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RESEARCH ARTICLE

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Antiparasitic activities of bimetallic *N*-heterocyclic carbene gold(I) complexes with ferrocene ligands: Relevance of chlorido and phosphino ligands

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Gold(I) complexes carrying imidazole-2-ylidene ligands and ferrocene substituents were prepared. Their activities against protozoal *Leishmania major* and *Toxoplasma gondii* parasites were analyzed. Certain gold(I) complexes with chlorido and 1,1′-bis(triphenylphosphino)ferrocene ligands revealed promising antiparasitic effects. The new chlorido complexes **5b** and **5c** showed high activities against *T. gondii* tachyzoites and *L. major* promastigotes while the new ferrocene-bridged bis-gold(I) complexes **8a** and **8b** were particularly active against *L. major* amastigotes and considerably selective as to toxicity results from Vero cells and macrophages.

KEYWORDS
antiparasitic drugs, ferrocene, gold, metal-based drugs, *N*-heterocyclic carbene

1 | INTRODUCTION

People who live in or visit (sub)tropical countries are at constant risk of infections with neglected tropical diseases (NTDs), and the current climate change will likely pave the way to new habitations for NTD parasites.[1] The search for efficient antiparasitic drugs is ongoing, in particular, the identification of antiparasitic compounds for the treatment of NTDs.[2] Several drugs based on metal compounds are already applied for the chemotherapy of human diseases, and the identification of new metal-based drugs is promising.[3] Auranofin, a simple gold complex, is being applied as a remedy for patients suffering from rheumatoid arthritis.[4] Antiparasitic gold complexes were also described.[5] Auranofin exhibited a dual mode of inhibition when bound to trypanothione reductase of the parasite *Leishmania infantum*.[6] A derivative of auranofin dubbed GoPI-sugar, a phosphole containing gold(I) complex bearing the carbohydrate ligand of auranofin, was found to be active against *Leishmania donovani* amastigotes.[7] Further, auranofin derivatives were

disclosed, which were active against cisplatin-resistant ovarian cancer.[8] Similarly, various heterobimetallic gold complexes containing additional platinum or iron centers were also able to overcome cisplatin-resistance.[9,10] Moreover, the number of anticancer and antiparasitic gold *N*-heterocyclic carbene (NHC) complexes is on the rise.[11] A simple chlorido-gold(I)-NHC complex showed high but unselective activity against *Trypanosoma cruzi* and *L. infantum* parasites as well as a moderate inhibition of the *Leishmania mexicana* cysteine protease CPB2.8dCTE.[12] A gold(I)-NHC complex with a caffeine derivative as NHC ligand exhibited considerable activities against *Leishmania amazonensis* and *Leishmania braziliensis* promastigotes without toxicity to macrophages.[13]

Our groups previously disclosed cationic gold(I)-NHC complexes with ferrocene-based ligands, which showed significant antitrypanosomal and parasite cytoskeleton damaging activities and a certain selectivity when compared with their toxicity against human cancer cells.[14] Such a high antiparasitic effect can be expected because ferrocene derivatives in their own right are known for their activities against various parasites.[15–17] In addition, heterocyclic compounds such as imidazoles showed antimicrobial and antiparasitic activities.[18,19] Various heterobimetallic ferrocene-based gold complexes were described, which displayed pronounced anticancer activities.[10,20] More recently, we also reported promising activities of heterobimetallic ferrocene-based gold(I)-NHC complexes against *Leishmania major* and *Toxoplasma gondii*.[21] We now evaluated the antiparasitic activity and its dependence on the ligand structure of a series of bimetallic ferrocene-based gold(I)-NHC complexes against the apicomplexan parasites *L. major* and *T. gondii* (Figure 1). Gornitzka et al. stated that neutral gold(I)-NHC complexes were more selective for *Plasmodium* parasites than cationic complexes.[22] Hence, a focus was set on the influence of the chlorido ligand in neutral complexes and phosphino ligands in cationic complexes on the antiparasitic activity of these gold complexes. The applied NHC ligands were based on the imidazole-bridged *cis*-stilbene motif. So we also investigated differences in terms of the used NHC ligands. Phenyl derivatives were compared with analogs featuring the biologically active nature-derived piperlongumine and combretastatin A4-type 3,4,5-trimethoxyphenyl/3-bromo-4,5-dimethoxyphenyl scaffolds.[23,24] The results are described in the following.

