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 Bruker 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](https://example.com/scheme.png)

**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): To a mixture of 1a (1.2 g, 10 mmol) in 50 mL chloroform was added tert-butyltrimethylsilyl 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$_2$O (3×50 mL) and dried with anhydrous Na$_2$SO$_4$. 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$_3$): $\delta = 1.38$ (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 50 mL ethanol. The mixture was stirred at room temperature for 2 h. Then the reaction mixture was evaporated and diluted with chloroform, washed by H$_2$O (3 $\times$ 50 mL) and dried with anhydrous Na$_2$SO$_4$ 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 (0.9 g, 90%). $^1$H NMR (300 MHz, CDCl$_3$): $\delta = 0.02$ (s, 4 H), 0.89 (s, 9 H), 4.87 (s, 2 H), 5.31 (s, 1 H), 6.70 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 0.5 h and then mixture was diluted by chloroform (30 mL), washed by H$_2$O (3 $\times$ 50 mL). After the isolated solution was dried with anhydrous Na$_2$SO$_4$, 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$_3$): $\delta = 0.06$ (s, 4 H), 1.23 (s, 9 H), 4.98 (s, 2 H), 7.06 ppm (s, 2 H).

4-(tert-Butyldimethylsilyloxy)benzylbromide (1e) $^1$: To a mixture of 1d (0.34 g, 1 mmol) in 30 mL THF was added LiBr (0.1 g, 1.2 mmol), the reaction mixture was refluxed for 20 h. After the mixture was diluted by chloroform (30 mL), washed by H$_2$O (3 $\times$ 50 mL) and dried with anhydrous Na$_2$SO$_4$, 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$_3$): $\delta = 0.21$ (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-butyl)methylsilyloxy-N,N'-bis(2-hydroxyethyl)-N,N'-dimethylphenyl (1f) $^1$: 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 overnight. After filtration, the crude 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$_6$): $\delta = 0.82$ (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): $\delta = 153.5, 129.6, 125.5, 120.3, 66.3, 64.5, 48.1, 25.5, 17.3, -4.03$ ppm. ESI-MS found m/z = 340.9 [M-Br].

4-Di(tert-butyl)methylsilyloxy-N,N'-bis(2-chloroethyl)-N,N'-dimethylphenyl (1) $^1$: A solution of 4-di(tert-butyl)methylsilyloxy-N,N'-bis(2-hydroxyethyl)-N,N'-dimethylphenyl (1f)
N'-dimethylphenyl (1f) (0.1 g, 0.20 mmol) in CH₂Cl₂ (20 mL) was slowly added dropwise to thioyl chloride (2 mL) in ice-water bath, then stirred at r. t. for 2 days. After evaporated, the residue was recrystallized with CH₃OH/Et₂O several times, then the desired product 1 was obtained (0.086 g, 85%). ¹H NMR (300 MHz, DMSO-d₆): 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); ¹³C NMR (DMSO-d₆, 600 MHz): δ 151.5, 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 C₁₈H₃₂Cl₂NOSi, 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 °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 °C for one night, then the mixture was centrifugated 12000 rpm at 4 °C for 20 min, dried and treated with 100 µL 1 M piperidine for 30 min at 90 °C. After stored and precipitated by ethanol at -20 °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 4: 0.5μg pBR322 + 50 μM compound 2 + 500 μM KF; lane 5: 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 IO₄⁻; 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
