SynOpen H. Nie et al. THIEME OPEN ACCESS Paper

Radiosynthesis and Tumor MicroPET/CT Imaging of ^{18}F-Fluoroethoxylerianin, an ^{18}F-Labeled Erianin Analogue

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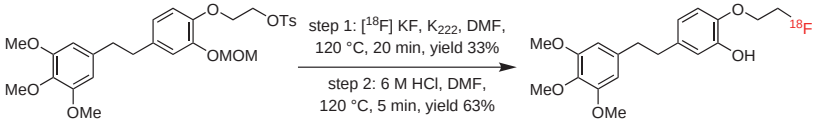


Received: 12.03.2022
Accepted after revision: 05.04.2022
Published online: 06.04.2022
DOI: 10.1055/a-1818-8330; Art ID: so-2022-d0009-op

Abstract Erianin is an active constituent of *Dendrobium candidum*. In this work, ^{18}F-fluoroethoxylerianin ([^{18}F]FEE), a ^{18}F-Labeled erianin analogue, was designed and synthesized to evaluate the properties of erianin and related analogues by *in vivo* PET imaging. The initial product was separated and purified by liquid-phase separation module Explora LC and simple homemade solid-phase extraction, and high purity [^{18}F]FEE was finally obtained. The radiochemical purity of [^{18}F]FEE was determined by Radio-TLC and Radio-HPLC. [^{18}F]FEE showed good stability in normal saline and serum, and could be quickly eliminated from mice. Cell experiments, biological distribution, and small-animal PET/CT further showed that [^{18}F]FEE had a high uptake rate in HepG2 tumor cells, and showed good imaging ability in a HepG2 tumor model. The results of this study indicate that the synthesized ^{18}F-labeled erianin analogue is an effective new probe for positron emission tomography (PET) imaging of HepG2 hepatocellular carcinoma, which provides an intuitive and reliable theoretical basis for the development of erianin as an anticancer drug.

Keywords fluorine-18, radiosynthesis, microPET/CT, tumors, erianin

Erianin {2-methoxy-5-(3,4,5-trimethoxyphenethyl)-phenol}, is a diphenzyl active pharmaceutical ingredient isolated from a well-known traditional Chinese medicine derived from *Dendrobium candidum* (Figure 1).[1] It possesses a variety of pharmacological properties such as antioxidant,[2] antibacterial,[3] antiviral,[4] anti-inflammatory, and antineoplastic activity.[5–7] Mechanistic studies revealed that erianin can induce apoptosis and autophagy of tumor cells and arrest the cell cycle in the G2/M phase by inhibiting the EPK,[8] JNK/c-Jun, AKT/mTOR,[9] and MAPK signaling pathways;[10] moreover, it can also inhibit tumor angiogenesis by inhibiting IDO expression[11] and blocking the VEGF/VEGFR2 signaling pathway.[12]

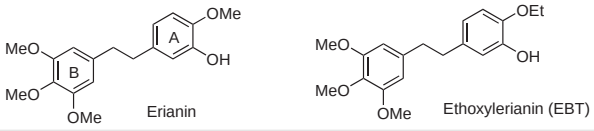


Figure 1 Structures of erianin and ethoxylerianin

Previously, modifications of erianin were widely studied by using twin drug, prodrug, and fluorination strategies, and it was found that ethoxylerianin (EBT) shows better anti-tumor activity than erianin.[13] Moreover, as an effective vascular disrupting agent, EBTP (the phosphate prodrug of EBT) is less toxic compared with Combrestatin A-4 phosphate prodrug (CA4P).[14] In a continuation of this work, it was desirable to evaluate the properties of EBT *in vivo* by positron emission tomography (PET). PET is an imaging method using positron-emitting radioisotope-labeled functional molecules to diagnose and monitor the development

