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# Complexation with Organometallic Ruthenium Pharmacophores Enhances the Ability of 4-Anilinoquinazolines Inducing Apoptosis

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## Electronic Supplementary Information

## Experimental Section

### *1. Materials*

$RuCl_3 \cdot 3H_2O$ (Ru > 36.7%) was purchased from Shenyang Jingke Reagent Co. (China), 4-(3'-chloro-4'-fluoroanilino)-6-hydroxy-7-methoxyquinazoline (AR grade) from Shanghai FWD Chemicals Co. (China), 1,2-dibromoethane, 1,3-dibromopropane from Beijing Ouhe Technology Co. (China), ethylenediamine from Beijing Xingjin Chemicals Co. (China). Organic solvents including absolute methanol, absolute ethanol, absolute ether, acetonitrile, dichloromethane and DMSO were all analytical grade and used directly without further purification.

Column chromatography silica gel was purchased from Qingdao Jiyida Silica Reagent Manufacture (China), and thin layer chromatography silica gel from Yantai Insitute of Chemical Industry Research (China). Trifluoroacetic acid (TFA) was purchased from Sigma, chromatographic grade acetonitrile from Tedia Company (China).

The protein tyrosine kinase epidermal growth factor receptor (EGFR), and the epidermal growth factor (EGF) were purchased from Sigma, and other biological agents including the ELISA kits for EGFR inhibitor screening from Cell Signaling Technology Inc. (USA). Sodium silicate nonahydrate ($Na_2SiO_3 \cdot 9H_2O$), cetyltrimethylammonium bromide (CTAB), iron nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), ethylene glycol, ammonium hexafluorotitanate ($(NH_4)_2TiF_6$), boric acid ($H_3BO_3$) were purchased from Shanghai General Chemical Reagent Manufacture (China). The deionized water used in the experiments was prepared by a Milli-Q system (Millipore, Milford, MA).

### *2. Synthesis and characterization of 4-anilinoquinazoline derivatives*

2.1 4-(3'-chloro-4'-fluoroanilino)-6-(2-(2-aminoethyl)aminoethoxy)-7-methoxyquinazoline (L1)

The compounds 4-(3'-chloro-4'-fluoroanilino)-6-hydroxy-7-methoxyquinazoline (2.0

g, 6.3 mmol) and potassium carbonate (5.0 g, 36 mmol) were mixed in DMF (30 mL). Then 1,2-dibromoethane (2 mL, 23 mmol) was added and the resulting mixture was heated at 80 °C for 5 h. After cooling to room temperature, the mixture was filtered in vacuum and the filtrate was collected and poured into water (130 mL), followed by extraction using ethyl acetate (30 mL × 4), organic layers were combined and dried over magnesium sulfate. After concentration, the residue was chromatographed by flash chromatography on Silica gel using ethyl acetate/ petroleum (5:1) as eluent to give 4-(3'-chloro-4'-fluoroanilino)-6-(2-bromoethoxy)-7-methoxyquinazoline (L1′) as yellow powder (2.0 g, 65.2%). Then the intermediate L1′ (0.8 g, 0.87 mmol) was dissolved in acetonitrile (30 mL) containing ethylenediamine (1 mL, 15 mmol). The solution was heated to 80 °C and refluxed for 2.5 h, and the solvent was evaporated in vacuum and the residue was recrystallized from water and ethanol to give L1 as white powder (0.55 g, 72.1%). mp 145 – 147 °C (from water and ethanol). Anal. (Found C, 51.82; H, 5.05; N, 15.18. $C_{19}H_{21}ClFN_5O_2 \cdot HCl$ requires C, 51.59; H, 5.01; N, 15.83%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-d$^6$, $Me_4Si$) 9.56 (1 H, s, C(2)H), 8.50 (1 H, s, Ph), 8.14 (1 H, s, Ph), 7.86 (1 H, s, Ph), 7.82 – 7.80 (1 H, m, Ph), 7.44 (1 H, t, *J* 8.8, 7.2 Hz, Ph), 7.21 (1 H, s, PhNH), 6.61 (1 H, br s, NH), 4.19 (2 H, m, $OCH_2$), 3.94 (3 H, s, $OCH_3$), 3.43 (2 H, br s, $NH_2$), 3.21 – 2.89 (4 H, m, $NCH_2$), 2.75 – 2.66 (2 H, m, $NCH_2$). ESI-MS: *m/z* (the most abundant isotopomer) 406.9 ($M + H^+$, $C_{19}H_{22}ClFN_5O_2$ requires 406.9).

2.2. 4-(3'-chloro-4'-fluoroanilino)-6-(3-(2-aminoethyl)aminopropoxy)-7-methoxyquinazoline (L2)

The compounds 4-(3'-chloro-4'-fluoroanilino)-6-hydroxy-7-methoxyquinazoline (2.0 g, 6.3 mmol) and potassium carbonate (5.0 g, 36 mmol) were mixed in acetone (100 mL). Then 1, 3-dibromopropane (3.2 mL, 23 mmol) was added and the resulting mixture was refluxed for 4.5 h. After cooling to room temperature, the mixture was filtered in vacuum and the filtrate was evaporated to give yellow oil, which was chromatographed by flash chromatography on Silica gel using ethyl acetate/ petroleum (5:1) as eluent to give

4-(3'-chloro-4'-fluoroanilino)-6-(3-bromopropoxy)-7-methoxyquinazoline (L2′) as yellow powder (1.93 g, 70.3%.). Then the intermediate L2′ (0.8 g, 0.87 mmol) was dissolved in acetonitrile (30 mL) containing ethylenediamine (1 mL, 15 mmol). The solution was refluxed for 2.5 h, and the solvent was evaporated in vacuum and the residue was recrystallized from water and ethanol to give L2 as white powder (0.5 g, 65.8%). mp 120 – 123 °C (from water and ethanol). Anal. (Found C, 52.44; H, 5.34; N, 14.53. $C_{20}H_{23}ClFN_5O_2 \cdot HCl$ requires C, 52.64; H, 5.30; N, 15.35%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-d$^6$, $Me_4Si$): 9.60 (1 H, s, C(2)H), 8.51 (1 H, s, Ph), 8.15 (1 H, s, Ph), 7.87 (1 H, m, Ph), 7.84 – 7.82 (1 H, m, Ph), 7.48 – 7.43 (1 H, t, $J$ = 9.0, 9.0 Hz, Ph), 7.22 (1 H, s, PhNH), 6.72 (1 H, br s, NH), 4.23 (3 H, s, $OCH_3$), 3.95 – 3.92 (4 H, m, $NCH_2$), 3.65 (2 H, br s, $NH_2$), 2.84 – 2.81 (2 H, m, $NCH_2$), 2.77 – 2.70 (6 H, m, $NCH_2$), 2.01 – 1.97 (2 H, m, $CH_2$). ESI-MS: *m/z* (the most abundant isotopomer) 420.8 ($M+H^+$, $C_{20}H_{23}ClFN_5O_2$ requires 420.9).

### 3. *General synthesis procedure for the Ru (arene) compounds 1 – 6*

The ruthenium arene dimer $[(\eta^6\text{-arene})RuCl_2]_2$ (arene = benzene, *p*-cymene and biphenyl) were prepared following the methods described in the literature.[1] The 4-anilinoquinazoline derivatives L1 or L2 (0.26 mmol) and corresponding ruthenium dimer $[(\eta^6\text{-arene})RuCl_2]_2$ (0.13 mmol) were dissolved in methanol (10 mL), and the resulting solution was stirred at 298 K for 4 h and filtered. Then ammonium hexafluorophosphate (0.39 mmol) was added to this mixture and further stirred for 10 min. The solution was slowly condensed in vacuum to *ca.* 3 mL, and stood at room temperature for precipitation. And the precipitate was collected by filtrating and washing with methanol and ether in succession to give the product.

