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A new design for nucleolipid-based Ru(III) complexes as anticancer agents

by

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TABLE OF CONTENTS

General synthetic methods

All the reagents were of the highest commercially available quality and were used as received. TLC analyses were carried out on silica gel plates from Merck (60, F254). Reaction products on TLC plates were visualized by UV light and then by treatment with a 10 % $Ce(SO_4)_2/H_2SO_4$ aq. solution. For column chromatography, silica gel from Merck (Kieselgel 40, 0.063-0.200 mm) was used. NMR spectra were recorded on Varian XR 200 and Varian Inova 500 spectrometers, as specified. All the chemical shifts are expressed in ppm with respect to the residual solvent signal. Peak assignments have been carried out on the basis of standard ^{1}H-^{1}H COSY and HSQC experiments. For the ESI MS analyses, a Waters Micromass ZQ instrument – equipped with an Electrospray source – was used in the positive and/or negative mode.

Synthesis of compound 3

Nucleoside **2** (197 mg, 0.732 mmol), dissolved in anhydrous DMF (1.0 mL), was reacted with imidazole (119 mg, 1.76 mmol) and TBDMSCl (132 mg, 0.878 mmol). The solution was stirred at room temperature for 1 h, then the solvent was removed under reduced pressure and the crude mixture was purified on a silica gel column, eluted with *n*-hexane/AcOEt (1:1, v/v). The desired product **3** was obtained in 80% isolated yield (224 mg, 0.586 mmol).

3: oil, *Rf* = 0.5 [*n*-hexane/AcOEt, 1:1, v/v].

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 10.53 (1H, bs, N-H); 7.73 (1H, d, *J* = 8.4 Hz, H-6); 5.79 (1H, d, *J* = 1.4 Hz, H-1'); 5.71 (1H, d, *J* = 8.0 Hz, H-5); 4.45 (2H, overlapped signals, H-2' and H-4'); 4.20 (1H, m, H-3'); 3.93 (2H, d, *J* = 5.2 Hz, H-5'$_a$ and H-5'$_b$), 0.92 (9H, s, SiC(CH_3)$_3$), 0.12 (6H, s, Si(CH_3)$_2$). **^{13}C-NMR ($CDCl_3$, 50 MHz):** 164.0 (C-4); 151.2 (C-2); 140.3 (C-6); 101.8 (C-5);

91.8 (C-1'); 81.7 (C-4'); 79.4 (C-2'); 65.9 (C-3'); 61.0 (C-5'); 25.8 (SiC(CH_3)$_3$); 18.2 (SiC(CH$_3$)$_3$); -5.5 and -5.6 (Si(CH_3)$_2$). **ESI-MS** (positive ions): for $C_{15}H_{25}N_5O_5Si$, calculated 383.1625; found m/z: 384.14 [M+H$^+$]; 406.06 [M+Na$^+$]; 422.00 [M+K$^+$]. **HRMS** (MALDI-TOF): calcd. for $C_{15}H_{25}N_5O_5SiNa$ = 406.1523; found *m/z*: 406.1544 [M + Na$^+$].

Synthesis of compound 4

Nucleoside **3** (100 mg, 0.261 mmol), dissolved in anhydrous CH_2Cl_2 (2.0 mL),, was reacted with DMAP (16 mg, 0.131 mmol), oleic acid (100 μl, 89 mg, 0.315 mmol) and DCC (81 mg, 0.392 mmol). The solution was stirred at room temperature for 2 h, then the solvent was removed under reduced pressure and the crude mixture was purified on a silica gel column, eluted with *n*-hexane/AcOEt (7:3, v/v). The desired product **4** was obtained in 74% isolated yield (125 mg, 0.193 mmol).

4: oil, *Rf* = 0.7 [*n*-hexane/AcOEt, 1:1, v/v].