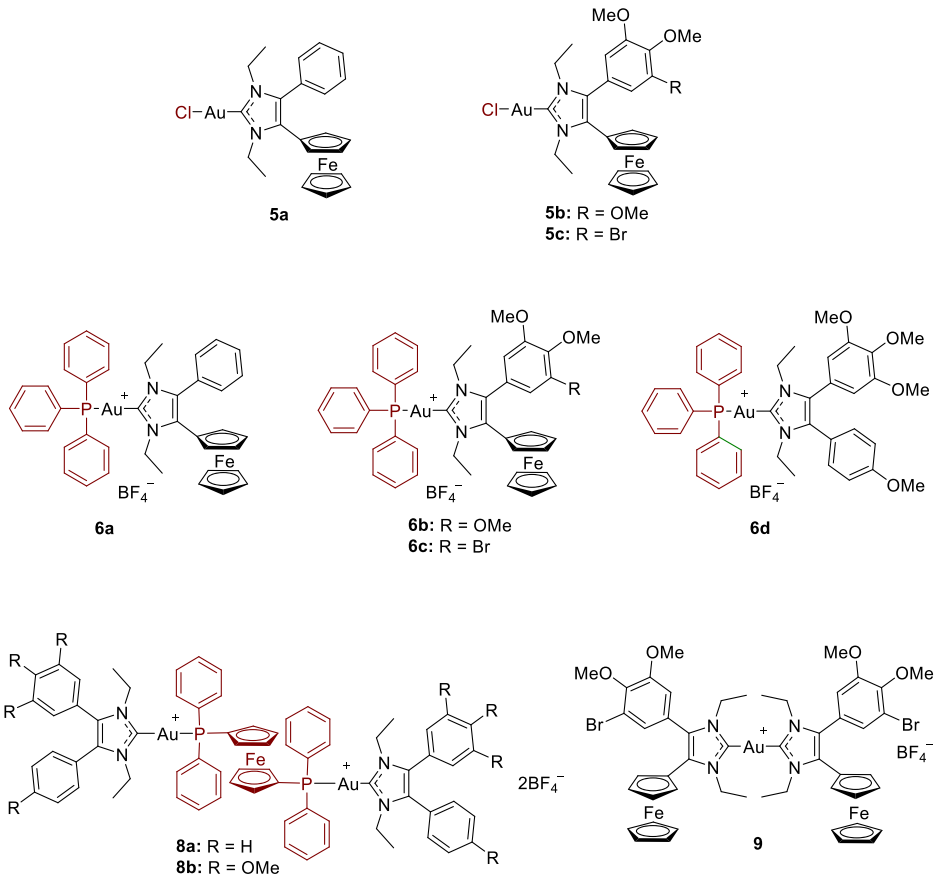


FIGURE 1 Structures of the bimetallic ferrocene-based NHC gold(I) complexes used in this study (chlorido and phosphino ligands are depicted in auburn color). The mononuclear complex **6d** was added to this study for comparison.

2 | EXPERIMENTAL SECTION

2.1 | General

Reagents and starting compounds were purchased from Alfa Aesar, Aldrich, and TCI. The known precursors, the TosMIC reagents **1a**–**1c**, the ferrocene derivatives **2a** and **2b**, the imidazolium salts **3a** and **3b**, and the known (imidazole-2-ylidene) complexes **4a**, **5a**, **6a**, **6d**, **7a**, and **7b** were synthesized following published procedures.[21,25–28] The synthesis of the new compounds **2c**, **3c**, **4b**, and **4c**, and the machines applied to obtain the mentioned analytical data can be found in the Supporting Information.

2.2 | Chemistry—Synthesis of gold complexes

2.2.1 | Chlorido-[1,3-diethyl-4-ferrocenyl-5-(3,4,5-trimethoxyphenyl)-imidazol-2-ylidene] gold(I) (**5b**)

A solution of **4b** (71 mg, 0.10 mmol) in CH_2Cl_2 was treated with AuCl (DMS) (30 mg, 0.10 mmol) and stirred at room temperature for 24 h in the dark. The mixture was filtered and the solvent was removed in vacuum. The residue was recrystallized from CH_2Cl_2/*n*-hexane. Yield: 55 mg (0.078 mmol, 78%); brown solid of mp 109–110°C; ν_{max} (ATR)/cm^{-1} 2971, 2937, 2869, 2834, 1581, 1508, 1462, 1411, 1375, 1346, 1327, 1286, 136, 1167, 1124, 1001, 886, 823, 782, 727, 674; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.30 (3 H, t, $J = 7.2$ Hz), 1.60 (3 H, t, $J = 7.2$ Hz), 3.82 (6 H, s), 3.91 (3 H, s), 4.0–4.1 (9 H, m), 4.2–4.3 (2 H, m), 4.6–4.7 (2 H, m), 6.46 (2 H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.2, 17.6, 44.2, 44.4, 56.4, 56.6, 61.1, 68.3, 69.0, 69.4, 69.6, 69.9, 72.7, 108.3, 123.9, 127.9, 129.7, 139.2, 153.6, 169.5; *m/z* (ESI, %) 712.15 (10) [M$^+$ − Cl + MeCN], 686.19 (100); Anal. Cald for $C_{26}H_{30}AuClFeN_2O_3$ (%): C, 44.18; H, 4.28; N, 3.96. Found (%): C, 44.33; H, 4.22; N, 3.90.

2.2.2 | Chlorido-[1,3-diethyl-4-(3-bromo-4,5-dimethoxyphenyl)-5-ferrocenylimidazol-2-ylidene]gold(I) (**5c**)

Analogously to the synthesis of **5b**, complex **5c** was obtained from **4c** (88 mg, 0.116 mmol) was dissolved and AuCl (DMS) (34 mg, 0.116 mmol) in CH_2Cl_2. Yield: 60 mg (0.079 mmol, 68%); brown solid of mp 190–192°C (dec.); ν_{max} (ATR)/cm^{-1} 3087, 2968, 2931, 2868, 2834, 1585, 1550, 1495, 1462, 1413, 1396, 1348, 1311, 1262, 1236, 1185, 1157, 1106, 1075, 1036, 999, 892, 840, 828, 815, 781, 753, 730, 700, 667; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (3 H, t, $J = 7.2$ Hz), 1.59 (3 H, t, $J = 7.2$ Hz), 3.80 (6 H, s), 3.92 (3 H, s), 4.0–4.1 (9 H, m), 4.2–4.3 (2 H, m), 4.62 (2 H, q, $J = 7.2$ Hz), 6.69 (1 H, s), 7.12 (1 H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.1, 17.7, 44.2, 44.4, 56.4, 60.9, 68.4, 69.2, 69.4, 69.6, 69.8, 72.4, 114.4, 118.2, 124.0, 125.6, 126.8, 127.3, 128.2, 128.4, 147.7, 153.9, 169.9; *m/z* (ESI, %) 555.0 (50), 523.1 (100); Anal. Cald for $C_{25}H_{27}AuBrClFeN_2O_2$ (%): C, 39.74; H, 3.60; N, 3.71. Found (%): C, 39.68; H, 3.53; N, 3.66.

2.2.3 | Triphenylphosphino-[1,3-diethyl-4-ferrocenyl-5-(3,4,5-trimethoxyphenyl)-imidazol-2-ylidene]gold(I) tetrafluoroborate (**6b**)

