, gradient 1:1→ 1:9, 100% EtOAc). The yield was 312 mg (23%).



NMR spectrum of “Crude mixture 1”:

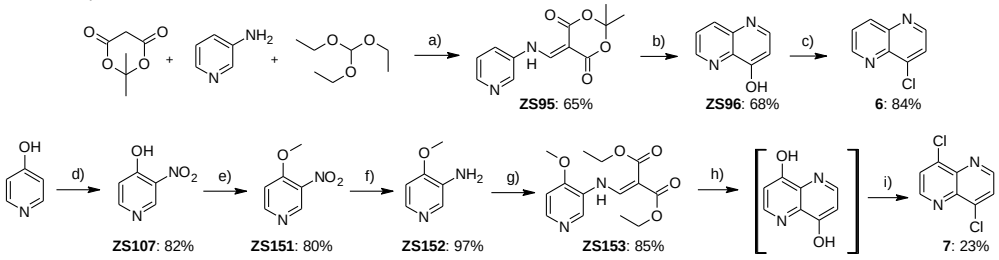


NMR spectrum of **7 (ZS94)**:



Naphthyridine core synthesis (compounds 6 and 7)

Key intermediate **6** (Scheme S1) was obtained in analogy to previously reported procedures (Scheme 1).[4,5] The three-component condensation product **ZS95** was subsequently cyclized in boiling diphenyl ether and the obtained **ZS96** was purified by sublimation. The procedure used by Brown[6] to synthesize another important intermediate, dichloride **7**, was modified in two key steps (Scheme 1). The first part of the synthesis followed Brown's procedure. Nitration of 4-pyridone gave rise to **ZS107**[2], which was then methylated to **ZS151**. Upon reduction of the nitro group the obtained amine **ZS152**[3], was subjected to condensation with diethyl ethoxymethylenemalonate affording **ZS153** in good yield over four steps. Formation of the second pyridine ring, and the subsequent decarboxylation step, were performed by prolonged refluxing of **ZS153** in concentrated hydrobromic acid, rather than using quinoline and vacuum-thermal decarboxylation[6], which sped up and eased the synthesis. Finally, both key intermediates **6** and **7** were obtained after $POCl_3$ treatment of respective hydroxy precursors (Scheme 1).



a) EtOH, reflux; b) Ph_2O, 260 °C, 50 min; c) $POCl_3$, 115 °C, 15 min; d) KNO_3, H_2SO_4, 100 °C, 60 min; e) MeI, acetone, 40 °C; f) H_2, Pd/C, 70 psi, MeOH; g) diethyl ethoxymethylenemalonate, EtOH, reflux; h) 1. Ph_2O, 260 °C, 50 min, 2. $POCl_3$, 120 °C, 4 h, 3. 48% HBr, reflux, 48 h; i) $POCl_3$, 120 °C, 4 h

Scheme S1. The synthesis of monochloro- (**6**) and dichloro-naphthyridine (**7**).

2. In vitro and in vivo EBOV assays:

In vitro:

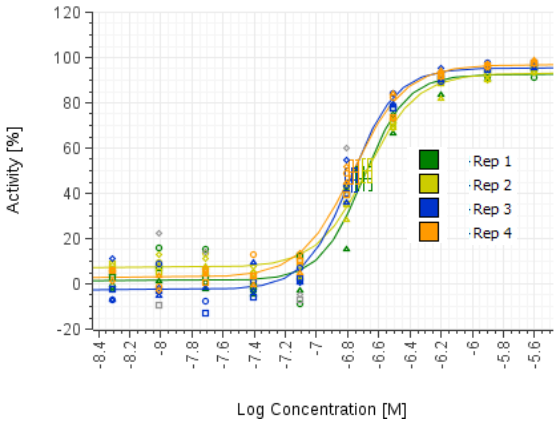
- Two hours prior to infection, compounds were dispensed via the HP D300 directly from 100% DMSO stocks into assay cell plates. DMSO was normalized in all wells to a final 1.0%.
- Compound activity was tested in a 10-point dose response with 2-fold step dilutions starting at 10 µM (final). Doses were repeated 4 times on a single plate (n=4).
- Sixteen wells/plate were treated as neutral (infected) controls.
- Additionally, 16 uninfected wells/plate were used as low signal controls.
- Cells were infected with EBOV (Kikwit) MOI = 0.5 (HeLa).
- Infection was stopped after 48h by fixing cells with a formalin solution.

Plate Statistics for Quality Control, standard compound:

Table S1.

Index	Cell line	Pathogen	MOI	Plate ID	Date	Well Masking	Nuclei - Number, NC	% Infection	Z' Factor	# of Analyzed Fields	EC50 (uM)	SD
1	HeLa	EBOV	0.5	AA00000913	2/3/2016	0.26%	5228	69.24	0.75	5	0.2	0.02
2	HeLa	EBOV	0.5	AA00000914	2/3/2016	0.00%	5281	70.19	0.78	5	0.2	0.01
3	HeLa	EBOV	0.5	AA00000915	2/4/2016	0.00%	4648	74.95	0.83	5	0.2	0.01
4	HeLa	EBOV	0.5	AA00000916	2/4/2016	0.00%	4454	72.66	0.78	5	0.2	0.01

Figure S1.



In vivo:

Animals: Mice Balb/c, 10 mice per test

Virus: Mouse adapted Ebolavirus (Zaire)

Dose of pathogen: 1000 pfu
Route of compound treatment: IP
Route of pathogen infection: IP

3. ADME data[7]

Table S2.

ADMET Properties	Units	Good	Moderate	Poor
Purity	%	> 90		
Solubility	pH 7.4 (μg/mL)	> 50	10 – 50	< 10
Stability-Microsomes	$T_{1/2}$ (min)	> 30	15 - 30	< 15
	CL_{int} (μL/min/mg)	< 46	46 - 92	> 92
Stability -Plasma	% Remaining at 1 hr	> 85	20 - 85	< 20
CYP450 Inhibition	% Inhibition at 3 μM	< 15	15 - 50	> 50
	IC_{50} (μM)	> 10	1 - 10	< 1
MDR1-MDCKII	P_{app} (a-b, 10^{-6} cm/s)	> 20	2 - 20	< 2
	Pgp Efflux Ratio	< 1.5	1.5 - 2.5	> 2.5

Permeability:

Comment [B1]: Rajini, what should be done about these large standard deviations?

Table S3: **2 (ZSML08)** Apparent Permeability with Two Technical Replicates for Each of Three Biological Replicates

Assay Date	REP 1	REP 2	REP 1	REP 2
7/27	PAPP A→B ± SD	PAPP A→B ± SD	PAPP B→A ± SD	PAPP B→A ± SD
ATENOLOL	0.39 ± 0.07	0.15 ± 0.05	0.43 ± 0.02	0.30 ± 0.01
PRAZOSIN	27.87 ± 0.5	27.62 ± 0.48	54.02 ± 1.32	48.58 ± 1.18
PROPRANOLOL	39.03 ± 0.73	38.26 ± 0.63	42.86 ± 1.31	41.56 ± 1.24
2 (ZSML08)	**45.82 ± 33.22**	**67.21 ± 40.14**	**55.57 ± 13.70**	**67.29 ± 10.20**

Table S4: **2 (ZSML08)** Apparent Permeability with Three Biological Replicates

Assay Date 8/9/2017	PAPP A→B ± SD	PAPP B→A ± SD
ATENOLOL	0.79 ± 0.15	0.59 ± 0.12
PRAZOSIN	30.02 ± 0.56	58.87 ± 1.32
PROPRANOLOL	37.74 ± 1.81	41.90 ± 0.81
2 (ZSML08)	**39.29 ± 25.40**	**43.16 ± 9.13**

These assays were run in the MDCK model for intestinal absorption. The standard deviation values for the apparent permeability constants for **2** are very large. The standards shown (atenolol, prazosin, and propranolol) were run on the same plate at the same time as **2**, and do not display large standard deviation values. The technical repeats shown in Table S3 are separate analytical runs of the same three biological replicates that were run on the same day. Table S4 shows the data from the three biological replicates and one analytical run that were run on a separate day from the samples used to generate the data in Table S3.

