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Synthesis and radiolabelling of $Re(CO)_3$-β-elemene derivatives as potential therapeutic radiopharmaceuticals

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β-Elemene, (1*S*, 2*S*, 4*R*)-(−)-(1-methy-1-vinyl-2,4-diisopropenyl cyclohexane) is an anticancer agent from the *Traditional Chinese Herb Medicinal*. Three novel $Re(CO)_3$-β-elemene derivatives including their radioactive conjugates containing *N*,*N*,*N* tridentate ligands and tricarbonyl rhenium (complex 12, 13, 14) were synthesized. Their structures were characterized by infrared (IR), ^{1}H-NMR and HRMS. Good radioactive yield (above 90%) and radioactive chemical purity with Re-188 (above 95%) were obtained for all of the three derivatives (complex 15, 16, 17). The antiproliferative activity of non-radioactive β-elemene-$Re(CO)_3$ derivatives on Lewis lung cancer cells and HeLa cell lines were evaluated by WST-1 methods. The result shows substantial decrease in IC_{50} values compared with the parent compound β-elemene. The synthesis and radiosynthesis of β-elemene tricarbonyl rhenium conjugates provide the possibility to find a new kind of potential radiopharmaceuticals on β-elemene.

Keywords: β-elemene; tricarbonyl rhenium; Re-188; antiproliferative activity; IC_{50}

Introduction

β-Elemene (Figure 1), (1*S*, 2*S*, 4*R*) 1-methy-1-vinyl-2,4-diisopropenyl cyclohexane, a natural sesquiterpene extracted from the *Traditional Chinese Herb Medicinal Curcuma wenyujin*[1]and is the main effective monomer of elemene emulsion. β-Elemene exhibits anticancer effects in human and murine tumor cells *in vitro* and *in vivo* and has substantial clinical activity against various tumors without severe side effects.[2–4] No bone marrow suppression and drug resistance have been observed in the clinical studies; on the contrary, patient immunity was improved during the therapy with β-elemene.

The nuclear properties of ^{188}Re [$T_{1/2}$ 16.9 h; 2.12 MeV (71.6%) and 1.96 MeV (25.1%) β emissions, 155 KeV [(15%) γ emissions] would make it an ideal therapeutic radioisotopes. In this paper, we synthesized three novel Re(CO)3-β-elemene derivatives including their radioactive conjugates containing *N*,*N*,*N* tridentate ligands and tricarbonyl rhenium (complex **12**, **13**, **14**); The antiproliferative activity and structure characterization of non-radioactive β-elemene-Re(CO)3 derivatives were described; The radioactive yield and radioactive chemical purity with Re-188 were obtained for all of the three derivatives (complex **15**, **16**, **17**). It is an attempt to radiolabelling β-elemene derivatives for potential therapeutic radiopharmaceuticals.

Results and discussion

The synthesis procedure for the important intermediates was shown in Scheme 1, the compound **1** was prepared according to the literature[5] with slight modification and careful control of reaction conditions in order to yield the 13-monosubstituted β-elemene chlorinated intermediate as the main product. The compound **2** was prepared similar to that of the reported procedure.[6]

Synthesis of various chelating systems with the spacer entities are presented in Schemes 2 and 3. We followed the recently published synthetic strategy[7] for the preparation of tridentate chelating systems with spacer groups comprising a terminal, primary amine functionality. Chelators with spacer entities were prepared as follows: for the synthesis of chelator **5,** mono-Boc-protected diaminohexane **3** was treated with 2 equiv of pyridine-2-carboxaldehyde and a slight excess of sodium triacetoxy borohydride for *in situ* reduction of the imine intermediate followed by Boc-deprotection with 6 N HCl (Scheme 2). Compound **8** was synthesized with similar procedure to that of compound **5** starting from mono-Boc-protected 2, 2′-(ethylenedioxyl)diethylamine **6** (Scheme 3).

These intermediates including commercial available compound di-(2-picoyl)-amine were coupled to the β-elemene chlorinated compound **1** under basic conditions to form the corresponding intermediates **9, 10, 11** in very good yields.

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The complex of $[N(CH_2CH_3)_4]_2[ReBr_3(CO)_3]$(**2**) was prepared according to a previously published procedure.[6,8] This complex is an important starting material for compounds containing the *fac*-$Re(CO)_3$ moiety since the three bromide ligands are very weakly bound. When the$[N(CH_2CH_3)_4]_2[ReBr_3(CO)_3]$ complex was dissolved in water, the three bromide ligands were quantitatively exchanged by three H_2O molecules to form the complex of *fac*-$[Re(CO)_3(H_2O)_3]^+$, which is stable in aqueous solution even when exposed to air for weeks.

These substrates (**9, 10, 11**) are easy to coordinate with $[N(CH_2CH_3)_4]_2[ReBr_3(CO)_3]$ to afford the non-radioactive compounds **12, 13, 14** (Scheme 4). All the compounds were characterized by infrared (IR), ^{1}H-NMR, HRMS, HPLC or elemental analysis.

