Supplementary Information

Hydroxylated Di- and Tri-styrylbenzenes a New Class of Antiplasmodial Agents: Discovery and Mechanism of Action†

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Spectral data of other compounds

Spectral data of compound 2-8 [1-2, 4-5] (Table 1):

(*E*)-4-(4-methoxystyryl)phenol (2)

Creamish solid (50% yield), m.p. 130-132°C, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 7.44 (2H, d, *J*= 8.5 Hz), 7.32 (2H, t, *J*= 7.9 Hz), 7.13 (1H, s), 7.10 (1H, d, *J*= 16.3 Hz), 7.06 (1H, s), 6.98 (1H, d, *J*= 16.3 Hz), 6.88 (2H, d, *J*= 8.5 Hz), 5.90 (1H, s), 3.87 (3H, s); ^{13}C NMR δ (75.4 MHz, $CDCl_3$), 159.5, 147.6, 134.9, 130.6, 130.0, 129.7, 127.8, 126.8, 114.5, 103.5 and 55.7

(*E*)-2-methoxy-4-(4-methoxystyryl)phenol (3)

White solid (56% yield), m.p. 164-167°C (lit. m.p. 163-166°C), ^{1}H NMR δ ($CDCl_3$, 300 MHz) 7.37 (2H, d, *J*= 8.48 Hz), 6.95-6.92 (2H, m), 6.84-6.81 (5H, m), 3.88 (3H, s), 3.76 (3H, s); ^{13}C NMR δ (75.4 MHz, $CDCl_3$) 159.0, 146.7, 145.2, 130.3, 127.4, 126.6, 126.1, 120.1, 118.6, 114.5, 114.1, 108.0, 55.9 and 55.3.

(*E*)-4-(2-(benzo[d][1,3]dioxol-5-yl)vinyl)-2-methoxyphenol (4)

White solid (50% yield), m.p. 153-156°C, ^{1}H NMR (CD_3COCD_3, 300 MHz), δ (ppm) 7.63 (1H, s), 7.10 (2H, d, *J* = 19.3 Hz), 6.97-6.91 (4H, m), 6.79 (2H, d, *J* = 8.0 Hz), 5.96 (2H, s), 3.85 (3H, s); ^{13}C NMR (75.4 MHz, CD_3COCD_3), δ (ppm) 148.3, 147.7, 147.0, 146.5, 132.5, 129.7, 127.1, 125.6, 120.9, 120.0, 115.0, 109.1, 108.2, 105.0, 100.8 and 55.3.

(*E*)-2-methoxy-4-(3,4,5-trimethoxystyryl)phenol (5)

White solid (45% yield), m.p. 123-125°C, ^{1}H NMR δ ($CDCl_3$, 300 MHz), 7.05-7.02 (2H, m), 6.94 (3H, d, *J*= 8.3 Hz), 6.73 (2H, s), 5.80 (1H, s), 3.95 (3H, s), 3.93 (6H, s), 3.89 (3H, s); ^{13}C NMR δ (75.4 MHz, $CDCl_3$), 153.8, 147.2, 146.0, 138.1, 133.8, 130.3, 128.3, 126.8, 120.8, 115.0, 108.7, 103.8, 61.3, 56.2 and 56.3.

(*E*)-2,6-dimethoxy-4-(4-methoxystyryl)phenol (6)

White solid (54% yield), m.p. 96-98°C, ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.46 (2H, d, *J* = 6.9 Hz), 6.98-6.90 (4H, m), 6.75 (2H, s), 5.60 (1H, s), 3.96 (6H, s), 3.85 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ (ppm) 159.2, 147.3, 134.6, 130.3, 129.4, 127.5, 126.9, 126.5, 114.2, 103.2, 56.4 and 55.4.

(*E*)-4-(4-fluorostyryl)-2-methoxyphenol (7)

White solid (59% yield), m.p.128-130°C, ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.47 (2H, t, *J* = 6.0 Hz), 7.07-7.03 (4H, m), 6.93-6.90 (3H, m), 5.70 (1H, s), 3.95 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ (ppm) 147.1, 146.1, 134.2, 130.2, 128.9, 127.6, 120.8, 116.1, 115.8, 115.0, 108.6 and 56.3.

(*E*)-4-(4-chlorostyryl)-2-methoxyphenol (8)

White solid (60% yield), m.p. 119-122°C (lit. m.p. 121-124), ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.32 (2H, d, *J* = 8.4 Hz), 7.23 (2H, d, *J* = 8.4 Hz), 6.97-6.93 (3H, m), 6.86-6.82 (2H, m), 5.70 (1H, s), 3.86 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ (ppm) 147.0, 146.2, 136.7, 132.8, 129.8, 129.5, 128.8, 127.7, 125.7, 120.9, 114.9, 108.7 and 55.9.