*Compound* ***1***. Yellow powder (26.1 mg, 13.1%). mp 212 – 216 °C (from methanol). Anal. (Found C, 38.68; H, 3.67; N, 9.02. $C_{25}H_{27}Cl_2F_7N_5O_2PRu \cdot H_2O$ requires C, 38.33; H, 3.73; N, 8.94%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-d$^6$, $Me_4Si$): 9.88 (1 H, s, C(2)H), 8.61 (1 H, s, Ph), 8.12 (1 H, d, $J$ = 5.2 Hz, Ph), 7.98 (1 H, s, Ph), 7.77 (1 H, s, Ph),

7.49 (1 H, t, *J* = 9.2, 9.2 Hz, Ph), 7.31 (1 H, s, PhNH), 6.84 (1 H, br s, NH), 6.26 (1 H, br s, NH), 5.79 (6 H, s, Ph), 4.02 (3 H, s, $OCH_3$), 3.96 – 3.92 (2 H, m, $OCH_2$), 3.86(1 H, br, s, NH), 3.96 – 3.51 (2 H, m, $NCH_2$), 2.71 – 2.65 (2 H, m, $NCH_2$), 2.42 – 2.28 (2 H, m, $NCH_2$). $^{13}$C NMR: $\delta_C$ (300 MHz, DMSO-$d^6$, $Me_4Si$): 156.78, 155.46, 155.12, 153.54, 148.22, 147.93, 145.94, 137.37, 128.91, 124.16, 122.95, 119.56, 117.31, 117.04, 109.40, 108.29, 103.82, 89.02, 88.25, 85.16, 84.54, 84.03, 83.27, 82.68, 68.14, 56.68, 53.97, 52.05, 46.20. ESI-MS: *m/z* (the most abundant isotopomer) 620.3 ($M^+$, $C_{25}H_{27}Cl_2FN_5O_2Ru$ requires 620.1); 584.3 ($[M - Cl]^+$, $C_{25}H_{27}ClFN_5O_2Ru$ requires 584.2).

*Compound* ***2.*** Yellow powder (76.8 mg, 36.2%). mp 199 – 201 °C (from methanol). Anal. (Found C, 42.06; H, 4.46; N, 7.99. $C_{29}H_{35}Cl_2F_7N_5O_2PRu\cdot CH_3OH$ requires C, 42.21; H, 4.61; N, 8.20%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-$d^6$, $Me_4Si$): 9.63 (1 H, s, C(2)H), 8.54 (1 H, s, Ph), 8.13 (1 H, dd, *J* = 7.2, 1.6 Hz, Ph), 7.94 (1 H, s, Ph), 7.82 – 7.80(1 H, m, Ph), 7.47 (1 H, t, *J* 9.6 Hz, 9.6 Hz, Ph), 7.31 (1 H, s, Ph), 6.60 (1 H, br s, NH), 6.53 (1 H, br s, NH), 5.79 (1 H, m, Ph), 5.68 (1 H, d, *J* = 6.4 Hz, Ph), 5.63 (1 H, t, *J* = 4.8, 4.8 Hz, Ph), 5.57 (1 H, d, *J* = 6.0 Hz, Ph), 4.01 (3 H, s, $OCH_3$), 3.81 (1 H, br s, NH), 2.87 – 2.83 (2 H, m, $OCH_2$), 2.71 – 2.67 (2 H, m, $NCH_2$), 2.33 – 2.27 (2 H, m, $NCH_2$), 2.23 (3 H, s, $CH_3$), 2.09 (1 H, m, CH), 2.01 – 1.99 (2 H, m, $NCH_2$), 1.23 (6 H, d, *J* = 6.0 Hz, $2CH_3$). $^{13}$C NMR: $\delta_C$ (600 MHz, DMSO-$d^6$, $Me_4Si$): 156.61, 154.91, 154.50, 153.40, 152.89, 148.13, 147.76, 137.31, 123.97, 122.82, 119.37, 117.15, 109.21, 108.09, 104.72, 103.40, 96.59, 83.48, 82.37, 81.34, 67.46, 56.51, 54.62, 51.44, 45.81, 30.51, 22.81, 22.19, 17.36. ESI-MS: *m/z* (the most abundant isotopomer) 676.1 ($M^+$, $C_{29}H_{35}Cl_2FN_5O_2Ru$, requires 676.1); 640.1($[M - Cl]^+$, $C_{29}H_{35}ClFN_5O_2Ru$, requires 640.2).

*Compound* ***3***. Yellow powder (54.7 mg, 25.3%). mp 187 – 190 °C (from methanol). Anal. (Found: C, 43.18; H, 3.93; N, 7.69. $C_{31}H_{31}Cl_2F_7N_5O_2PRu\cdot H_2O$ requires C, 43.32; H, 3.87; N, 8.15%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-$d^6$, $Me_4Si$): 9.63 (1 H, s, C(2)H), 8.55 (1 H, s, Ph), 8.17 (1 H, dd, *J* = 6.8, 2.0 Hz, Ph), 7.90 (1 H, s, Ph), 7.67 –

7.65 (2 H, m, Ph), 7.31 (1 H, s, PhNH), 6.71 (1 H, br s, NH), 6.43 (1 H, br s, NH), 6.29 – 6.24 (3 H, m, Ph), 6.08 – 6.03 (3 H, m, Ph), 5.94 – 5.86 (4 H, m, Ph), 4.03 (3 H, s, $OCH_3$), 3.92 (2 H, m, $OCH_2$), 3.81 (1 H, br s, NH), 3.27 – 3.18 (2 H, m, $NCH_2$), 2.73 – 2.69 (2 H, m, $NCH_2$), 2.32 – 2.30 (1 H, m, NCH), 2.05 – 2.02 (1 H, m, NCH). $^{13}C$ NMR: $\delta_C$ (600 MHz, DMSO-$d^6$, $Me_4Si$): 156.60, 154.95, 154.49, 153.42, 152.88, 148.03, 147.77, 140.67, 137.21, 134.68, 123.96, 122.78, 119.38, 119.25, 117.15, 117.01, 109.25, 108.15, 103.58, 96.85, 85.76, 84.16, 83.85, 82.04, 80.32, 67.83, 56.57, 56.49, 53.80, 52.05. ESI-MS: *m/z* (the most abundant isotopomer) 696.1 ($M^+$, $C_{31}H_{31}Cl_2FN_5O_2Ru$ requires 696.1); 661.2 ($[M - Cl]^+$, $C_{31}H_{31}ClFN_5O_2Ru$ requires 661.1).

*Compound* ***4***. Yellow powder (34.4 mg, 17.2%). mp 224 – 228 °C (from methanol). Anal. (Found: C, 38.43; H, 3.82; N, 8.64. $C_{26}H_{29}Cl_2F_7N_5O_2PRu·2H_2O$ requires C, 38.29; H, 4.08; N, 8.59%). $^1H$ NMR: $\delta_H$ (400 MHz, DMSO-$d^6$, $Me_4Si$): 9.69 (1 H, s, C(2)H), 8.54 (1 H, s, Ph), 8.18 – 8.16 (1 H, m, $J$ = 6.8, 6.8, 2.8 Hz, Ph), 7.98 (1 H, d, $J$ = 8.4 Hz, Ph), 7.85 – 7.81 (1 H, m, Ph), 7.30 (1 H, s, Ph), 7.25 (1 H, s, PhNH), 6.89 (2 H, br s, NH), 5.81 (6 H, s, Ph), 4.01 (3 H, s, $OCH_3$), 3.93 – 3.90 (2 H, m, $NCH_2$), 3.87 (1 H, br s , NH), 2.91 – 2.83 (1 H, m, NCH), 2.73 – 2.70 (2 H, m, $NCH_2$), 2.62 – 2.57 (1 H, m, NCH), 2.40 – 2.27 (2 H, m, $NCH_2$), 2.02 – 1.99 (2 H, m, $CH_2$). $^{13}C$ NMR: $\delta_C$ (600 MHz, DMSO-$d^6$, $Me_4Si$): 156.62, 154.95, 154.49, 153.40, 152.88, 148.05, 147.77, 137.21, 128.78, 124.01, 122.83, 119.35, 119.22, 117.11, 116.96, 109.25, 108.13, 103.65, 88.87, 88.81, 83.87, 67.97, 56.54, 53.76, 51.89, 46.07. ESI-MS: *m/z* (the most abundant isotopomer) 634.1 ($M^+$, $C_{26}H_{29}Cl_2FN_5O_2Ru$ requires 634.1); 599.1($[M - Cl]^+$, $C_{26}H_{29}ClFN_5O_2Ru$ requires 599.1).