of diseases and to study the process of biological metabolism *in vivo* as well as assessing the efficacy of potential drugs. In oncology, PET tracers can enable the visualization of tumors and provide information on targeted biologic pathways.[15,16] Compared with other positron nuclides, such as ^{11}C, with only 20.4 min half-life, ^{18}F shows a longer half-life ($t_{1/2}$ = 109.7 min), lower positron energy (635 KeV) and a smaller van der Waals radius (1.35 Å) similar to that of hydrogen (1.20 Å).[17] It causes lower radiation damage to normal tissue without affecting the activity of labeled compounds.[18] There are many advantages in using ^{18}F- labeled PET tracers, such as wide availability, efficient tissue penetration and appropriate metabolic stability. Therefore, ^{18}F is widely used in the preparation of PET probes for clinical purposes.[19,20]

In this work, we envisaged that fluoethoxylerianin (FEE) and its ^{18}F-labeled analogue ([^{18}F]FEE) would be structurally very similar to EBT (Figure 2), showing identical biological properties for imaging studies. The tosylate functionality is very useful in ^{18}F labeling since it allows introduction of the ^{18}F isotope in high radiochemical yield through nucleophilic fluorination. Herein, we report the synthesis of both FEE and [^{18}F]FEE in eight steps, the fluorine atom being introduced by direct nucleophilic fluorination from the same tosylate precursor. Erianin, EBT and novel compound FEE were evaluated for their anti-tumor activities against HepG2 human hepatic cancer cell line and showed IC_{50} values of 72.8±2.3 nM, 60.4±7.8 nM, and 78.0±9.6 nM, respectively.

The ^{18}F-labeled erianin analogue may provide a new option for tumor positron emission tomography. In this paper, a method for radiolabeling and purification of this complex is presented. The *in vitro* stability and plasma protein binding ability of the new compound were investigated. Preliminary positron emission tomography studies were performed in mouse tumor models to demonstrate the potential of the ^{18}F-labeled erianin derivative as a molecular imaging probe.

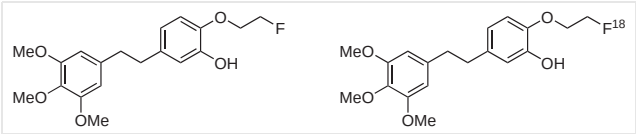


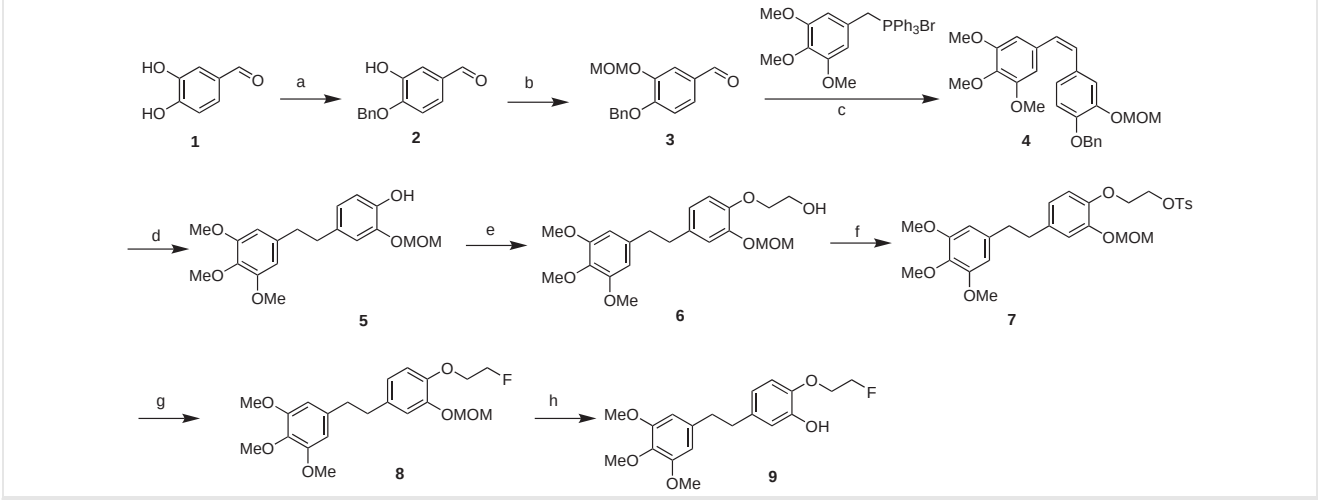
Figure 2 Fluoroethoxylerianin (FEE) and its ^{18}F isotope analogue [^{18}F]FEE