(*E*)-4-(4-bromostyryl)-2,6-dimethoxyphenol (10)

White solid (42% yield), m.p. 130-133°C, ^{1}H NMR δ ($CDCl_3$, 300MHz), 7.49 (2H, d, *J*= 8.3 Hz), 7.38 (2H, d, *J*= 8.3 Hz), 7.05 (2H, dd, *J*= 8.5 Hz), 6.76 (2H, s), 5.63 (1H, s), 3.96 (6H, s); ^{13}C NMR δ (75.4 MHz, $CDCl_3$), 147.6, 136.8, 135.5, 132.2, 130.0, 129.0, 128.1, 125.9, 121.3, 103.9 and 56.7. HRMS-ESI: m/z [M+H]+ for $C_{16}H_{16}BrO_3$, calculated 335.0283; observed 335.0160.

Spectral data of compound 12[6], 13-19[4] (Table 1):

4,4'-((1*E*,1'*E*)-1,4-phenylenebis(ethene-2,1-diyl))bis(benzene-1,2-diol) (12)

Grey solid (54% yield), m.p. 380-382°C, ^{1}H NMR (CD_3COCD_3: DMSO-d_6 (7:3), 300 MHz), δ (ppm) 8.91 (4H, s), 7.51 (4H, s), 7.12 (1H, s), 7.06 (4H, s), 6.96 (1H, s), 6.91 (2H, d, *J* = 10.2 Hz), 6.78 (2H, d, *J* = 10.2 Hz); ^{13}C NMR (75.4 MHz, CD_3COCD_3: DMSO-d_6 (7:3), δ (ppm) 146.9, 146.3, 137.1, 129.7, 129.1, 127.0, 125.4, 119.3, 116.3 and 113.9.

4,4'-((1*E*,1'*E*)-1,4-phenylenebis(ethene-2,1-diyl))bis(2-methoxyphenol) (13)

Grey solid (68% yield), m.p 266-270°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 9.16 (2H, s), 7.52 (4H, s), 7.20 (2H, s), 7.18-7.02 (4H, d, *J* = 13.5 Hz), 7.01 (2H, d, *J* = 8.2 Hz), 6.79 (2H, d, *J* = 8.2 Hz), 3.83 (6H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 148.3, 147.1, 136.7, 129.2, 128.8, 127.4, 125.5, 120.6, 116.8, 110.2 and 55.3. HRMS-ESI: m/z [M+H]+ for $C_{24}H_{22}O_4$, calculated 375.1591; observed 375.1591.

4,4'-((1*E*,1'*E*)-1,4-phenylenebis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (14)

Greenish brown solid (67% yield), m.p. 246-250°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 8.45 (2H, s), 7.46 (4H, s), 7.06 (2H, d, *J* = 16.4 Hz), 7.05 (2H, d, *J* = 16.4 Hz), 6.82 (4H, s), 3.74 (12H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 148.6, 136.8, 136.3, 129.1, 128.1, 125.9, 124.6, 104.7 and 55.7. HRMS-ESI: m/z [M+H]+ for $C_{26}H_{26}O_6$, calculated 435.1802; observed 435.1828.

4,4'-((1*E*,1'*E*)-(2-fluoro-1,4-phenylene)bis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (15)

Yellow solid (46% yield), m.p. 225-227°C, ^{1}H NMR (CD_3COCD_3, 300 MHz), δ (ppm) 7.72 (1H, t, *J* = 8.0 Hz), 7.49 (2H, s), 7.38-7.32 (2H, m), 7.27-7.21 (3H, m), 7.15 (1H, s), 6.96 (4H, s), 3.89 (12H, s); ^{13}C NMR (75.4 MHz, CD_3COCD_3), δ (ppm) 148.8, 139.6, 139.5, 137.3, 132.0,

131.0, 129.0, 128.7, 127.7, 125.3, 124.7, 123.2, 118.4, 113.3, 113.0, 105.1 and 56.5. HRMS-ESI:m/z [M+H]+ for $C_{26}H_{25}O_6F$, calculated 453.1708; observed 453.1705.

4,4'-((1*E*,1'*E*)-(2,5-difluoro-1,4-phenylene)bis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (16)

Yellowish green solid (19% yield), m.p. 229-232°C, ^{1}H NMR (DMSO, 300 MHz), δ (ppm) 8.7 (2H, s), 7.59 (2H, t, *J* = 9.3 Hz), 7.29 (2H, d, *J* = 16.6 Hz), 7.08 (2H, d, *J* = 16.6 Hz), 6.91 (4H, s), 3.83 (12H, s); ^{13}C NMR (75.4 MHz, DMSO), δ (ppm) 148.1, 136.5, 132.8, 130.9, 127.1, 116.3, 112.7, 104.6 and 56.0. HRMS-ESI: m/z [M+H]+ for $C_{26}H_{24}0_6F_2$, calculated 471.1614; observed 471.1613.

