**Supporting material**

**Tetraoxanes as inhibitors of Apicomplexan parasites *Plasmodium falciparum* and *Toxoplasma gondii* and anti-cancer molecules**

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**Scheme S1**: Reaction pathway for synthesis of derivative **21**.

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| --- | --- | --- | --- |
| **Table 1S.** **Calculated pKa and logP values for derivatives 21, 22 and 23.a** | | | |
| **Compound** | **21** | **22** | **23** |
| **pKa** | 10.16 | 12.08 | 12.14 |
| **logP** | 1.70 | 3.12 | 1.63 |
| a For pKa calculations Epik, version 2.9, Schrödinger, LLC, New York, NY, 2014. Were used. For log P calculations QikProp, version 4.1, Schrödinger, LLC, New York, NY, 2014 were used. | | | |

**Synthesis**

**4-hydroxycyclohexanecarboxylic acid** **(13) [[1]](#endnote-1)**

Mixture of 4-hydroxybenzoic acid (15.0 g, 108.6 mmol) and 5% Rh-Al2O3 (1g) in MeOH (100 mL) was shaken in Parr-shaker in hydrogen atmosphere (50 psi) at r.t. After 24 hours hydrogen was exchanged with Ar, mixture was filtered through celite and solvent was removed under reduce pressure. Product was obtained as mixture of *cis*/*trans* isomers. Yield 15.39 g (98 %), m.p. = 120 - 123 oC. (lit. t.t. = 126 - 128 oC). IR (ATR): 3437s, 2934s, 2857m, 2601w, 1702s, 1443w, 1368w, 1312m, 1242w, 1203w, 1058m, 1026w, 949w, 913w, 736w, 587w cm-1. 1H-NMR (200 MHz, CDCl3, *δ*):4.52 (bs, OH), 3.98 – 3.84 (m, He-COH), 3.72 – 3.54 (m, Ha-COH), 2.54 – 2.16 (m), 2.14 – 1.86 (m), 1.84 – 1.60 (m), 1.58 -1.16 (m).

**Benzyl 4-hydroxycyclohexanecarboxylate (14)****[[2]](#endnote-2)**

Obtained as cis/trans mixture with 2:1 ratio of axial:equatorial hydroxyl group (1H NMR).

Mixture of **13** (10.0 g, 69.4 mmol) and anhydrous K2CO3 (19.1 g, 138.2 mmol) in DMF (18 mL) was warmed to 55 oC and benzyl chloride (10.48 mL, 90.8 mmol) was added in drops and stirring was continued at same temperature. After 12 hours reaction was cooled to room temperature, water (25 mL) was added and mixture was extracted with CH2Cl2 (4 × 30 mL). Combined organic layers were washed once with sat. NaHCO3 (15 mL), once with brine (15 mL) and dried over anh. Na2SO4. Crude product (white powder, 49.28 g) was used without further purification in next reaction step. Analytical sample was obtained after column chromatography purification (flash, SP Biotage, SiO2-column, Flash 12+M, Hexane/EtOAc = 6:4). IR (ATR): 3405m, 3033w, 2938s, 2863w, 1732s, 1496w, 1454w, 1385m, 1311w, 1236m, 1169s, 1136w, 1070m, 1033m, 967m, 907w, 749m, 699m cm-1. 1H-NMR (200 MHz, CDCl3, *δ*):7.40-7.30 (m, 5H-Ar), 5.12 (s, Ar-C*H2*), 3.95 – 3.85 (m, *He*-COH), 2.52-2.36 (m, H­a-CO2Bn), 2.12-1.86 (m, 3H), 1.80-1.52 (m, 5H). 13C-NMR (50 MHz, CDCl3, *δ*): 175.10, 136.14, 128.51, 128.11, 127.98, 66.77, 66.04, 41.26, 31.94, 23.58.

**Benzyl-4-oxocyclohexanecarboxylate (15)**2, [[3]](#endnote-3)

Mixture of alcohol **14** (25.0 g, 106.7 mmol) and PCC (34.44 g, 160.0 mmol) in CH2Cl2 (150 L) was stirred at r.t 2 hours. Suspension was transferred on SiO2 column and product was collected after eluting with CH2Cl2 (600 mL). Solvent was removed under reduce pressure and product was obtained after column chromatography purification (flash, SP Biotage, SiO2-column, 40+M, eluent hexane / EtOAc gradient 85/15 → 7/3) as pale green-yellow oil. Yield 9.57g (67%) IR (ATR): 3033w, 2954m, 1710s, 1453m, 1384m, 1303m, 1210s, 1158s, 1028w, 1004m, 965w, 746s, 698s, 495w, 421w cm-1. 1H-NMR (200 MHz, CDCl3, *δ*):7.36 (s, Ph), 5.16 (s, Ar-C*H2*), 2.90-2.70 (m, Ha-CO2Bn), 2.56-1.92 (m, 8H). 13C-NMR (50 MHz, CDCl3, *δ*): 210.02, 173.94, 135.72, 128.62, 128.36, 128.13, 66.49, 40.62, 39.62, 28.42.

