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New Antiparasitic Bis-Naphthoquinone Derivatives

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A series of bis-naphthoquinone derivatives prepared by condensation of aryl aldehydes with lawsone were tested for antiparasitic activities against *Toxoplasma gondii* and *Trypanosoma brucei* parasites. Monofluorophenyl derivative **1a**, 3,4-difluorophenyl analog **1c** and furyl compound **1l** exhibited significant activity against *T. gondii* cells and appear to be new promising drug candidates against this parasite. The 3,4,5-trifluorophenyl derivative **1g** and the isovanillyl derivative **1j** displayed selective activity against *Leishmania major* amastigotes.

Keywords: lawsone, neglected tropical diseases, fluorine, furan, synthesis, biological activity.

Introduction

Parasitic diseases still represent an enormous worldwide medical challenge.[1] Neglected tropical diseases (NTDs) pose a particularly great danger both for local people and travelers in many tropical and subtropical countries.[2] Due to the climate change, further regions of the world will likely be affected by these parasitic diseases in the future.[2] There are only a few drugs available against NTDs because big pharma is reluctant to develop new and more potent drugs against diseases that mainly poor people suffer from.[3] Vice versa, poor people affected by NTDs are in want of affordable drugs, which could, however, arise from tapping into natural resources. Many natural product derived drug candidates, active against various NTDs, have emerged.[3] In addition, natural products constitute sustainable, cheap materials.

Lawsone, for instance, is a natural 2-hydroxy-1,4-naphthoquinone isolated from the Henna plant (*Lawsonia inermis*) which is being applied for the management of skin diseases in South Asia as a component of the local Ayurveda and Unani folk medicine.[4,5] Lawsone (2-hydroxy-1,4-naphthoquinone) is also a useful starting material for the preparation of various related quinones with proven bioactivity such as lapachol or atovaquone.[4,5] Arylmethylene-substituted bis-naphthoquinone derivatives have been reported to exhibit only moderate activities against human cancer cells which makes them interesting candidates for the design of selective antiparasitic drugs.[6] Indeed, a small series of substituted bis-2-hydroxy-1,4-naphthoquinones including **1a** (*Figure 1*) was obtained with distinct activity against *Leishmania* parasites.[7] Herein, we report on expanded series of such new derivatives (e.g., fluoroarenes, vanillins, furans) with optimized activity and selectivity against various parasites including *Toxoplasma gondii*, *Trypanosoma brucei* and *Leishmania*.

Results and Discussion

The known compounds **1a** and **1b** and the new analogs **1c**–**1l** were obtained as solids from the one-pot condensation reaction of two equivalents of 2-hydroxy-1,4-naphthoquinone (lawsone) with the corresponding aryl aldehyde and a catalytic amount of β-alanine in warm acetic acid (*Scheme 1*).[7,8] The structures of the target compounds **1a**–**1l** are shown in *Figure 1*.
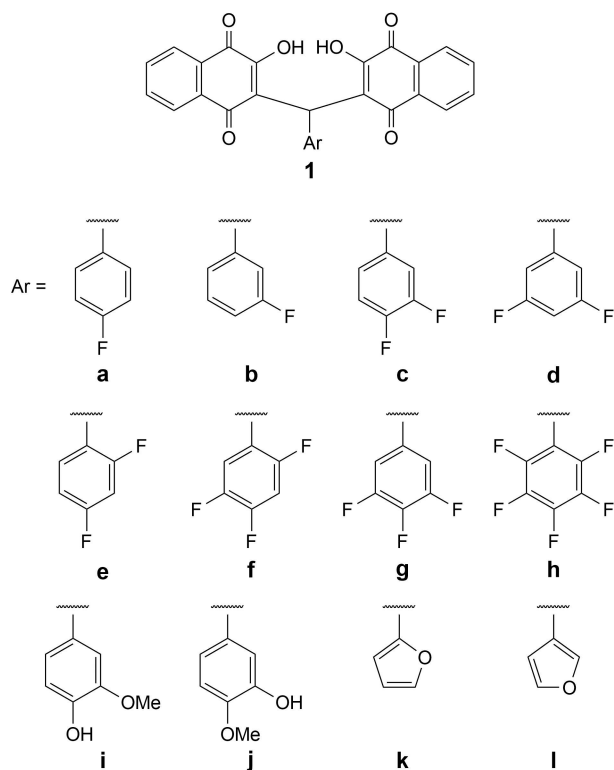
Figure 1. Structures of target compounds **1a**–**1l**.
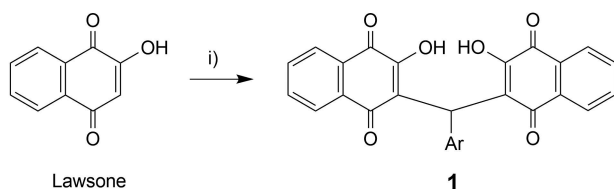
Scheme 1. Synthesis of bis-naphthoquinone derivatives. Reagents and conditions: i) Aryl aldehyde (0.5 equiv.), cat. β-alanine, AcOH, 50 °C, 2 h, 30–62 %.