Even with the large stand deviation values, the apparent permeability values for **2** indicate moderate to high permeability.

4. Fluorescence and UV-Vis spectra of binding to HSA and AGP

Compound 1

AGP:

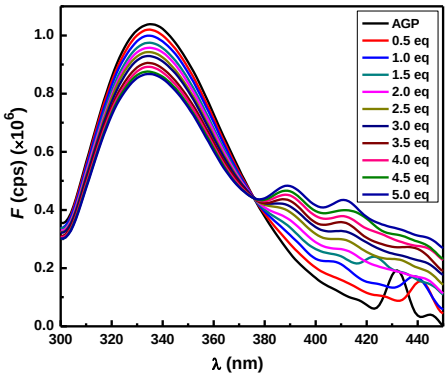


Figure S2. Changes in AGP fluorescence emission spectra upon addition of **1** (1-5 molar equivalents); T=298.0 K±0.1 °C, 30 mM PBS, pH=7.35.

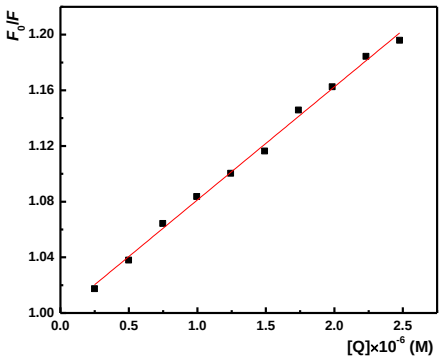


Figure S3. The Stern-Volmer plot for binding of **1** to AGP. $y = 1 + 81248.0\,x$.

K_{sv}=(8.12±0.07)×10^4 M^{-1}

HSA:

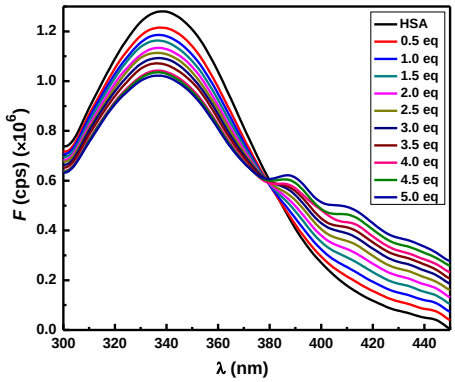


Figure S4. Changes in HSA fluorescence emission spectra upon addition of **1** (1-5 molar equivalents); T=298.0 K±0.1 °C, 30mM PBS, pH=7.35.

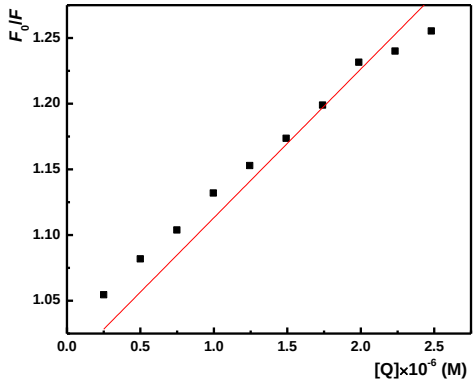


Figure S5. The Stern-Volmer plot for binding of **1** to HSA. $y = 1 + 113185.8\,x$.

K_{sv}=(1.13±0.04)×10^5 M^{-1}

Compound 2

AGP:

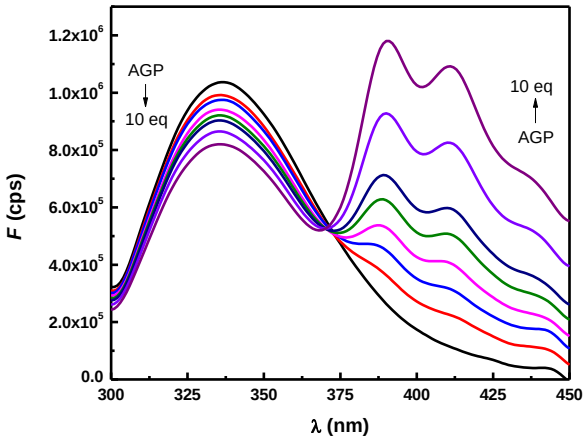


Figure S6. Changes in AGP fluorescence emission spectra upon addition of **2** (1-10 molar equivalents); T=298.0 K±0.1 °C, 30 mM PBS, pH=7.42.

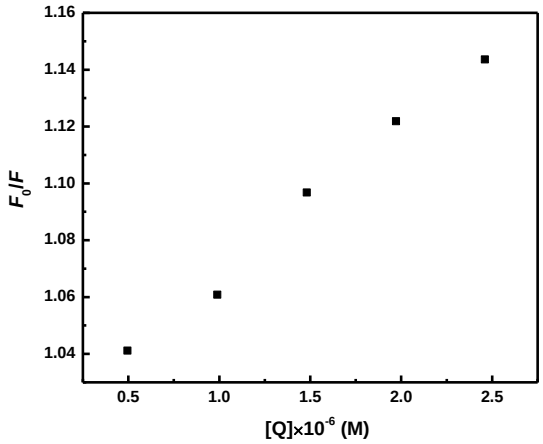


Figure S7. The Stern-Volmer plot for binding of **2** to AGP. y = 1 + 61187.3 x.

K_{sv}=(6.12±0.20)×10^4 M^{-1}

HSA:

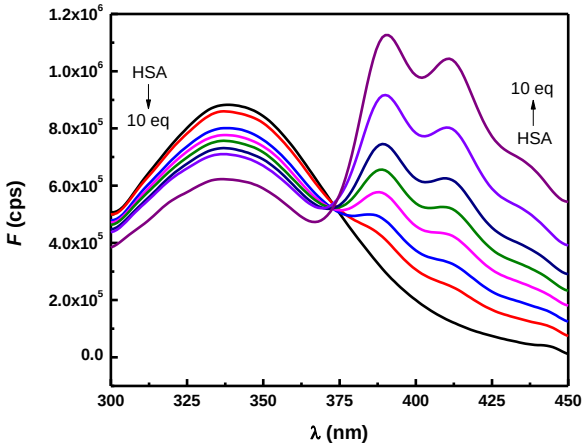


Figure S8. Changes in HSA fluorescence emission spectra upon addition of **2** (1-10 molar equivalents); T=298.0 K±0.1 °C, 30mM PBS, pH=7.42.