*Compound* ***5.*** Yellow powder (73.8 mg, 34.3%). mp 176 – 180 °C (from methanol). Anal. (Found: C, 41.07; H, 4.30; N, 7.70. $C_{30}H_{37}Cl_2FN_5O_2PRu·2.5H_2O$ requires C, 40.92; H, 4.81; N, 7.95%). $^1H$ NMR: $\delta_H$ (400 MHz, DMSO-$d^6$, $Me_4Si$): 9.65 (1 H, s, C(2)H), 8.54 (1 H, d, $J$ = 8.54 Hz, Ph), 8.15 (1 H, dd, $J$ = 6.8, 2.8 Hz, Ph), 7.95 (1 H, s, Ph), 7.84 – 7.79 (1 H, m, Ph), 7.47 (1 H, t, $J$ = 9.2, 9.2 Hz, Ph), 7.31 (1 H, s, PhNH), 6.65 (1 H, br s, NH), 6.55 (1 H, br s, NH), 5.80 (1 H, m, Ph), 5.69 (1 H, d, $J$ = 6.4 Hz,

Ph), 5.70 – 5.64 (1 H, m, Ph), 5.57 (1 H, d, $J$ = 6.0 Hz, Ph), 4.01 (3 H, s, $OCH_3$), 3.95 – 3.90 (2 H, m, $OCH_2$), 3.83 (1 H, br s, NH), 2.89 – 2.82 (2 H, m, $NCH_2$), 2.78 – 2.69 (2 H, m, $NCH_2$), 2.24 (3 H, s, $CH_3$), 2.17 – 2.11 (2 H, m, $NCH_2$), 2.09 (1 H, s, CH), 1.21 (6 H, d, $J$ = 6.8 Hz, $CH_3$), 1.18 – 1.16 (2 H, m, $CH_2$). $^{13}$C NMR: $\delta_C$ (600 MHz, DMSO-$d^6$, $Me_4Si$): 156.60, 154.90, 154.49, 153.39, 152.87, 148.11, 147.76, 137.21, 123.89, 122.81, 119.24, 117.14, 109.31, 108.08, 104.74, 103.43, 96.57, 83.39, 82.37, 81.36, 67.46, 56.50, 54.57, 51.43, 45.81, 30.51, 22.81, 22.19, 21.96, 17.25. ESI-MS: *m/z* (the most abundant isotopomer) 690.2 ($M^+$, $C_{30}H_{37}Cl_2FN_5O_2Ru$ requires 690.1); 655.2 ($[M - Cl]^+$, $C_{30}H_{37}ClFN_5O_2Ru$ requires 655.2).

*Compound* ***6***. Yellow powder (46.7 mg, 21.1%). mp 159 – 162 °C (from methanol). Anal. (Found: C, 43.98; H, 3.92; N, 7.74. $C_{32}H_{33}Cl_2F_7N_5O_2PRu \cdot H_2O$ requires C, 44.00; H, 4.04; N, 8.02%). $^1$H NMR: $\delta_H$ (400 MHz, DMSO-$d^6$, $Me_4Si$): 9.77 (1 H, s, C(2)H), 8.57 (1 H, s, Ph), 8.17 – 8.12 (1 H, m, $J$ = 6.8, 2.8 Hz, Ph), 7.92 (1 H, d, $J$ = 4.0 Hz, Ph), 7.66 (1 H, dd, $J$ = 8.4, 1.2 Hz, Ph), 7.26 (1 H, s, Ph), 7.17 (1 H, s, PhNH), 6.73 (1 H, br s, Ph), 6.51 (1 H, br s, Ph), 6.30 (1 H, t, $J$ = 6.0, 6.0 Hz, Ph), 6.22 (1 H, d, $J$ = 6.0 Hz, Ph), 6.17 (1 H, d, $J$ = 6.0 Hz, Ph), 6.05 – 6.02 (1 H, m, Ph), 5.98 – 5.95 (1 H, m, Ph), 5.94 – 5.91 (1 H, m, Ph), 5.90 – 5.87 (1 H, m, Ph), 5.81 – 5.76 (3 H, m, Ph), 3.98 (3 H, s, $OCH_3$), 3.95 – 3.88 (2 H, m, $OCH_2$), 3.67 (1 H, br s, NH), 2.34 – 2.23 (6 H, m, $3NCH_2$), 2.18 – 2.15 (2 H, m, $CH_2$). $^{13}$C NMR: $\delta_C$ (600 MHz, DMSO-$d^6$, $Me_4Si$): 156.77, 155.13, 154.67, 153.09, 152.85, 148.62, 140.67, 136.99, 135.24, 134.60, 127.89, 127.20, 124.29, 123.10, 119.37, 117.13, 109.10, 107.17, 103.60, 97.37, 96.80, 85.73, 84.56, 84.26, 83.17, 80.86, 67.71, 56.52, 52.62, 50.31, 45.05, 44.81. ESI-MS: *m/z* (the most abundant isotopomer) 710.1 ($M^+$, $C_{32}H_{33}Cl_2FN_5O_2Ru$ requires 710.1); 675.1($[M - Cl]^+$, $C_{32}H_{33}ClFN_5O_2Ru$ requires 675.1).

### *3. X-ray crystallography*

Single-crystal X-ray diffraction analysis for [($\eta^6$-*p*-cymene)$Ru^{II}(L_1)Cl]PF_6$ (**2**) was carried out on a Rigaku Saturn 724 diffractometer (Rigaku Corporation, Japan) using

graphite monochromated MoKα radiation ($\lambda$ = 0.71073 Å) on a Rigaku Saturn 724 CCD area detector. All data were collected at 173 K, and structure solution and refinement were performed using the SHELXL-97. The crystallographic data are listed in Table S1, the selected bond lengths and angles in Table S2. Standard data relating to the X-ray crystal structure of compound **2** have been also deposited in the Cambridge Crystallographic Data Centre with the CCDC 924855.

### *4. High performance liquid chromatography (HPLC)*

An Agilent 1200 series quaternary pump and a Rheodyne sample injector with a 20 μL loop, an Agilent 1200 series UV-Vis DAD detector and Chemstation data processing system were used. The mobile phases were water containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). The separation of hydrolytic adducts of the ruthenium compounds and reaction mixtures of ruthenium compounds with 9-ethylguanine were carried out on an Agilent Eclipse XDB-C18 reversed-phase column (4.6×150 mm, 5 μm, Agilent Technologies). The gradient (B) was: 10% to 80% from 0 to 20 min, keeping at 80% to 22 min, and finally resetting to 10% at 25 min.