The antiparasitic activity of the compounds **1a**–**1l** was initially tested against *Toxoplasma gondii* and *Trypanosoma brucei* parasites and compared with the toxicity against normal kidney epithelial Vero cells (*Table 1*). The best performing compounds **1a** and **1c** exhibited good and similar activities against *T. gondii* cells with **1c** showing a better selectivity for *T. gondii* cells over Vero cells. Like **1a**, the pentafluorophenyl derivative **1h** was more toxic to normal Vero cells than to *T. gondii* cells. Atovaquone, which is commonly applied for the treatment of toxoplasmosis, was used as positive control and displayed the highest activity and selectivity.[9] However, the toxicity of the most bis-naphthoquinones (except for **1a** and **1h**) to Vero cells

was distinctly lower when compared with atovaquone. In contrast to this varied spectrum of activities against *T. gondii*, all compounds, except **1a** and **1i** which displayed low double-digit IC_{50} values, were virtually inactive against *T. b. brucei* cells. Pentamidine, an approved drug for the treatment of sleeping sickness, was applied as positive control with activity in the nano-molar concentration range.[10]

The compounds **1a**–**1l** were also tested against *Leishmania major* promastigotes and amastigotes in comparison to normal Vero cells (*Table 2*). Compounds **1a** and **1h** showed similar moderate activities against the promastigotes ($EC_{50} = 35.0$ μM for **1a** and 35.5 μM for **1h**). Compounds **1c** and **1f** were most active against the amastigotes ($EC_{50} = 23.7$ μM for **1c** and 22.6 μM for **1f**) and more active than the known compound **1a**. However, these compounds showed only poor selectivity. In contrast to that, compounds **1g** and **1j** displayed only slightly weaker activities than **1c** and **1f** against the amastigotes ($EC_{50} = 34.1$ μM for **1g** and 37.9 μM for **1j**) but reasonable selectivity when compared with their effects on Vero cells (SI = 4.18 for **1g** and more than 2.64 for **1j**). The activity of the known compound **1a** against *L. major* promastigotes ($EC_{50} = 35.0$ μM) was much lower when compared with its published activities against *L. amazonensis* ($IC_{50} = 0.6$ μM) and *L. braziliensis* promastigotes ($IC_{50} = 0.8$ μM).[7] Amphotericin B was applied as a proper positive control for the tests with *L. major* parasites despite of its severe side-effects.[11] In this study, the toxicity of amphotericin B to Vero cells was distinctly higher when compared with **1g** and **1j**, for example, which did not harm Vero cells at doses of 100 μM and still showed moderate activity against amastigotes.

Conclusions

A series of bis-naphthoquinone derivatives with variance in the aryl residue at the bridging methylene carbon were prepared and tested on various protozoal parasites against which they showed distinctly different activities. We identified compounds that were remarkably active against *T. gondii* cells (e.g., the fluorophenyl derivatives **1a** and **1c**, and the furan **1l**), and sufficiently selective for *L. major* amastigotes (e.g., the fluorophenyl derivative **1g** and isovanillyl derivative **1j**) to warrant further drug optimization studies. It should be noted that in our tests with different parasites we could not confirm the excellent activities published for **1a**.[7] Although this compound was among the most active ones of this series, its toxic

Table 1. Effective concentrations EC_{50} of compounds **1a**–**1l** when applied to cells of *Toxoplasma gondii* and their inhibitory concentrations IC_{50} when applied to *Trypanosoma brucei brucei* cells as well as IC_{50} values for Vero (African green monkey kidney epithelial) cells.[a] Amphotericin B (AmB), pentamidine, and atovaquone (ATO) were applied as positive controls.

Compound	IC_{50} (Vero, [µM])	EC_{50} (*T. gondii*, [µM])	SI (Vero/*T. gondii*)[b]	IC_{50} (*T. b. brucei*, [µM])	SI (Vero/*T. b. brucei*)[c]
1a	6.78	11.64	0.58	15.6	0.43
1b	53.48	26.63	2.0	>10	–
1c	17.63	13.95	1.26	>10	–
1d	27.54	34.53	0.80	>100	–
1e	39.37	27.94	1.41	>10	–
1f	24.06	23.86	1.01	>10	–
1g	142.3	48.94	2.91	–	–
1h	6.88	21.47	0.32	72.3	0.10
1i	>100	109.0	–	27.8	>3.60
1j	>100	59.28	–	–	–
1k	57.41	25.33	2.27	>100	–
1l	68.25	23.22	2.94	>10	–
AmB	7.7	–	–	–	–
Pentamidine	–	–	–	0.042[d]	.
ATO	9.5	0.07	136	–	–

[a] Values are the means of at least three independent experiments (SD ±15%). They were derived from concentration–response curves obtained by measuring the percentage of vital cells relative to untreated controls after 72 h. [b] Selectivity index (EC_{50}/IC_{50}) calculated from the corresponding EC_{50} (*T. gondii*) and IC_{50} (Vero) values. [c] Selectivity index calculated from the corresponding IC_{50} (*T. b. brucei*) and IC_{50} (Vero) values. [d] Value is taken from ref. [8].