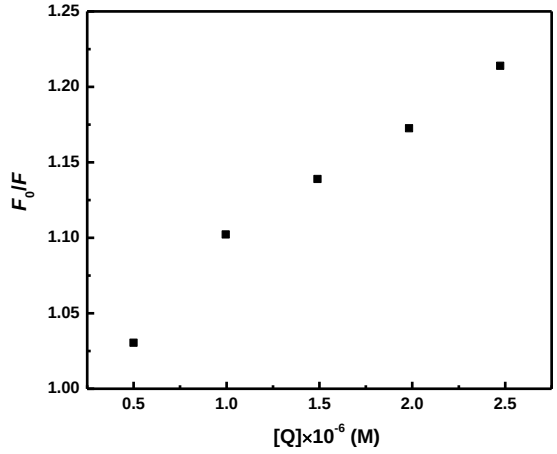


Figure S9. The Stern-Volmer plot for binding of **2** to HSA. $y = 1 + 88495.3\,x$.

K_{sv}=(8.85±0.30)×10^4 M^{-1}

5. In vivo Mouse Pharmacokinetics.

Compound **2** was dissolved in water and administered intraperitoneally at 20 mg/kg/day dose for 7 consecutive days. Blood was collected from 3 mice, previously anaesthetized with chloroform, 24 hours after the last dose, via cardiac puncture. Samples were immediately centrifuged and serum stored at -20 °C until the moment of analysis. Human serum was collected from a healthy volunteer and stored in refrigerator at 4 °C. Total concentrations of compound in mice samples were determined by precipitation of proteins by addition of two volume equivalents of acetonitrile (50 µL of sample and 100 µL of acetonitrile), following 15 seconds on vortex and 30 min in ultrasound bath. After centrifugation of denatured proteins (10 minutes, 13400 rpm), supernatants were injected. Calibration curves were

prepared using blank human serum. Compound standard solutions were prepared in water. Human serum was spiked with stock solutions; final solutions were incubated at 37 °C for 1 h and treated with acetonitrile in the same manner. Concentrations of compound in mice serum were quantitated using a Waters Acquity UPLC H-Class (WAT-176015007) (Milford, MA,USA) with Poroshell 120 EC-C18 column (4.6 × 50mm, 2.7µ, S.N. USCFU07797) and interfaced to mass detector (Waters TQ (Tandem Quadrupole, WAT-176001263)). Single ion recording experiment (SIR) was used, by monitoring ions: $[M+H]^{1+}$ (515), for the compound, and $[M+2]^{+}$, $[M+16]^{+}$, $[M+32]^{+}$, $[M-2]^{+}$, $[M+14]^{+}$, $[M+30]^{+}$, $[M+18]^{+}$, $[M+34]^{+}$ for the possible metabolites.[8] Column temperature was maintained at 40 °C and mobile phase flow rate at 0.3 mL/min. The mobile phase consisted of ultrapure water (TKA Germany MicroPure water purification system, 0.055 µS/cm) containing 0.2 vol.% formic acid (solvent A) and acetonitrile (solvent B). For detection of total concentration, limit of detection (LOD) was 0.08 ppm (S/N = 3:1), limit of quantitation (LOQ) was 0.17 ppm (S/N = 6:1) and R^2=0.9939 (calibration curve was performed in triplicate).

Table S3. Data for the calibration curve for **2**.

Salt concentration, ppm	Base concentration, ppm	Area
0.1	0.08	746
0.22	0.17	2387
0.25	0.19	2707
0.5	0.39	3354
0.75	0.58	4157
1.0	0.78	5400
1.5	1.17	7164
5.0	3.90	29409

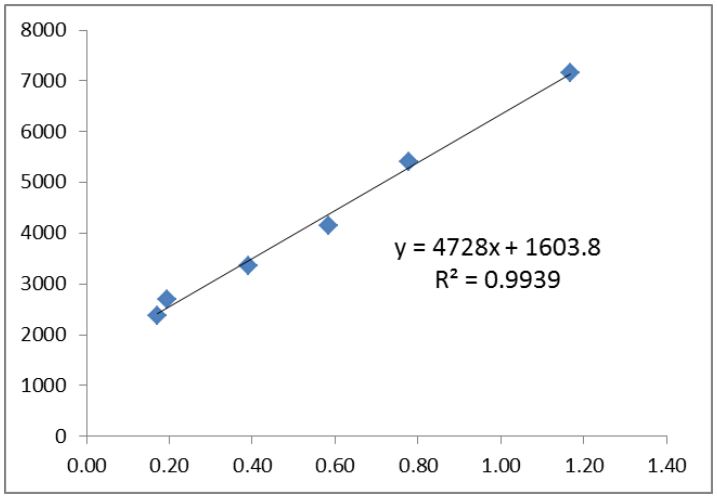
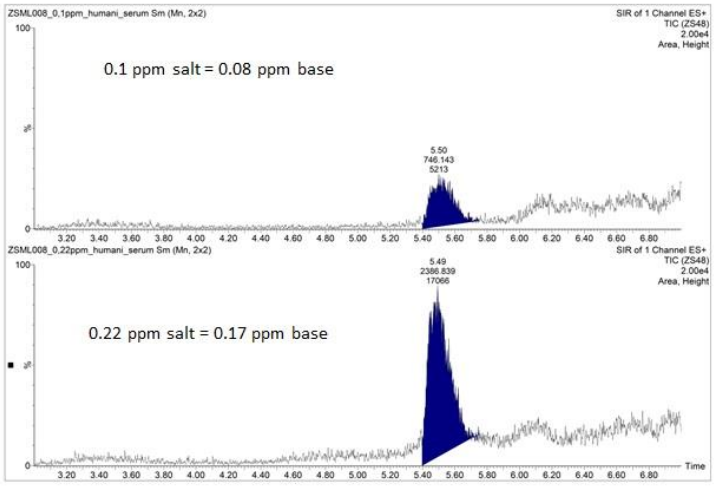


Figure S10. The calibration curve for **2**.



LOD (limit of detection) = 0.08 ppm
S/N (signal/noise) = 6/1

LOQ (limit of quantification) = 0.17 ppm
S/N (signal/noise= 10/1

Figure S11. Limit of detection and limit of quantification for **2 (ZSML08)**.

Pursuit of possible **2** metabolites chromatograms (designation in the upper right corner of each chromatogram):

