Supporting Information for

Fluoride as An Inducible DNA Cross-linking Agent for New Antitumor Prodrug

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Apparatus

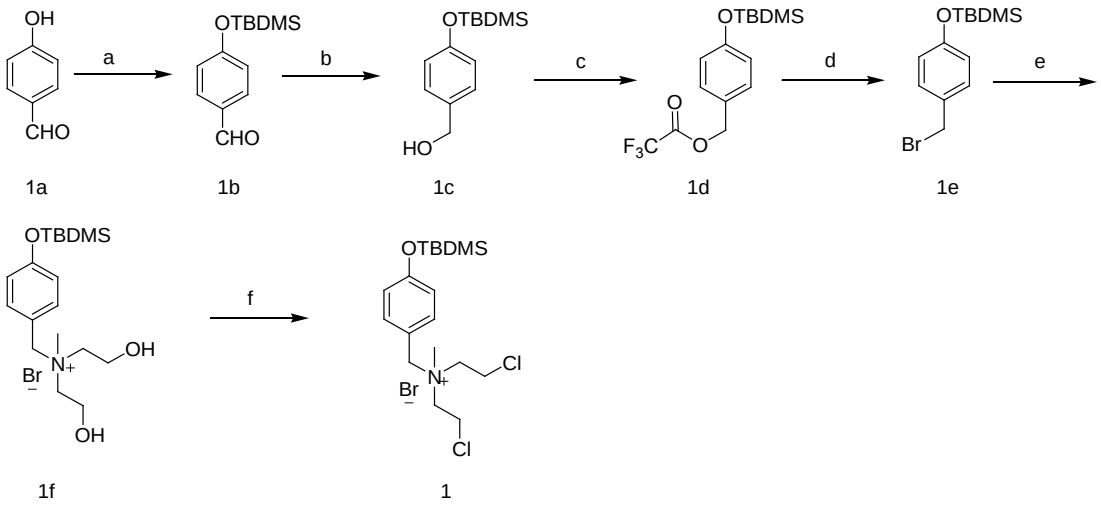
^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 spectrometers, respectively. HRMS were recorded on a Brucker APEX IV (7.0 T).

Materials

All solvents and reagents were commercially available and used without further purification unless for special needs: MEM (HyClone, Thermo Scientific), fetal bovine serum (FBS, HyClone), penicillin and streptomycin (Invitrogen), MTT (Sigma), and propidium iodide (Sigma-Aldrich). HeLa cells were purchased from China Center for Type Culture Collection.

Synthesis experiments

General information: Unless otherwise specified, chemicals were purchased from Alfa Aesar or Sigma-Aldrich and were used as received without further purification.



Scheme S1. Synthesis of compound **1**.a) *tert*-Butyldimethylsilyl chloride, chloroform, RT; b) sodium borohydride, ethanol, RT; c) TFA, THF; d) lithium bromide, THF; e) *N*-methyldiethanolamine, chloroform; f) thionyl chloride, RT.

Synthesis of compound 1

4-(*tert*-Butyldimethylsilyloxy)benzaldehyde (1b) [1]: To a mixture of **1a** (1.2 g, 10 mmol) in 50mL chloroforms was added *tert*-butyldimethylsilyl chloride (1.8 g, 12 mmol), TEA (1 ml), the reaction mixture was stirred at room temperature for 12 h. The mixture was diluted by DCM, washed by H_2O (3×50 mL) and dried with anhydrous Na_2SO_4. The mixture was evaporated and the residue was subjected to column chromatography on silica gel with 0–5% DCM in hexane to give the desired

product **1b** as a white solid (1.9 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.38 (s, 4 H), 3.08 (s, 9 H), 7.26 (s, 2 H), 11.97 ppm (s, 1 H).

4-(*tert*-Butyldimethylsilyloxy)phenylmethanol (1c) [1] **:** $NaBH_4$ (0.28 g, 7.5 mmol) was added to a mixture of **1b** (1.2 g, 5 mmol) in 50mL ethanol. The mixture was stirred at room temperature for 2h. Then the reaction mixture was evaporated and diluted with chloroform, washed by H_2O (3 × 50 mL) and dried with anhydrous Na_2SO_4 respectively. The solution was evaporated and the residue was subjected to column chromatography on silica gel with ethyl acetate/cyclohexane (1: 5, v/v) as eluent to get the desired product as a white solid **1c** (0.9 g, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.02 (s, 4 H), 0.89 (s, 9H), 4.87(s, 2 H), 5.31(s, 1 H), 6.70 (s, 2 H), 7.06 ppm (s, 2 H).