Table 2. Effective concentrations EC_{50} of compounds **1a**–**1l** when applied to promastigotes and amastigotes of *Leishmania major*.[a] Amphotericin B (AmB) was applied as positive control.

Compound	EC_{50} (promastigotes, [µM])	EC_{50} (amastigotes, [µM])	SI (Vero/promastigotes)[b]	SI (Vero/amastigotes)[c]
1a	35.0	32.4	0.19	0.21
1b	116.0	48.0	0.46	1.12
1c	92.5	23.7	0.19	0.74
1d	89.1	77.2	0.31	0.36
1e	83.0	36.4	0.48	1.08
1f	76.5	22.6	0.32	1.06
1g	108	34.1	1.32	4.18
1h	35.5	30.1	0.19	0.23
1i	>200	106.0	–	>0.94
1j	97.4	37.9	>1.03	>2.64
1k	101	33.5	0.57	1.71
1l	94.1	53.7	1.27	0.73
AmB	0.83	0.47	9.6	16.4

[a] Values are the means of at least three independent experiments (SD ±15%). They were derived from concentration–response curves obtained by measuring the percentage of vital cells relative to untreated controls after 72 h. [b] Selectivity index (IC_{50}/EC_{50}) calculated from the corresponding IC_{50} values for Vero cells and the EC_{50} values against *L. major* promastigotes. [c] Selectivity index calculated from the corresponding IC_{50} values for Vero cells and the EC_{50} values for *L. major* amastigotes.

effect on normal epithelial Vero cells is unfavorable for an antiparasitic drug. It might hold, however, like the new compound **1h**, some potential as an antitumoral drug candidate.

Experimental Section

General

All starting compounds were purchased from Aldrich. The known compounds **1a** and **1b** were prepared according to literature procedures.[7,12] The following instruments were used: melting points (uncorrected), Gallenkamp; IR spectra, PerkinElmer Spectrum One FT-

IR spectrophotometer with ATR sampling unit; nuclear magnetic resonance spectra, Bruker Avance 300 spectrometer; chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard; mass spectra, Varian MAT 311A (EI), Q Exactive (ESI, HRMS, solutions of MeCN); microanalyses, PerkinElmer 2400 CHN elemental analyzer. All tested compounds are $>95\%$ pure by elemental analysis.

2,2′-[(4-Fluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1a**). Yield: 69 mg (0.15 mmol, 30%). MS: 454 (21) [M$^+$], 436 (21), 408 (31), 252 (100), 224 (22), 196 (27), 174 (63), 105 (68), 76 (46).

2,2′-[(3-Fluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1b**). Yield: 68 mg (0.15 mmol, 30%). MS: 454 (83) [M$^+$], 436 (57), 408 (84), 280 (33), 251 (100), 174 (70), 105 (87), 76 (56).

2,2′-[(3,4-Difluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1c**) – *General Procedure.* 2-Hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), 3,4-difluorobenzaldehyde (71 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) were dissolved in AcOH (8 mL). The reaction mixture was stirred at 50 °C for 2 h. The reaction mixture was poured on water (30 mL), the precipitate was collected and dried in vacuum. Yield: 70 mg (0.15 mmol, 30%). Yellow solid. M.p. 109–110 °C. ATR-IR: 3340, 1642, 1593, 1515, 1460, 1431, 1362, 1336, 1269, 1211, 1162, 1115, 1044, 1013, 977, 918, 887, 868, 816, 756, 723, 693. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.10 (1 H, s), 7.0–7.2 (3 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 37.4 (CH), 110.7 (quinone-C3), 116.7–117.6 (m, fluoroaryl-C2, C5), 122.0 (fluoroaryl-C6), 124.3, 126.4, 126.5, 126.7, 127.3, 129.4, 132.7, 133.3 (aryl-carbons), 135.3–135.6 (m, fluoroaryl-C1), 147.6 (fluoroaryl-C3), 154.3 (C—OH), 181.3 (CO), 184.1 (CO). MS: 472 (63) [M$^+$], 454 (55), 426 (72), 270 (100), 242 (22), 214 (36), 174 (86), 105 (86) 76 (52). HR-ESI-MS: calc. for C$_{27}$H$_{15}$O$_6$F$_2$, [M+H]$^+$, 473.08312; found 473.08213.