**Cyclohexane-1,1-diyl dihydroperoxide** (**17)**

Into mixture of cyclohexanone (980.0 mg, 10.0 mmol) and Re2O7 (242.2 mg, 0.5 mmol, 5 mol %) in CH3CN (25mL), 50% solution of H2O2 (1.12 mL, 40.0 mmol) was added and stirring was continued at r.t. 1 hour. Reaction was transferred on the SiO2 column and was eluted with EtOAc. Fractions with crude product were combined, washed once with brine and dried over anh. Na2SO4 at 0 oC. Solvent was removed under reduce pressure and product was isolated after column chromatography (Lobar, SiO2-column C, eluent hexane / EtOAc = 7/3). Yield 890.2 mg (60%), colourless oil. IR (film): 3419s, 2946s, 2863s, 1712m, 1634w, 1454s, 1391s, 1278m, 1161m, 1098m, 1064s, 947m, 927m, 849m cm-1. IR(CCl4): 3424s, 2948s, 2865s, 1746m, 1722m, 1452s, 1393s, 1349m, 1162s, 951s, 922m cm-1. 1H-NMR (200 MHz, CDCl3, *δ*): 9.60 (bs, 2 × HOO), 2.0 – 1.8 (m, 4 H), 1.6 – 1.4 (m, 6 H). 13C-NMR (50 MHz, CDCl3, *δ*): 110.94, 29.41, 25.18, 22.31.

**Benzyl 7,8,15,16-tetraoxadispiro[5.2.5.2]hexadecane-3-carboxylate (18)**

Into ice-cold (ice-water mixture) solution of *gem*-dihydroperoxide **17** (412.7 mg, 2.53 mmol) and ketone **15** (374.8 mg, 2.53 mmol) in CH2Cl2 (5 mL), under intensive stirring, precooled freshly prepared solution of conc. H2SO4 / CH3CN (1 / 10, v / v, 1.82 mL) was added dropwise. After one hour stirring at same temperature, reaction was quenched with water, and layers were separated. Water layer was extracted with CH2Cl2 (3 × 50 mL), combined organic layers were washed with sat. NaHCO3 (3 × 20 mL), brine (2 × 10 mL) and dried over anh. Na2SO4. Solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO2-column, eluent hexane / EtOAc, gradient 97/3 → 9/1). Yield 241.9 mg (26 %), as amorphous powder. m.p. = 70-73 ºC. IR (ATR): 3033w, 2939s, 2863m, 1734s, 1496w, 1449s, 1357m, 1274m, 1254m, 1169m, 1066s, 947w, 925w, 750w, 699w cm-1. 1H-NMR (200 MHz, CDCl3, *δ*): 7.31 (bs, Ph), 5.12 (s, Ph-C*H2*), 2.89 (bs, 1H), 2.60-2.10 (m, 3H), 2.10-1.30 (m, 15H). 13C-NMR (50 MHz, CDCl3, *δ*): 174.37, 135.96, 128.56, 128.20, 128.04, 108.39, 107.22, 66.20, 41.57, 31.76. 30.35, 28.02, 25.27, 24.54, 23.80, 22.62, 21.92. (+)ESI-HRMS (*m*/*z*): Calculated for [M + Na]+ 385.16216, found 385.16216. Combustion analysis (C20H26O6): Calculated C 66.28, H 7.23; found C 65.82, H 6.96. HPLC purity: method A: RT 3.141, area 96.25 %; method B: RT 1.372, area 96.82 %.