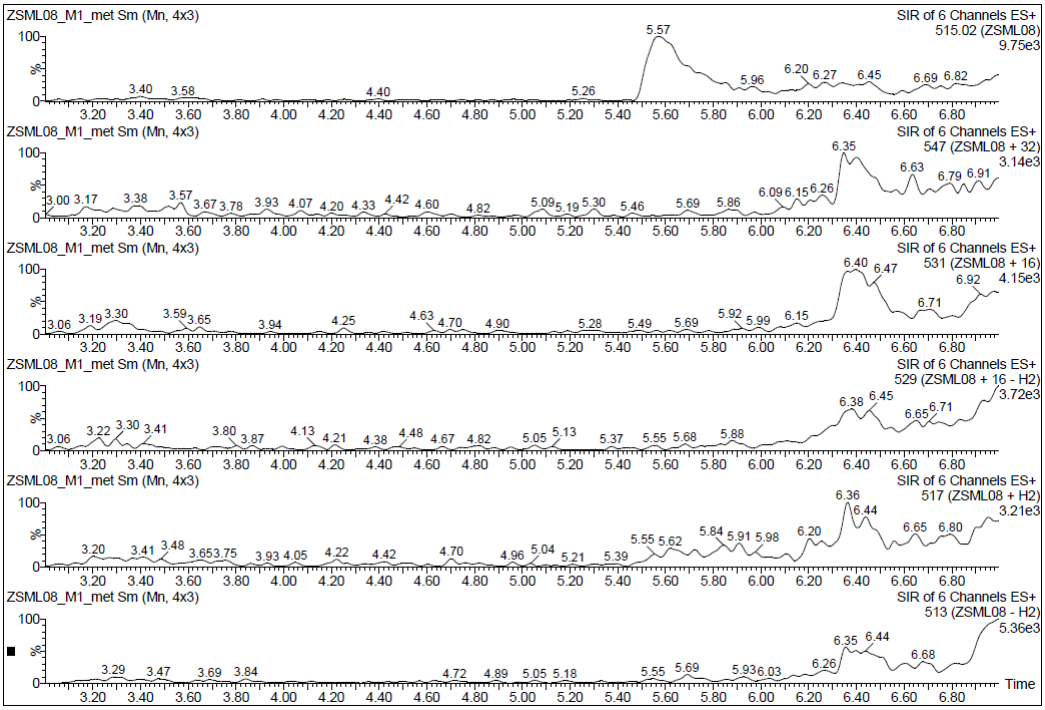


Figure S12. Possible **2 (ZSML08)** metabolites chromatograms.

A similar small study was performed for our previously published **1** compound. The dose used was 10 mg/kg. A greater number of samples were collected, on the fifth and on the seventh day of the experiment, at given times after a dose was administered. Therefore, not all chromatograms will be presented. Instead only a representative one will. **1** was present at concentrations between 0.46 and 1.03 µM.

Table S4. Concentration of **1** in different mice.

Day	Sample	Area	Concentration, µM
5th	15 min, mouse 1	684	1.03
5th	15 min, mouse 2	<LOD	<LOD
5th	30 min, mouse 1	<LOD	<LOD
5th	30 min, mouse 2	480	0.70
5th	45 min, mouse 1	<LOD	<LOD
5th	45 min, mouse 2	<LOD	<LOD
5th	90 min, mouse 1	386	0.55
5th	90 min, mouse 2	340	0.48
5th	180 min, mouse 1	425	0.61
5th	180 min, mouse 2	329	0.46

7th	5 min, mouse 1	<LOD	<LOD
7th	5 min, mouse 2	<LOD	<LOD
7th	20 min, mouse 1	<LOD	<LOD
7th	20 min, mouse 2	<LOD	<LOD
7th	60 min, mouse 1	415	0.60
7th	60 min, mouse 2	<LOD	<LOD
7th	120 min, mouse 1	449	0.65
7th	120 min, mouse 2	<LOD	<LOD
7th	240 min, mouse 1	<LOD	<LOD
7th	240 min, mouse 2	327	0.46

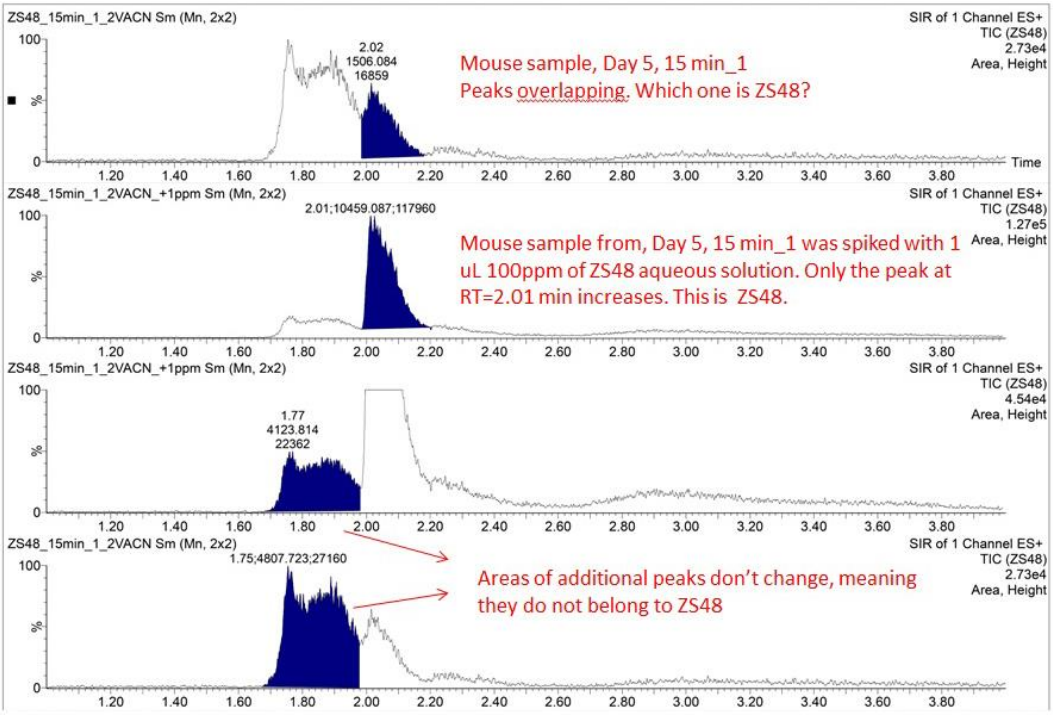


Figure S13. Chromatographs for a sample taken on the 5th day, 15 minutes after the dose, from one mouse: From top down: original chromatogram; spiking the sample with **1 (ZS48)** to identify the correct peak.

The concentrations were calculated on the basis of the calibration curve:

Table S5. Data for the calibration curve for **1**.

Salt concentration, ppm	Base concentration, ppm	Area
0.1	0.08	-
0.2	0.15	234
0.5	0.39	530
0.7	0.54	679
1.0	0.77	1025
1.5	1.16	1562
2.0	1.54	2160
3.0	2.31	2980
4.0	3.09	4004
5.0	3.86	-

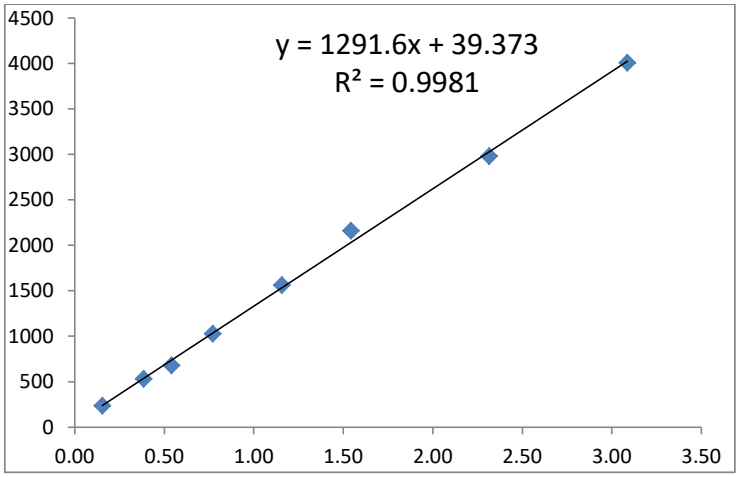


Figure S14. The calibration curve for **1**.

The limit of detection (LOD) limit of quantification (LOQ) were below 0.08 ppm (1/15 signal/noise at that concentration).

Note: "ZSML008" which appears on certain chromatographs here and in the paper is a mistake by the operator who accidentally typed that instead of "ZSML08".