2,2′-[(3,5-Difluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1d**). Compound **1d** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), 3,5-difluorobenzaldehyde (71 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (8 mL). Yield: 90 mg (0.21 mmol, 42%). Yellow solid. M.p. 116–118 °C. ATR-IR: 3327, 1649, 1624, 1594, 1459, 1365,

1337, 1300, 1266, 1213, 1158, 1115, 1045, 990, 919, 853, 795, 725, 686. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.14 (1 H, s), 6.6–6.7 (1 H, m), 6.7–6.8 (2 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 37.6 (CH), 101.8–102.5 (m, fluoroaryl-C4), 110.7 (quinone-C3), 111.1–111.5 (m, fluoroaryl-C2, C6), 121.4, 126.4, 126.7, 127.3, 129.4, 132.7, 133.1, 133.3, 135.3 (aryl carbons), 142.7–142.9 (m, fluoroaryl-C1), 154.5 (C—OH), 161.2–164.6 (m, fluoroaryl-C3, C5), 181.3 (CO), 184.0 (CO). MS: 472 (92) [M$^+$], 454 (69), 426 (100), 382 (23), 359 (29), 326 (18), 298 (31), 269 (31), 214 (23), 105 (62), 104 (61), 76 (38). HR-ESI-MS: calc. for C$_{27}$H$_{15}$O$_6$F$_2$, [M+H]$^+$, 473.08312; found 473.08267.

2,2′-[(2,4-Difluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1e**). Compound **1e** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), 2,4-difluorobenzaldehyde (71 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (8 mL). Yield: 138 mg (0.29 mmol, 58%). Yellow solid. M.p. 169–170 °C. ATR-IR: 3336, 1647, 1593, 1502, 1461, 1428, 1364, 1338, 1297, 1274, 1258, 1211, 1161, 1139, 1090, 1044, 1012, 967, 938, 903, 848, 816, 798, 782, 722, 693, 679, 646, 608. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.23 (1 H, s), 6.7–6.8 (2 H, m), 7.1–7.2 (1 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 32.1 (CH), 103.1–103.6 (m, fluoroaryl-C3), 110.5–110.8 (m, fluoroaryl-C5, quinone-C3), 121.3, 126.3, 127.2, 129.3, 130.2–130.5 (m), 132.8, 133.1, 135.2 (aryl carbons), 154.0 (C—OH), 161.0–164.0 (m, fluoroaryl-C2, C4), 181.3 (CO), 183.8 (CO). MS: 472 (4) [M$^+$], 452 (100), 426 (53), 279 (27), 251 (32), 174 (26), 105 (60), 76 (48). HR-ESI-MS: calc. for C$_{27}$H$_{15}$O$_6$F$_2$, [M+H]$^+$, 473.08312; found 473.08247.

2,2′-[(2,4,5-Trifluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1f**). Compound **1f** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), 2,4,5-trifluorobenzaldehyde (80 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (8 mL). Yield: 130 mg (0.27 mmol, 54%). Yellow solid. M.p. 167–169 °C. ATR-IR: 3347, 1647, 1593, 1513, 1461, 1424, 1364, 1334, 1276, 1261, 1212, 1188, 1151, 1098, 1043, 1011, 970, 938, 911, 870,3 840, 816, 796, 762, 723, 688, 658, 638. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.20 (1 H, s), 6.8–6.9 (1 H, m), 7.0–7.1 (1 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 32.0 (CH), 104.9–105.3 (m, fluoroaryl-C3), 117.8–118.1 (m, fluoroaryl-C6), 120.7 (fluoroaryl-C6), 126.4, 127.3, 129.2, 132.8, 133.2, 135.3 (aryl carbons), 148.0 (fluoroaryl-C5), 151.0 (fluoroaryl-

C4), 153.9 (C—OH), 157.5 (fluoroaryl-C2), 181.2 (CO), 183.6 (CO). MS: 490 (9) [M$^+$], 470 (100), 444 (76), 298 (37), 288 (31), 269 (47), 104 (72), 76 (54). HR-ESI-MS: calc. for $C_{27}H_{14}O_6F_3$, [M+H]$^+$, 491.07370; found: 491.07311.

2,2′-[(3,4,5-Trifluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1g**). Compound **1f** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), 3,4,5-trifluorobenzaldehyde (80 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (8 mL). Yield: 120 mg (0.25 mmol, 50%). Yellow solid. M.p. 116–117 °C. ATR-IR: 3318, 1647, 1620, 1593, 1527, 1460, 1444, 1363, 1338, 1260, 1213, 1159, 1040, 998, 971, 919, 879, 868, 852, 817, 797, 725, 700. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.08 (1 H, s), 6.9–7.0 (2 H, m), 7.7–7.8 (4 H, m), 8.1–8.2 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 37.5 (CH), 110.7 (quinone-C3), 112.4–112.7 (m, fluoroaryl-C2, C6), 121.3, 126.4, 126.5, 126.7, 127.3, 129.3, 132.7, 133.1, 133.3, 135.3 (aryl carbons), 148.9 (fluoroaryl-C4), 152.5 (fluoroaryl-C3, C5), 154.3 (C—OH), 181.3 (CO), 183.9 (CO). MS: 490 (62) [M$^+$], 472 (51), 444 (100), 416 (11), 400 (14), 359 (27), 344 (12), 288 (26), 232 (14), 174 (20), 147 (12), 104 (38), 76 (21). HR-ESI-MS: calc. for $C_{27}H_{14}O_6F_3$, [M+H]$^+$, 491.07370; found: 491.07262.