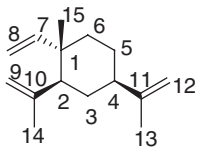
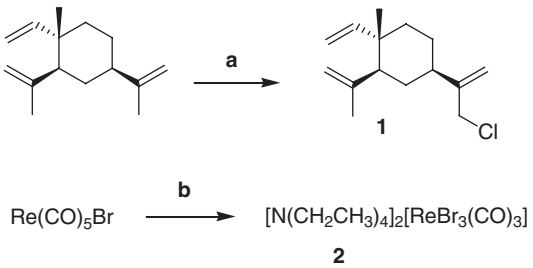


Figure 1. Chemical Structure of β-elemene.



Scheme 1. Reagents and conditions: (a)NaClO, CH_3COOH, CH_2Cl_2, 0°C; (b)$N(Et)_4Br$, $CH_3OCH_2CH_2OCH_2CH_2OCH_3$, 120°C.

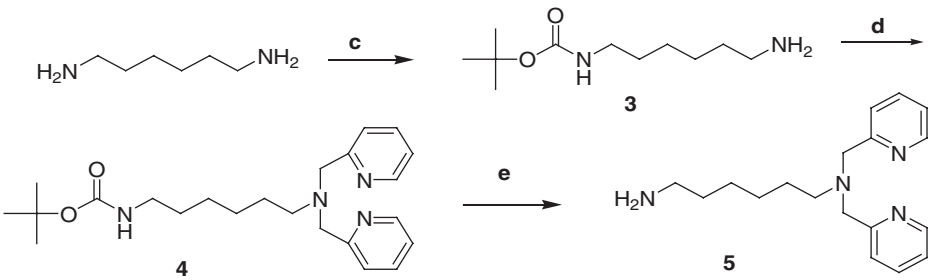
The rhenium complexes with oxidation states+V/+III have a higher tendency to reoxidized. Therefore, kinetically more inert complexes containing rhenium in the low oxidation state+I have received more attention, recently[9–11] *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ may be an ideal candidate agent for labelling biomolecules. The kinetic inertness of the Re(+I) oxidation state opens a new way for exploring the more oxidation sensitive ^{188}Re for therapy.

Radiosynthesis of compounds **15**, **16**, **17** was performed at 70°C for 50 min with *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ as starting material and the method is according to the published procedure.[12–14] The complex of *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ was synthesized with an overall radiochemical yield of 92%, and with a radiochemical purity of over 95% after a Sep-Pak® silica cartridge separation.

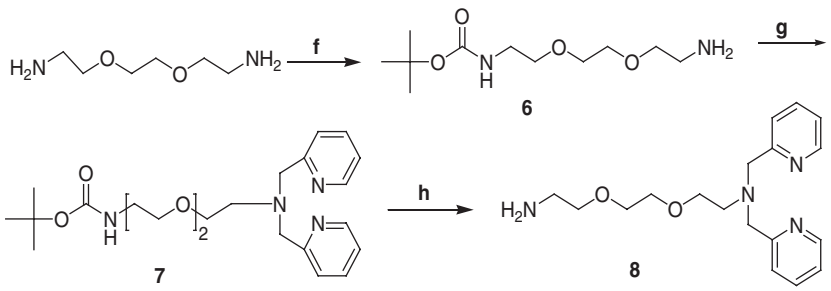
The ligand concentrations in the reactions were in 10^{-6}–10^{-4} M range. At these concentrations, labelling yields >90% in respect to *fac*-$[^{188}Re(OH_2)_3(CO)_3]^+$ and specific activities of up to 200 GBq/μmol ligand (based on initial activity of 18 GBq/mL $^{188}ReO_4^-$) could be achieved (Scheme 5).

The formation of the ^{188}Re tricarbonyl complexes was monitored by RP-HPLC, and showed generally slightly shift to longer retention time (for **15**, R_f = 21.21 min; for **16**, R_f = 21.13 min; for **17**, R_f = 21.19 min) compared with that of free precursor $^{188}Re(CO)_3Br$ (R_f = 3.46 min) and β-elemene derivatives (for **12**, R_f = 21.17 min; for **13**, R_f = 20.07 min; for **14**, R_f = 20.18 min) (Figure 2). Formation of small amount of by-product was observed in all of the three cases. The radioactive chemical purity of the compounds **15, 16, 17** were >95% after purified by HPLC. Stability of the Re-188 complexes was evaluated. The HPLC purified product showed no evidence of degradation in PBS or human plasma over a period of 24 h at 37°C.