**7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-yl methanol (19)****[[4]](#endnote-4)**

Flame dried two-neck round bottom flask was charged, under Ar atmosphere, with LiAlH4 (280.0 mg, 7.3 mmol) and dry THF (20 mL), and solution of ester **18** (2.4 g, 4.55 mmol) in dry THF (20 mL) was added dropwise under intensive stirring, at r.t. After 2 hours reaction was quenched with EtOAc, water was added and emulsion was transferred into separatory funnel. Water layer was acidified (pH = 2 ) with dilute HCl (1:1, v/v), layers were separated and water layer was extracted with EtOAc (3 × 20 mL). Combined organic layer were dried over anh. Na2SO4, solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO2-column, eluent heptane / EtOAc = 8 / 2). Yield 1.4 g (82%). Colourless foam, melts at 116 -118 oC. IR (KBr): 3320m, 3009w, 2940s, 2861s, 1443m, 1360w, 1339w, 1310w, 1273w, 1250w, 1159w, 1094w, 1068m, 1045m, 984w, 941w, 918m, 897w, 881w, 850w cm-1. 1H NMR (500 MHz, CDCl3, *δ*): 3.5 (d, *J* = 6.2 Hz, C*H2*-OH), 3.12 (bs, 1H), 2.45-2.15 (m, 2H), 1.85-1.70 (m, 3H), 1.70-1.35 (m, 12H), 1.35-1.20 (m, 2H). 13C NMR (125 MHz, CDCl3, *δ*): 108.29, 108.16, 67.41, 39.44, 31.80, 30.90, 29.52, 28.53, 25.35, 24.95, 24.45, 22.17, 21.88. (+)ESI-HRMS (*m*/*z*): Calculated for [M + NH4]+ 276.18055, found 276.18041. Combustion analysis (C13H22O5): Calculated C 60.45, H 8.58, found C 60.47, H 8.18.

**3-(Azidomethyl)-7,8,15,16-tetraoxadispiro[5.2.5.2]hexadekane (20)**4

Into solution of **19** (1.38 g, 5.34 mmol) in dry Py (11 mL) methanesulfonyl chloride (495 μL, 6.4 mmol) was added at r.t. under intensive stirring. After 2 hours, reaction was quenched with water / EtOAc mixture, transferred into separatory funnel. Water layer was acidified (pH = 5) with dilute HCl (1:1, v/v), layers were separated and water layer was extracted with EtOAc (4 × 25 mL). Combined organic layer were dried over anh. Na2SO4, filtered of and solvent was removed under reduce pressure. Obtained crude product was used in next reaction step without further purification. Mixture of mesylate and NaN3 (3.47 g, 53.4 mmol) in DMF (20 mL) was stirred at 50 oC over 12 hours, cooled at r.t. and poured in to EtOAc / water mixture. Layers were separated and water layer was extracted with EtOAc (4 × 25 mL). Combined organic layers were washed with brine (2 × 25 mL), dried over anh. Na2SO4, filtered of and solvent was removed under reduce pressure. Product was isolated after column chromatography purification (dry-flash, SiO2-column, eluent heptane / EtOAc = 9 / 1). Yield 1.45 g (97%). Colourless foam melts at 86-87 oC. Spectra are in accordance to literature data. IR (KBr): 2993w, 2946m, 2868w, 2096s, 1714w, 1445m, 1358w, 1338w, 1292m, 1258m, 1213w, 1183w, 1183w, 1155w, 1137w, 1091w, 1067w, 1047m, 1016w, 952w, 915m, 883w, 850w, 817w cm-1.1H NMR (200 MHz, CDCl3, *δ*): 3.18 (d, *J* = 6.2 Hz CH2, 2 H), 3.14 (bs, 1 H), 2.27 (bs, 2 H), 1.80 – 1.26 (m, 16 H). 13C NMR (50 MHz, CDCl3, *δ*): 108.39, 107.73, 56.74, 37.04, 31.65, 30.81, 29.48, 28.46, 25.29, 22.05. HPLC purity: method A: RT 3.140, area 96.998 %; method B: RT 1.371, area 96.81 %.