^{1}H-NMR (CDCl$_3$, 200 MHz): δ 7.70 (1H, d, *J* = 8.2 Hz, H-6); 6.05 (1H, d, *J* = 3.8 Hz, H-1'); 5.76 (1H, dd, *J* = 1.4 and 8.2 Hz, H-5); 5.34 - 5.32 (2H, m, H-9 and H-10 oleic acid); 5.23 (1H, dd, *J* = 3.2 and 3.8 Hz, H-2'); 4.23 - 4.21 (2H, m, H-3' and H-4'); 3.91 (2H, d, *J* = 4.8 Hz, H-5'$_a$ and H-5'$_b$), 2.39 (2H, dd, *J* = 6.8 and 7.8 Hz, 2x H-2 oleic acid); 2.03 - 2.01 (4H, m, 2x H-8 and 2x H-11 oleic acid); 1.64 - 1.62 (2H, m, H-3 oleic acid), 1.28 (20H, overlapped signals, aliphatic protons of oleic acid); 0.92 (9H, s, SiC(CH_3)$_3$), 0.87 (3H, t, *J* = 6.6 and 6.6 Hz, CH_3 oleic acid); 0.12 (6H, s, Si(CH_3)$_2$).**^{13}C-NMR (CDCl$_3$, 50 MHz):** δ 172.4 (C=O); 163.1 (C-4); 150.3 (C-2); 139.8 (C-6); 130.0 and 129.6 (C-9 and C-10 oleic acid); 102.9 (C-5); 87.2 (C-1'); 80.2 (C-4'); 79.5 (C-2'); 64.6 (C-3'); 61.0 (C-5'); 33.8, 31.8, 29.7, 29.6, 29.4, 29.2, 29.0, 28.9, 27.1, 24.6,

22.6 (aliphatic carbons of oleic acid); 25.8 (SiC(CH$_3$)$_3$); 18.2 (SiC(CH$_3$)$_3$); 14.0 (CH$_3$ oleic acid); -5.5 and -5.7 (Si(CH$_3$)$_2$). **ESI-MS** (positive ions): for $C_{33}H_{57}N_5O_6Si$, calcd. 647.4078; found m/z: 648.12 [M+H$^+$]; 670.01 [M+Na$^+$]; 686.07 [M+K$^+$]. **HRMS** (MALDI-TOF): calcd. for $C_{33}H_{57}N_5O_6SiNa$ = 670.3976; found *m/z*: 670.3989 [M + Na$^+$].

Synthesis of compound 5

Nucleoside **4** (31 mg, 0.048 mmol) was dissolved in anhydrous AcOEt (0.7 mL) and then Pd/C 10% p.p. (10 mg, 0.01 mmol) was added. The solution was stirred at room temperature for 12 h under 1 atm pressure of H_2, then the solvent was removed under reduced pressure and the crude was filtered on a short column of silica gel, eluted with AcOEt. The desired product **5** was obtained in almost quantitative yields (30 mg, 0.048 mmol).

5: oil, *Rf* = 0.2 [*n*-hexane/AcOEt, 3:2, v/v].

^{1}H-NMR (CDCl$_3$, 200 MHz): δ 8.04 (1H, d, *J* = 8.4 Hz, H-6); 6.03 (1H, d, *J* = 4.8 Hz, H-1'); 5.72 (1H, d, *J* = 8.4 Hz, H-5); 5.37 - 5.33 (2H, overlapped signals, H-9 and H-10 oleic acid); 5.0 (1H, dd, *J* = 4.8 and 5.2 Hz, H-2'); 4.22 - 4.18 (2H, m, H-4'); 3.93 (2H, m, H-5'$_a$ and H-5'$_b$), 3.68 (1H, dd, *J* = 6.4 and 5.8 Hz, H-3'); 2.36 (2H, t, *J* = 7.4 Hz, 2x H-2 oleic acid); 2.03 - 2.01 (4H, m, 2x H-8 and 2x H-11 oleic acid); 1.60 - 1.57 (2H, m, H-3 oleic acid), 1.28 - 1.25 (20H, overlapped signals, aliphatic protons of oleic acid); 0.94 - 0.91 (12H, overlapped signals, CH$_3$ oleic acid and SiC(CH_3)$_3$), 0.14 (6H, s, Si(CH_3)$_2$).**^{13}C-NMR (CDCl$_3$, 50 MHz):** δ 173.2 (C=O); 162.7 (C-4); 150.2 (C-2); 140.8 (C-6); 130.0 and 129.6 (C-9 and C-10 oleic acid); 102.4 (C-5); 86.2 (C-1'); 81.5 (C-4'); 80.2 (C-2'); 61.9 (C-5'); 56.6 (C-3'); 33.9, 31.8, 29.6, 29.4, 29.2, 29.0, 27.1, 24.6, 22.5 (aliphatic carbons of oleic acid); 25.8 (SiC(CH$_3$)$_3$); 18.0 (SiC(CH$_3$)$_3$); 14.0 (CH$_3$