4-(*tert*-Butyldimethylsilyloxy)benzyl 2,2,2-trifluoroacetate (1d) [1]**:** TFA (1 ml, 1.2 mmol) was added to a mixture of **1c** (0.24 g, 1 mmol) in 30 mL THF. The reaction mixture was refluxed for 0.5h and then mixture was diluted by chloroform (30 mL), washed by H_2O (3 × 50 mL). After the isolated solution was dried with anhydrous Na_2SO_4, it was evaporated and the residue was subjected to column chromatography on silica gel with ethyl acetate/cyclohexane (1:10, v/v) as eluent to get the desired product **1d** as a yellow oil (0.23 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.06 (s, 4 H), 1.23(s, 9H), 4.98(s, 2 H), 7.06(s, 2 H), 7.36 ppm (s, 2 H).

4-(tert-Butyldimethylsilyloxy)benzylbromide (1e) [1]**:** To a mixture of **1d** (0.34 g, 1 mmol) in 30mL THF was added LiBr (0.1 g, 1.2 mmol), the reaction mixture was refluxed for 20h. After the mixture was diluted by chloroform (30 ml), washed by H_2O (3 × 50 mL) and dried with anhydrous Na_2SO_4, the solution was evaporated and the residue was subjected to column chromatography on silica gel with ethyl acetate/cyclohexane (1:10, v/v) as eluent to get the desired product **1e** as a yellow solid (0.23 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.21 (s, 4 H), 0.98 (s, 9 H), 4.56 (s, 2 H), 6.86 (s, 2 H), 7.16 ppm (s, 2 H).

4-Di*tert*-butylmethylsilyloxy-*N*, *N*'-bis(2-hydroxyethyl)-*N*, *N*'-dimethylphenyl (1f) : A solution of 4-(*tert*-butyldimethylsilyloxy)benzylbromide (**1e**) (0.15 g, 0.5 mmol) and *N*-methyldiethanolamine (0.12 g, 1 mmol) in chloroform (40 mL) was refluxed for overnight. After filtration, the rude product was recrystallized by ethanol and ethylether for several times. The product **1f** was obtained as a white solid (0.21 g, 90%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.82 (s, 4 H), 0.81(s, 9 H), 2.83(s, 3 H), 3.17-3.37 (m, 4 H), 3.76 (s, 4 H), 4.49 (s, 2 H), 5.20 (s, 2 H), 6.80 (s, 2 H), 7.35 ppm (s, 2 H). ^{13}C NMR (DMSO-d_6, 600 MHz): δ 153.5, 129.6, 125.5, 120.3, 66.3, 64.5, 48.1, 25.5, 17.3, -4.03 ppm. ESI-MS found m/z = 340.9 $[M\text{-}Br]^-$.

4-Di*tert*-butylmethylsilyloxy-*N*, *N*'-bis(2-chloroethyl)-*N*, *N*'-dimethylphenyl (1) : A solution of 4-di*tert*-butylmethylsilyloxy-*N*, *N*'-bis(2-hydroxyethyl)-*N*,

N′-dimethylphenyl (**1f**) (0.1 g, 0.20 mmol) in CH_2Cl_2 (20 mL) was slowly added dropwise to thinoyl chloride (2 mL) in ice-water bath, then stirred at r. t. for 2 days. After evaporated, the residue was recrystallized with CH_3OH/Et_2O several times, then the deaired product **1** was obtained (0.086 g, 85%). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.23 (s, 4 H), 0.96 (s, 9H), 3.07 (s, 3 H), 3.69-3.96 (m, 4H), 4.18 (s, 4 H), 4.72 (s, 2 H), 7.16 ppm (s, 2 H); ^{13}C NMR (DMSO-d_6, 600 MHz): δ151.5, 130.6, 126.5, 119.3, 65.3, 63.5, 46.1, 23.5, 16.3, -4.09 ppm. HRMS-ES (*m/z*) $[M-Br]^+$ calcd. For $C_{18}H_{32}Cl_2NOSi$, 376.1625; found, 376.1624

DNA experiments:

General Information: Plasmid DNA (pBR322) was purchased from Fermentas Co., Ltd. The DNA oligonucleotide was purchased from Takara BIO Co.Ltd., with the 5′-terminus fluorolabeled with TAMRA.

General protocol for alkaline agarose gel electrophoresis:

See reference 2.

Cross-linking of DNA oligonucleotide by compounds:

Cross-linking reaction was carried out in a volume of 10 μL containing 5 μM selective 5′-terminus fluoro-labeled oligonucleotide, 10 mM KF, 10 mM phosphate buffer (pH = 7.4) and 1 mM compound. The mixture was incubated at 37 ^{o}C for 3 h. Then the solution was mixed with 10 μL formamide deionized to the final volume of 20 μL. The residue was took out and then analyzed by a 20% denaturing polyacrylamide electrophoresis. The cross-linking products of compounds **1** and **2** with oligonucleotide were produced follow the same protocol mentioned as above.

Piperidine treatment

The cross-linked products were isolated by following procedures: first, ethanol and sodium acetate/acetic acid buffer (pH = 5) at -20 ^{o}C for one night, then the mixture was centrifugated 12000 rpm at 4 ^{o}C for 20 min, dried and treated with 100 μL 1 M piperidine for 30 min at 90 ^{o}C. After stored and precipitated by ethanol at -20 ^{o}C for one night, the DNA product fragments were then obtained and further dissolved it in formamide deionized. The cross-linking results were analyzed by polyacrylamide gel electrophoresis under denaturing conditions.