Materials and Methods

The synthesis of fluoroethoxylerianin (**9**, FEE) and its ^{18}F-isotope labeling procursor (**7**) are outlined in Scheme 1. 3,4-Dihydroxybenzaldehyde (**1**) was protected with benzyl and methoxymethyl groups sequentially to give aldehyde intermediate (**3**).[21–23] A mixture of (Z/E)-stilbenes (**4**) was obtained from the Wittig reaction of aldehyde intermediate (**3**) and triphenyl(3,4,5-trimethoxy-benzyl) phosphonium bromide,[24] and subsequent hydrogenation gave dibenzyl intermediate (**5**). The hydroxyl group of compound (**5**) was etherified with 2-bromoethanol to afford hydroethylerianin intermediate (**6**), which was converted into tosylate (**7**) by reacting with *p*-toluene sulfonyl chloride.

Radiosynthesis

An Explora GN Radiopharmaceutics automatic synthesis module was used to prepare the ^{18}F labeled erianin analogue, and a product with 200 mCi activity was obtained in yields of 25–30%. The total preparation time was 70 minutes, and the specific activity of the [^{18}F]FEE reached 8.0 × 10^5 mCi $mmol^{-1}$.



Scheme 1 The synthesis of fluoroethylerianin (**9**, FEE) and its ^{18}F-isotope labeling precursor (**7**). Reagents and conditions: (a) NaH (1.1 eqiuv), DMF, BnCl (1.1 equiv); (b) MOMBr (1.2 equiv), DIPEA (3 equiv), CH_2Cl_2; (c) t-BuOK (4 equiv), THF; (d) H_2 (1 atm), Pd/C (10 %), THF; (e) $BrCH_2CH_2OH$ (1.2 equiv), K_2CO_3 (3 equiv), DMF; (f) TsCl (1.2 equiv), Et_3N (3 equiv), DMAP (cat.) (0.1 equiv), CH_2Cl_2; (g) KF-K2.2.2 complex, DMF; (h) 6 M HCl, DMF.

Quality Control of Radiochemical Purity

In order to control the quality of the [^{18}F]FEE, Radio-TLC was used to determine the labelling efficiency. The radiochemical purity of the [^{18}F]FEE was detected by radio-high-performance liquid chromatography (radio-HPLC) with 276 nm and radioactivity detectors. The product concentration was calculated by using a correlation curve of UV peak area and concentration. The specific activity of the product was obtained from the ratio of the radioactive activity to the concentration.

MicroPET Imaging in HepG2 Tumor-Bearing Mice

About 3 × 10^6 HepG2 cells were subcutaneously implanted into the right upper limb of nude mice, and the tumor was grown to 50 mm. *In vivo* biological distribution and imaging of the tumor-bearing mice were carried out. Three HepG2 tumor-bearing mice were injected with [^{18}F]FEE (0.1–0.2mCi) in a tail vein and the mice were anesthetized with 2% isoflurane 30, 60 and 90 min after injection, followed by micro-PET imaging. Assessment of the biological distribution of [^{18}F]FEE in normal mice was carried out in the same manner.

Statistical Analysis

All results are expressed as mean ±SD ($\bar{x}$±SD) derived using SPSS software. A P value <0.05 was considered statistically significant.

Anti-tumor Assay of Erianin, EBT and FEE Against the HepG2 Cell-Line

Human hepatic cancer cell line HepG2 (ATCC, Rockville, MD) was cultured in DMEM medium containing 10% FBS (purchased from Gibco, Thermo Fisher Scientific), penicillin (100 units mL^{-1}), and streptomycin (100 µg mL^{-1}). Cells were incubated in a humidified atmosphere with 95% air and 5% CO_2 at 37 °C.