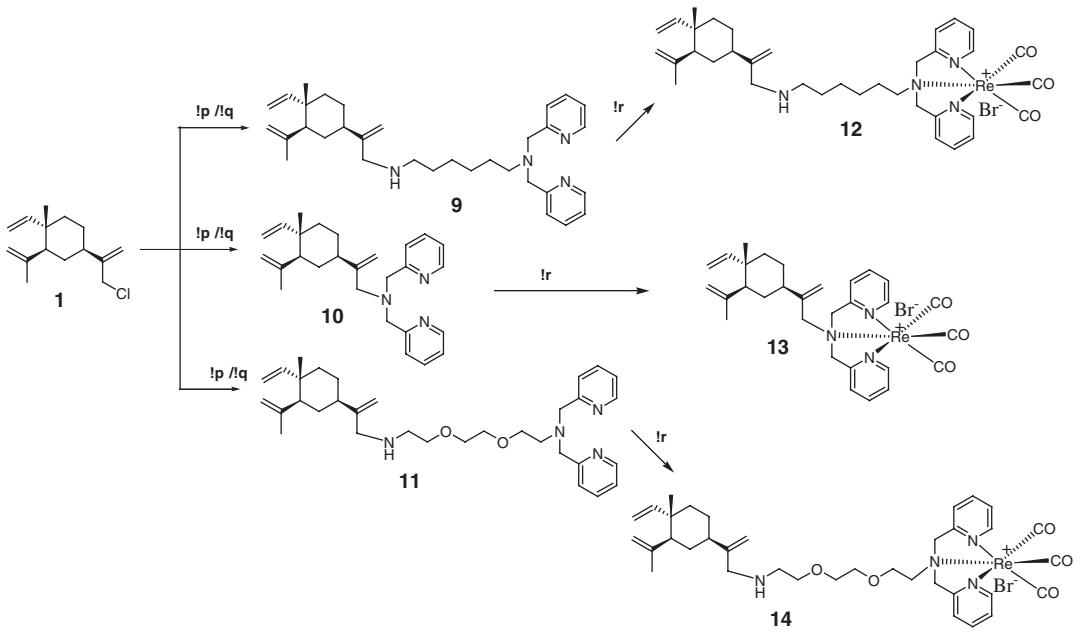
The antiproliferative effect of non-radioactive $Re(CO)_3$-β-elemene derivatives in mice Lewis lung cancer cells (LLC) and human HeLa cervix carcinoma cells were evaluated by WST-1 method.[15] MTT, WST-1 and XTT method could be used to detect the cell viability. WST-1, the tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (Beyotime), can form



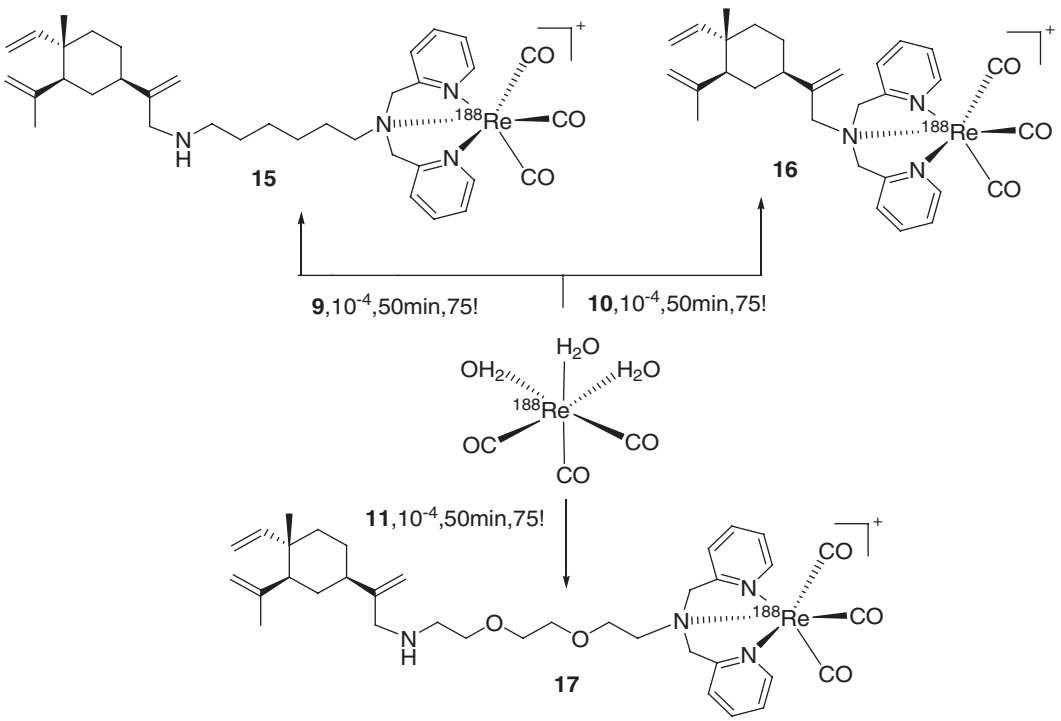
Scheme 2. Reagents and conditions: (c) Boc_2O, $CHCl_3$, 0°C; (d) pyridine-2-carboxaldehyde, $C_2H_4Cl_2$, 2 h, rt; $(CH_3COO)_3BHNa$, rt; (e)HCl 6 N, rt,3 h.



Scheme 3. Reagents and conditions: (f) Boc_2O, $CHCl_3$, 0°C; (g) pyridine-2-carboxaldehyde, $C_2H_4Cl_2$, 2 h, rt; $(CH_3COO)_3BHNa$, rt; (h) HCl 6 N, rt, 3 h.



Scheme 4. Reagents and conditions: (i) NaOH, CH_3CN, 63°C, 8 h; (ii) **5** or di-(2-picolyl)amine or **8;** and (iii) **2** in CH_3OH, rt, 40 min.