4,4'-((1*E*,1'*E*)-(2,5-dimethoxy-1,4-phenylene)bis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (17)

Yellowish green solid (50% yield), m.p. 245-248°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 9.17 (2H, s), 7.24-7.21 (6H, d, *J* = 10.4 Hz), 7.13 (2H, s), 7.00 (2H, s), 6.79 (2H, s), 3.89 (6H, s), 3.84 (6H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 150.7, 147.7, 146.5, 129.1, 125.6, 119.6, 115.7, 110.0, 108.8, 56.0 and 55.5. HRMS-ESI: m/z [M+H]+ for $C_{26}H_{26}O_6$, calculated 435.1802; observed 435.1800.

4,4'-((1*E*,1'*E*)-anthracene-9,10-diylbis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (18)

Brown solid (14% yield), m.p. 212-216°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 8.62 (2H, s), 8.43 (4H, d, *J* = 6.4 Hz), 8.03 (2H, d, *J* = 16.2 Hz), 7.57-7.54 (4H, d, *J* = 7.3 Hz), 7.11 (4H, s), 6.84 (2H, d, *J* = 16.2 Hz), 3.87 (12H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 148.2, 137.7, 136.0, 132.3, 129.0, 127.4, 126.3, 125.3, 121.8, 104.5 and 56.1. HRMS-ESI: m/z [M+H]+ for $C_{34}H_{30}O_6$, calculated 535.2115; observed 535.2123.

4,4'-((1*E*,1'*E*)-[1,1'-biphenyl]-4,4'-diylbis(ethene-2,1-diyl))bis(2,6-dimethoxyphenol) (19)

Yellowish brown solid (62% yield), m.p. 277-280°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 8.58 (2H, s), 7.74 (4H, d, *J*= 7.6 Hz), 7.65 (4H, d, *J* = 7.6 Hz), 7.19 (4H, s), 6.93 (4H, s), 3.83 (12H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 148.1, 138.0, 136.6, 135.8, 129.1, 127.5, 126.6, 125.1, 104.2 and 56.0. HRMS-ESI: m/z [M+H]+ for $C_{32}H_{30}O_6$, calculated 511.2115; observed 511.2141.

Spectral data of compound 21-22[3], 23[6] 24-26[3] (Table 1):

4-((*E*)-4-((*E*)-4-hydroxystyryl)styryl)-2-methoxyphenol (21)

Yellow green solid, m. p. 255-257°C, ^{1}H NMR (DMSO-d_6:$CDCl_3$ (1.0: 1.0), 300 MHz), δ (ppm) 9.35 (1H, s), 8.87 (1H, s), 7.49 (4H, s), 7.40 (2H, d, *J* = 8.2 Hz), 7.10 (3H, d, *J* = 17.6 Hz), 7.00-6.96 (3H, m), 6.93 (1H, d, *J* = 7.3 Hz), 6.87 (2H, t, *J* = 8.2 Hz), 3.94 (3H, s); ^{13}C NMR (75.4 MHz, (DMSO-d_6:$CDCl_3$ (1.0: 1.0), δ (ppm) 156.8, 147.1, 146.8, 135.6, 134.8, 128.2, 127.6, 127.5, 127.0, 125.6, 124.5, 124.2, 119.5, 115.0, 114.9, 108.6 and 55.1. HRMS-ESI: m/z $[M+H]^+$ for $C_{23}H_{20}O_3$, calculated 345.1485; observed 345.1436.

4-((*E*)-4-((*E*)-4-hydroxystyryl)styryl)-2,6-dimethoxyphenol (22)

Light brown solid, m. p. 180-182°C, ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm) 9.61 (1H, s), 8.54 (1H, s), 7.53 (4H, s), 7.44 (2H, d, *J* = 7.7 Hz), 7.19 (3H, d, *J* = 16.6 Hz), 7.03 (1H, d, *J* = 16.6 Hz), 6.98 (2H, s), 6.79 (2H, d, *J* = 7.5 Hz), 3.82 (6H, s); ^{13}C NMR (75.4 MHz, DMSO-d_6), δ (ppm) 158.2, 149.0, 137.2, 137.1, 136.6, 129.5, 129.0, 128.9, 128.7, 128.5, 127.2, 126.3, 125.7, 116.4, 105.1 and 56.9. HRMS-ESI: m/z $[M+H]^+$ for $C_{24}H_{22}O_4$, calculated 375.1590; observed 375.1532.

4-((*E*)-4-((*E*)-4-hydroxy-3-methoxystyryl)styryl)benzene-1,2-diol (23)

Light green solid (38% yield), m.p. 238-240°C, ^{1}H NMR ($CDCl_3$: DMSO-d_6 (7:3), 300 MHz), δ (ppm) 8.0 (1H, s), 7.85 (1H, s), 7.83 (1H, s), 7.28 (1H, d, *J* = 8.6 Hz), 7.24 (3H, s), 7.17-7.18

(1H, m), 6.85 (2H, s), 6.80 (1H, s), 6.74 (2H, d, *J* = 5.1 Hz), 6.69 (2H, d, *J* = 5.5 Hz), 6.65 (2H, *J* = 5.5 Hz), 3.73 (3H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$: DMSO-d_6 (7:3), δ (ppm) 147.9, 146.8, 145.3, 136.6, 129.6, 128.8, 128.6, 128.5, 126.7, 125.8, 125.6, 124.7, 124.1, 120.5, 119.4, 119.2, 115.9, 115.7, 113.4, 109.3 and 56.2.