### *5. Electrospray ionization mass spectroscopy (ESI-MS)*

The positive-ion ESI mass spectra for the hydrolytic products and 9-EtG adduct of the ruthenium compounds were obtained with a Micromass Q-TOF (Waters) equipped with a Masslynx (ver. 4.0) data processing system for analysis and post processing. For the online LC-ESI-MS assays, an Agilent 1200 system was interfaced with the mass spectrometer, using the same column and gradients as described above for the HPLC assays with a flow rate of 1 mL/min and a splitting ratio of 1/10 into mass spectrometer. The spray voltage and the cone voltage were 2.8~3.8 kV and 55~70 V, respectively. The desolvation temperature was 393 K and the source temperature 373 K. Nitrogen was used as both cone gas and desolvation gas with a flow rate of 50 L $h^{-1}$ and 500 L $h^{-1}$, respectively. The collision energy was set up to 10

V. The spectra were acquired in the range of 200~2500 m/z. The mass accuracy of all measurements was within 0.001 *m/z* unit, and all *m/z* values are the mass-to-charge ratios of the most abundant isotopomer for observed ions.

### *6. Elemental analysis and NMR spectroscopy*

Elemental analysis was performed on a Flash EA 1112 element analysis instrument (ThermoQuest). $^{1}$H NMR spectra were obtained on Bruker Avance 400 spectrometer (Germany), and $^{13}$C NMR spectra were obtained on Bruker DMX 300 and Bruker Avance 600 spectrometer (Germany).

### *7. Hydrolysis of compounds 1 – 6 and interaction of compound 2 with 9-ethylguanine*

The kinetic studies on the hydrolysis of compounds **1** – **6** were carried out using UV-2550 spectrometer (Shimadzu, Japan). Firstly, the compound being tested was dissolved in DMSO with a concentration 2 mM, aliquot (10 μL) of the DMSO solution was then added to 190 μL deionized water in quartz cuvette and the UV-Vis spectra of the mixture was immediately recorded by scanning over the wavelength region from 200 – 500 nm at 5 minute intervals. The wavelength corresponding to the maximum changes in absorbance of each hydrolysis reaction was selected for measurement of the rate constant. The samples used for the kinetic study were prepared by the same procedure as described above, and the absorbance at selected wavelengths was then recorded at 20 s intervals. The absorbance/time data for each hydrolysis reaction were computer-fitted to the first-order rate equation [Eq. (1)], which gave the $k_{H_2O}$ value ($k$) for each aquation, where $C_0$ and $C_1$ are computer-fitted constants, $A$ is the absorbance corresponding to time t.

$$A = C_0 t + C_1 e^{-kt} \qquad (1).$$

For the identification of the hydrolysis products of compound **2**, the sample was prepare by diluting methanol solution of **2** (10 mM) with deionized water to give a

final concentration of 1 mM. The solution was incubated at 298 K for 2 h, aliquot of which was then analysed by HPLC coupled to ESI-MS.

For the identification of the reaction products of compound **2** (0.2 mM) with 9-ethylguanine (9-EtG), an aliquot of methanol solution of **2** (10 mM) was mixed with 3-fold excess of 9-EtG in methanol, and the mixture was stirred at 298 K for 2 h. Then the resulting mixture was analysed by HPLC coupled to ESI-MS.

***8. Enzyme-linked immunosorbent assay (ELISA)***

Enzyme-linked immunosorbent assay (ELISA), a widely used *in vitro* screening method for enzyme inhibitors[2] was applied to characterise the inhibition potency of ruthenium arene compounds containing 4-anilinoquinazoline ligands towards the enzyme epidermal growth factor receptor (EGFR). The receptor tyrosine kinase solution in 50% glycerol, containing 50 mM HEPES (pH 7.6), 150 mM NaCl, 0.1% Triton and 1 mM dithiothreitol (DTT) was purchased from SIGMA Chemical Company; Signal Transduction Protein (Tyr66) biotinylated peptide, Phospho-Tyrosine Mouse mAb (P-Tyr-100), HTScan® Tyrosine Kinase Buffer (4×), Adenosine-triphosphate (ATP), DL-Dithiothreitol (DTT) were purchased from Cell Signalling Company; HRP-labeled Goat Anti-Mouse lgG (H+L) was purchased from Zhongshan Golden bridge Biotechnology Co. Ltd (China), Bovine Serum Albumin (BSA), 3, 3', 5, 5'-Tetramethylbenzidine (TMB) from Xinjingke Biotechnology Co. Ltd (China), and Streptavidin from Tianjin Biotechnology Co. Ltd (China); 96-well plates were purchased from Beijing BioDee BioTech Co. Ltd.

The ELISA screening was performed following the instruction provided by the supplier of the assay kits (No. 7909, Cell Signalling Technology, Inc). An aliquot (10 μL) of the enzyme solution was added to 415 μL DTT kinase buffer which is consist of 5 1.25 M DTT and 4× HTScan® Tyrosine Kinase Buffer (240 mM HEPES (pH 7.5), 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 μM $Na_3VO4$). Each test compound was dissolved in dimethylsulfoxide (DMSO) to give a 4 mM solution which was diluted with 0.05% Tween-20 in deionised water to give a 40 μM solution. The ATP/peptide mixture was

prepared by addition of 10 μL of 10 mM ATP to 125 μL of 6 μM substrate peptide, and then diluted with $D_2O$ to 250 μL.

An aliquot (12.5 μL) of the solution of a tested compound was mixed with as-prepared EGFR solution (12.5 μL) and incubated at 298 K for 5 minutes, followed by addition of 25 μL of ATP/substrate mixture, and then the resulting mixture was incubated at 310 K for 1 h. The phosphorylation reaction was terminated by the addition of 50 μL/well stop buffer (50 mM EDTA, pH 8).

Each well of a microtitre plate was coated with 100 μL of 10 μg $mL^{-1}$ streptavidin in carbonate-bicarbonate buffer and incubated overnight at 277 K, and then blocked with 1.5% bovine serum albumin (BSA) in PBS/T (PBS solution contain 0.05% Tween-20) at 310 K for 2 h, followed by three times of washing with PBS/T prior to use. Then, 25 μL/well of each enzymatic reaction mixture and 75 μL/well of $D_2O$ were added to the plate (in triplicate) for incubation at 310 K for 1 h. Following three times of washing with PBS/T, 100 μL of primary antibody (Phospho-Tyrosine Mouse mAb, 1:1000 in PBS/T with 1.5% BSA) was added to each well and the plate was incubated at 310 K for another 1 h. The plate was again washed three times with PBS/T, and then 100 μL of secondary antibody (HRP-labelled Goat Anti-Mouse lgG, 1:1000 in PBS/T with 1.5% BSA) was added to each well for 1 h of incubation at 310 K, followed by three times of washing with PBS/T. Finally, 100 μL of TMB substrate was added to each well and the plate was incubated at 310 K for 15 min, and then the reaction was stopped by addition of 100 μL of 2M $H_2SO_4$ to each well, and the plate was read on the ELISA plate reader (SpectraMax M5 Molecular Devices Corporation) at 450 nm to determine the OD values.

All reported $IC_{50}$ values were averages of three independent experiments and expressed as mean ± SD (standard deviation).

***9. In vitro anti-proliferation assays***

The human breast cancer cell line MCF-7 was obtained from the Centre for Cell Resource of Shanghai Institute for Biological Sciences, Chinese Academy of Science, and the human bronchial epithelial (HBE) cell line was a gift provided by Dr. Li Xu at

the Institute of Chemistry, CAS. MCF-7 cells were maintained in RPMI 1640 (Invitrogen, USA) media supplemented with 10% foetal calf serum (HyClone, USA). On requested, an aliquot of 100 ng $mL^{-1}$ epidermal growth factor (Sigma, USA) was added into the media. The cells were grown at 310 K in a humidified atmosphere containing 5% $CO_2$ for 2 – 3 days prior to screening experiments.