Cell viability was quantified with a Cell Counting Kit-8 (CCK-8, EnoGene). Cells were seeded at 8900 cells to each well in 96-well plates. The compounds were incubated with cells at different concentrations. After 72 hours treatment, CCK8 solution 10 µL was added to each well. The absorbance at 450 nm was measured with a microplate reader (MK3, Thermo Fisher) after 4 hours incubation at 37 °C. IC_{50} values of the compounds on HepG2 cancer cells were calculated using GraphPad Prism 5 (GraphPad Software).

Results and Discussion

Radiosynthesis of [^{18}F]FEE

As shown in Scheme 2, The tosylate precursor (**7**) underwent nucleophilic fluorination with KF-K2.2.2,[25,26] followed by deprotection with 6 M HCl to give fluoroethoxylerianin (FEE). Using the *in situ* prepared [^{18}F]-KF-K2.2.2 complex, the ^{18}F-labeled fluoroethoxylerianin ([^{18}F]FEE, **10**) was prepared in one pot from the same tosylate precursor **7**.

Quality Control of [^{18}F]FEE

A radiochemical purity of more than 95% was obtained by semi-preparative HPLC and an in-house prepared solid-phase extraction unit. The labeling efficiency was measured by using the reference standard sample of [^{18}F]FEE, with a retention time of about 6 min (Figure 3).

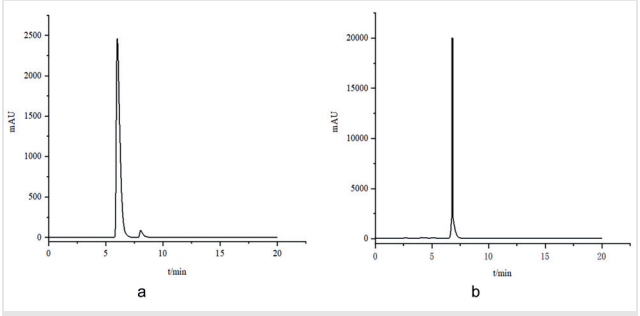
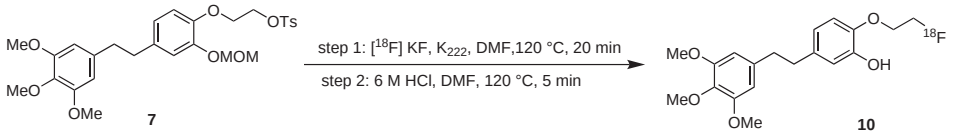


Figure 3 (a) HPLC analysis result for standard compound (**9**), retention time R_t = 6 min. (b) Radio-HPLC analysis result for [^{18}F]FEE, retention time R_t = 6.8 min.

[^{18}F]FEE MicroPET/CT Imaging of HepG2 Tumor Model

Figure 4 shows the whole-body PET imaging of HepG2 tumor model with [^{18}F]FEE at 30, 60 and 90 minutes after injection. Whole-body positron emission tomography (PET) data from the same animal scanned showed a clear area of tumor in the right upper limb compared to the uninoculated left upper limb tissue (Figure 4a). The biodistribution of HepG2 tumor-bearing mice, as already demonstrated by positron emission tomography, was observed with rapid removal of the tracer from blood and non-target tissues through the liver and kidney, with the highest radioactive concentration in tumor tissues at 60 minutes. Moreover, the uptake and removal rates of radioactive tracer in tumor tissue were not significantly different from those in the liver region. Figure 4b shows the ratio of tracer [^{18}F]FEE uptake in the tumor to contralateral muscle (T/M) during the PET scan analyses. The removal rate of tracer in tumor tissue



Scheme 2 Synthesis of ^{18}F-fluoroethoxylerianin (**10**, [^{18}F]FEE)

was lower than that in muscle, so the ratio of T/M showed an increasing trend. These results indicate that the tracer [^{18}F]FEE can be significantly detected by positron emission tomography in HepG2 tumor models.