4-((*E*)-4-((*E*)-4-hydroxy-3,5-dimethoxystyryl)styryl)benzene-1,2-diol (24)

Yellow solid, m. p. 102-104°C, ^{1}H NMR (CD_3COCD_3, 300 MHz), δ (ppm) 8.03 (1H, s), 7.54 (4H, s), 7.48 (2H, s), 7.16-7.10 (4H, m), 7.02-6.95 (3H, m), 6.85 (1H, d, *J* = 8.1 Hz), 3.89 (6H, s); ^{13}C NMR (75.4 MHz, CD_3COCD_3), δ (ppm) 161.7, 149.4, 146.7, 138.2, 138.1, 137.6, 131.3, 130.1, 129.8, 127.8, 127.1, 126.7, 120.5, 116.8, 114.3, 105.6 and 57.1. HRMS-ESI: m/z $[M+H]^+$ for $C_{24}H_{22}O_5$, calculated 391.1540; observed 391.1538.

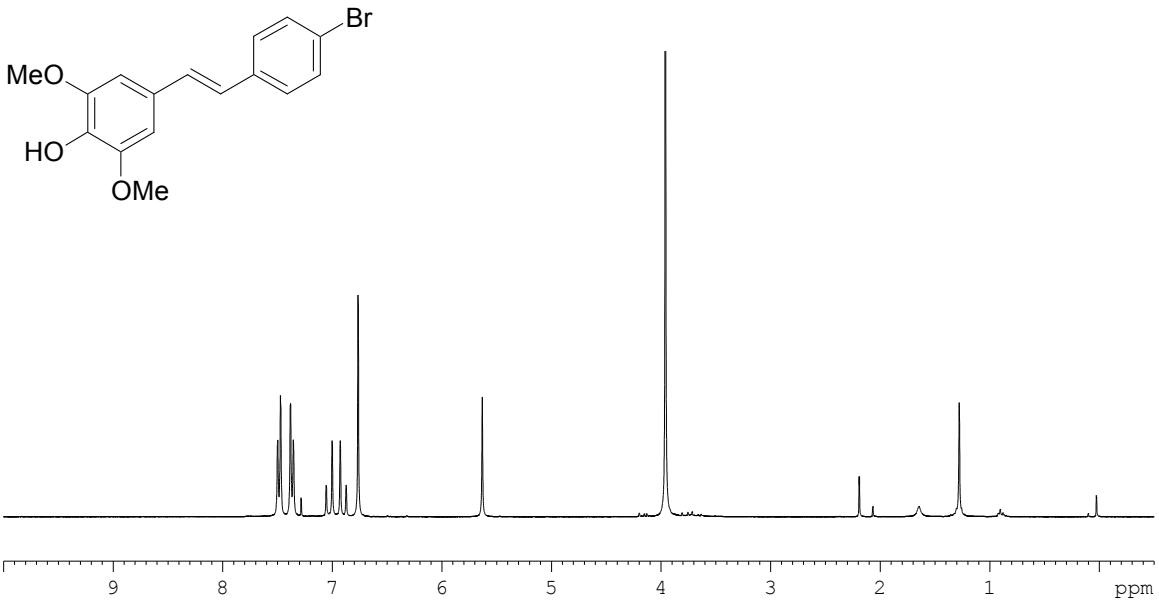
4-((*E*)-4-((*E*)-4-hydroxy-3-methoxystyryl)styryl)-2,6-dimethoxyphenol (25)

Creamish solid, m. p. 190-192°C, ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.49 (4H, s), 7.28 (1H, s), 7.11-7.04 (4H, m), 7.00 (1H, s), 6.94 (1H, d, *J* = 8.1Hz), 6.78 (2H, s), 5.69 (1H, s), 5.60 (1H, s), 3.97 (9H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ (ppm) 147.6, 147.1, 146.9, 137.2, 137.1, 129.9, 129.5, 129.1, 128.9, 128.8, 126.9, 120.9, 115.0, 108.6, 103.7, 56.7 and 55.9. HRMS-ESI: m/z $[M+H]^+$ for $C_{25}H_{24}O_5$, calculated 405.1696; observed 405.1679.