The $IC_{50}$ values, this is the concentration of tested compounds that inhibit 50% of cell growth of MCF-7 cell line were determined using the sulforhodamine B (SRB) technique. Cells were plated at a density of 6500 cells/well in 150 μL media in 96-well plates and grew in the absence or the presence of EGF for 24 h. The stock solutions (2 or 4 mM) of all tested compounds were made up fresh in 10% DMSO and saline before diluted down in media to give the required concentration for addition to the cells. The final concentration of DMSO in media was 0.5%. Cells were then exposed to each tested compound at various concentrations for 48 h and cell growth measured using SRB assay following the procedure reported by Skehan et al..[3] Briefly, after 48 h exposure to the tested compounds, cells were fixed with 50 μL of cold trichloroacetic acid (50%) per well (96-well plate) for 60 min at 310 K. After washed five times with tap water, the cells were stained for 30 min at 298 K with 0.5% acetic acid containing 0.4% sulforhodamine B (SRB, Sigma). Then, each plate was rinsed five times with 1% acetic acid and allowed to air dry. The resulting coloured residue was dissolved in 200 μL of Tris base (10 mM) and optical density (OD) value for each well was measured using a microplate reader (SpectraMax M5 Molecular Devices Corporation) at the wavelength of 570 nm. The inhibition rate (IR) was calculated based on the equation as followings:

$$IR\ (\%) = [1 - (OD_{compound} - OD_{blank}) / (OD_{control} - OD_{blank})] \times 100\ \%.$$

All reported values were averages of three independent experiments and expressed as mean ± SD (standard deviation).

The in vitro anti-proliferative activity of compounds **2** and **4** on healthy HBE cell line was characterized using the same method described above.

***10. Fluorescence microscopy***

Induction of apoptosis in human breast cancer cell line MCF-7 was evaluated by confocal microscope after DAPI (4′, 6-diamidino-2-phenylindole) staining. Briefly, $1\times10^5$ cells per well were seeded on cover glasses in a 24-well plate and allowed to attach for 16 h at 310 K. Cells were then treated with the test compounds at 310 K for 24 h. After removing the supernatant and washing the cells with PBS three times, the cells were fixed with the pre-cool methanol for 15 min. After that, the cells were treated with 1 μg $mL^{-1}$ DAPI (Sigma) in deionised water in dark. After washing the cells with PBS, the cover glasses were mounted on microscopy slides with glycerol. Fluorescence images were obtained on a Carl Zeiss LSM 510 META confocal microscope (Oberkochen, Germany) at excitation wavelength of 790 nm and emission wavelength of 461nm.

***11. Annexin v / propidium iodide (PI) double staining assay***

To further verify the induction of apoptosis by the synthesized ruthenium arene compounds, MCF-7 cells were seeded in a density of $2\times10^5$ per well in a 6-well plate and allowed to attach for 16 h, then the cells were exposed to different tested compounds at 310 K for 24 h. The supernatant was removed, and cells were detached by trypsinization after washing by PBS. The cells were transferred to FACS tubes after washing by PBS and centrifuged at 1000 rpm for 3 min. After re-suspension in 0.5 mL binding buffer, the cells were incubated with 5 μL Annexin-V conjugate for 5 min, followed by addition of 5 μL PI prior to the FACS analysis. The FACS assays were preformed on a Calibur flow cytometer (BD，Franklin Lakes, New Jersey, US), of which the FL1 channel was used to record the intensity of annexin V-FITC staining and FL2 channel to record the intensity of PI staining. The data were quantified by Sell Quest software (BD, Franklin Lakes, New Jersey, US).

**References**

1 1. R. E. Morris, R. E. Aird, P. del Socorro Murdoch, H. Chen, J. Cummings, N. D. Hughes, S.

Parsons, A. Parkin, G. Boyd, D. I. Jodrell and P. J. Sadler, *J. Med. Chem.*, 2001, **44**, 3616-3621.

2 E. Engvall and P. Perlmann, *J. Immunol.*, 1972, **109**, 129-135.

3 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer. Inst.*, 1990, **82**, 1107-1112.

**Table S1.** Crystal structure data for compound **2**.

| | |
|---|---|
| Formula | $C_{29}\ H_{35}\ Cl_2\ F_7\ N_5\ O_2\ P\ Ru$ |
| Molecular weight | 821.56 |
| Crystal system | Monoclinic |
| space group | P2(1)/c |
| *a* (Å) | 11.698(2) |
| *b* (Å) | 18.520(4) |
| *c* (Å) | 32.653(7) |
| *β* (º) | 94.53(3) |
| *V*(Å$^3$) | 7052(2) |
| Z | 8 |
| Crystal size (mm) | 0.22 x 0.18 x 0.06 |
| Crystal description | plate |
| Crystal color | yellow |
| $D_x$ (Mg/m$^3$) | 1.548 |
| $\mu$(mm$^{-1}$) | 0.713 |
| *T* (K) | 173(2) |
| Wavelength (Å) | 0.71073 |
| Theta range for data collection | 1.25 to 25.00 deg. |
| Data collection mode | ω scan |
| $\theta_{max}$(º) | 27.48 |
| No. of integrated refl. | 43399 |
| $R_{int}$ | 0.0756 |
| Final R and $R_w$ | 0.1027 0.2490 |
| No. of parameters | 863 |
| $\Delta\rho_{max}$ $\Delta\rho_{min}$ (e Å$^{-3}$) | 1.073 -1.149 |

**Table S2.** Selected bond lengths (Å), angles and torsion (º) for compound **2**

| | | | |
|---|---|---|---|
| Ru(1)-C(2) | 2.212(10) | Ru(1)-C(5) | 2.194(10) |
| Ru(1)-C(3) | 2.196(11) | Ru(1)-C(6) | 2.168(10) |
| Ru(1)-C(4) | 2.162(11) | Ru(1)-C(7) | 2.182(10) |
| Ru(1)-N(1) | 2.123(8) | Ru(1)-N(2) | 2.164(7) |
| Ru(1)-Cl(1) | 2.401(2) | N(2)-C(13) | 1.484(11) |
| C(1)-C(2) | 1.509(15) | C(6)-C(7) | 1.408(14) |
| C(2)-C(3) | 1.418(16) | C(2)-C(7) | 1.437(15) |
| C(3)-C(4) | 1.420(15) | C(5)-C(8) | 1.561(16) |
| C(4)-C(5) | 1.393(15) | C(8)-C(9) | 1.472(17) |
| C(5)-C(6) | 1.389(15) | C(8)-C(10) | 1.519(17) |
| C(11)-C(12) | 1.515(13) | C(13)-C(14) | 1.504(12) |
| N(1)-C(11) | 1.477(12) | N(2)-C(12) | 1.467(11) |
| | | | |
| N(1)-Ru(1)-C(2) | 127.6(4) | N(1)-Ru(1)-C(5) | 111.8(4) |
| N(1)-Ru(1)-C(3) | 164.8(4) | N(1)-Ru(1)-C(6) | 91.2(4) |
| N(1)-Ru(1)-C(4) | 147.5(4) | N(1)-Ru(1)-C(7) | 97.4(4) |
| N(2)-Ru(1)-C(2) | 92.4(3) | N(2)-Ru(1)-C(5) | 168.9(4) |
| N(2)-Ru(1)-C(3) | 101.3(3) | N(2)-Ru(1)-C(6) | 146.8(3) |
| N(2)-Ru(1)-C(4) | 131.9(4) | N(2)-Ru(1)-C(7) | 111.5(3) |
| N(1)-Ru(1)-Cl(1) | 84.9(2) | N(2)-Ru(1)-Cl(1) | 85.1(2) |
| C(4)-Ru(1)-Cl(1) | 88.7(3) | C(5)-Ru(1)-Cl(1) | 95.9(3) |
| | | | |
| C(6)-Ru(1)-C(2)-C(7) | -29.5(6) | Cl(1)-Ru(1)-C(2)-C(7) | -152.5(5) |
| N(1)-Ru(1)-C(2)-C(7) | 44.3(8) | N(2)-Ru(1)-C(2)-C(7) | 122.8(6) |
| Ru(1)-C(2)-C(3)-C(4) | -52.6(8) | N(1)-Ru(1)-C(3)-C(2) | -11.8(17) |