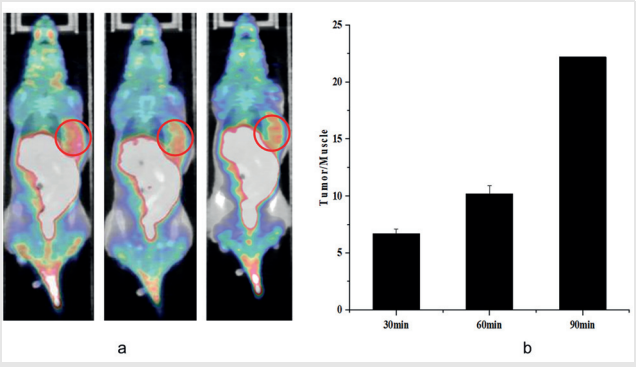


Figure 4 (a) PET/CT imaging of [^{18}F]FEE in HepG2 tumor model at 30 min (Left), 60 min (Middle) and 90 min (Right) (tumors delineated with a red circle); (b) Tumor/Muscle ratio of HepG2 tumor model.

In this paper, a novel precursor compound **7** and its ^{19}F-fluoroethoxylerianin analogue **9** were successfully synthesized. On this basis, by using a radiopharmaceutical synthesis module, the synthesis of ^{18}F labeled erianin was successfully completed. Moreover, purification of the labeled product was accomplished by semi-preparative HPLC and solid-phase extraction. *In vitro* evaluation showed that the tracer was stable and remained intact in serum for up to 4 h. In PET studies, the ^{18}F labeled erianin analogue showed potential as a tracer candidate. However, the compound needs to undergo detailed validation studies to determine the true nature of its target region uptake. The results of this study will contribute to the further development of ^{18}F labeled erianin derivatives as molecular imaging probes.

All solvents and starting materials were obtained from Tansoole and used directly without further purification. The reactions were monitored by thin-layer chromatography (TLC) using a 0.25 mm pre-coated silica gel plate, and visualized under UV light. The products were purified by flash column chromatography on silica gel (200–300 mesh). Structure characterization and purity assessment were assessed by NMR spectroscopy, HRMS and HPLC. NMR spectra were collected with a Bruker 500 MHz spectrometer and analysed by MestReNova using TMS or deuterated solvent as reference. HRMS was recorded with a Waters GCT Premier mass spectrometer. The purity of the target compound was assessed with an Agilent-1200. Semi-preparative HPLC was performed with an Agilent Technologies 1260 Infinity chromatograph.

4-(Benzyloxy)-3-hydroxybenzaldehyde (2)[21,23]

To an ice-bath cooled solution of 3,4-dihydroxybenzaldehyde (10.0 g, 72.40 mmol) in DMF (100 mL) was added sodium hydride (1.91 g, 79.64 mmol). After stirring for 1 h, benzyl chloride (10.08 g, 79.64 mmol) was added dropwise. Then, the reaction was warmed to room temperature gradually and stirred for 20 h at room temperature. The reaction mixture was poured onto ice-water (1000 mL), the pH was adjusted to 2–3 with 1 M HCl and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was recrystallized from petrol ester/ethyl acetate (4:1, v/v) to afford compound (**2**) (11.28 g, yield 68%) as a white solid, mp 72.8–74.3 °C.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.83 (s, 1 H), 7.54 (d, *J* = 1.6 Hz, 1 H), 7.50–7.40 (m, 6 H), 7.10 (d, *J* = 8.0 Hz, 1 H), 6.43 (s, 1 H), 5.20 (s, 2 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 190.88, 151.97, 146.43, 135.53, 129.90, 128.90, 128.79, 128.16, 127.66, 114.76, 110.37, 71.37.