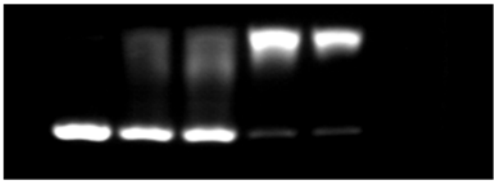


Figure S1. Cross-linking results of compound 2 with different fluoride concentration. lane 1: 0.5μg pBR322 control ; lane 2: 0.5 μg pBR322 + 50 μM compound **2** + 50 μM KF; lane 3 0.5μg pBR322 + 50 μM compound **2** + 100 μM KF; lane 3 : 0.5μg pBR322 + 50 μM compound **2** + 500 μM KF; lane 4: 0.5μg pBR322 + 50 μM compound **2** + 1 mM KF.

Figure S2. Cross-linking results of compound 2 with different anion selectivity. lane 1: 0.5μg pBR322 control ; lane 2: 0.5μg pBR322 + 50 μM compound **2** + 10 mM IO_4^-; lane 3 0.5μg pBR322 + 50 μM compound **2** + 10 mM OCl^-; lane 4 : 0.5μg pBR322 + 50 μM compound **2** + 10 mM Br^-; lane 5: 0.5μg pBR322 + 50 μM compound **2** + 10 mM I^-. lane 6: 0.5μg pBR322 + 50 μM compound **2** + 10 mM KF.

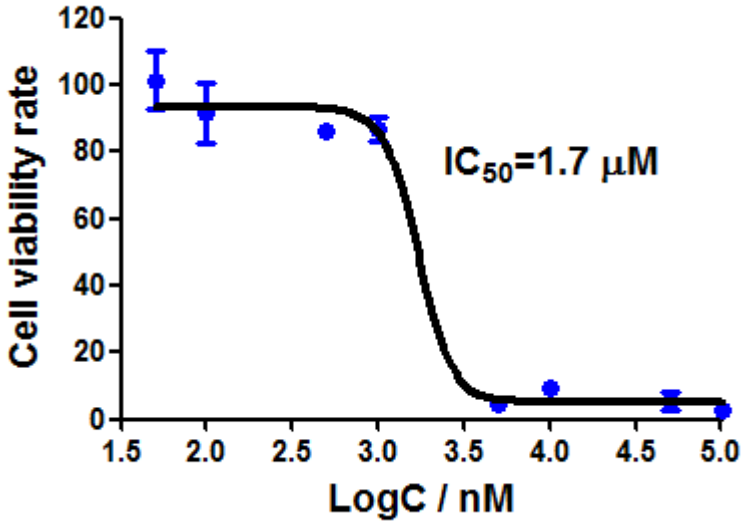


Figure S3. Cell viability in the presence of **2** at different concentrations (50 nM-100 μM) with 100 μM fluoride. The data were obtained through MTT assay and presented as mean± SD (n= 3).

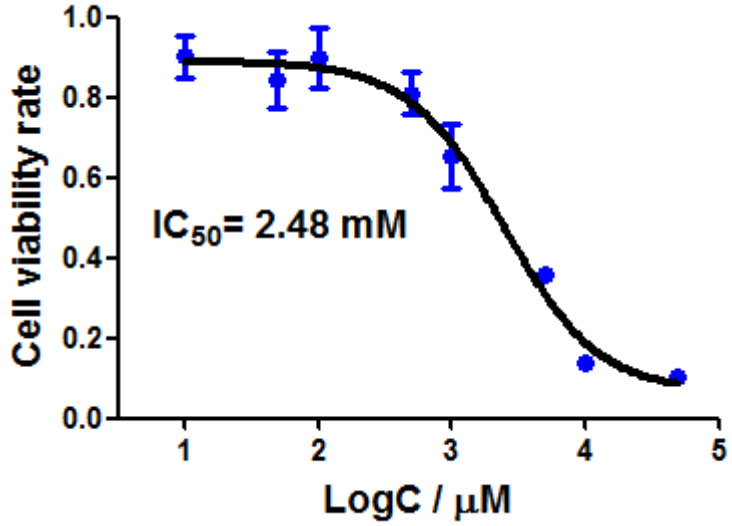


Figure S4. Cell viability in the presence of fluoride at different concentrations (10 μM-50mM). The data were obtained through MTT assay and presented as mean± SD (n= 3).

Reference

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2 P. Wang, R. P. Liu, X. J. Wu, H. J. Ma, X. P. Cao, P. Zhou, J. Y. Zhang, X. C. Weng, X. L. Zhang, J.Qi, X. Zhou, L. H. Weng, *J. Am. Chem. Soc.* 2003, **125**, 1116-1117.