oleic acid); -5.7 (Si(CH_3)$_2$). **ESI-MS** (positive ions): for $C_{33}H_{59}N_3O_6Si$, calcd. 621.4173; found m/z: 622.15 [M+H^+]; 644.07 [M+Na^+]; 660.13 [M+K^+]. **HRMS** (MALDI-TOF): calcd. for $C_{33}H_{59}N_3O_6SiNa$ = 644.4071; found *m/z*: 644.4092 [M + Na^+].

Synthesis of compound 6

Nucleolipid **5** (48 mg, 0.077 mmol), dissolved in anhydrous CH_2Cl_2 (1.0 mL), was treated with DMAP (28 mg, 0.231 mmol), 2-(pyridin-4-yl)acetic acid hydrochloride (20 mg, 0.116 mmol) and DCC (40 mg, 0.193 mmol). The solution was stirred at room temperature for 12 h, then the solvent was removed under reduced pressure and the crude was purified on a silica gel column, eluted with *n*-hexane/AcOEt (1:4, v/v). The desired product **6** was obtained in 99% isolated yield (56 mg, 0.076 mmol).

6: oil, *Rf* = 0.2 [*n*-hexane/AcOEt, 2:3, v/v].

^{1}H-NMR (CD_3OD, 200 MHz): δ 8.47 (2H, d, *J* = 4.4 Hz, 2x H_α Py); 7.82 (1H, d, *J* = 8.0 Hz, H-6); 7.39 (2H, d, *J* = 5.8 Hz, 2x H_β Py); 5.81 (1H, d, *J* = 5.4 Hz, H-1'); 5.70 (1H, d, *J* = 8.2 Hz, H-5); 5.35 (2H, overlapped signals, H-9 and H-10 oleic acid); 4.72 (1H, dd, *J* = 6.2 and 7.2 Hz, H-2'); 4.35 (1H, m, H-4'); 3.93 (5H, overlapped signals, H-5'$_a$ and H-5'$_b$, H-3', CH_2 Py), 2.36 (2H, dd, *J* = 7.4 and 7.0 Hz, 2x H-2 oleic acid); 2.02 (4H, m, 2x H-8 and 2x H-11 oleic acid); 1.60 (2H, m, H-3 oleic acid), 1.29-1.25 (20H, overlapped signals oleic acid); 0.93-0-90 (12H, overlapped signals, CH_3 oleic acid and SiC(CH_3)$_3$), 0.07 (6H, s, Si(CH_3)$_2$). **^{13}C-NMR (CD_3OD, 50 MHz):** δ 174.7 (C=O ester), 171.8 (C=O amide); 165.8 (C-4); 152.2 (C-2); 150.2 (C_α Py); 146.8 (C_γ Py); 143.2 (C-6); 130.9 (C_β Py); 126.2 (C-9 and C-10 oleic acid); 103.2 (C-5); 89.9 (C-1'); 80.6 (C-4'); 79.4 (C-2'); 63.2 (C-5'); 55.7 (C-3'); 42.8 (CH_2Py); 34.7, 34.6, 33.0, 30.8, 30.6,

30.4, 30.3, 30.2, 30.1, 28.1, 26.7, 26.0, 25.7, 23.7, 19.2 (aliphatic carbons of oleic acid); 26.5 (SiC(CH_3)$_3$); 18.0 (SiC(CH_3)$_3$); 14.5 (CH_3 oleic acid); -5.2 and -5.3 (Si(CH_3)$_2$). **ESI-MS** (positive ions): for $C_{40}H_{64}N_4O_7Si$, calculated 741.0443; found m/z: 741.19 [M+H^+]; 763.17 [M+Na^+]; 768.02 [M+K^+]. **HRMS** (MALDI-TOF): calcd. for $C_{40}H_{64}N_4O_7SiNa$ = 763.4442; found *m/z*: 763.4492 [M + Na^+].