4-(Benzyloxy)-3-(methoxymethoxy)benzaldehyde (3)[21,23]

To a solution of compound **2** and DIPEA (19.16 g, 148.26 mmol) in dichloromethane (100 mL), bromomethyl methyl ether (7.41 g, 59.30 mmol) was added dropwise. After stirring at room temperature for 6 h, the reaction mixture was diluted with dichloromethane (300 mL) and washed with brine and water. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was recrystallized from ethanol/petroleum ether (2:1, v/v) to yield compound **3** (10.48 g, yield 83%) as a white solid, mp 103.6–104.9 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.85 (s, 1 H), 7.69 (d, *J* = 2.0 Hz, 1 H), 7.56-7.32 (m, 6 H), 7.04 (d, *J* = 8.3 Hz, 1 H), 5.30 (s, 2 H), 5.26 (s, 2 H), 3.55 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 190.72, 154.42, 147.27, 136.05, 130.34, 128.73, 128.23, 127.18, 126.70, 116.36, 113.18, 95.54, 70.86, 56.42.

(Z)-5-(4-Benzyl-3-(methoxymethoxy)-styryl)-1,2,3-trimethoxybenzene (4)

Under a nitrogen atmosphere, to an ice-bath cooled suspension of triphenyl(3,4,5-trimethoxybenzyl)-phosphonium bromide (22.16 g, 42.34 mmol) in dry THF (250 mL), was added t-BuOK (17.27 g, 153.95 mmol). After stirring for 30 min, a solution of compound (3) (11.54 g, 42.34 mmol) in dry THF (150 mL) was added dropwise. After addition, the reaction was warmed to room temperature and stirred for 12 h. The reaction mixture was poured into water (1000 mL), extracted with ethyl acetate (3 × 300 mL), the combined organic phases were washed with brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was recrystallized from anhydrous ethanol (200 mL) to afford pure *cis*-isomer (8.43 g, yield 57%) as a white solid, mp 106.3–107.4 °C.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.47 (d, *J* = 7.5 Hz, 2 H), 7.37 (ddd, *J* = 24.0, 14.5, 7.0 Hz, 4 H), 7.10 (dd, *J* = 8.5, 2.0 Hz, 1 H), 6.99–6.88 (m, 3 H), 6.74 (s, 2 H), 5.31 (s, 2 H), 5.20 (s, 2 H), 110.78, 106.13, 94, 60.93, 56.21, 56.10.3.93 (s, 6 H), 3.89 (s, 3 H), 3.59 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.29, 149.31, 147.75, 137.85, 137.10, 133.44, 132.28, 129.58, 129.10, 128.84, 128.36, 128.26, 123.16, 114.58.

HRMS (ESI): *m*/*z* calcd for $C_{26}H_{28}O_6$ [M + H]$^+$: 437.1954; found: 437.1954.

2-(Methoxymethoxy)-4-(3,4,5-trimethoxyphenethyl)phenol (5)

To a solution of compound **4** (8.43 g, 19.31 mmol) in THF (100 mL) was added 10%Pd/C (850 mg), the reaction was stirred for 6 h at room temperature under 1 atm. Hydrogen. After completion of reaction, the reaction mixture was filtered through a pad of silica gel and the filtrate was concentrated *in vacuo* to afford compound **5** (6.43 g, yield 96%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.79 (dd, *J* = 8.5, 2.0 Hz, 3 H), 6.39 (s, 2 H), 5.90 (s, 1 H), 5.17 (s, 2 H), 3.84 (d, *J* = 1.5 Hz, 9 H), 3.53 (s, 3 H), 2.84 (s, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.02, 148.05, 147.17, 139.18, 136.93, 136.18, 121.40, 115.93, 115.44, 106.30, 94.12, 60.90, 56.12, 56.07, 36.87, 36.66.

HRMS (ESI): *m*/*z* calcd for $C_{19}H_{24}O_6$ [M + NH_4]$^+$: 366.1917; found: 366.1914.