"TIC (ZS48)" which appears on certain chromatographs here and in the paper refers to the total ion current and other mass spectrometry parameters set up for the method used for **ZS48**, and the same MS method was used for **ZSML08** as well.

6. pKa determination.

The compounds were dissolved in 25.00 mL 0.1 M NaCl in MeOH:H_2O = 1:1 (*v*:*v*). Each solution was titrated with 0.0764 M NaOH (MeOH:H_2O = 1:1, *v*:*v*), increment volume 2.0 µL. The titrations were performed in an Ar atmosphere at T=25±1ºC;
Software package HYPERQUAD2008 was used to interpret data.[9]

Aparatus: Potentiometric titrations were performed using CRISON pH-Burette 24 2S equipped with CRISON 50 29 micro-combined pH electrode (CRISON INSTRUMENTS, S.A. Spain). The electrode was calibrated by means of a strong acid – strong base titration in 0.1 M NaCl in MeOH:H_2O (1:1, *v*:*v*), using GLEE – GLass Electrode Evaluation software;[10] standard potential E^0=386.1±0.6 mV, slope 58.4±0.3 mV, and pK_W 13.78±0.01 values are obtained as mean values of five (**2**) or three (**4**, **13**) titrations.

2 (ZSML08):

C_{ZSML08} = 5.7514 × 10^{-4} M (0.1 M NaCl in MeOH:H_2O = 1:1)

Table S6. 2 titration data.

experiment	pK_{a1}	pK_{a2}	pK_{a3} ± SD	pK_{a4} ± SD	pK_{a5} ± SD	pK_{a6} ± SD
1	pK_a out of titration range	pK_a out of titration range	4.56 ± 0.01	5.49± 0.01	6.56± 0.01	7.64± 0.01
2			4.66 ± 0.01	5.64± 0.01	6.65± 0.01	7.78± 0.01
3			4.46 ± 0.01	5.62± 0.02	6.60± 0.01	7.71± 0.01
4			4.97 ± 0.08	5.59± 0.08	6.68± 0.06	7.60± 0.08
5			4.83 ± 0.05	5.64± 0.04	6.77± 0.05	7.79± 0.06
<pK_a>			**4.56 ± 0.08**	**5.60 ± 0.06**	**6.65 ± 0.07**	**7.70 ± 0.07**

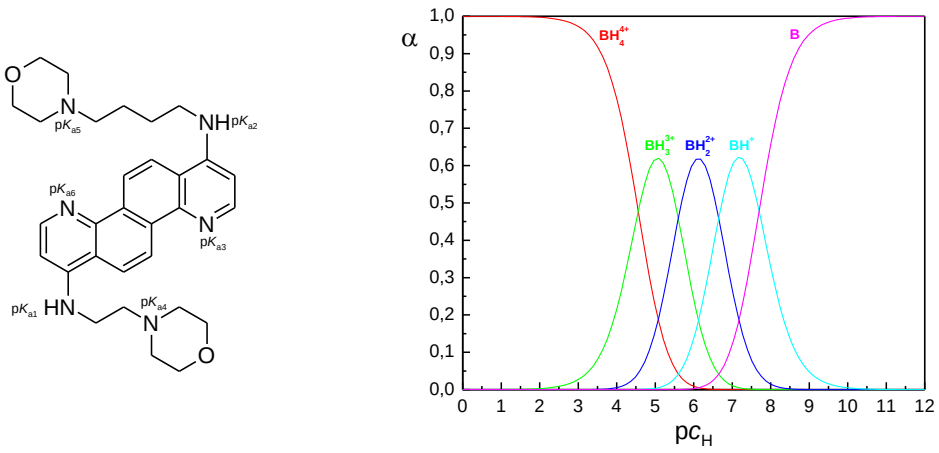


Figure S15. 2 titration data.

13 (ZS102):

Table S7. 13 titration data.

C_{ZS102} = 6.829 × 10^{-4} M

experiment	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4} ± SD	pK_{a5} ± SD	pK_{a6} ± SD
1	of titrati	of titrati	of titrati	5.49 ± 0.05	8.51 ± 0.06	10.96 ± 0.06

2	5.48 ± 0.05	8.50 ± 0.06	11.08 ± 0.06
3	5.57 ± 0.06	8.50 ± 0.03	10.87 ± 0.03
<pK_a>	**5.51 ± 0.06**	**8.50 ± 0.01**	**10.97 ± 0.11**

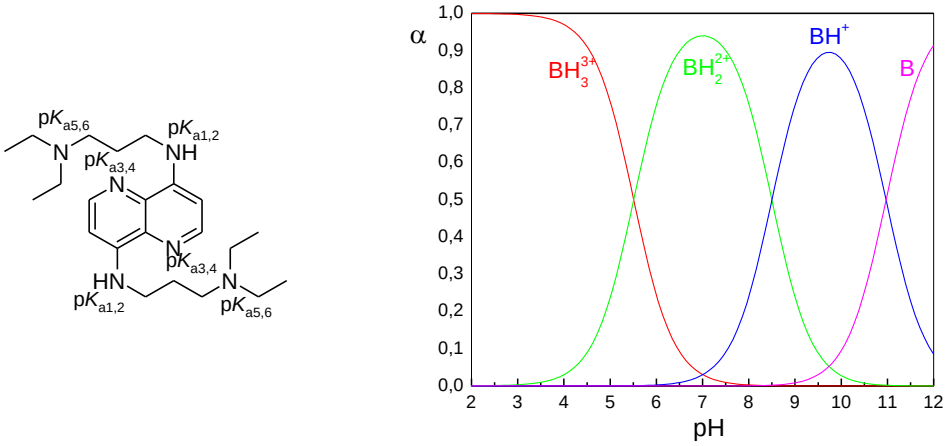


Figure S16. 13 titration data.

4 (ZS103):

Table S8. 4 titration data.

***C*$_{ZS103}$ = 4.608 × 10^{-4} M**

experiment	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4} ± SD	pK_{a5} ± SD	pK_{a6} ± SD
1	pK_a out of titration range	pK_a out of titration range	pK_a out of titration range	5.85 ± 0.08	8.69 ± 0.07	10.69 ± 0.04
2				5.74 ± 0.08	8.65 ± 0.08	10.80 ± 0.04
3				5.73 ± 0.09	8.61 ± 0.07	10.74 ± 0.06
<pK_a>				**5.77 ± 0.07**	**8.65 ± 0.04**	**10.74 ± 0.06**

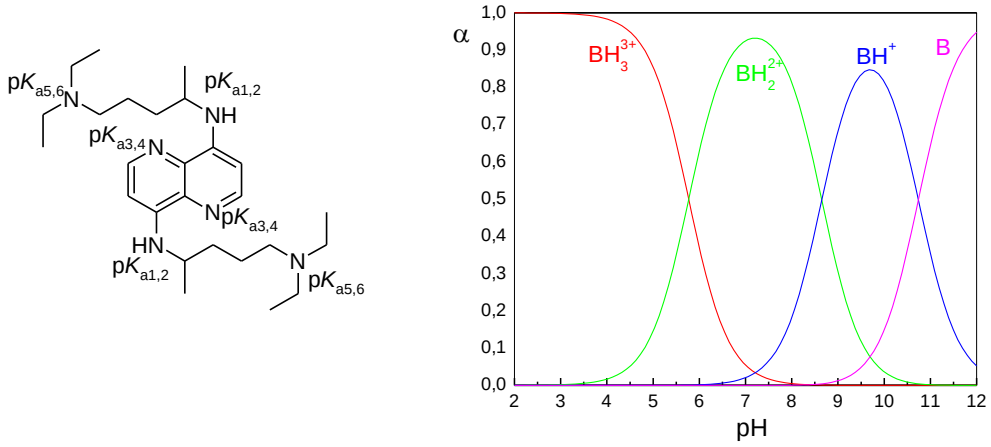


Figure S17. 4 titration data.