Synthesis of compound 7

Nucleolipid **6** (56 mg, 0.076 mmol), dissolved in anhydrous THF (1.0 mL), was treated with Et_3N·x 3HF (50 μL, 0.31 mmol). The solution was stirred at room temperature for 3 h, then the solvent was removed under reduced pressure and the crude was purified on a silica gel column, eluted with AcOEt/CH_3OH (95:5, v/v). The desired product **7** was obtained in 86% isolated yield (41 mg, 0.065 mmol).

7: oil, *Rf* = 0.2 [AcOEt/CH_3OH, 9:1, v/v].

^{1}H-NMR ($CDCl_3$, 200 MHz): δ 8.55 (2H, d, *J* = 4.4 Hz, 2x H_α Py); 7.68 (1H, d, *J* = 8.0 Hz, H-6); 7.29 (2H, d, *J* = 6.2 Hz, 2x H_β Py); 5.71 (1H, d, *J* = 7.8 Hz, H-5); 5.45-5.24 (5H, overlapped signals, H-1', CH_2Py, H-9 and H-10 oleic acid); 4.75 (1H, m, H-2'); 4.23 (1H, m, H-3'); 3.64 (3H, overlapped signals, H-5'$_a$ and H-5'$_b$, H-4'); 2.34 (2H, t, *J* = 7.4 and 7.4 Hz, CH_2C=O); 1.99-1.27 (26H, overlapped signals oleic acid); 0.89 (3H, t, CH_3 oleic acid). **^{13}C-NMR ($CDCl_3$, 50 MHz):** δ 173.4 (C=O ester), 170.3 (C=O amide); 163.1 (C-4); 150.3 (C-2); 149.7 (C_α Py); 144.0 (C_γ Py); 143.3 (C-6); 130.1 (C_β Py); 129.6 (C-9 and C-10 oleic acid); 103.2 (C-5); 93.5 (C-1'); 79.5 (C-4'); 78.6 (C-2'); 60.2 (C-5'); 54.9 (C-3'); 42.8 (CH$_2$Py); 33.8, 31.9, 29.7, 29.3, 29.1, 27.2, 24.6, 22.7 (aliphatic carbons of oleic acid); 14.1 (CH$_3$ oleic acid). **ESI-MS** (positive ions):

for $C_{34}H_{50}N_4O_7$, calculated 626.3679; found m/z: 627.38 [M+H$^+$]. **HRMS** (MALDI-TOF): calcd. for $C_{34}H_{50}N_4NaO_7$ = 649.3577; found *m/z*: 649.3601 [M + Na$^+$].

Synthesis of compound 8

7 (17 mg, 0.027 mmol), dissolved in anhydrous CH_2Cl_2 (0.7 mL) was reacted with DMAP (2.0 mg, 0.016), $BnO(CH_2CH_2O)_6CH_2COOH$ (15 mg, 0.035 mmol) - prepared by us as described in ref. [1] - and DCC (8.3 mg, 0.040 mmol). The solution was stirred at room temperature for 2 h, then the solvent was removed under reduced pressure and the crude was purified on a silica gel column, eluted with *n*-hexane/acetone (2:3, v/v). The desired product **8** was obtained in 70% isolated yield (20 mg, 0.019 mmol).

8: oil, *Rf* = 0.3 [AcOEt/CH_3OH, 9:1, v/v].