A solution of **5b** (21 mg, 0.03 mmol) in acetone (10 ml) was treated with $NaBF_4$ (7 mg, 0.058 mmol) and PPh_3 (11 mg, 0.040 mmol), and stirred at room temperature for 24 h. The suspension was filtered and the solvent of the filtrate was removed in vacuum. The residue was recrystallized from CH_2Cl_2/*n*-hexane. Yield: 26 mg (0.026 mmol, 85%); amber solid of m.p. 120–121°C (dec.); ν_{max} (ATR)/cm^{-1} 2967, 2939, 2831, 1582, 1509, 1465, 1437, 1413, 1328, 1238, 1125, 1098, 1048, 1032, 1000, 915, 886, 838, 748, 728, 712, 694; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (3 H, t, $J = 7.2$ Hz), 1.67 (3 H, t, $J = 7.2$ Hz), 3.83 (6 H, s), 3.91 (3 H, s), 4.0–4.2 (9 H, m), 4.2–4.3 (2 H, m), 4.69 (2 H, q, $J = 7.2$ Hz), 6.52 (2 H, s), 7.4–7.6 (15 H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.7, 18.4, 44.3, 44.6, 56.5, 61.0, 68.5, 69.1, 69.2, 69.5, 69.6, 69.7, 72.0, 108.3, 123.3, 128.5, 129.2, 129.3, 129.6, 129.7, 130.8, 132.1, 133.9, 134.1, 139.2, 153.7, 184.5; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 38.6; ^{11}B NMR (96.3 MHz, $CDCl_3$) δ −0.87; *m/z* (ESI, %) 933.2 (10) [M$^+$], 554.2 (10), 415.2 (100), 399.1 (20); Anal. Cald for $C_{44}H_{45}AuBF_4FeN_2O_3P$ (%): C, 51.79; H, 4.45; N, 2.75. Found (%): C, 51.87; H, 4.52; N, 2.80.

2.2.4 | Triphenylphosphino-[1,3-diethyl-4-(3-bromo-4,5-dimethoxyphenyl)-5-ferrocenylimidazol-2-ylidene]gold (I) tetrafluoroborate (**6c**)

Analogously to the synthesis of **6b**, complex **6c** was obtained from **5c** (62 mg, 0.08 mmol), $NaBF_4$ (17 mg, 0.154 mmol) and PPh_3 (28 mg, 0.106 mmol) in acetone (10 ml). Yield: 80 mg (0.075 mmol, 94%); amber solid of m.p. >150°C (dec.); ν_{max} (ATR)/cm^{-1} 2974, 2940, 1587, 1555, 1496, 1465, 1437, 1413, 1345, 1311, 1266, 1237,

1021, 997, 888, 817, 780, 747, 711, 693; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (3 H, t, $J = 7.2$ Hz), 1.64 (3 H, t, $J = 7.2$ Hz), 3.90 (6 H, s), 3.93 (3 H, s), 4.0–4.1 (9 H, m), 4.2–4.3 (2 H, m), 4.67 (2 H, q, $J = 7.2$ Hz), 7.00 (1 H, s), 7.04 (1 H, s), 7.3–7.5 (15 H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.6, 18.3, 44.3, 44.7, 56.6, 56.7, 60.8, 68.5, 69.3, 69.5, 69.7, 71.9, 114.6, 114.9, 117.7, 125.1, 126.9, 127.1, 128.1, 128.8, 129.3, 129.6, 129.7, 129.8, 132.3, 134.0, 134.1, 147.7, 154.1, 154.3, 184.4; ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 40.4; ^{11}B NMR (96.3 MHz, $CDCl_3$) δ −0.89; *m/z* (ESI, %) 983.8 (35) [M^+], 980.8 (80) [M^+], 721.0 (100), 523 (27); Anal. Cald for $C_{43}H_{42}AuBBrF_4FeN_2O_2P$ (%): C, 48.30; H, 3.96; N, 2.62. Found (%): C, 48.41; H, 4.01; N, 2.69.

2.2.5 | Ferrocenyl-1,1′-bis [diphenylphosphino-(1,3-diethyl-4,5-diphenylimidazol-2-ylidene)gold(I)] bis-tetrafluoroborate (**8a**)

A solution of **7a** (56 mg, 0.11 mmol) in acetone (10 ml) was treated with $NaBF_4$ (23 mg) and 1,1′-bis(diphenylphosphino)ferrocene (36 mg, 0.063 mmol) and stirred at room temperature for 1.5 h. The suspension was filtered and the solvent of the filtrate was removed in vacuum. The residue was recrystallized from acetone/*n*-hexane. Yield: 90 mg (0.054 mmol, 86%); yellow solid of mp 148°C (dec.); ν_{max}/cm^{-1}: 3055, 2975, 1483, 1463, 1437, 1413, 1380, 1345, 1311, 1049, 1022, 926, 820, 750, 696; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.3–1.5 (12 H, m), 4.2–4.4 (15 H, m), 4.9–5.0 (2 H, m), 7.2–7.6 (40 H, m); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 17.4, 17.5, 44.3, 44.7, 75.0, 75.1, 127.2, 127.4, 128.9, 129.3, 129.4, 129.5, 129.6, 130.4, 130.6, 131.8, 132.0, 132.2, 133.3, 133.5, 182.6; ^{31}P NMR (121.5 MHz, $CDCl_3$): δ 36.0; ^{11}B NMR (96.3 MHz, $CDCl_3$): δ −0.80; *m/z* (ESI, %) 1026.2 (15) [M – imidazol-2-ylidene-Au]$^+$, 749.6 (100), 338.2 (20), 277.1 (40); Anal. Cald for $C_{72}H_{68}Au_2B_2F_8FeN_4P_2$ (%): C, 51.64; H, 4.09; N, 3.35. Found (%): C, 51.74; H, 4.14; N, 3.39.

2.2.6 | Ferrocenyl-1,1′-bis {diphenylphosphino-[1,3-diethyl-4-anisyl-5-(3,4,5-trimethoxyphenyl)imidazol-2-ylidene] gold(I)} bis-tetrafluoroborate (**8b**)