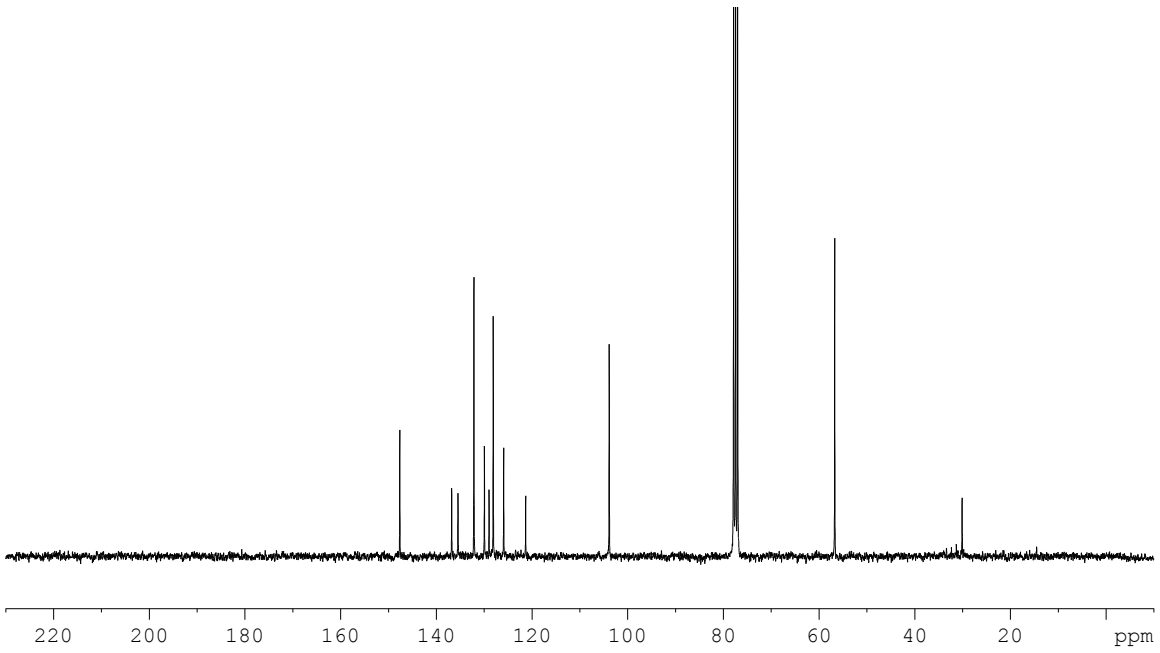
2,6-dimethoxy-4-((*E*)-4-((*E*)-styryl)styryl)phenol (26)

Creamish solid, m. p. 162-164°C, ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm) 7.56 (7H, d, *J* = 11.2 Hz), 7.41 (2H, t, *J* = 7.5 Hz), 7.14 (2H, s), 7.10 (2H, q, *J* = 16.2 Hz), 6.79 (2H, s), 5.62 (1H, s), 3.97 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$), δ (ppm) 147.6, 137.7, 137.2, 136.8, 135.3, 129.4, 129.1, 128.8, 128.7, 128.0, 127.3, 127.0, 126.9, 103.8 and 56.7.