**1-(7,8,15,16-Tetraoxadispiro[5.2.5.2]hexadec-3-yl)methanamine (21)**4

Flame dried two-neck round bottom flask was charged, under Ar atmosphere, with LiAlH4 (217.8 mg, 5.74 mmol) and dry diethyl ether (15 mL), and solution of azide **20** (1.18 g, 4.16 mmol) in dry diethyl ether (15 mL) was added dropwise under intensive stirring, at r.t. After 1 hour reaction was quenched with water and 10 % NaOH solution, K,Na-tartarate (4.8 g, 17.22 mmol, 3 eq. calculated to LiAlH4) was added and mixture was stirred at r.t. until organic layer become clear (12 – 15 hours. Layers were separated, water layer was extracted with diethyl ether (3 × 25 mL) and combined organic layer were washed with brine (2 × 15 mL) and dried over anh. Na2SO4. Mixture was filtered of, solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO2-column, eluent EtOAc / MeOH / NH3aq = 8 / 1 / 1). Yield 0.4g (69 %) as pale yellow amorphous powder, mp. 75-77 oC. Spectra were in accordance to literature data. IR (KBr): 3378m, 3340m, 3010w, 2941s, 2862s, 1720w, 1443m, 1362w, 1341w, 1275w, 1253w, 1160w, 1096w, 1069m, 1049m, 984w, 942w, 919m, 896w, 851w, 824w cm-1. 1H NMR (200 MHz, CDCl3, *δ*): 3.11 (bs, 1H) 2.58 (d, *J* = 6.0 Hz, C*H2*-NH2), 2.26 (bs, 2H), 1.90-1.11 (m, 18 H). 13C NMR (50 MHz, CDCl3, *δ*): 108.26, 47.58, 40.20, 31.68, 31.0, 29.52, 28.73, 25.78, 25.31, 21.96. HPLC purity: method A: RT 3.139, area 97.24 %; method B: RT 1.369, area 96.93 %.

***N*-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-ylmethyl)-4,5-dihydro-1*H*-imidazol-2-amine (22)**

Mixture of **21** (100.0 mg, 0.39 mmol), **I** (142.2 mg, 0.58 mmol) and Et3N (0.5 mL) in methanol (3 mL) was stirred in inert atmosphere (Ar) at 70 oC 3 days. Solvent was evaporated, residue was dissolved in CH2Cl2, 10% NaOH solution was added and mixture was intensively shaken. Layers were separated, organic layer was washed once with water, once with brine and dried over anh. Na2SO4. Solvent was removed under reduce pressure and product was isolated after column chromatography purification (Lobar, SiO2-column A, eluent CH2Cl2/ CH3OH sat. NH3 = 1 / 1). Yield 91 mg (72%), amorphous powder m.p. = 153-156 °C. IR (ATR): 2939s, 2862m, 1693s, 1636m, 1551m, 1446m, 1366m, 1252m, 1157w, 1068m, 952w, 844w, 804w, 714w, cm-1. 1H NMR (500 MHz, T=340.2 K, DMSO-d6, *δ*): 3.38 (s, N-CH2CH2-N), 2.98 (d, *J* = 6,6 Hz, CH2-N), 1.86 (bs, 3H), 1.70 – 1.40 (m, 9H), 1.28 - 1.23 (m, 1H), 1.18 – 1.06 (m, 2H). 13C NMR (125 MHz, *δ*) 160.38, 107.09, 46.83, 45.81, 35.91, 28.67, 28.29, 24.87, 24.10, 21.06. (+)ESI-HRMS (*m*/*z*): Calculated for [M + H+] 325.20016, found 326.20896. Combustion analysis (C16H27N3O4 ): Calculated C 59.06, H 8.36, N 12.91; found C 59.43, H 8.71, N 12.61. HPLC purity: method A: RT 3.139, area 95.88 %; method B: RT 1.367, area 96.77 %.

**1-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-ylmethyl)guanidine (23)**

Mixture of **21** (100.0 mg, 0.39 mmol), S-methylthiourea sulphate (162.0 mg, 0.58 mmol) and Et3N (0.5 mL) in methanol (3 mL) was stirred at 70 oC, in inert atmosphere (Ar), 3 days. Into warm solution, methanol was added (10 mL) and warm mixture was filtered, solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO2-column, gradient CH2Cl2 / MeOH, = 9/1 → 1/1). Yield 108.6 mg (93 %). Pale yellow oil, become solid with time, melts at 36 oC. IR (ATR): 3360s, 2943m, 2865w, 1674s, 1579s, 1415m, 1254m, 1061w, 1010m, 766w, 651w, 618w cm-1. 1H NMR (500 MHz, DMSO-d6, *δ*): 5.29 (bs, 2×NH2), 2.95 (d, *J* = 6.9, CH2-N), 1.75-1.39 (m, 13H), 1.33 – 1.10 (m, 6H). 13C NMR (125 MHz, 340,1 K, DMSO-d6): 157.76, 107.44, 107.38, 45.02, 35.62, 30.16, 28.56, 24.86, 24.38, 21.31. (+)ESI-HRMS (*m*/*z*): Calculated for [M + H]+ 299.18451, found 300.19232 Combustion analysis (C14H25N3O4): Cqalculated C 56.17, H 8.42, N 14.04; found C 55.87, H 8.02, N 13.74. HPLC purity: method A: RT 3.133, area 78.69 %; method B: RT 1.071, area 80.69 %.