Analogous to the synthesis of **8a**, complex **8b** was obtained from **7b** (70 mg, 0.11 mmol), $NaBF_4$ (23 mg) and 1,1′-bis(diphenylphosphino)ferrocene (36 mg, 0.063 mmol) in acetone (10 ml). Yield: 112 mg (0.058 mmol, 92%); yellow solid of mp 199°C; ν_{max}/cm^{-1}: 2936, 2833, 1605, 1581, 1516, 1504, 1463, 1436, 1414, 1351, 1330, 1291, 1248, 1177, 1123, 1049, 1022, 886, 837, 808, 745, 694; ^{1}H NMR (300 MHz, Acetone-d_6): δ 1.3–1.5 (12 H, m), 3.73 (6 H, s), 3.77 (6 H, s), 3.78 (6 H, s), 3.83 (6 H, s), 4.3–4.5 (8 H, m), 4.5–4.6 (4 H, m), 4.9–5.0 (4 H, m), 6.75 (4 H, s), 7.01 (4 H, d, $J = 8.9$ Hz), 7.4–7.7 (24 H, m); ^{13}C NMR (75.5 MHz, Acetone-d_6): δ 17.8, 18.0, 18.1, 18.2, 44.9, 45.1, 45.3, 45.5, 55.8, 56.7, 60.7, 74.7, 75.9, 109.3, 109.5, 115.2, 115.3, 120.3, 120.6, 123.6, 123.9, 130.3, 132.5, 132.8, 132.9, 133.2, 133.3, 133.4, 134.4, 139.9, 140.0, 154.5, 161.5, 161.6, 182.9; ^{31}P NMR (121.5 MHz, Acetone-d_6): δ 36.3; ^{11}B NMR (96.3 MHz, Acetone-d_6): δ −0.53; *m/z* (ESI, %) 1147.0 (15) [M – imidazol-2-ylidene-Au]$^+$, 1145.5 (35), 987.8 (95), 868.9 (98), 750.0 (65), 397.0 (100); Anal. Cald for $C_{80}H_{84}Au_2B_2F_8FeN_4O_8P_2$ (%): C, 50.18; H, 4.42; N, 2.93. Found (%): C, 50.23; H, 4.37; N, 2.88.

2.2.7 | Bis-[1,3-diethyl-4-(3-bromo-4,5-trimethoxyphenyl)-5-ferrocenyl-imidazol-2-ylidene]gold(I) (**9**)

A solution of **3c** (113 mg, 0.17 mmol) in acetone (15 ml) was treated with $NaBF_4$ (29 mg, 0.27 mmol) and stirred at room temperature for 24 h. After filtration over $MgSO_4$, the solvent was removed from the filtrate and the residue was dried in vacuum. The crude product was dissolved in CH_2Cl_2/MeOH (1:1, 30 ml) and Ag_2O (42 mg, 0.18 mmol) was added. After stirring at room temperature for 5 h, Au (DMS)Cl (25 mg, 0.09 mmol) was added. The mixture was stirred at room temperature for 24 h, filtered, and the filtrate was concentrated in vacuum. The residue was redissolved in CH_2Cl_2 and filtered over $MgSO_4$/celite. The filtrate was concentrated and the residue was dried in vacuum. Yield: 116 mg (0.09 mmol, 53%); brown solid of mp >110°C (dec.); ν_{max} (ATR)/cm^{-1} 2967, 2933, 2876, 2855, 1587, 1554, 1495, 1463, 1411, 1345, 1312, 1264, 1237, 1185, 1156, 1030, 998, 887, 857, 816, 780, 692, 667; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (6 H, t, $J = 7.2$ Hz), 1.68 (6 H, t, $J = 7.2$ Hz), 3.86 (6 H, s), 3.92 (6 H, s), 4.0–4.1 (18 H, m), 4.2–4.3 (4 H, m), 4.68 (4 H, q, $J = 7.2$ Hz), 6.83 (2 H, s), 7.08 (2 H, s); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.5, 18.1, 44.1, 44.4, 56.4, 56.5, 60.9, 68.4, 69.2, 69.4, 69.5, 72.1, 114.6, 118.0, 125.3, 127.0, 129.0, 129.3, 147.7, 154.1, 183.0; *m/z* (ESI, %) 1242.1 (100) [M^+], 527.1 (80); Anal. Cald for $C_{50}H_{54}AuBBr_2F_4Fe_2N_4O_4$ (%): C, 45.14; H, 4.09; N, 4.21. Found (%): C, 45.22; H, 4.15; N, 4.31.

2.3 | Evaluation of gold complexes against *T. gondii*

Vero cells (ATCC® CCL81™, USA) after serial passages were used for the cultivation of *T. gondii* tachyzoites

(obtained from Dr. Saeed El-Ashram, State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China). The cells were cultivated in complete RPMI 1640 supplied with 10% FBS in a humidified 5% CO_2 atmosphere at 37°C (in 96-well plates with 5×10^3 cells/well in volumes of 200 µl). After 24 h, cells were inoculated with parasite tachyzoites in a ratio of 5:1. After 5 h, gold complexes were added with different concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg ml^{-1}) as described previously.[29] Atovaquone (ATO) was applied as positive control. After 72 h, an inverted microscope was used for identifying cell viability via following equation:

$$\text{Inhibition}(\%) = (1 - \text{infected cells of experimental}/\text{infected cells of control}) \times 100.$$

Effects of test compounds on parasite growth were expressed as IC_{50} values (concentration that caused a 50% reduction in viable cells) based on three independent experiments.

2.4 | Evaluation of gold complexes against *L. major* cell

As reported previously, *L. major* promastigotes isolated from a Saudi person (February 2016), and *L. major* amastigotes isolated from infected BALB/c mice, were cultured and used for compound testing.[30,31] BALB/c mice were received from the Pharmaceutical College of the King Saud University, KSA, and kept according to the ethical guidelines of the committee of research ethics, Deanship of Scientific Research, Qassim University, permission number 20–03-20. Both forms of *L. major* amastigotes and promastigotes were cultivated in 96 well plates by using RPMI 1640 with 10% fetal bovine serum (FBS) at 26°C in a humidified 5% CO_2 atmosphere. For assessing the activity against promastigotes (2×10^5/well), different concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg ml^{-1}) of gold complexes were added to each well (in a final volume of 200 µl/well) followed by incubation for 72 h.