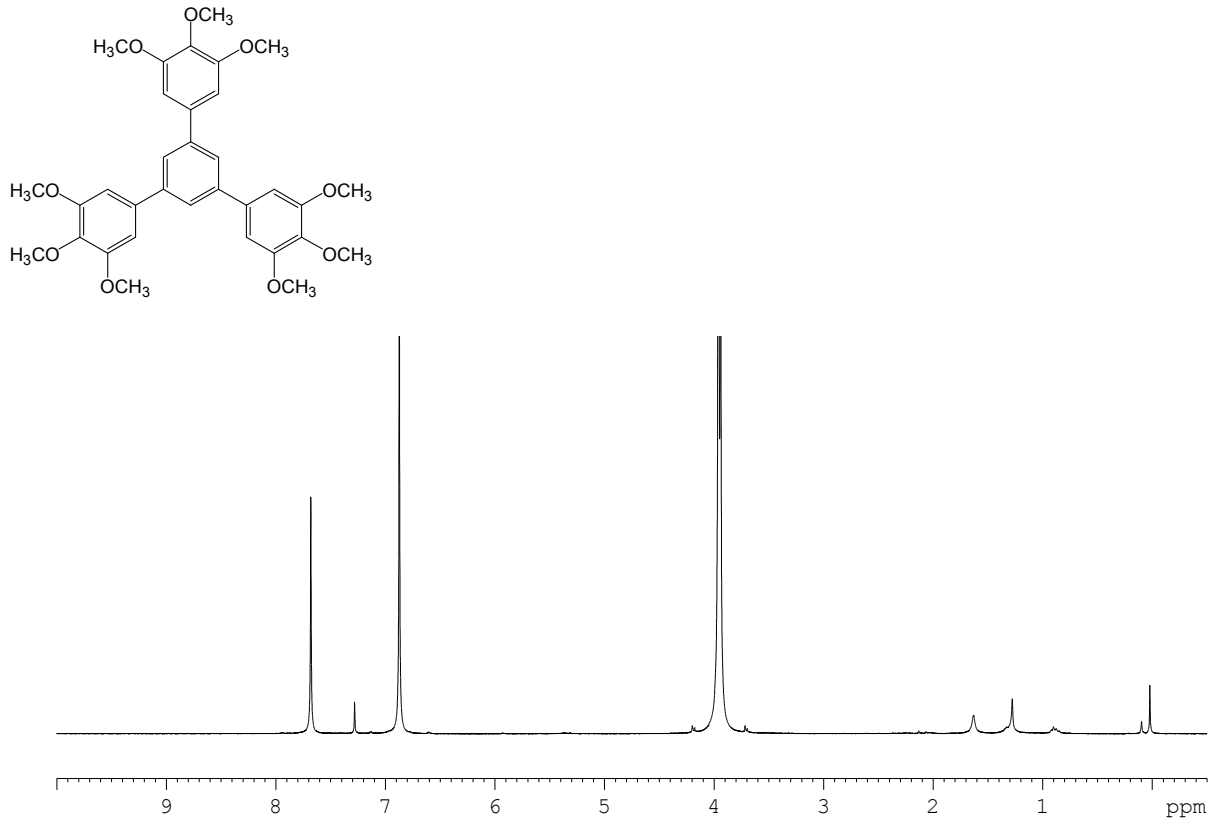
NMR spectra of novel compounds



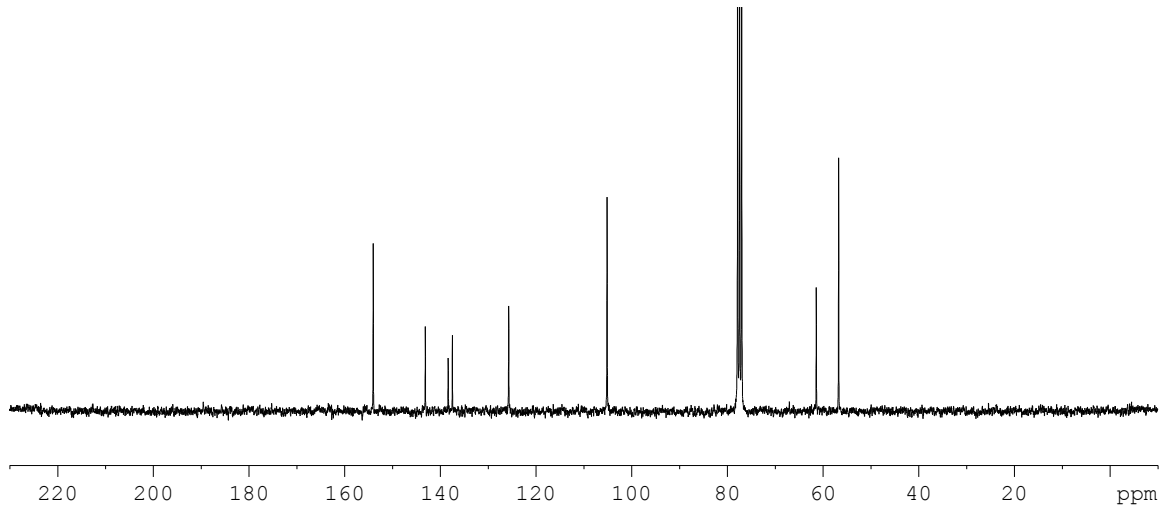
^{1}H NMR (in $CDCl_3$) spectrum of (*E*)-4-(4-bromostyryl)-2,6-dimethoxyphenol (**10**)



^{13}C NMR (in $CDCl_3$) spectrum of (*E*)-4-(4-bromostyryl)-2,6-dimethoxyphenol (**10**)



^{1}H NMR (in $CDCl_3$) spectrum of 3,3'',4,4'',5,5''-hexamethoxy-5'-(3,4,5-trimethoxyphenyl)-1,1':3',1''-terphenyl (**28**)



^{13}C NMR (in $CDCl_3$) spectrum of 3,3'',4,4'',5,5''-hexamethoxy-5'-(3,4,5-trimethoxyphenyl)-1,1':3',1''-terphenyl (**28**)

Microscopic studies of Hydroxylated Distyrylbenzene 11, and Octupolar Stilbenoid 27, in malaria parasite