Scheme 5. Radiosynthesis of complex **15**, **16**, **17**.

water-soluble tetrazolium salts after incubated with active cells but not the MTT-formazan crystals. The results are shown in Table 1. Their antiproliferative activities of compound **12**, **13** and **14** were improved significantly in comparison to β-elemene ($p<0.01$).

Octanol/water partition coefficient of β- elemene-^{188}Re(CO)$_3$

To evaluate the aqueous solubility of the complex, octanol/water partition coefficient was determined. The results were shown in Table 2. Compared with that of β-elemene, which was reported as $P=199.5\pm4.12$.[16] The oil–water partition coefficients of β-elemene-^{188}Re(CO)$_3$(H$_2$O)$_3^+$ complexes was improved more than 20 times than the parent β-elemene.

Experimental

Materials and instruments

β-elemene was obtained from WenTe Research Institute of Oleum Curcumae Wenchowensis in Yue Qing city, Zhejinag

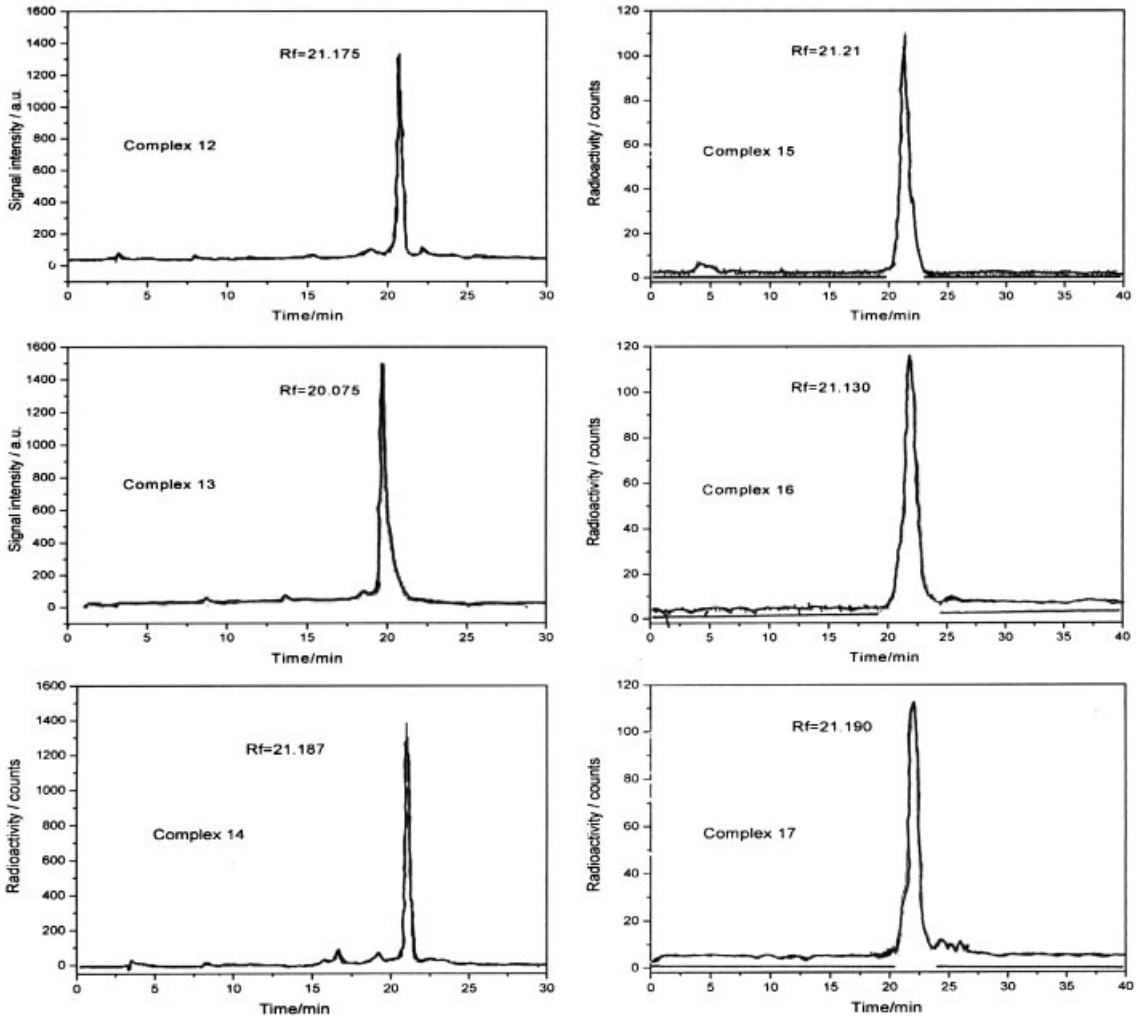


Figure 2. HPLC analyses of the complex **12–14** and radioactive HPLC-traces of the $^{188}Re(CO)_3$-β-elemene radiotracers **15–17**.