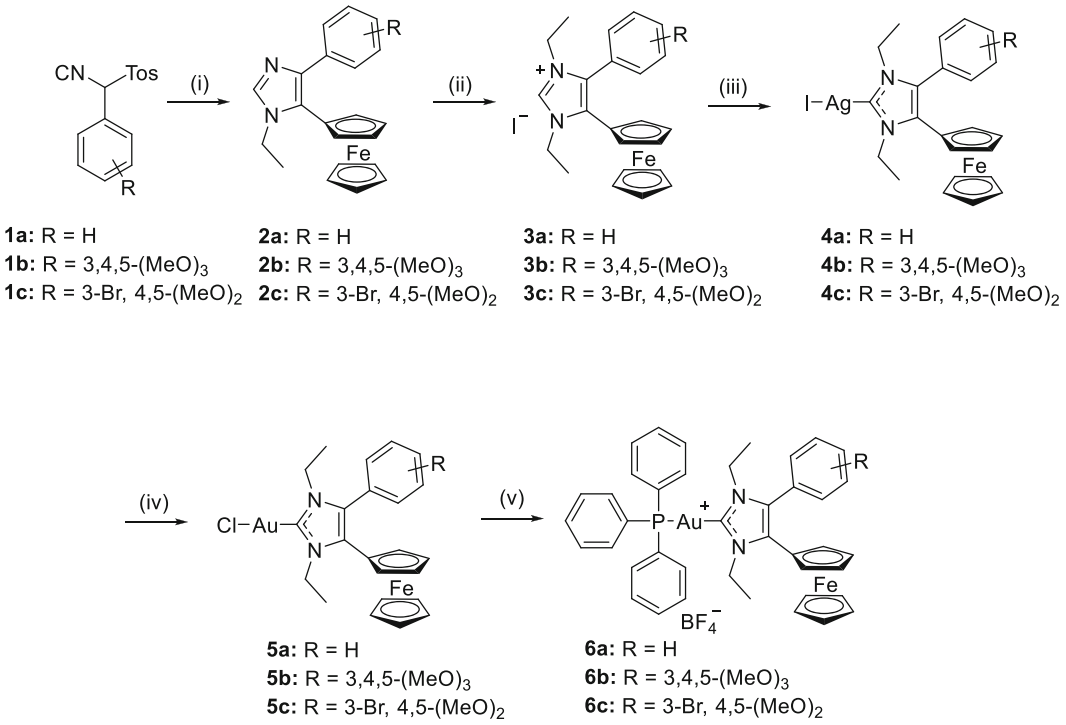
In addition, mouse macrophage cells were used as host cells for the development of amastigotes and the assessment of the activity of the gold complexes against amastigotes. The cells were cultivated in complete RPMI 1640 supplied with 10% FBS at 37°C in a humidified 5% CO_2 atmosphere (in 96-well plates with 5×10^3 cells/well in volumes of 200 µl). After incubation for 24 h, the cells were inoculated with the parasites in a ratio of 10:1. After 5 h, gold complexes were added with different concentrations (50, 25, 12.5, 6.25, 3.13, 1.65, and 0.75 µg ml^{-1}) followed by incubation for 72 h. Amphotericin B (AmB) was applied as positive control. For the detection of viable parasites, MTT (tetrazolium salt of (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide)) was added and evaluated spectrophotometry by ELISA reader (spectrophotometer) at 570 nm. Effects of test compounds on parasite growth were expressed as IC_{50} values (concentration that caused a 50% reduction in viable cells) based on three independent experiments.

2.5 | Toxicity evaluation of gold complexes by MTT assay

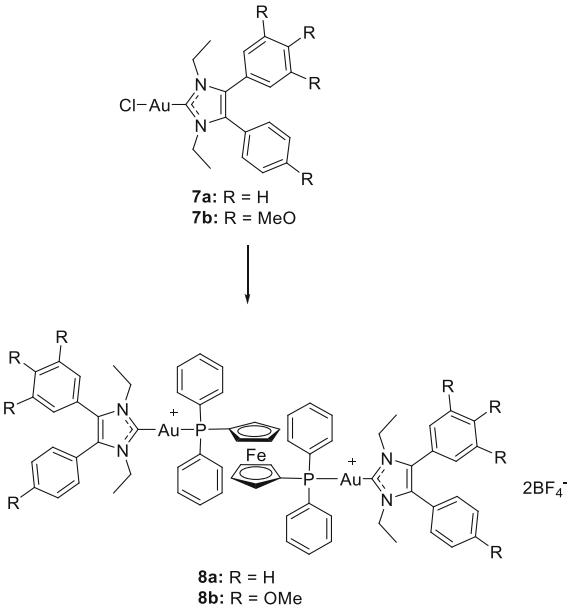
Vero cells and macrophages were cultured and applied for MTT tests of the gold complexes as described previously.[31,32] In order to assess the cytotoxicity of the gold complexes at different concentrations (100, 33, 11, 3.7, 1.2, 0.4, 0.14 and 0.04 µg ml^{-1}), 96-well plates were used for the incubation of the Vero cells (5×10^3 cells/well/200 µl) in RPMI 1640 medium supplemented with 10% FBS at 37°C and 5% CO_2. After incubation with the gold complexes for 72 h, the supernatant was removed and 0.05 ml of RPMI 1640 medium containing MTT (5 mg ml^{-1}) was added to each well followed by incubation for 4 h. Thereupon, the supernatant was removed and 0.1 ml DMSO was added to each well. After 15 min with occasional shaking, the colorimetric analysis ($\lambda = 540$ nm) of the samples using a FLUOstar OPTIMA spectrophotometer was carried out. IC_{50} values were used to express the cytotoxic effects of the test compounds (concentration that caused a 50% reduction in viable cells). Obtained IC_{50} values resulted from three independent experiments.

3 | RESULTS AND DISCUSSION

Complexes **5a–c** and **6a–c** including the new complexes **5b**, **5c**, **6b**, and **6c** were prepared as described before (Scheme 1).[21,26] The reaction of ferrocenecarboxaldehyde with ethyl amine followed by treatment with the TosMIC reagents **1a–c**, respectively, formed the *N*-ethyl-imidazoles **2a–c** in good yields. After alkylation with ethyl iodide, the iodide salts **3a–c** were converted to the silver complexes **4a–c** by treatment with Ag_2O. Finally, transmetalation with Au (DMS)Cl generated the gold complexes **5a–c** in good yields. The phosphino complexes **6a–c** were obtained from **5a–c** upon reaction with PPh_3 and $NaBF_4$. Analogously to the synthesis of complexes **6a–c**, the new bis-gold(I)-NHC complexes **8a** and **8b** were obtained from the reaction of



SCHEME 1 Reagents and conditions: (i) Ferrocenecarboxaldehyde, 2-M $EtNH_2$/THF, AcOH, EtOH, reflux, 1 h, then K_2CO_3, EtOH, reflux, 2 h, 55%; (ii) EtI, MeCN, 85°C, 24 h, 100%; (iii) Ag_2O, CH_2Cl_2, rt, 24 h, 89%; (iv) Au (DMS)Cl, CH_2Cl_2, rt, 24 h, 68–78%; (v) PPh_3, $NaBF_4$, acetone, rt, 24 h, 85–94%