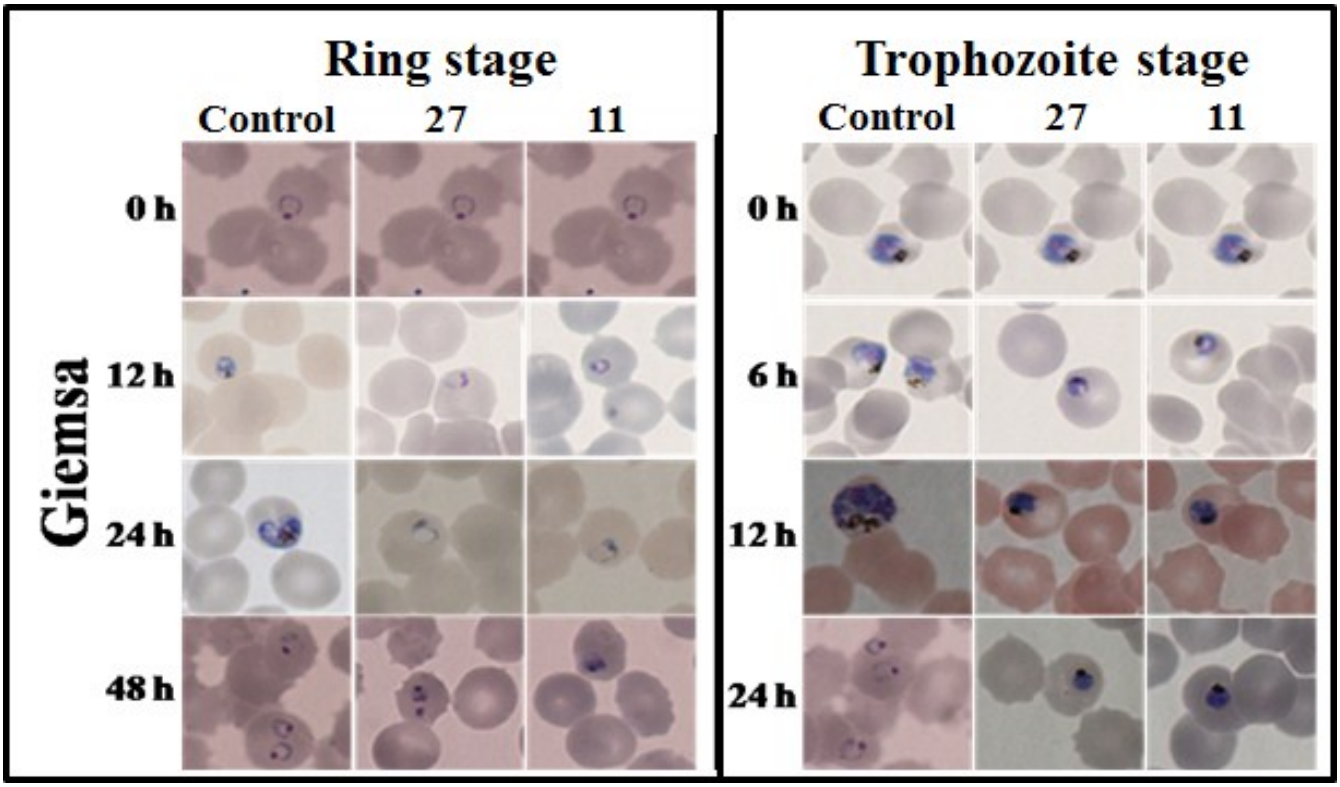


Figure S1a: Giemsa staining showed stressed and shrunken "crisis forms in **27 &11** treated cultures. Drug treated ring and trophozoite stages lagged behind their respective controls in terms of ring to trophozoite and trophozoite to schizont transitions.