1 (ZS48):

Table S9. 1: titration data.

***C*$_{ZS48}$ = 3.099 × 10^{-4} M (0.1 M NaCl u MeOH:H$_2$O = 1:1)**

experiment	p*K*$_{a1}$	p*K*$_{a2}$	p*K*$_{a3}$ ± SD	p*K*$_{a4}$ ± SD	p*K*$_{a5}$ ± SD	p*K*$_{a6}$ ± SD
1	p*K*$_a$ van pH opsega titracija	p*K*$_a$ van pH opsega titracija	4.37 ± 0.01	5.26 ± 0.01	5.97 ± 0.01	7.41 ± 0.01
2			4.60 ± 0.01	5.17 ± 0.01	6.02 ± 0.01	7.36 ± 0.01
3			4.39 ± 0.01	5.25 ± 0.01	5.94 ± 0.01	7.33 ± 0.01
4			4.34 ± 0.01	5.26 ± 0.01	5.91 ± 0.01	7.31 ± 0.01
<p*K*$_a$>			**4.37 ± 0.03**	**5.24 ± 0.04**	**5.96 ± 0.05**	**7.35 ± 0.04**

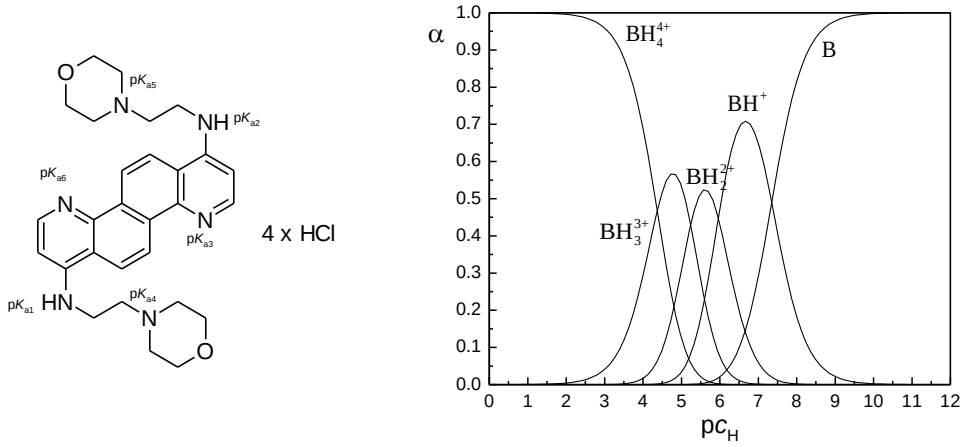
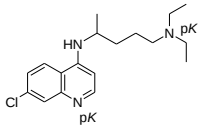


Figure S18. 1 titration data.

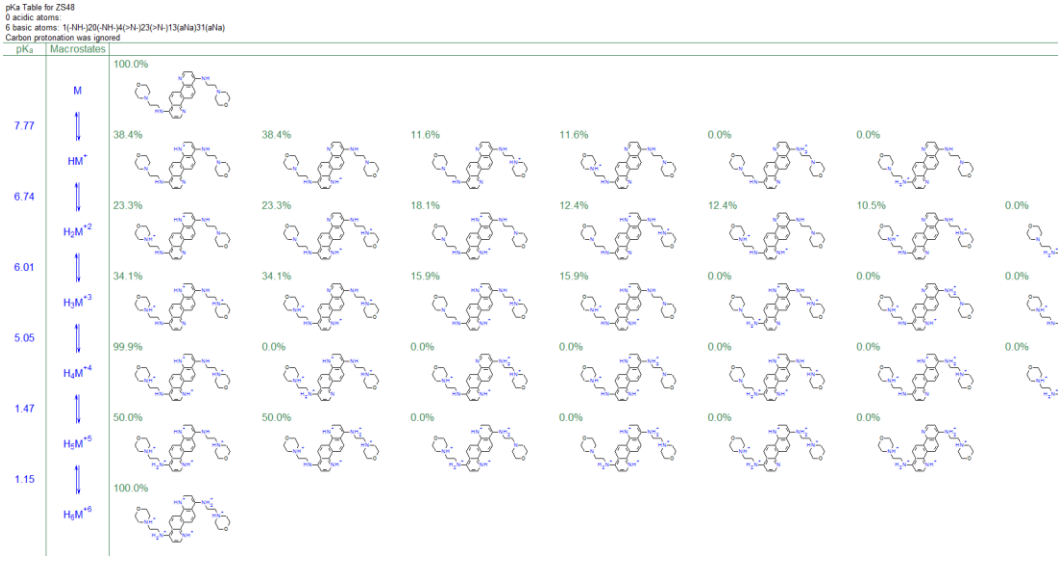
Chloroquine:

Table S10. Chloroquine titration data.

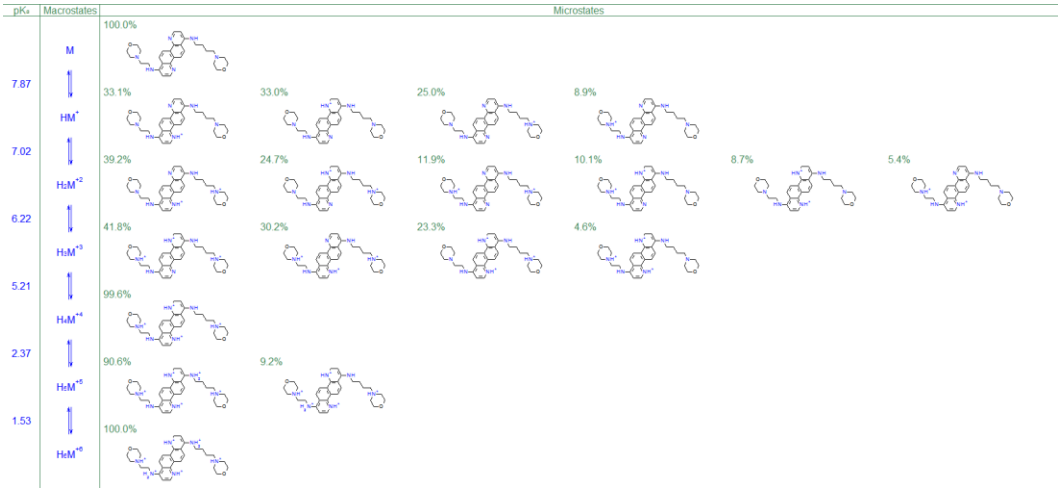
	experiment	pK_{a1}±SD	pK_{a2}±SD
	1.	7.42±0.01	9.08±0.01
	2.	7.49±0.01	9.24±0.01
	3.	7.46±0.01	9.18±0.01
	4.	7.44±0.01	9.20±0.01
c=1.024·10^{-3} M	<pK_a>	**7.45±0.01**	**9.18±0.01**