Table 1. The antiproliferative activity of $Re(CO)_3$-β-elemene derivatives in LLC and HeLa cell lines

	IC50(μM)	
Compounds	HeLa	LLC
β-elemene	236.2 ± 3.2	346.1 ± 41.5
Compound **12**	10.9 ± 1.2	5.0 ± 1.9
Compound **13**	11.2 ± 1.5	5.1 ± 1.3
Compound **14**	10.5 ± 2.9	4.8 ± 2.3

Table 2. Oil–water partition coefficient of **12-14**

Complexes	*P*	Log *P*
Complex 12	11.50 ± 1.05	1.06 ± 0.02
Complex 13	13.20 ± 1.05	1.12 ± 0.02
Complex 14	9.48 ± 1.07	0.98 ± 0.03

province (purity 98%). Other materials were purchased from Fluka Co. and Sinopharm Chemical Reagent Co. Ltd. The NMR data were obtained using a Bruker DRX 500 MHz FT spectrometer. The chemical shifts as δ are reported in ppm relative to TMS. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer. Mass spectral data were collected using positive mode on a Finnigan LCQ classic mass spectrometer. Elemental analysis was performed using a Perkin-Elmer Series III analyser.

A general procedure for the formation of 13-chloro-β-elemene (compound 1)

To a solution of β-elemene (2 g, 9.79 mmol) in ice acetic acid (2 mL), sodium hypochlorite (13.5 mL) was added over 4 h period under stirring at ice cold, then the mixture was stirred for 2 h at room temperature and extracted with EtOAc. The combined organic extracts were washed with water, dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* to yield the mixture as yellow oil (2 g, 60%), which was used for the next step without further purification.

The procedure for the formation of $[N(CH_2CH_3)_4]_2[ReBr_3(CO)_3]$ (compound 2)

Powdered $N(Et)_4Br$ (158.2 mg) was stirred in 14 mL of 2,5,8-trioxanonane (diglyme) under dry argon and heated to 80°C. Then solid $[ReBr(CO)_5]$ (118.2 mg) was added into the mixture and kept at 120°C for 8 h, during which a pale yellow precipitate

formed. The mixture was filtered while hot and washed with several portions of cold diglyme, diethyl ether and dried at 100°C in vacuum. The resulting pale yellow solid was then slurried in 3 mL absolute ethanol to remove unreacted NEt_4Br. Filtration and drying in vacuum yielded the product as a pale yellow powder (130 mg, 73%).

General procedure[17] for the formation of fac-[^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$

Powdered $BH_3 \cdot NH_3$ (5.0 mg) was added into a 10 mL glass vial (flushed with N_2 for 10 min firstly). The vial was capped with a rubber stopper and an aluminum seal and then filled with CO gas for 20 min. The radiolabelling procedure was performed by adding a mixture of 6 μL of phosphoric acid (85%) and 1 mL of $^{188}ReO_4$ (18 GBq/mL) into the vial and incubating in a water bath at 70–80°C for 15 min. A 10 mL syringe was used to keep the balance of H_2 gas. The chelating efficiency was determined by TLC, using a silica GF_{254} glass plate as stationary phase and CH_3OH:HCl (36%) = 99:1 as mobile phase. In this system, colloidal $^{188}ReO_2$ stays at the origin ($R_f = 0$), the R_f of [^{188}Re(CO)$_3$(H$_2$O)$_3$]$^+$ is 0.4–0.6, and the free ^{188}Re perrhenate has an R_f of 0.8–1.0. The yield of the product is better than 92%, and with a radiochemical purity of over 95% after a Sep-Pak® silica cartridge separation.

The antiproliferative evaluation by WST-1 method

LLC and HeLa cells were maintained in DMEM with 10% inactivated fetal bovine serum. The cell lines were grown in logarithmic growth at 37°C in a humidified atmosphere consisting of 5% CO_2 and 95% air. The cells were harvested using 0.25% trypsin-EDTA and seeded 5×10^3 cells in each well of a 96-well plate and incubated for 12 h. Then the Re(CO)$_3$-*β*-elemene derivatives and the parent compound *β*-elemene were added to wells of the plate at concentrations (100, 10, 1 μmol/L) and continued to culture in CO_2 incubator for 24 h. Ten microliter WST-1 solution was added to wells, absorbance values were taken using a 96-well Opsys Microplate Reader at 450 nm. IC_{50} were calculated according to absorbance.

IC_{50} were calculated with SPSS 11.5 statistical software, differences between mean values were analyzed with students' *t*-tests. Differences were considered significant when $p < 0.01$. Data are presented as mean ± SD.

Partition coefficient study

Usually the final partition coefficient value was expressed as log *P*. Log *P* of *β*-elemene -^{188}Re(CO)$_3$ was determined by measuring the distribution of radioactivity in 1-octanol and PBS. A 5 μL sample of *α*-elemene-^{188}Re(CO)$_3$ in PBS was added to a vial that contains 1 mL 1-octanol and 1 mL PBS. After vortexing for 5 min, the vial was centrifuged for 5 min to ensure complete separation of layers. Then, 5 μL of each layer was pipetted into other test tubes, and log *P* values were calculated using the formula: Log *P* = Log (counts in octanol/counts in water).[18]

Synthesis of compound 3

Compound **3** was synthesized according to a previously published procedure,[19] starting from 1,6-diaminohexane. A colorless oil, 94% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.30–1.37 (m, 4H), 1.40–1.51 (m, 11H), 1.60–1.68 (m, 4H, NH_2 and CH_2), 2.68 (t, $J = 6.96$ Hz, 2H), 3.11 (q, $J = 6.41$ Hz, 2H), 4.59 (s, 1H); IR (KBr) *ν*: 3344, 2925, 1699, 1527, 1174 cm^{-1}; EI-HRMS: calcd for $C_{11}H_{24}N_2O_2$ 216.1838, found 216.1808.