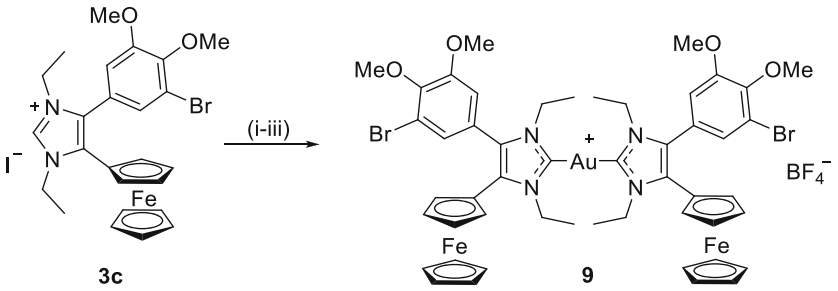


SCHEME 2 Reagents and conditions: 1,1′-bis (triphenylphosphino)ferrocene, $NaBF_4$, acetone, rt, 1.5 h, 86–92%

the known chlorido-gold(I) complexes **7a** and **7b** with 1,1′-bis(triphenylphosphino)ferrocene and $NaBF_4$ (Scheme 2).[27,28] For comparison, the new 3-bromo-4,5-dimethoxyphenylimidazolylidene-based bis-carbene complex **9** was prepared from **3c** (Scheme 3).

At first, the complexes **5a–c**, **6a**, **6c**, **6d**, **8a**, **8b**, and **9** (Figure 1) were evaluated for their activity against *T. gondii* tachyzoites (Table 1). The chlorido complex **5b** exhibited a high activity ($IC_{50} = 0.11$ μM) accompanied by a considerable selectivity (SI = 11.9). Only known bis-carbene derivatives showed comparable activities against and selectivities for *T. gondii* ($IC_{50} = 0.013–0.046$ μM, SI = 14.4–28.1), which were distinctly more active than their new bromo-substituted bis-carbene analog **9**.[21] In contrast, the phosphino complexes **6a–d** and **8a–b** displayed lower activities and selectivities here. The triphenylphosphino complexes **6a** and **6c** showed similar activities, however, **6c** was less toxic to Vero cells than **6a**. The non-ferrocene derivative **6d** exhibited activities and toxicities similar to **6c**. Only **6b** was much less active against *T. gondii* and Vero cells, even less active than **5a**. The ferrocene ring obviously does not play a considerable role for the biological activities of these triphenylphosphino complexes. However, the distinct activity differences between the couples of **5a** and **6a** on the one hand, and **5b** and **6b** on the other hand, are remarkable, shedding light on the interplay between the NHC ligand and the chlorido or triphenylphosphino ligands. While chlorido complex **5a** is less active than the

SCHEME 3 Reagents and conditions: (i) $NaBF_4$, acetone, rt, 24 h, 100%; (ii) Ag_2O, CH_2Cl_2/MeOH (1:1), rt, 5 h; (iii) Au (DMS)Cl, CH_2Cl_2/MeOH (1:1), rt, 24 h, 53%



3c 9

TABLE 1 IC_{50} (inhibitory concentrations in μM) of test compounds when applied to cells of the Vero cell line (African green monkey kidney epithelial) and *Toxoplasma gondii* cells

Compd.	IC_{50} (*T. gondii*)	IC_{50} (Vero)	SI (Vero/*T. gondii*)[a]
5a	1.46 ± 0.32	2.11 ± 0.33	1.44
5b	0.11 ± 0.03	1.34 ± 0.28	11.9
5c	0.46 ± 0.06	1.56 ± 0.31	3.37
6a	0.75 ± 0.09	0.42 ± 0.05	0.56
6b	6.08 ± 0.88	7.74 ± 1.02	1.27
6c	0.75 ± 0.10	1.61 ± 0.21	2.15
6d	0.50 ± 0.06	2.10 ± 0.28	4.16
8a	0.24 ± 0.04	0.48 ± 0.07	2.01
8b	0.63 ± 0.07	1.18 ± 0.30	1.89
9	0.45 ± 0.06	0.40 ± 0.07	0.88

Note: IC_{50} values are means of at least three experiments (± SD), derived from concentration–response curves obtained from the percentage of vital cells in relation to untreated controls after 72 h.
[a]Selectivity index (SI) calculated from the corresponding IC_{50} values for the Vero cells and the IC_{50} values against *T. gondii*.

TABLE 2 IC_{50} (inhibitory concentrations in μM) of indicated complexes in *Leishmania major* promastigotes and amastigotes

Compd.	IC_{50} (*L. major* promastigotes)	IC_{50} (*L. major* amastigotes)	SI (Vero/promastigotes)[a]	SI (Vero/amastigotes)[a]
5a	0.16 ± 0.02	1.78 ± 0.29	13	1.18
5b	0.040 ± 0.01	0.21 ± 0.03	33.9	6.3
5c	0.022 ± 0.00	1.06 ± 0.17	69.4	1.48
6a	0.32 ± 0.05	1.40 ± 0.22	1.3	0.3
6b	0.098 ± 0.02	3.72 ± 0.54	79.0	2.08
6c	2.52 ± 0.42	2.43 ± 0.38	0.64	0.66
6d	0.032 ± 0.01	0.50 ± 0.08	65.6	4.16
8a	0.11 ± 0.03	<0.12	4.36	>4.0
8b	0.30 ± 0.04	<0.10	3.90	>11.8
9	0.23 ± 0.04	0.98 ± 0.11	1.77	0.41

Note: IC_{50} values are means of at least three experiments (± SD), derived from concentration–response curves obtained from the percentage of vital cells in relation to untreated controls after 72 h.
[a]Selectivity index (SI) calculated from the corresponding IC_{50} values for Vero cells and *L. major*.

triphenylphosphino complex **6a**, which is its analog in terms of the NHC ligand, the chlorido complex **5b** is much more active than its NHC-analogous triphenylphosphino complex **6b**.