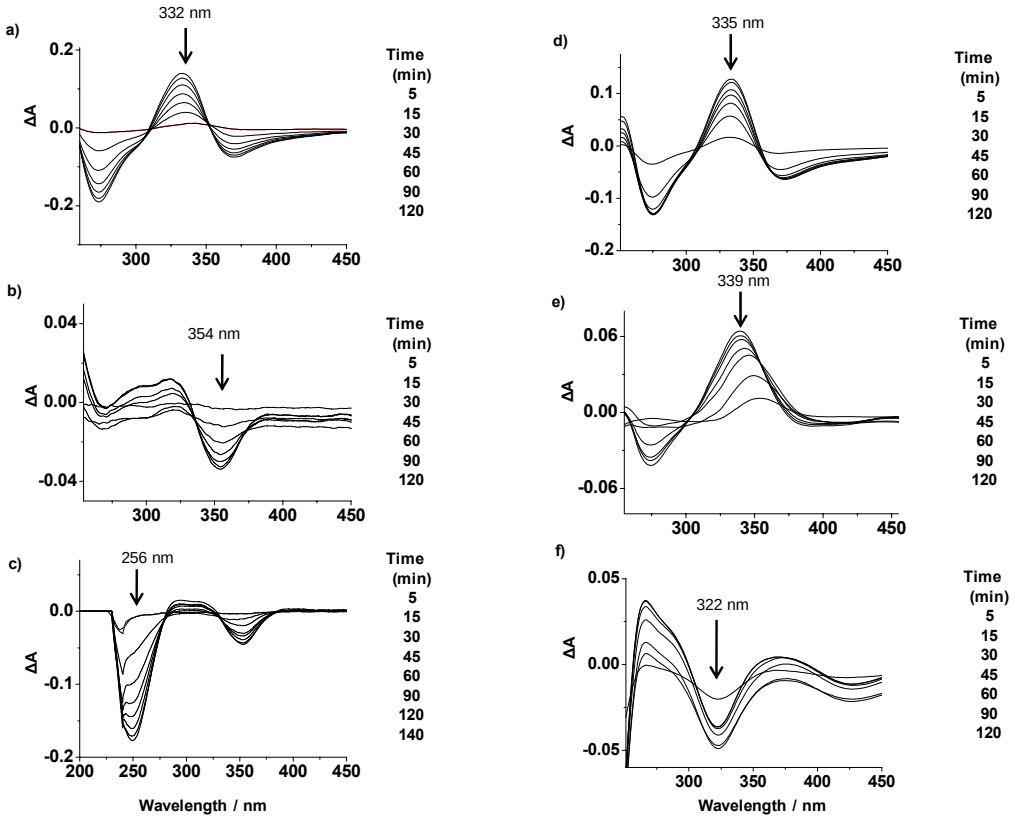


**Figure S1**. Time evolution of UV-Vis differential spectra for the aquation of (a) $[(\eta^6\text{-ben})Ru(L_1)Cl]PF_6$ (**1**), (b) $[(\eta^6\text{-}p\text{-cym})Ru(L_1)Cl]PF_6$ (**2**), (c) $[(\eta^6\text{-bip})Ru(L_1)Cl]PF_6$ (**3**), (d) $[(\eta^6\text{-ben})Ru(L_2)Cl]PF_6$ (**4**), (e) $[(\eta^6\text{-}p\text{-cym})Ru(L_2)Cl]PF_6$ (**5**), and (f) $[(\eta^6\text{-bip})Ru(L_2)Cl]PF_6$ (**6**) in aqueous solution at 298 K. The down arrows indicate the wavelengths selected for subsequent kinetic studies; $\Delta A = A_t - A_0$ ($A_t$ = absorbance at time $t$, $A_0$ = absorbance at the time 0.5 min, i.e. immediately after dilution of methanol solutions with water).

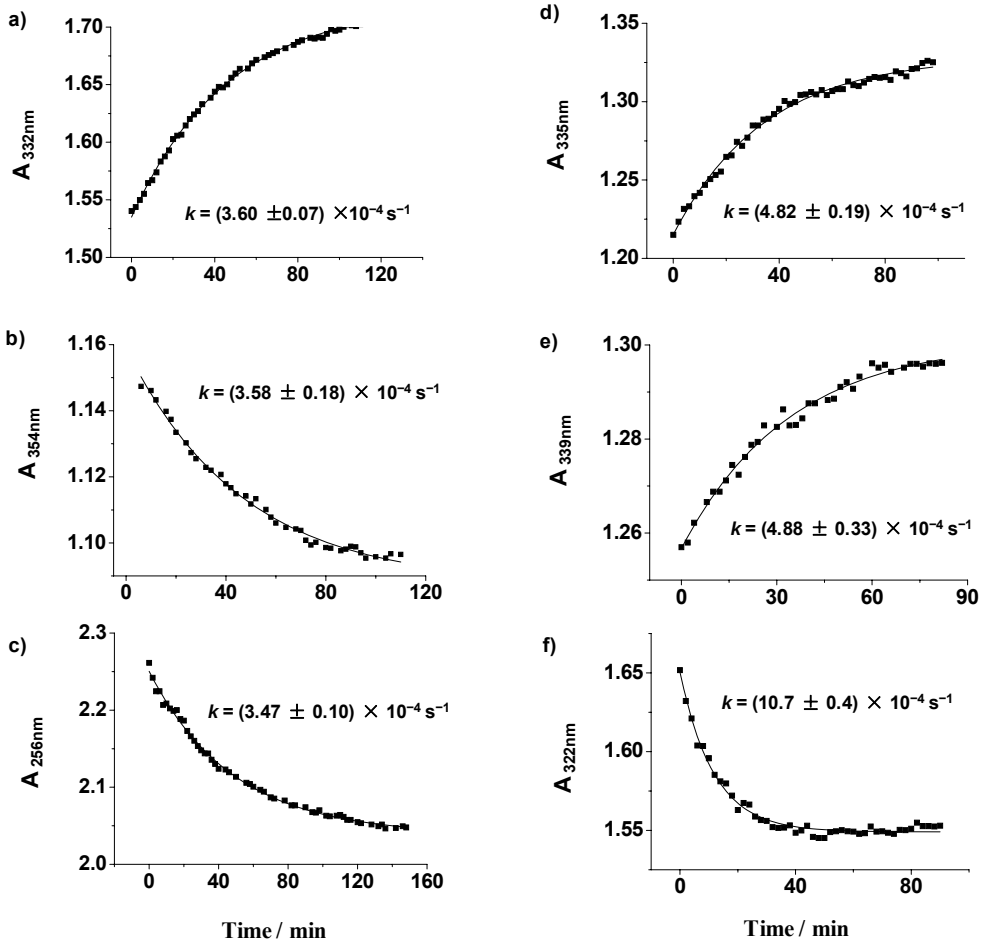


**Figure S2**. Time-dependence of the absorbance at selected wavelengths for the hydrolysis of 0.1 mM (a) $[(\eta^6\text{-ben})Ru(L_1)Cl]PF_6$ (**1**), (b) $[(\eta^6\text{-}p\text{-cym})Ru(L_1)Cl]PF_6$ (**2**), (c) $[(\eta^6\text{-bip})Ru(L_1)Cl]PF_6$ (**3**), (d) $[(\eta^6\text{-ben})Ru(L_2)Cl]PF_6$ (**4**), (e) $[(\eta^6\text{-}p\text{-cym})Ru(L_2)Cl]PF_6$ (**5**), and (f) $[(\eta^6\text{-bip})Ru(L_2)Cl]PF_6$ (**6**) in aqueous solution at 298 K. The full lines represent computer fits giving the first order rate constants shown in the insets and listed in Table 1 in the main text.