Synthesis of compound 4

Pyridine-2-carboxaldehyde (3.1018 g; 28.9 mmol; 2.1 equiv) dissolved in 40 mL of dry 1,2-dichloroethane was added to a solution of compound **3** (2.8778 g; 13.3 mmol; 1 equiv) in 50 mL of dry 1, 2-dichloroethane and stirred for 2 h at room temperature. The reaction solution was cooled to 0°C before slow addition of sodium triacetoxy borohydride (6.1783 g; 29.1 mmol; 2.3 equiv) in portions. After 24 h of stirring at room temperature, water was added to quench residual sodium triacetoxy borohydride and the reaction mixture extracted twice with CH_2Cl_2. The organic layer was extracted with water and brine, dried over Na_2SO_4, evaporated, and dried in a vacuum. A colorless oil, 75% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.18–1.31 (m, 4H), 1.40–1.48 (m, 11H), 1.50–1.56 (m, 2H), 2.53 (t, $J = 7.20$ Hz, 2H), 3.07 (d, $J = 6.22$ Hz, 2H), 3.81 (s, 4H), 4.75 (s, 1H), 7.14 (t, $J = 5.75$ Hz, 2H), 7.54 (d, $J = 7.582$ Hz, 2H), 7.66 (dt, $^1J = 7.67$ Hz, $^2J = 1.71$ Hz, 2H), 8.52 (d, $J = 4.77$ Hz, 2H); IR (KBr) *ν*: 3368, 3061, 2931, 1709, 1171, 760 cm^{-1}; EI-HRMS: calcd for $C_{23}H_{34}N_4O_2$ 398.2682, found 398.2679.

Synthesis of compound 5

The Boc-deprotection of compound **4** (692 mg, 1.73 mmol; 1 equiv) was achieved quantitatively in 6 N HCl (15 mL) after 4 h at room temperature as determined by TLC/ninhydrin. After addition of 10 N NaOH to obtain a pH value of 5, the reaction mixture was evaporated and dried in a vacuum. Methanol was added and the mixture was filtered to remove the precipitated salt colorless oil, 92% yield; ^{1}H NMR (CDCl3, TMS, 500 MHz), δ: 1.20–1.29 (m, 4H), 1.35–1.41 (m, 2H), 1.50–1.55 (m, 2H), 1.75 (s, 2H), 2.52 (t, $J = 7.29$, 2H), 2.62 (t, $J = 7.06$ Hz, 2H), 3.79 (s, 4H), 7.12 (t, $J = 5.20$ Hz, 2H), 7.52 (d, $J = 7.80$ Hz, 2H), 7.63 (dt, $^1J = 7.71$ Hz, $^2J = 1.66$ Hz, 2H), 8.50 (d, $J = 4.13$ Hz, 2H); IR (KBr) *ν*: 2924, 2028, 1917 cm^{-1}; EI-HRMS: calcd for $C_{18}H_{26}N_4$ 298.2157, found 298.2157.

Synthesis of compound 6

Compound **6** was synthesized with similar procedure to that of compound **3** starting from 2,2′-(ethylenedioxyl)diethylamine. A colorless oil, 92% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.39 (s, 9H), 2.31–2.49 (m, 2H), 2.84 (s, 2H), 3.27 (s, 2H), 3.47–3.57 (m, 8H), 5.26 (s, 1H); IR (KBr) *ν*: 584.3, 1708.9, 2972.9, 3342.6 cm^{-1}; ESI-HRMS: calcd for $C_{11}H_{25}N_2O_4$ 249.1814, found 249.1809.

Synthesis of compound 7

Compound **7** was synthesized with similar procedure to that of compound **4**. A colorless oil, 80% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.39 (s, 9H), 2.45 (s, 1H), 2.86 (t, $J = 5.60$ Hz, 2H), 3.26–3.34 (m, 2H), 3.51–3.59 (m, 6H), 3.63 (t, $J = 6.00$ Hz, 2H), 3.93 (s, 4H), 7.15 (t, $J = 5.60$ Hz, 2H), 7.54 (d, $J = 7.60$ Hz, 2H), 7.65 (dt, $^1J = 7.60$ Hz, $^2J = 1.60$ Hz, 2H), 8.53 (d, $J = 4.40$ Hz, 2H); IR (KBr) *ν*: 1364, 1701, 2358, 2931, 3499 cm^{-1}; EI-MS: calcd for $C_{23}H_{34}N_4O_4$ 430.3, found 430.4.

Synthesis of compound 8

Compound **8** was synthesized with similar procedure to that of compound **5**. A colorless oil, 87% yield; ^{1}H NMR ($CDCl_3$, TMS,

500 MHz), δ: 2.64 (s, 2H), 2.76–2.85 (m, 4H), 3.48–3.68 (m, 8H), 3.87 (s, 4H), 7.12 (t, J = 6.40 Hz, 2H), 7.51 (d, J = 7.60 Hz, 2H), 7.62 (dt, ^1J = 7.60 Hz, ^2J = 1.60 Hz, 2H), 8.49 (d, J = 4.80 Hz, 2H); IR (KBr) ν: 1858, 1999, 2987, 3458 cm^{-1}; ESI-HRMS: calcd for $C_{18}H_{27}N_4O_2$ 331.2134, found 331.2123.