Finally, the complexes were tested for their activity against *L. major* promastigotes and amastigotes (Table 2). Except for **6c**, all complexes showed considerable activities against *L. major* promastigotes. The chlorido

complexes **5b** and **5c** were especially active ($IC_{50} = 0.022–0.04$ μM) against the promastigotes, and considerably selective for these parasite cells (SI = 33.9–69.4). In terms of antiamastigote activity, the bis-gold (I) complexes **8a** and **8b** as well as the chlorido complex **5b** exhibited high activities with IC_{50} values below 0.21 μM. The observed activities against the amastigotes are of high relevance because clinically applied drugs against leishmaniasis showed strong effects on amastigotes, while other promising antileishmanials also displayed a higher efficacy in *L. major* amastigotes when compared with their effects on promastigotes.[33,34] **5c** was less active than **5b** against the amastigotes indicating an activity reducing effect of the bromo substituent here. This effect was also observed for the other bromo-substituted complexes **6c** and **9**. While **6c** was less active against promastigotes and amastigotes, bis-carbene complex **9** showed slightly higher activity against promastigotes ($IC_{50} = 0.23$ μM) than previously published close bis-carbene analogs ($IC_{50} = 0.37–0.42$ μM) again, but it was less active against the amastigotes.[21] Interestingly, the 3,4,5-trimethoxyphenyl derivative **6b** showed high activity against promastigotes, which is in stark contrast to its low activities against amastigotes, Vero cells, and *T. gondii* parasites. This distinct selectivity for *L. major* promastigotes may have specific reasons, which remain to be elucidated. The replacement of a *meta*-methoxy group by a bromine atom in analogous gold(I) biscarbene complexes reportedly led to slight differences in activity in certain cancer cells as well as in *T. gondii* and *L. major* parasites, too.[21,25] When compared with the bis-gold (I) complexes **8a** and **8b**, the bis-carbene complex **9** exhibited an activity against the promastigotes, which was comparable with the activities of complexes **8a** and **8b**. However, complex **9** was distinctly less active than **8a** and **8b** against the amastigotes, which is not unexpected given its relatively low activity against amastigotes when compared with other closely related bis-carbene complexes published recently by our groups.[21]

The *L. major* amastigotes are intracellular parasite forms, which live in host cells such as macrophages. Thus, the toxicity of the test compounds to macrophages was also studied in order to identify drug selectivities for the amastigotes when compared with macrophages (Table 3). The complexes **5a**, **6a–d**, and **9** were only slightly less toxic to the macrophages than to the amastigotes. In contrast to that, complexes **8a** and **8b** revealed much higher selectivities. In particular, complex **8a** showed an SI value of >10; that is, this compound is at least more than 10 times more active against amastigotes than against macrophages.

TABLE 3 IC_{50} (inhibitory concentrations in μM) of complexes when applied to macrophages

Compd.	IC_{50} macrophages[a]	SI macrophages/amastigotes[b]
5a	2.76	1.55
6a	1.61	1.15
6b	4.70	1.26
6c	4.21	1.73
6d	1.17	2.34
8a	1.25	>10.4
8b	0.68	6.8
9	1.28	1.31

[a]Means of three experiments (SD ± 15%), obtained from concentration–response curves of the percentage of treated cells in relation to untreated controls after 72 h.
[b]Selectivity index (SI) from the corresponding IC_{50} values for the macrophages and amastigotes.

4 | CONCLUSIONS

Encouraging results were obtained from the investigation of the efficacy of a series of chlorido and phosphino NHC gold(I) complexes in pathogenic protozoal *T. gondii* and *L. major* parasites, although there is no clear evidence that the ferrocene rings of the active molecules contribute significantly to the antiparasitic activity. The new chlorido complexes **5b** and **5c** exhibited high activities against *T. gondii* tachyzoites and *L. major* promastigotes, while the new 1,1′-bis(triphenylphosphino)ferrocene-bridged bis-gold(I) complexes **8a** and **8b** were particularly active against *L. major* amastigotes. This effect is typical of clinically applied antileishmanial drugs and of other currently investigated drug candidates, which underlines the relevance of the activities of complexes **8a** and **8b** against these parasite cells. The toxicity of **8a** and **8b** to macrophages was relatively low indicating a good selectivity profile of these compounds. Mechanistic studies are necessary to reveal the reason for these interesting differences. Possible modes of action may include the formation of reactive oxygen species and the interaction with cysteine residues of relevant antiprotozoal drug targets. The combination of the most active gold complexes, in particular, of compounds **8a** and **8b**, with clinically applied drugs such as amphotericin B, miltefosine, or pentamidine appears promising in terms of a reduction of working doses and possible side-effects.

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AUTHOR CONTRIBUTIONS

Ibrahim S. Al Nasr: Conceptualization; funding acquisition; investigation; methodology; project administration; resources; validation. **Markus Weise:** Investigation. **Waleed S. Koko:** Conceptualization; funding acquisition; investigation; methodology; supervision. **Tariq A. Khan:** Investigation; methodology; validation. **Rainer Schobert:** Conceptualization; funding acquisition; supervision; writing-review and editing. **Bernhard Biersack:** Conceptualization; investigation; methodology; project administration; resources; supervision; validation; writing-original draft.

DATA AVAILABILITY STATEMENT

Original data can be obtained from the authors upon request.