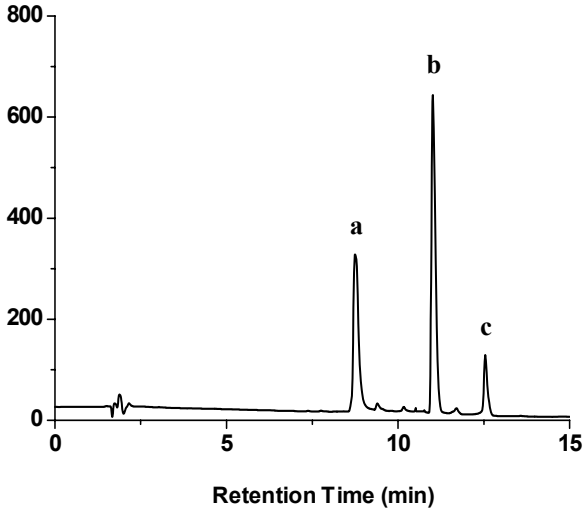


**Figure S3**. HPLC chromatogram with UV detection at 254 nm of compound **2** (1 mM) in aqueous solution incubated at 298 K for 2 h. Peak assignment: a, $t_r$ = 8.75 min, *m/z* 640.2, aqua adduct $[(\eta^6\text{-}p\text{-cym})Ru(L_1)(H_2O)]^{2+}$; b, $t_r$ = 11.01 min, *m/z* 674.7, the intact cation $[\mathbf{2} - PF_6]^+$; c, $t_r$ = 12.54 min, *m/z* 753.7, TFA adduct $[(\eta^6\text{-}p\text{-cym})Ru(CF_3COO)(L_1)]^+$. The mass spectra for each fraction are shown in Figure S4.

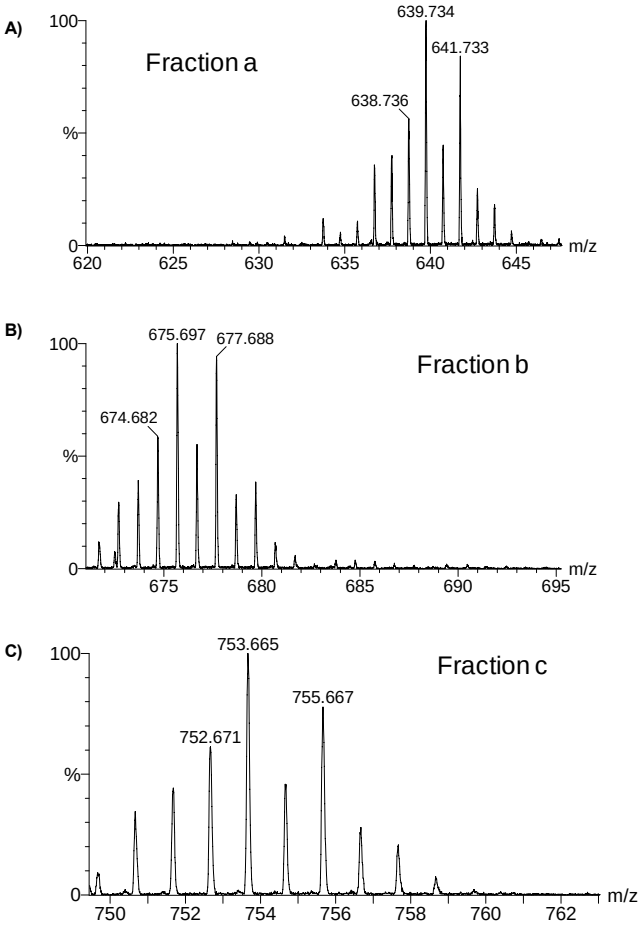


**Figure S4**. Mass spectra for HPLC fractions shown in Figure S3. The results indicate that the three HPLC fractions correspond to (A) the aqua adduct $[(\eta^6\text{-}p\text{-cym})Ru(L_1)(H_2O) - H]^+$, calcd. 640.1, found 639.7, (B) the intact cation [**2**$^+$] calcd. 674.7, found 675.7 and (C) the TFA adduct $[(\eta^6\text{-}p\text{-cym})Ru(CF_3COO)(L_1)]^+$ calcd. 754.1, found 753.7, which was formed by the substitution of the water molecule by TFA in the HPLC solvent, respectively.