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 8.65 (2H, bs, 2x H_α Py); 7.37-7.25 (8H, overlapped signals, aromatic proton of Bn, H-6, 2x H_β Py); 5.75 (1H, d, *J* = 8.0 Hz, H-5); 5.30-5.27 (5H, overlapped signals, H-1', C*H*$_2$Py, H-9 and H-10 oleic acid); 4.76 (1H, m, H-2'); 4.55 (2H, s, C*H*$_2$ Bn); 4.39 (1H, m, H-3'); 4.18-4.30 (5H, overlapped signals, H-5'$_a$ and H-5'$_b$, H-4', OC*H*$_2$C=O); 3.77-3.63 (24H, OC*H*$_2$C*H*$_2$O); 2.33 (2H, dd, *J* = 8.0 and 7.0 Hz, C*H*$_2$C=O); 1.93-1.08 (26H, overlapped signals oleic acid); 0.87 (3H, dd, *J* = 6.0 and 7.0 Hz, CH_3 oleic acid). **^{13}C-NMR ($CDCl_3$, 125 MHz):** δ 173.2, 172.3 (C=O ester); 169.6 (C=O amide); 162.5 (C-4); 156.7 (C_α Py); 150.0 (C-2); 142.8 (C_γ Py); 138.1 (quaternary carbon of Bn); 128.3, 127.6, 127.5 (C_β Py, C-9 and C-10 oleic acid); 103.2 (C-5); 93.3 (C-1'); 80.9 (C-4'); 79.6 (C-2'); 73.1 (*C*H_2Bn); 70.4, 70.3 (overlapped signals, O*C*H_2*C*H_2O); 69.3 (O*C*H_2C=O); 61.9 (C-5'); 54.7 (C-3'); 49.0 (*C*H_2Py); 33.8, 29.6, 29.3, 29.2, 29.1, 29.0, 25.5, 24.8, 24.5, 22.6 (aliphatic carbons of oleic acid); 14.0 (*C*H_3 oleic

acid). **ESI-MS** (positive ions): for $C_{55}H_{82}N_4O_{15}$, calculated 1039.2574; found m/z: 1041.27 [M+H$^+$]; 1063.20 [M+Na$^+$]; 1079.24 [M+K$^+$]. **HRMS** (MALDI-TOF): calcd. for $C_{55}H_{82}N_4NaO_{15}$ = 1061.5674; found *m/z*: 1061.5705 [M + Na$^+$].

Synthesis of compound 1

Nucleolipid **8** (18 mg, 0.017 mmol), dissolved in anhydrous CH_2Cl_2 (0.5 mL), was treated with $[RuCl_4(DMSO)_2]^-Na^+$ (7.0 mg, 0.017 mmol) – prepared as described in ref. [2] - and the resulting solution was taken under stirring at 40 °C. TLC monitoring on alumina plates showed in 4 h the total disappearance of the starting material; the reaction mixture was therefore taken to dryness, giving the desired product **1** in quantitative yields, obtained in a pure form (23 mg, 0.017 mmol).

1: amorphous solid, *Rf* = 0.3 [acetone/*n*-hexane, 7:3, v/v].

^{1}H NMR ($CDCl_3$, 500 MHz): significant signals at δ 7.55-7.30 (broad, overlapped signals: protons of Bn and H-6); 4.96-3.86 (broad, overlapped signals: H-5, protons of ribose, H-9 and H-10 protons of oleic acid and protons of the polyether chain); 2.75-0.91 (overlapped signals, aliphatic protons of oleic acid); -1.98 (very broad signals, protons of Py); -9.59 (very broad, 2x C*H*$_3$ DMSO). **^{13}C NMR ($CDCl_3$, 125 MHz):** significant signals at δ 128.2, 127.9, 127.4 (carbons of Bn, C-9 and C-10 oleic acid); 49.3 (*C*H$_2$Py); 33.9, 31.3, 29.1, 28.8, 25.0, 24.7, 22.1 (sp^3 carbons of oleic acid); 13.5 (-*C*H$_3$ oleic acid). **ESI-MS** (negative ions): for $C_{57}H_{88}Cl_4N_4O_{16}RuS$, calculated 1358.3714; found m/z: 1283.37 [M$^-$-DMSO]; 1358.89 [M$^-$].

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