General procedure for the preparation of compounds 9–11

A solution of Cl-β-elemene, (2 mmol), **5** or di-(2-picolyl)amine or **8** (4 mmol), and sodiun hydroxide (8 mmol) in 10 mL dry acetonitrile was refluxed for 8–10 h. Then water (10 mL) was added and the mixture was extracted with ethyl ether (4 × 30 mL). The comp-bined organic extracts were dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated *in vacuo*. The residue was purified on a silica gel column with dichloromethane-methanol as eluent to give a target product.

Compound 9

A brown oil, 80% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 0.97 (s, 3H), 1.19–1.32 (m, 4H), 1.35–1.60 (m, 8H), 1.62–1.75 (m, 2H), 1.69 (s, 3H), 1.96–2.12 (m, 2H), 2.55 (t, J = 6.88 Hz, 2H), 2.89 (t, J = 7.78 Hz, 2H), 3.61 (s, 2H), 3.84 (s, 4H), 4.55 (s, 1H), 4.80 (s, 1H), 4.87–4.94 (m, 2H), 5.15 (s, 1H), 5.17 (s, 1H), 5.79 (dd, ^1J = 17.71 Hz, ^2J = 10.54 Hz, 1H), 7.17 (t, J = 5.6 Hz, 2H), 7.50 (d, J = 7.79 Hz, 2H), 7.67 (dt, ^1J = 7.67 Hz, ^2J = 1.64 Hz, 2H), 8.53 (d, J = 4.19 Hz, 2H); IR (KBr) ν: 2928, 2362, 1388 cm^{-1}; ESI-HRMS calcd for $C_{33}H_{49}N_4$ 501.3957, found 501.3922.

Compound 10

A brown oil, 87% yield; ^{1}H-NMR ($CDCl_3$, TMS, 500 MHz), δ: 0.90 (s, 3H), 1.47–1.27 (m, 6H), 1.61 (s, 3H), 1.92–1.89 (m, 1H), 2.07–2.04 (m, 1H), 3.06 (q, 2H, J = 14.0 Hz), 3.73 (q, J = 14.1 Hz, 4H), 4.47 (s, 1H), 4.73 (s, 1H), 4.84 (dd, J = 5.82 Hz, J = 1.08 Hz, 1H), 4.85 (d, J = 1.3 Hz, 1H), 4.89 (s, 1H), 5.06 (s, 1H), 5.77 (dd, J = 10.48 Hz, J = 7.38 Hz, 1H), 7.09–7.07 (m, H), 7.50–7.47 (m, H), 7.60 (dt, J = 7.65 Hz, J = 1.57 Hz, 2H), 8.45 (d, J = 4.3 Hz, 2H); IR (KBr): 3080, 1640 cm^{-1}; ^{13}C-NMR ($CDCl_3$, TMS, 125 MHz), δ: 17.39, 25.31, 27.68, 33.73, 40.57, 40.74, 42.56, 53.74, 59.71, 60.80, 110.53, 111.63, 112.71, 122.62, 123.41, 137.10, 148.14, 149.61, 150.94, 152.06, 160.46; ESI-HRMS calcd for $C_{33}H_{49}N_4$ 401.2831, found 401.2842.

Compound 11

A brown oil, 81% yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 0.97 (s, 3H), 1.35–1.63 (m, 6H), 1.67 (s, 3H), 1.97–2.16 (m, 2H), 2.89 (s, 2H), 3.22 (t, J = 4.32 Hz, 2H), 3.46–3.51 (m, 2H), 3.57–3.65 (m, 4H), 3.69 (s, 2H), 3.81–3.95 (m, 6H), 4.54 (s, 1H), 4.80 (s, 1H), 4.86–4.94 (m, 2H), 5.18 (s, 1H), 5.32 (s, 1H), 5.78 (dd, ^1J = 17.85 Hz, ^2J = 6.85 Hz, 1H),7.17 (t, J = 6.65 Hz, 2H), 7.38 (d, J = 7.45 Hz, 2H), 7.66 (t, J = 7.64 Hz, 2H), 8.55 (d, J = 3.97 Hz, 2H); IR (KBr) ν: 765, 1591, 1639, 2928, 3371 cm^{-1}; ESI-HRMS calcd for $C_{33}H_{49}N_4$ O_2 533.3856, found 533.3845.