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REFERENCES

[1] A. K. Mitra, A. R. Mason, *Trop. Med. Infect. Dis.* **2017**, *2*, 36.
[2] P. M. Cheuka, G. Mayoka, P. Mutai, K. Chibale, *Molecules* **2017**, *22*, 58.
[3] M. G. Ferraro, M. Piccolo, G. Misso, R. Santamaria, C. Irace, *Pharmaceutics* **2022**, *14*, 954.
[4] M. Yamashita, *Int. Immunopharmacol.* **2021**, *101*, 108272.
[5] S. Medici, M. PEana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, M. A. Zoroddu, *Coord. Chem. Rev.* **2015**, *284*, 329.
[6] A. Ilari, P. Baiocco, L. Messori, A. Fiorillo, A. Boffi, M. Gramiccia, T. di Muccio, G. Colotti, *Amino Acids* **2012**, *42*, 803.
[7] L. Feng, S. Pomel, P. L. de Late, A. Taravaud, P. M. Loiseau, L. Maes, F. Cho-Ngwa, C. A. Bulman, C. Fischer, J. A. Sakanari, P. D. Ziniel, D. L. Williams, E. Davioud-Charvet, *Molecules* **2020**, *25*, 5075.
[8] I. Landini, L. Massai, D. Cirri, T. Gamberi, P. Paoli, L. Messori, E. Mini, S. Nobili, *J. Inorg. Biochem.* **2020**, *208*, 111079.
[9] M. Serratrice, L. Maiore, A. Zucca, S. Stoccoro, I. Landini, E. Mini, L. Massai, G. Ferraro, A. Merlino, L. Messori, M. A. Cinellu, *Dalton Trans.* **2016**, *45*, 579.
[10] N. Lease, V. Vasilevski, M. Carreira, A. de Almeida, M. Sanaú, P. Hirva, A. Casini, M. Contel, *J. Med. Chem.* **2013**, *56*, 5806.
[11] M. Mora, M. C. Gimeno, R. Visbal, *Chem. Soc. Rev.* **2019**, *48*, 447.
[12] L. Massai, L. Messori, N. Micale, T. Schirmeister, L. Maes, D. Fregona, M. A. Cinellu, C. Gabbiani, *Biometals* **2017**, *30*, 313.
[13] K. Minori, L. B. Rosa, R. Bonsignore, A. Casini, D. C. Miguel, *ChemMedChem* **2020**, *15*, 2146.
[14] I. Winter, J. Lockhauserbäumer, G. Lallinger-Kube, R. Schobert, K. Ersfeld, B. Biersack, *Mol. Biochem. Parasitol.* **2017**, *214*, 112.
[15] K. Heinze, H. Lang, *Organometallics* **2013**, *32*, 5623.
[16] M. Patra, G. Gasser, *Nat. Rev. Chem.* **2017**, *1*, 0066.
[17] B. S. Ludwig, J. D. G. Correia, F. E. Kühn, *Coord. Chem. Rev.* **2019**, *396*, 22.
[18] O. S. Adeyemi, A. O. Eseola, W. Plass, O. Atolani, T. Sugi, Y. Han, G. E. Batiha, K. Kato, O. J. Awakan, T. D. Olaolu, C. O. Nwonuma, O. Alejolowo, A. Owolabi, D. Rotimi, O. T. Kayode, *Parasitol. Res.* **2020**, *119*, 1925.
[19] L. Zhang, X.-M. Peng, G. L. V. Damu, R.-X. Geng, C.-H. Zhou, *Med. Res. Rev.* **2014**, *34*, 340.
[20] M. C. Gimeno, H. Goitia, A. Laguna, M. E. Luque, M. D. Villacampa, C. Sepúlveda, M. Meireles, *J. Inorg. Biochem.* **2011**, *105*, 1373.
[21] W. S. Koko, J. Jentzsch, H. Kalie, R. Schobert, K. Ersfeld, I. S. Al Nasr, T. A. Khan, B. Biersack, *Arch. Pharm.* **2020**, *353*, e1900363.
[22] C. Hemmert, A. P. Ramadani, L. Boselli, A. F. Álvarez, L. Paloque, J.-M. Augereau, H. Gornetzka, F. Benoit-Vical, *Bioorg. Med. Chem.* **2016**, *24*, 3075.
[23] J. F. Peixoto, Y. J. Ramos, D. de Lima Moreira, C. R. Alves, L. F. Goncalves-Oliveira, *Parasitol. Res.* **2021**, *120*, 2731.
[24] R. Schobert, B. Biersack, A. Dietrich, K. Effenberger, S. Knauer, T. Müller, *J. Med. Chem.* **2010**, *53*, 6595.
[25] J. K. Muenzner, B. Biersack, H. Kalie, I. C. Andronache, L. Kaps, D. Schuppan, F. Sasse, R. Schobert, *ChemMedChem* **2014**, *9*, 1195.
[26] J. K. Muenzner, B. Biersack, A. Albrecht, T. Rehm, U. Lacher, W. Milius, A. Casini, J.-J. Zhang, I. Ott, V. Brabec, O. Stuchlikova, I. C. Andronache, L. Kaps, D. Schuppan, R. Schobert, *Chem. A Eur. J.* **2016**, *22*, 18953.
[27] S. I. Bär, M. Gold, S. W. Schleser, T. Rehm, A. Bär, L. Köhler, L. R. Carnell, B. Biersack, R. Schobert, *Chem. A Eur. J.* **2021**, *27*, 5003.
[28] W. Liu, K. Bensdorf, M. Proetto, U. Abram, A. Hagenbach, R. Gust, *J. Med. Chem.* **2011**, *54*, 8605.
[29] H. Jelali, I. Al Nasr, W. Koko, T. Khan, E. Deniau, M. Sauthier, N. Hamdi, J. Heterocycl, *Chem* **2021**. https://doi.org/10.1002/jhet.4396
[30] E. Osorio, G. Arango, N. Jiménez, F. Alzate, G. Ruiz, D. Gutiérrez, S. Robledo, *J. Ethnopharmacol.* **2007**, *111*, 630.
[31] I. Al Nasr, R. Hanachi, R. Said, S. Rahali, B. Tangour, S. I. Abdelwahab, A. Farasani, M. M. E. Taha, A. Bidwai, W. S. Koko, T. A. Khan, R. Schobert, B. Biersack, *Bioorg. Chem.* **2021**, *114*, 105099.
[32] W. S. Koko, M. A. Mesaik, S. Yousaf, M. Galal, M. I. Choudhary, *J. Ethnopharmacol.* **2008**, *118*, 26.
[33] E. Calvo-Álvarez, N. A. Guerrero, R. Álvarez-Velilla, C. F. Prada, J. M. Requena, C. Punzón, M. Á. Llamas, F. J. Arévalo,

L. Rivas, M. Fresno, Y. Pérez-Pertejo, R. Balana-Fouce, R. M. Requera, *PLoS Negl. Trop. Dis.* **2012**, *6*, e1927.

[34] D. Santos, P. Veiga-Santos, T. Ueda-Nakamura, B. P. D. Filho, D. B. Sudatti, É. M. Bianco, R. C. Pereira, C. V. Nakamura, *Mar. Drugs* **2010**, *8*, 2733.

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