2,2′-[(Pentafluorophenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1h**). Compound **1h** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), pentafluorobenzaldehyde (98 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (6 mL). Yield: 160 mg (0.30 mmol, 61%). Yellow solid. M.p. 110–111 °C. ATR-IR: 3327, 1648, 1594, 1522, 1499, 1364, 1339, 1275, 1213, 1117, 1045, 999, 977, 911, 785, 724, 699. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.17 (1 H, s), 7.59 (2 H, s), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 29.1 (CH), 113.8–114.2 (m, fluoroaryl-C1), 119.7, 126.4, 126.9, 127.2, 129.2, 132.7, 133.2, 135.4 (aryl carbons), 137.8–139.2 (m, fluoroaryl-C3, C5), 141.5–142.2 (m, fluoroaryl-C2, C6), 143.7–144.6 (m, fluoroaryl-C2, C6), 147.1–148.6 (m, fluoroaryl-C4), 153.4 (C—OH), 181.1 (CO), 183.4 (CO). MS: 526 (79) [M$^+$], 506 (66), 480 (100) 434 (20), 359 (36), 334 (64), 305 (51), 174 (38), 105 (78), 104 (79), 76 (47). HR-ESI-MS: calc. for $C_{27}H_{12}O_6F_5$, [M+H]$^+$, 527.05486; found 527.05347.

2,2′-[(4-Hydroxy-3-methoxyphenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1i**). Compound **1i** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), vanillin (76 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (6 mL). Yield: 80 mg (0.17 mmol, 34%). Yellow solid. M.p. 107–108 °C. ATR-IR: 3294, 1645, 1592, 1580, 1513, 1460, 1431, 1362, 1336, 1252, 1206, 1155, 1122, 1030, 972, 915, 867, 813, 796, 722. ^{1}H-NMR (300 MHz, CDCl$_3$): 3.79 (3 H, s), 5.52 (1 H, s), 6.7–6.8 (3 H, m), 7.6–7.8 (4 H, m), 7.9–8.1 (6 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 37.6 (CH), 56.0 (MeO), 110.7 (quinone-C3), 111.3 (phenyl-C2), 114.1 (phenyl-C5), 121.1 (phenyl-C6), 122.9, 126.3, 126.5, 127.2, 129.6, 132.8, 133.2, 135.1 (aryl carbons), 144.6 (phenyl-C4), 146.5 (phenyl-C3), 154.5 (quinone-C—OH), 181.4 (CO), 184.6 (CO). MS: 482 (8) [M$^+$], 464 (5), 308 (45), 280 (100), 263 (14), 249 (27), 237 (19), 209 (12), 174 (73), 146 (16), 105 (64), 76 (27). HR-ESI-MS: calc. for $C_{28}H_{19}O_8$, [M+H]$^+$, 483.10744; found 483.10624.

2,2′-[(3-Hydroxy-4-methoxyphenyl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1j**). Compound **1j** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), isovanillin (76 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (6 mL). Yield: 80 mg (0.17 mmol, 34%). Yellow solid. M.p. 114–116 °C. ATR-IR: 3334, 1646, 1592, 1579, 1510, 1460, 1441, 1362, 1336, 1259, 1211, 1128, 1045, 1023, 981, 916, 885, 868, 798, 740, 723, 692, 656. ^{1}H-NMR (300 MHz, CDCl$_3$): 3.83 (3 H, s), 5.53 (1 H, s), 6.14 (1 H, s), 6.3–6.4 (3 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 37.0 (CH), 55.9 (MeO), 110.4 (quinone-C3), 114.6 (phenyl-C5), 119.6 (phenyl-C2), 122.7 (phenyl-C6), 126.3, 126.5, 127.2, 129.6, 131.0, 132.7, 133.2, 135.0 (aryl carbons), 145.5 (phenyl-C4), 154.8 (quinone-C—OH), 181.3 (CO), 184.7 (CO). MS: 482 (5) [M$^+$], 308 (47), 280 (100), 265 (22), 249 (27), 237 (25), 209 (17), 174 (87), 146 (23), 105 (97), 76 (42). HR-ESI-MS: calc. for $C_{28}H_{19}O_8$, [M+H]$^+$, 483.10744; found 483.10706.