pKa values were assigned based on calculations[11,12]**:**

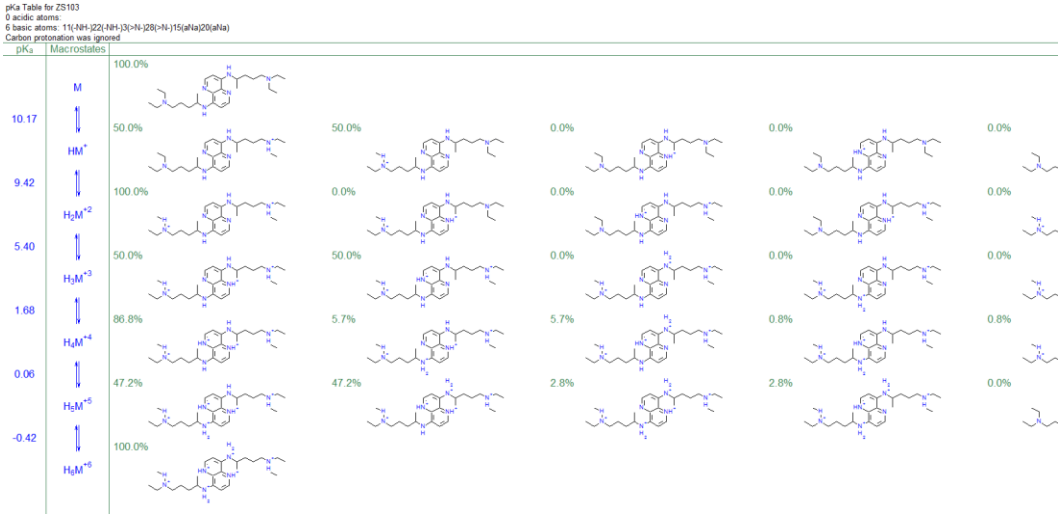
1:


pKa Table for ZS48
0 acidic atoms:
6 basic atoms: 1(-NH-)20(-NH-)4(>N-)23(>N-)13(aNa)31(aNa)
Carbon protonation was ignored
pKa
Macrostates
M
HM+
H2M+2
H3M+3
H4M+4
H5M+5
H6M+6
7.77
6.74
6.01
5.05
1.47
1.15
100.0%
38.4%
38.4%
11.6%
11.6%
0.0%
0.0%
23.3%
23.3%
18.1%
12.4%
12.4%
10.5%
0.0%
34.1%
34.1%
15.9%
15.9%
0.0%
0.0%
0.0%
99.9%
0.0%
0.0%
0.0%
0.0%
0.0%
0.0%
50.0%
50.0%
0.0%
0.0%
0.0%
0.0%
100.0%

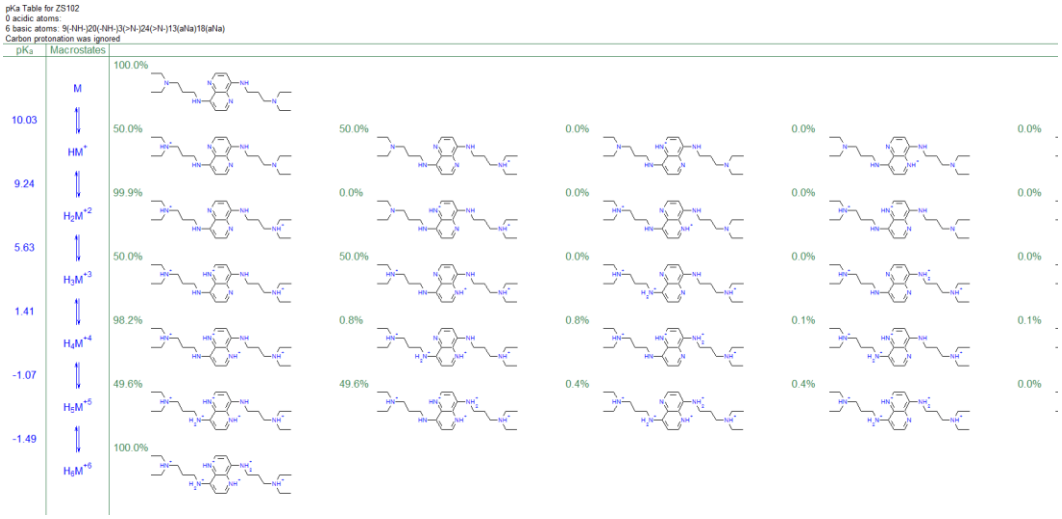
2:


pKa
Macrostates
Microstates
M
HM+
H2M+2
H3M+3
H4M+4
H5M+5
H6M+6
7.87
7.02
6.22
5.21
2.37
1.53
100.0%
33.1%
33.0%
25.0%
8.9%
39.2%
24.7%
11.9%
10.1%
8.7%
5.4%
41.8%
30.2%
23.3%
4.6%
99.6%
90.6%
9.2%
100.0%

4:



13:



Order of protonation:

Considering the basicity of N atom from the side chains (the morpholine *N* in **1** and **2** vs. $-NEt_2$ in **4** and **13**) and the size of the aromatic systems, it is expected that aromatic *N* gets protonated first within **1** and **2** (morpholine *N* in the second step), but not within **4** and **13**, where much higher basicity of $-NEt_2$ dictates it's protonation as the first step. This is due to the oxygen electron withdrawing effects of the morpholine side chain which lowers the basicity of *N* comparing to $-NEt_2$ but also due to positive charge delocalization within the aromatic system. The effect of system stabilization due to positive charge delocalization is much more pronounced within **1** and **2** comparing to **4** and **13** as the size of the aromatic system is doubled.

The ADMET Predictor software confirmed the proposed protonation order.[9,10]

7. Zebrafish model toxicity assessment.

Evaluation of toxicity of **2 (ZSML08)**, **1 (ZS48)** and **CQ** on the zebrafish embryos model was carried out according to general rules of the OECD Guidelines for the Testing of Chemicals.[13] All experiments involving zebrafish were performed in compliance with the European directive 2010/63/EU and the ethical guidelines of the Guide for Care and Use of Laboratory Animals of the Institute of Molecular Genetics and Genetic Engineering, University of Belgrade.

Wild type zebrafish (*Danio rerio*) were kindly provided by Dr Ana Cvejic (Wellcome Trust Sanger Institute, Cambridge, UK), housed in a temperature- and light-controlled facility under 28 °C and standard 14:10-hour light-dark photoperiod, and regularly fed with commercial dry flake food (TetraMin™ flakes; Tetra Melle, Germany) twice a day and *Artemia nauplii* once daily. Zebrafish embryos were produced by pair-wise mating, collected and distributed into 24-well plates containing 10 embryos per well and 1 mL embryos water (0.2 g/L of Instant Ocean® Salt in distilled water), and raised at 28 °C. For assessing lethal and developmental toxicity, embryos at the 6 hours post fertilization (hpf) stage were treated with seven concentrations (2.5, 5, 10, 20, 30, 40 and 50 μM) of **2 (ZSML08)**, **1 (ZS48)** and **CQ**. DMSO (0.1%) was used as negative control. Experiments were performed three times using 40 embryos per concentration.