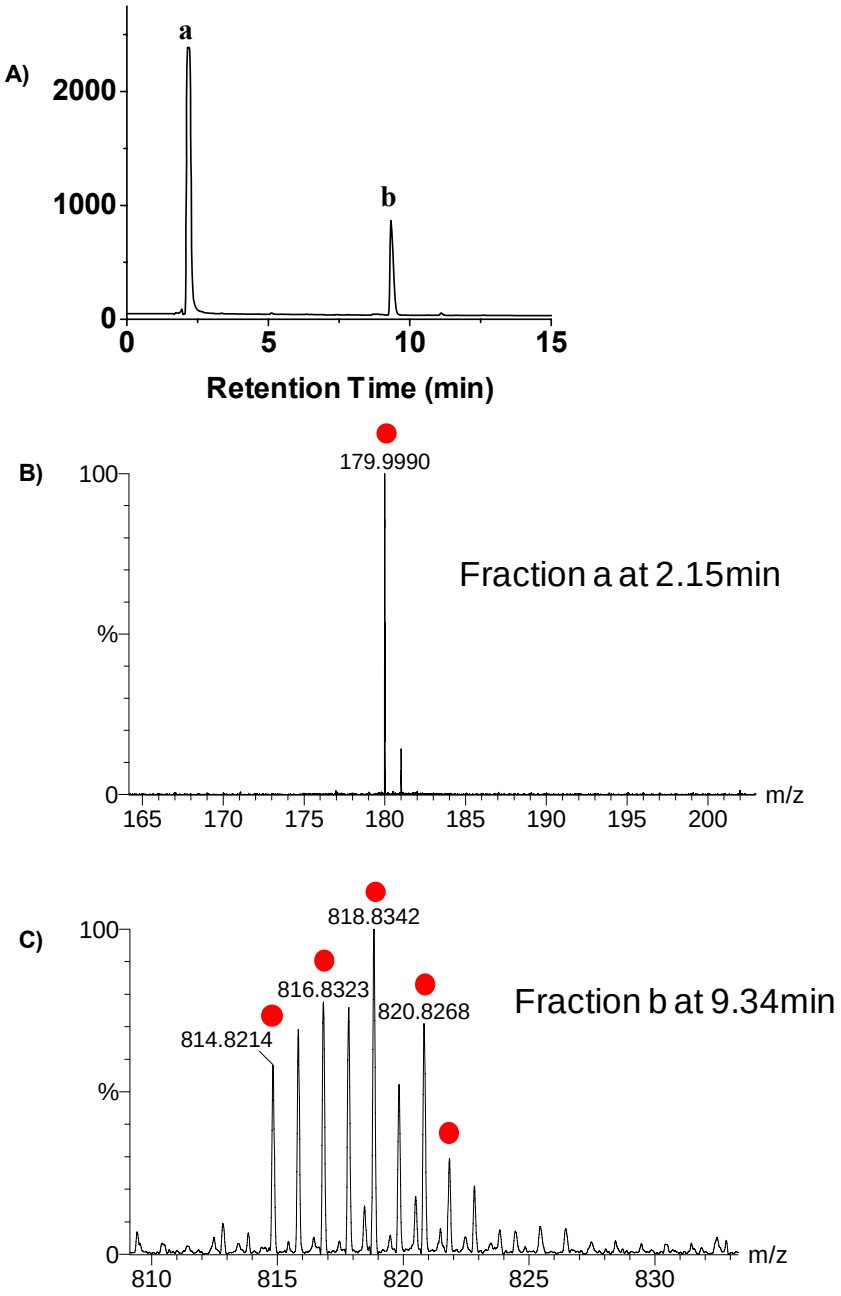


**Figure S5**. (A) HPLC chromatogram with UV detection at 254nm for the reaction mixture of compound **2** (200 μM) with 3-fold excess of 9-ethylguanine (9-EtG) in aqueous solution at 298 K for 2 h. Peak assignment: a, 9-EtG; b, adduct of **2** binding to 9-EtG. (B, C) Mass spectra for the HPLC fractions shown in (A), indicating that the two fractions contain $[9\text{-EtG} + H]^+$ $t_r$ = 2.15 min, (*m/z* found 180.0, the simulative peaks are labeled as red spot) and $[(\eta^6\text{-}p\text{-cym})Ru(L_1)(9\text{-EtG}) - H]^+$ $t_r$ = 9.34 min, (*m/z* found 820.2, and the simulative peaks are labeled as red spots), respectively.

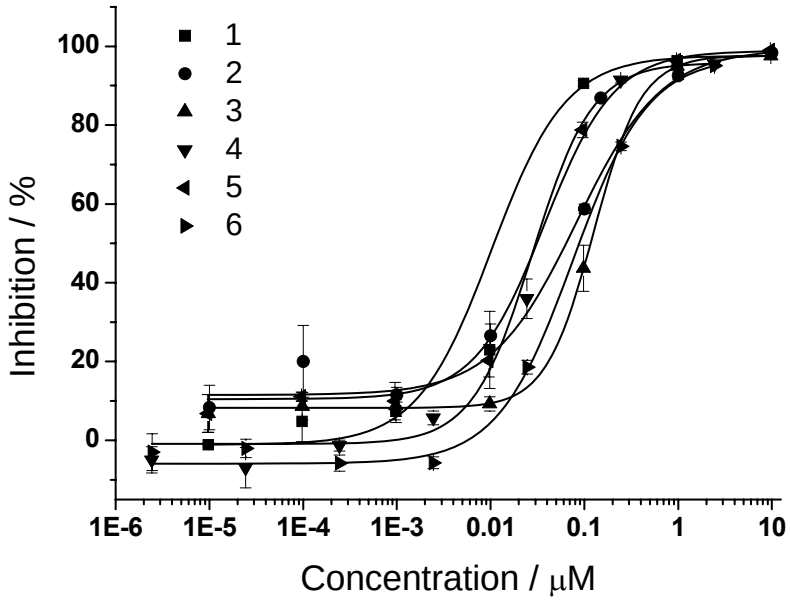


**Figure S6**. Dose-dependent inhibition curves of inhibition efficiency of the ruthenium arene compounds **1** – **6** against the activity of EGFR. *Points:* mean ± SD of triplicate determinations. Lines: computer-fitted curves.

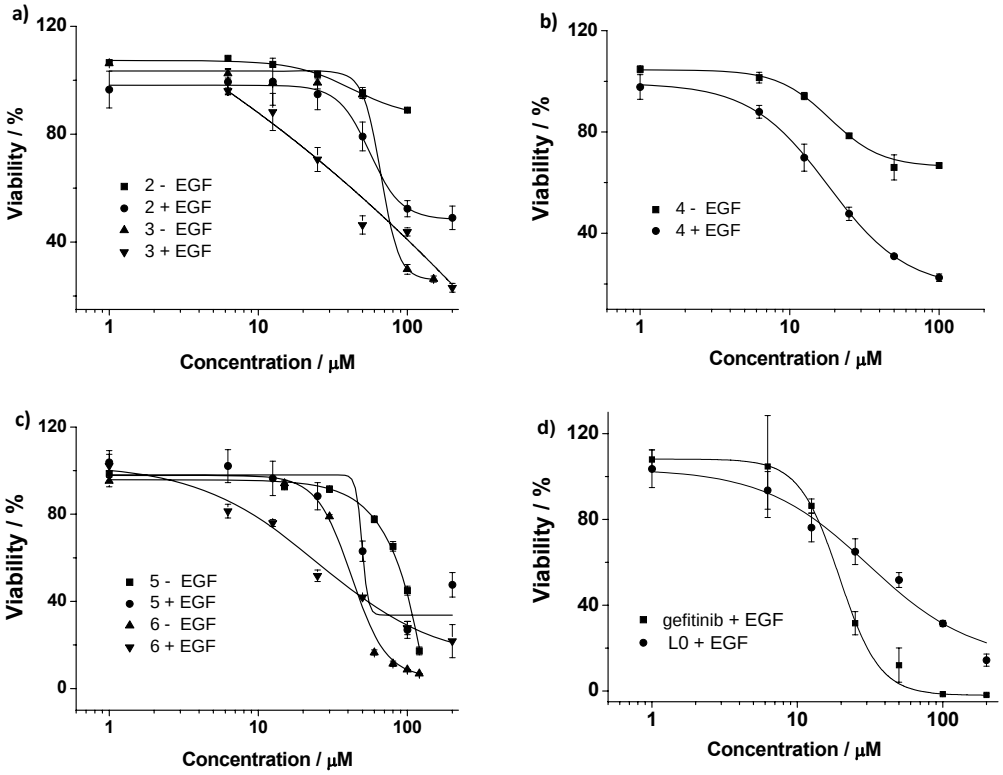


**Figure S7**. Dose-dependent inhibition curves of (a, b, c) compounds **2** – **6** and (d) gefitinib and L0 on the proliferation of MCF-7 cancer cells in the absence or the presence of EGF (10 nM).

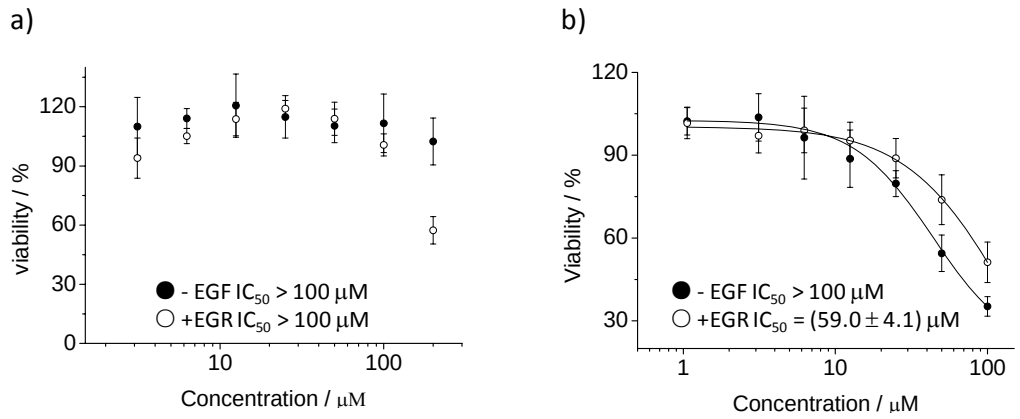


**Figure S8**. Dose-dependent inhibition curves of compounds **2** (a) and **4** (b) on the proliferation of HEB normal cell line in the absence (-) or the presence (+) of EGF (10 nM).