2,2′-[(Furan-2-yl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1k**). Compound **1k** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), furfural (57 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (6 mL). Yield: 80 mg (0.19 mmol, 38%). Yellow solid. M.p. 118–120 °C. ATR-IR: 3329, 1647, 1592, 1580, 1501, 1460, 1363, 1336, 1261, 1212, 1160, 1142, 1092, 1074, 1043, 1011, 970, 883, 869, 819, 797, 761, 722. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.09 (1 H, d, J=3.3), 6.20 (1 H, s), 6.2–6.3 (1 H, m), 7.3–7.4 (1 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m), 8.2–8.3 (2 H, br. s). ^{13}C-NMR

(75.5 MHz, CDCl$_3$): 31.8 (CH), 107.2 (furyl-C3), 110.5 (quinone-C3), 120.7, 126.4, 127.2, 129.6, 132.6, 133.3, 135.0 (aryl carbons), 141.8 (furyl-C5), 150.5 (furyl-C2), 154.8 (C—OH), 181.2 (CO), 184.3 (CO). MS: 426 (43) [M$^+$], 224 (42), 174 (100), 146 (22), 105 (78), 77 (23). HR-ESI-MS: calc. for C$_{25}$H$_{15}$O$_7$, [M+H]$^+$, 427.08123; found 427.08009.

2,2′-[(Furan-3-yl)methylene]bis(3-hydroxynaphthalene-1,4-dione) (**1l**). Compound **1l** was prepared analogously to **1c** from 2-hydroxy-1,4-naphthoquinone (174 mg, 1.00 mmol), furyl-3-aldehyde (57 mg, 0.5 mmol) and a catalytic amount of β-alanine (15 mg) in AcOH (6 mL). Yield: 100 mg (0.24 mmol, 48%). Yellow solid. M.p. 167–170 °C. ATR-IR: 3328, 1646, 1592, 1579, 1502, 1460, 1362, 1336, 1264, 1219, 1158, 1071, 1044, 1024, 967, 920, 891, 872, 793, 756, 739, 722, 682, 658. ^{1}H-NMR (300 MHz, CDCl$_3$): 6.0–6.1 (1 H, m), 6.3–6.4 (1 H, m), 7.3–7.4 (1 H, m), 7.6–7.8 (4 H, m), 8.0–8.1 (4 H, m). ^{13}C-NMR (75.5 MHz, CDCl$_3$): 28.9 (CH), 111.5 (quinone-C3), 121.9, 122.2, 126.4, 127.1, 129.6, 132.6, 133.3, 135.0 (aryl carbons), 140.2 (furyl-C2), 143.0 (furyl-C5), 154.9 (C—OH), 181.2 (CO), 184.8 (CO). MS: 426 (14) [M$^+$], 252 (21), 224 (66), 196 (57), 174 (100), 146 (24), 139 (27), 105 (96), 76 (42). HR-ESI-MS: calc. for C$_{25}$H$_{15}$O$_7$, [M+H]$^+$, 427.08123; found 427.08056.

Toxoplasma gondii *Culture Conditions*

Serial passages of the cell lines Vero (ATCC® CCL81™, USA) were used for the cultivation of *T. gondii* tachyzoites of the RH strains (a gift from Dr. Saeed El-Ashram, State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China). Vero cells were cultured by using RPMI 1640 medium (Sigma, USA) with heat-inactivated 10% fetal bovine serum (FBS, Invitrogen, USA) in a humidified 5% CO$_2$ atmosphere at 37 °C. 96-Well plates (5×10^3 cells/well in 200 μL RPMI 1640 medium) were used for the cultivation of the Vero cells and then incubated at 37 °C and 5% CO$_2$ for one day, followed by removal of medium and washing the cells with phosphate buffered saline (PBS). Then, RPMI 1640 medium with 2% FBS containing tachyzoites (RH strains) of *T. gondii* at a ratio of 5 (parasite): 1 (Vero cells) was added. After incubation at 37 °C and 5% CO$_2$ for 5 h, cells were washed with PBS and then overlaid with medium containing tested compounds or atovaquone (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 μg mL^{-1}).

After incubation at 37 °C and 5% CO$_2$ for 72 h, the cells were stained with 1% toluidine blue after washing with PBS and fixation in 10% formalin. The cells were examined under an inverted photomicroscope (MCD-400, Leica, Japan) to determine the infection index (number of infected cells from 200 tested cells) of *T. gondii*.[13,14]

Leishmania major *Promastigotes and Amastigotes*

Promastigotes of *L. major* were isolated from a Saudi male patient in February 2016 and maintained at 26 °C in Schneider's Drosophila medium (Invitrogen, USA) supplemented with 10% heat inactivated FBS (Invitrogen, USA) and antibiotics in a tissue culture flask with weekly transfers. Promastigotes were cryopreserved in liquid nitrogen at concentrations of 3×10^6 parasite/ml. The virulence of *L. major* parasites was maintained by passing in female BALB/c mice by injecting hind footpads with 1×10^6 stationary-phase promastigotes. After 8 weeks, *L. major* amastigotes were isolated from mice. Isolated amastigotes were transformed to promastigote forms by culturing at 26 °C in Schneider's medium supplemented with 10% FBS and antibiotics. For infection, amastigote-derived promastigotes with less than five *in vitro* passages were used. Male and female BALB/c mice were obtained from Pharmaceutical College, King Saud University, Kingdom of Saudi Arabia, and maintained in specific pathogen-free facilities.