**1-phenyl-3-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-ylmethyl)urea (24)**

Mixture of **21** (150.0 mg, 0.58 mmol) and phenyl-isocyanate (63.4 μL, 0.58 mL) in CH2Cl2 (10 mL), was stirred at r.t., in inert atmosphere (Ar), 45 min. Solvent was evaporated and product was isolated after column chromatography purification (dry-flash, SiO2-column, gradient hexane / EtOAc = 1/1 → EtOAc → EtOAc / MeOH = 7/3). Yield 215 mg (98 %). Colourless foam softens at 188-191°C. IR (ATR): 3389m, 3304m, 3182w, 3150w, 3042w, 2934m, 2861m, 2363m, 1647s, 1601s, 1559s, 1499m, 1442m, 1357w, 1314m, 1248s, 1156w, 1055w, 952w, 927w, 757m, 728w, 698m cm-1. 1H NMR (500 MHz, DMSO-d6, *δ*): 8.37, (s, NH-Ph), 7.50-7.42 (m, 2H, Ar), 7.25-7.15 (m, 2H, Ar), 6.90-6.85 (m, 1H, Ar), 6.10 (bs, HN), 3.17 (d, *J* = 5.25, C*H2*-NH), 3.05-2.95 (m, 2H), 2.30-2.10 (m, 1H), 1.75-1.0 (m, 16H). 13C NMR (125 MHz, DMSO-d6, *δ*): 155.23, 140.53, 128.61, 120.90, 117.51, 107.81, 107.71, 43.93, 36.73, 31.11, 30.29, 28.99, 28.06, 27.93, 25.68, 25.11, 24.66, 21.83, 21.42. (+)ESI-HRMS (*m*/*z*): Calculated for [M + H]+ 376.19982, found 377.20742. Combustion analysis (C20H28N2O5×0.5 H2O): Calculated C 62.32, H 7.58, N 7.27; found C 62.63, H 7.71, N 7.56. HPLC purity: method A: RT 3.141, area 96.56 %; method B: RT 1.368, area 96.97 %.

**1-phenyl-3-(7,8,15,16-tetraoxadispiro[5.2.5.2]hexadec-3-ylmethyl)thiourea (25)**

Mixture of **21** (78.2 mg, 0.30 mmol) and phenyl-isothiocyanate (35.57 μL, 0.30 mL) in CH2Cl2 (15 mL), were stirred in inert atmosphere at r.t. 2 hours. Solvent was removed under reduce pressure and product was isolated after column chromatography purification (dry-flash, SiO2-column, gradient hexane / EtOAc = 9/1 → EtOAc). Yield 87.3 mg (73%). Colourless foam melts at 143-191°C. IR (KBr): 3326m, 3240m, 2948m, 2920m, 2885w, 2858w, 1736w, 1593m, 1550s, 1513s, 1447m, 1390w, 1345m, 1313m, 1256m, 1233m, 1190m, 1069s, 983w, 947w, 917w, 886w, 750w, 695w, cm-1. 1H NMR (500 MHz, CDCl3, *δ*): 7.81 (s, Ph-NH), 7.49-7.43 (m, 2H, Ar), 7.38-7.30 (m, 2H, Ar), 7.25-7.19 (m, 1H, Ar), 6.13-6.06 (m, NH-C=S), 3.54 (bs, C*H2*-NH), 3.08 (bs, 1H), 2.26 (bs, 2H), 1.89-1.40 (m, 14H), 1.32-1.18 (m, 2H). 13C NMR (125 MHz, CDCl3, *δ*): 180.99, 135.92, 130.34, 127.54, 125.35, 108.31, 107.80, 50.43, 36.25, 31.68, 30.81, 29.53, 28.41, 26.21, 25.54, 25.32, 22.09. (+)ESI-HRMS (*m*/*z*): Calculated for [M + Na]+ 392.17698, found 415.16570. Combustion analysis (C20H28N2O4S): Calculated C 61.20, H 7.19, N 7.14, S 8.17; found C 60.88, H 7.27, N 7.02, S 8.39. HPLC purity: method A: RT 3.138, area 96.34 %; method B: RT 1.374, area 96.40 %.

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