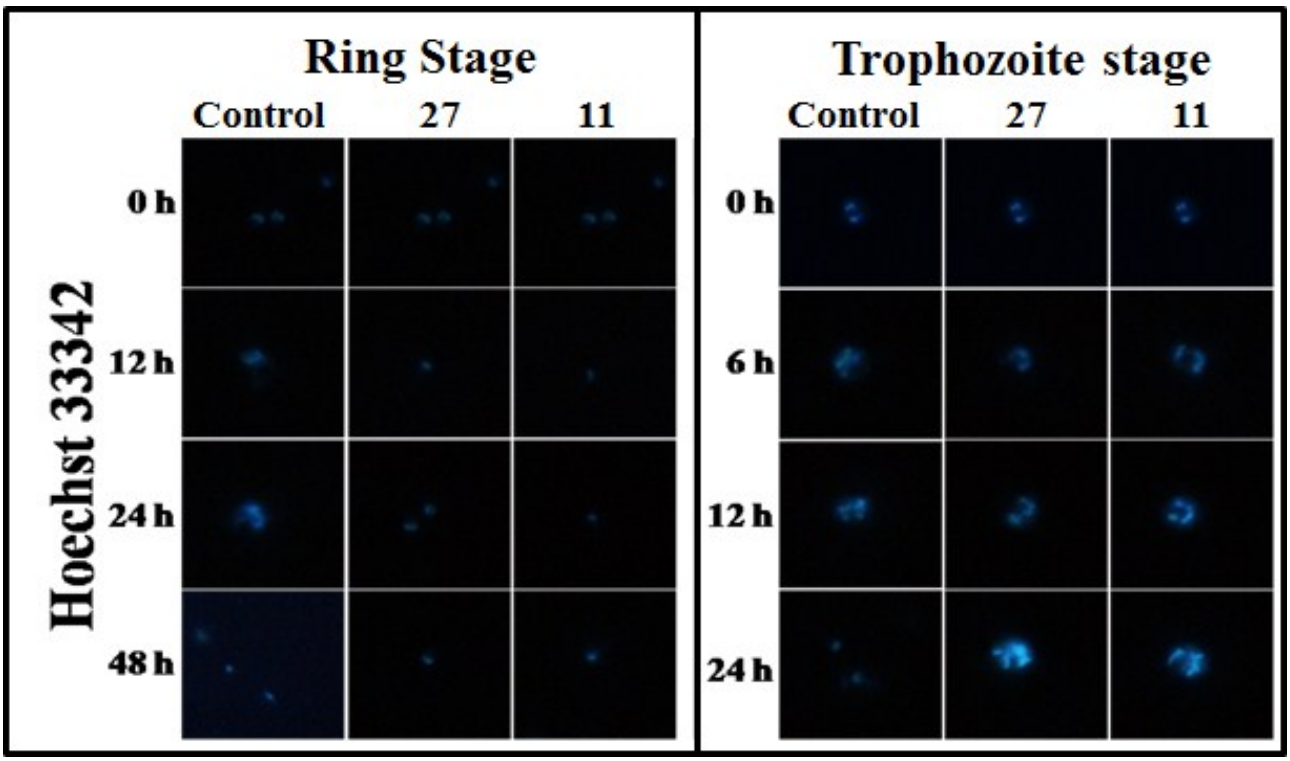


Figure S1b: Increased fluorescence of Hoechst 33342 in treated trophozoites (24 h) suggests DNA condensation and fragmentation in malaria parasites.

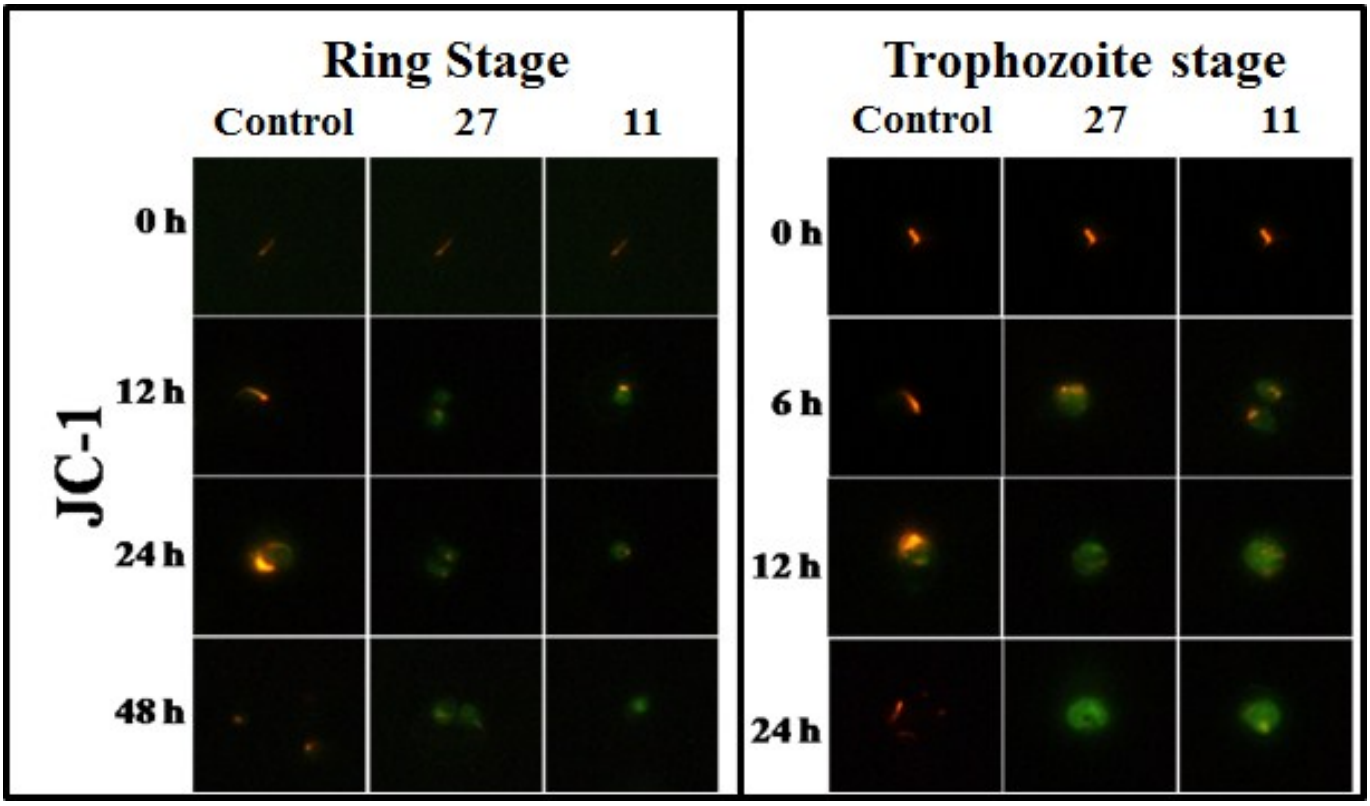


Figure S1c: JC-1 staining indicates loss of mitochondrial membrane potential in **27** and **11** treated ring and trophozoites stages.

References

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