In order to evaluate the activity of tested compounds against *L. major* promastigotes, promastigotes from logarithmic-phase cultured in phenol red-free RPMI 1640 medium (Invitrogen, USA) with 10% FBS were suspended on 96-wells plates to yield 10^6 cells mL^{-1} (200 μL/well) after hemocytometer counting. Compounds were added to obtain the final concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 μg mL^{-1}). Negative control wells containing cultures with DMSO (1%) and without compound and positive control wells containing cultures with decreasing concentration of amphotericin B (reference compound, 50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 μg mL^{-1}) were used. Plates were incubated at 26 °C for 72 h to evaluate the antiproliferative effect. The number of viable promastigotes were assessed by colorimetric method using the tetrazolium salt colorimetric assay (MTT). It measures the reduction of the MTT component into an insoluble formazan product. This colored product was solubilized by adding detergent solution to lyse the cells. The samples were analyzed by using a

microplate absorbance spectrophotometer (xMark, Bio-Rad, USA) at 570 nm.[14]

In order to evaluate the activity of tested compounds against amastigotes in macrophages, peritoneal macrophages from female BALB/c mice (6–8 weeks of age) were collected by aspiration, then 5×10^4 cells/well were seeded on 96-wells plates in RPMI 1640 medium with 10% FBS for 4 h at 37 °C in 5% CO_2 atmosphere to promote cell adhesion. Medium was discarded and washed with PBS. 200 µL of solution containing *L. major* promastigotes (at a ratio of 10 promastigotes: 1 macrophage in RPMI 1640 medium with 10% FBS) were added per well. Plates were incubated for 24 h at 37 °C in humidified 5% CO_2 atmosphere to allow infection and amastigote differentiation. Then, the infected macrophages were washed three times with PBS to remove the free promastigotes and overlaid with fresh RPMI 1640 medium containing compounds at final concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg mL^{-1}) and cells were incubated at 37 °C in humidified 5% CO_2 atmosphere for 72 h. Negative control containing cultures with DMSO (1%) and without compounds and positive control wells containing cultures with decreasing concentration of amphotericin B (reference compound, 50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg mL^{-1}) were used. The percentage of infected macrophages was evaluated microscopically after removing medium, washing, fixation, Giemsa staining.[14]

Trypanosoma Cell Line and Culture Conditions

Cells of the *T. b. brucei* bloodstream trypomastigote cell lines Lister 427 were maintained in HMI-9 medium, pH 7.5, supplemented with 10% FBS in a humidified 5% CO_2 atmosphere at 37 °C.[15]

Alamar Blue (AB) Assay

The AB assay was used to identify viable cells after treatment with drug candidates.[16–19] This assay was based on the irreversible reaction of the blue dye resazurin and NADH to pink resofurin in intact cells. *T. b. brucei* cells (8000/well) were seeded on 96-well microplates, treated with the tested compounds (dissolved in DMSO) and incubated for 72 h (5% CO_2, 95% humidity, 37 °C). 10 µL of the AB reagent (500 mM resazurin sodium salt in PBS) were added and incubated for further 4 h at 37 °C. The fluorescence (extinction at 544 nm, emission at 590 nm) was measured on an Omega Fluostar (BMG Labtech) fluorescence plate reader.

Cytotoxicity Assay

MTT assay was carried out for cytotoxicity evaluation of compounds. Briefly, Vero cells were cultured in 96-well plates (5×10^3 cells/well/200 µL) for 24 h in RPMI 1640 medium with 10% FBS and 5% CO_2 at 37 °C. Cells were washed with PBS and treated with tested compounds for 72 h at varying concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg mL^{-1}) in medium with 10% FBS. As negative control, cells were treated with medium only. Thereafter, the supernatant was removed and 50 µL RPMI 1640 medium containing 14 µL MTT (5 mg mL^{-1}) were added and incubated for 4 h. After that, the supernatant was removed and 150 µL DMSO were added in order to dissolve the formazan. Microplate absorbance spectrophotometer was applied for colorimetric analysis ($\lambda = 540$ nm). Cytotoxic effects were expressed by IC$_{50}$ values (concentration that caused a 50% reduction in viable cells).[14,20]

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Author Contribution Statement

B. B. prepared the tested compounds and wrote the article. I. S. N., T. K. and J. J. carried out the antiparasitic assays. W. S. K., K. E. and R. S. provided the material, supervised the work and proofread the article.

References

[1] M. Cholewinski, M. Derda, E. Hadas, 'Parasitic diseases in humans transmitted by vectors', *Ann. Parasitol. Hum. Comp.* **2015**, *61*, 137–157.