General procedure for the preparation of compounds 12–14

Complexes **12–14** were prepared according to the following general procedure: 2 mmol $[N(CH_2CH_3)_4]_2[ReBr_3(CO)_3]$, and 2 mmol corresponding derivative (**9, 10, 11**) was dissolved in CH_3OH and stirred for 30 min. The mixture was evaporated and dried in a vacuum and the production was recrystallized with *n*-hexane: dichloromethane = 1:2

Compound 12

A white solid, 78% yield, m.p. 161.7–162.1°C; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 0.99 (s, 3H), 1.23–1.33 (m, 2H), 1.38–1.46 (m, 2H), 1.47–1.59 (m, 3H), 1.62–1.83 (m, 4H), 1.70 (s, 3H), 2.10–2.21 (m, 4H), 2.27–2.33 (m, 1H), 3.05 (t, J = 7.16 Hz, 2H), 3.70 (q, J = 14.00 Hz, 2H), 3.81 (t, J = 8.08 Hz, 2H), 4.57 (s, 1H), 4.73 (d, J = 16.75 Hz, 2H), 4.81 (s, 1H), 4.87–4.89 (m, 1H), 4.91 (s, 1H), 5.26 (s, 1H), 5.45 (s, 1H), 5.59 (d, J = 17.02 Hz, 2H), 5.82 (dd, J = 17.49 Hz, 10.85, 1H), 7.23 (t, J = 6.71 Hz, 2H), 7.84 (dt, J = 7.74 Hz, 0.38, 2H), 7.93 (d, J = 7.81 Hz, 2H), 8.67 (d, J = 5.4 Hz, 2H). IR (KBr) ν: 3472, 2926, 2027, 1912 cm^{-1}; anal. calcd for $C_{36}H_{48}BrN_4O_3Re \cdot CH_2Cl_2$: C 47.49, H 5.39, N 5.99; found C 47.29, H 5.64, N 6.26.

Compound 13

A white solid, 82 % yield; ^{1}H-NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.03 (s, 3H, CH_3), 1.51–1.58 (m, 3H), 1.68 (s, 3H, CH_3), 1.72–1.82 (m, 2H), 1.97–2.02 (m, 1H), 2.17–2.19 (m, 1H), 2.30–2.39 (m, 1H), 4.36–4.49 (m, 4H), 4.61 (s, 1H), 4.85 (s, 1H), 4.91–4.99 (m, 2H), 5.53 (s, 1H), 5.62 (s, 1H), 5.77–5.89 (q, 1H), 6.05 (q, J = 18.53 Hz, 2H), 7.18 (t, J = 6.39 Hz, 2H), 7.80 (t, J = 7.63 Hz, 2H), 8.00 (d, J = 7.64 Hz, 2H), 8.63 (d, J = 5.34 Hz, 2H); IR (KBr): 3080, 2027, 1910 cm^{-1};^{13}C-NMR ($CDCl_3$, TMS, 125 MHz), δ: 17.23, 25.64, 28.00, 34.65, 40.44, 40.82, 44.52, 52.92, 67.78, 67.92, 74.48, 110.86, 113.00, 121.66, 125.85, 126.36, 140.96, 147.12, 148.02, 150.49, 151.07, 161.84, 161.95, 196.22, 196.86; anal. calcd for $C_{30}H_{36}BrN_4O_3Re \cdot CH_2Cl_2$: C 46.99, H 4.73, N 7.31; found C 46.63, H 4.69, N 7.08.

Compound 14

A white solid, 75 % yield; ^{1}H NMR ($CDCl_3$, TMS, 500 MHz), δ: 1.00 (s, 3H), 1.38–1.47 (m, 2H), 1.48–1.61(m, 2H), 1.74 (s, 3H), 1.65–1.93 (m, 10H), 2.08–2.31(m, 6H), 3.01–3.09 (m, 2H), 3.62–3.74 (m, 2H), 3.76–3.85 (m, 2H), 4.58 (s, 1H), 4.73 (d, J = 16.80 Hz, 2H), 4.81 (s, 1H), 4.87 (s, 1H), 4.91 (d, J = 8.8 Hz, 1H), 5.27 (s, 1H), 5.48 (s, 1H), 5.64 (d, J = 16.80 Hz, 2H), 5.82 (dd, J = 17.49 Hz, 10.85, 1H), 7.23 (t, J = 6.80 Hz, 2H), 7.84 (t, J = 7.60 Hz, 2H), 7.95 (d, J = 7.60 Hz, 2H), 8.68 (d, J = 6.80 Hz, 2H). IR (KBr) ν: 1914, 2028, 2928, 3437 cm^{-1}. Calcd for $[C_{36}H_{48}N_4O_5Re]Br \cdot 1/3CH_2Cl_2$: C 47.89, H 5.38, N 6.15; found C 47.80, H 5.45, N 6.11.

Radiochemical synthesis of 15–17

Complexes **15–17** were prepared according to the following general procedure:[17,20–22] *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ was added to 100 μL **9** or **10** or **11**(10^{-4} mol/L). The mixture was incubated at 70°C for 50 min. HPLC analyses of the complexes **15–17** revealed yields between 90 and 96%. Radioactive chemical purity with Re-188 (above 95%) was obtained for all of the three derivatives

Conclusion

Three novel β-elemene Re complexes have been prepared and characterized successfully. Their antiproliferative activity *in vitro* on LLC and HeLa cell lines were increased significantly compared with that of the parent β-elemene by WST-1 methods. The radiolabelling of these three β-elemene Re free derivatives with *fac*-$[^{188}Re(CO)_3(H_2O)_3]^+$ was straightforward and efficient. Their further biological evaluation for radioactive

target compounds *in vivo* is under way and will be reported in due course.

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