Apical endpoints for the toxicity evaluation (Table S11) were recorded at 24, 48, 72, 96 and 120 hpf using an inverted microscope (CKX41; Olympus, Tokyo, Japan). Dead embryos were counted and discarded every 24 h. At 120 hpf, embryos were inspected for heartbeat rate, anesthetized by addition of 0.1% (w/v) tricaine solution (Sigma-Aldrich, St. Louis, MO), photographed and killed by freezing at -20°C for ≥ 24 h. None of the lethal and teratogenic effects (listed in table S11) were registered.

Toxicity was evaluated by determination of the lethal concentration (LC_{50}), defined as the treatment concentration resulting in the 50% mortality of embryos over a period of 120 hpf. The LC_{50} value was determined by the program ToxRatPro (ToxRat®, Software for the Statistical Analysis of Biotests, ToxRat Solution GmbH, Alsdorf, Germany, Version 2.10.05) using probit analysis with linear maximum likelihood regression.

In order to analyze compound **1** and **2** for possible hepatotoxic effect *in vivo*, the transgenic *Tg*(*l-fabp*:EGFP) zebrafish embryos with the fluorescently labeled liver were treated with tested compounds at 6 hpf when the liver has not yet been formed (assessing the effect on the liver development), and at 72 hpf when the liver is fully functional (assessing the effect on the liver function). Embryos were treated with the doses of **1** and **2** in the range of 10 to 40 μM, and of CQ in the range of 10 to 100 μM. Experiments were performed three times using 6 embryos per concentration. The hepatotoxicity was determined according to the change of liver area index compared to the control group, calculated as the ration between liver area and embryonic lateral area x 100%, as reported by Zhang et al (2017).[14]

In order to analyze antiviral **1** and **2** for possible myelotoxicity *in vivo*, its effect on neutrophil occurrence in transgenic *Tg*(*mpx*:EGFP) zebrafish embryos with fluorescently labeled neutrophils was explored, enabling direct visualization of applied treatment on the neutrophils. Embryos staged at 6 hpf were treated with the doses of **1** and **2** in the range of 10 to 40 μM, and after 72-h treatment neutrophils were imaged using fluorescence microscope (Olympus BX51, Applied Imaging Corp., San Jose, CA, USA). Experiments were performed two times using 20 embryos per concentration.

All mentioned measurements were performed by using Image J program (National Institutes of Health, USA).

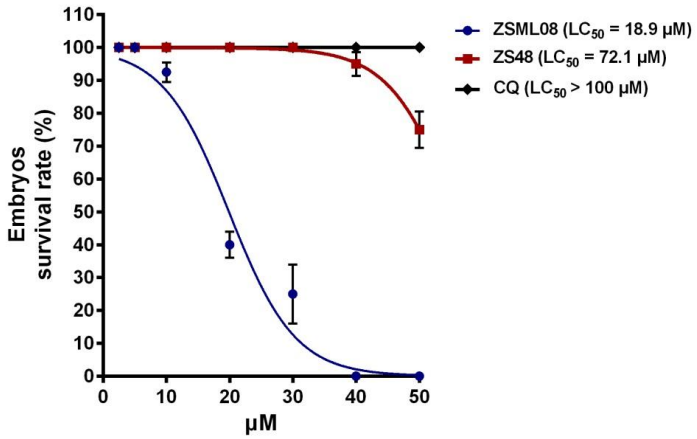


Figure S19. The dose-response survival curve of zebrafish embryos at 120 hpf upon antiviral **2 (ZSML08)**, **1 (ZS48)** and **CQ** treatments.

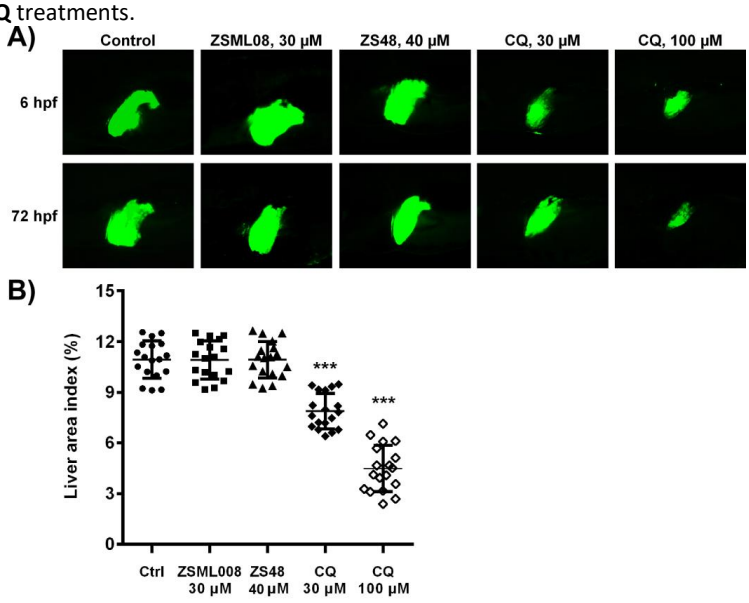


Figure S20. The hepatotoxicity of **2 (ZSML08)**, **1 (ZS48)** and **CQ** evaluated in *Tg*(*l-fabp*:EGFP) zebrafish embryos with fluorescently labeled the liver. A. *In vivo* imaging of the liver morphology of 120-h old embryos exposed to tested compounds at 6 hpf and

Table S11. Lethal and teratogenic effects inspected in zebrafish (*Danio rerio*) embryos upon **2 (ZSML08)**, **1 (ZS48)** and **CQ** treatments at different hours post fertilization (hpf).

Category	Developmental endpoints	Exposure time (hpf)

		24	48	72	96/120
Lethal effect	Egg coagulation[a]	●	●	●	●
	No somite formation	●	●	●	●
	Tail not detached	●	●	●	●
	No heartbeat		●	●	●
Teratogenic effect	Malformation of head	●	●	●	●
	Malformation of eyes[b]	●	●	●	●
	Malformation of sacculi/otoliths[c]	●	●	●	●
	Malformation of notochord	●	●	●	●
	Malformation of tail[d]	●	●	●	●
	Scoliosis	●	●	●	●
	Heartbeat frequency		●	●	●
	Blood circulation		●	●	●
	Pericardial edema	●	●	●	●
	Yolk edema	●	●	●	●
	Liver morphology and color			●	●
	Yolk absosrption	●	●	●	●
	Growth retardation[e]	●	●	●	●

[a]No clear organs structure is recognized.

[b]Malformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.

[c]Presence of no, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).

[d]Tail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparation.

[e]Growth retardation was recorded by comparing with the control embryos in development or size (before hatching, at 24 hpf and 48 hpf) or in a body length (after hatching, at and onwards 72 hpf) using by optical comparation using a inverted microscope (CKX41; Olympus, Tokyo, Japan).

8. Tolerability assay in mice

Groups of six healthy female C57Bl/6 mice were once daily treated intraperitoneally with a dose of the compound dissolved in 0.9% aqueous NaCl, for 7 days. Individual mouse behavior and appearance was monitored two times a day for 28 days after the last dose. Compounds proved to be non-toxic if all mice survived 28 days after administration and showed normal appearance and behavior. The study followed the International Guiding Principles for biomedical research involving animals, and was reviewed by a local Ethics Committee and approved by the Veterinary Directorate at the Ministry of Agriculture and Environmental Protection of Serbia (decision no. 323-07-02444/2014-05/1).

9. References

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