[2] A. K. Mitra, A. R. Mason, 'Neglected tropical diseases: Epidemiology and global burden', *Trop. Med. Infect. Dis.* **2017**, *2*, 36.

[3] P. M. Cheuka, G. Mayoka, P. Mutai, K. Chibale, 'The role of natural products in drug discovery and development

against neglected tropical diseases', *Molecules* **2017**, *22*, 58.

[4] A. S. Borade, B. N. Kale, R. V. Shete, 'A phytopharmacological review on *Lawsonia inermis* (Linn.)', *Int. J. Pharm. Life Sci.* **2011**, *2*, 536–541.

[5] R. Pradhan, P. Dandawate, A. Vyas, S. Padhye, B. Biersack, R. Schobert, A. Ahmad, F. H. Sarkar, 'From body art to anticancer activities: perspectives on medicinal properties of Henna', *Curr. Drug Targets* **2012**, *13*, 1777–1798.

[6] P. Sadhukan, S. Saha, K. Sinha, G. Brahmachari, P. C. Sil, 'Selective pro-apoptotic activity of novel 3,3′-(aryl/alkylmethylene)bis(2-hydroxynaphthalene-1,4-dione) derivatives on human cancer cells via the induction reactive oxygen species', *PLoS One* **2016**, *11*, e0158694.

[7] M. V. de Araújo, P. S. O. de Souza, A. C. de Queiroz, C. B. B. da Matta, A. B. Leite, A. E. da Silva, J. A. A. de Franca, T. M. S. Silva, C. A. Camara, M. S. Alexandre-Moreira, 'Synthesis, leishmanicidal activity and theoretical evaluations of a series of substituted bis-2-hydroxy-1,4-naphthoquinones', *Molecules* **2014**, *19*, 15180–15195.

[8] I. Winter, J. Lockhauserbäumer, G. Lallinger-Kube, R. Schobert, K. Ersfeld, B. Biersack, 'Anti-trypanosomal activity of cationic *N*-heterocyclic carbene gold(I) complexes', *Mol. Biochem. Parasitol.* **2017**, *214*, 112–120.

[9] N. Konstantinovic, H. Guegan, T. Stäjner, S. Belaz, F. Robert-Gangneux, 'Treatment of toxoplasmosis: current options and future perspectives', *Food Waterborne Parasitol.* **2019**, *12*, e00036.

[10] D. Steverding, 'The development of drugs for treatment of sleeping sickness: a historical review', *Parasit. Vectors* **2010**, *3*, 15.

[11] E. Torres-Guerrero, M. R. Quintanilla-Cedillo, J. Ruiz-Esmenjaud, R. Arenas, 'Leishmaniasis: a review', *F1000Research* **2017**, *6*, 750.

[12] Y. Li, B. Du, X. Xu, D. Shi, S. Ji, 'A green and efficient synthesis of 13-aryl-5,7,12,14-tetrahydrodibenzo[*b,i*]xanthene-5,7,12,14(13*H*)-tetrone derivatives in ionic liquid', *Chin. J. Chem.* **2009**, *27*, 1563–1568.

[13] K. M. Choi, J. Gang, J. Yun, 'Anti-*Toxoplasma gondii* RH strain activity of herbal extracts used in traditional medicine', *Int. J. Antimicrob. Agents* **2008**, *32*, 360–362.

[14] I. Al Nasr, J. Jentzsch, I. Winter, R. Schobert, K. Ersfeld, W. S. Koko, A. H. Mujawah, T. A. Khan, B. Biersack, 'Antiparasitic activities of new lawsone Mannich bases', *Arch. Pharm. Chem. Life Sci.* **2019**, e1900128.

[15] E. Wirtz, S. Leal, C. Ochatt, G. A. Cross, 'A tightly regulated inducible expression system for conditional gene knock-outs and dominant-negative genetics in *Trypanosoma brucei*', *Mol. Biochem. Parasitol.* **1999**, *99*, 89–101.

[16] R. S. Twigg, 'Oxidation-reduction aspects of resazurin', *Nature* **1945**, *155*, 401–402.

[17] R. D. Fields, M. V. Lancaster, 'Dual-attribute continuous monitoring of cell proliferation/cytotoxicity', *Am. Biotechnol. Lab.* **1993**, *11*, 48–50.

[18] S. A. Ahmed, R. M. Gogal Jr., J. E. Walsh, 'A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes', *J. Immunol. Methods* **1994**, *170*, 211–224.

[19] S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, R. Pijnenborg, 'The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells', *Hum. Reprod.* **2007**, *22*, 1304–1309.

[20] W. S. Koko, M. A. Mesaik, S. Yousaf, M. Galal, M. I. Choudhary, 'In vitro immunomodulating properties of selected Sudanese medicinal plants', *J. Ethnopharmacol.* **2008**, *118*, 26–34.