2-(2-(Methoxymethoxy)-4-(3,4,5-trimethoxyphenethyl)phenoxy)ethan-1-ol (6)

To a solution of compound **5** (6.43 g, 19.32 mmol) and 2-bromoethanol (2.90 g, 23.18 mmol) in DMF (65 mL) was added K_2CO_3 (6.15 g, 38.63 mmol). The reaction was heated to 80 °C and stirred for 5 h. After cooling to room temperature, the reaction mixture was poured into water (100 mL) and extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with brine and water, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to afford compound **6** (6.38 g, yield 88%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.96 (d, *J* = 1.5 Hz, 1 H), 6.89 (d, *J* = 8.5 Hz, 1 H), 6.84–6.77 (m, 1 H), 6.39 (s, 2 H), 5.19 (s, 2 H), 4.17–4.09 (m, 2 H), 3.94–3.90 (m, 2 H), 3.84 (s, 9 H), 3.53 (s, 3 H), 2.85 (s, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ= 153.04, 147.26, 147.17, 137.38, 135.79, 122.62, 117.71, 115.60, 105.43, 95.87, 77.32, 77.07, 76.81, 71.69, 61.27, 60.86, 56.32, 56.06, 38.39, 37.37.

HRMS (ESI): *m*/*z* calcd for $C_{21}H_{28}O_7$ [M + Na]$^+$: 415.1733; found: 415.1731.

2-(2-(Methoxymethoxy)-4-(3,4,5-trimethoxyphenethyl)phenoxy)ethyl 4-Methylbenzenesulfonate (7, the precursor of ^{18}F-FEE)

To an ice-cooled solution of compound **6** (6.38 g, 16.38 mmol), triethylamine (4.97 g, 49.15 mmol) and DMAP (200 mg, 1.64 mmol) in CH_2Cl_2 (100 mL) was added TsCl (3.75 g, 19.66 mmol) in four portions. After addition, the reaction was stirred for 1 h at 0 °C when TLC indicated the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with water (3 × 50 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel to afford compound **7** (7.16 g, yield 80%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.83 (d, *J* = 8.5 Hz, 2 H), 7.35 (d, *J* = 8.0 Hz, 2 H), 6.94 (d, *J* = 1.5 Hz, 1 H), 6.77 (d, *J* = 1.5 Hz, 2 H), 6.38 (s, 2 H), 5.13 (s, 2 H), 4.37 (dd, *J* = 6.5, 3.5 Hz, 2 H), 4.21 (dd, *J* = 5.0, 4.5 Hz, 2 H), 3.84 (s, 9 H), 3.50 (s, 3 H), 2.84 (s, 4 H), 2.46 (s, 3 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.04, 147.11, 146.77, 144.94, 137.37, 136.10, 132.87, 129.88, 127.97, 122.50, 118.09, 115.42, 105.39, 95.81, 77.33, 77.08, 76.83, 68.17, 67.28, 60.85, 56.28, 56.06, 38.35, 37.34, 21.65, 14.21.

HRMS (ESI): *m*/*z* calcd for $C_{28}H_{34}SO_9$ [M + Na]$^+$: 569.1821; found:569.1820.

5-(4-(2-Fluoroethoxy)-3-(methoxymethoxy)phenethyl)-1,2,3-trimethoxybenzene (8)

To a solution of compound **7** (0.50 g, 914.69 μmol) and Kryptofix[2.2.2] (0.69 g, 1.83 mmol) in DMF (5 mL) preheated to 120 °C was added KF (0.21 g, 3.66 mmol). After stirring for 5 min, TLC analysis indicated that the reaction was complete. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel column chromatography to yield compound **8** (0.12 g, yield 33%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.98 (d, *J* = 2.5 Hz, 1 H), 6.88 (d, *J* = 8.0 Hz, 1 H), 6.81 (dd, *J* = 8.5, 2.0 Hz, 1 H), 6.39 (s, 2 H), 5.20 (s, 2 H), 4.76 (dt, *J* = 47.5, 4.0 Hz, 2 H), 4.26 (dt, *J* = 27.5, 4.0 Hz, 2 H), 3.84 (s, 9 H), 3.54 (s, 3 H), 2.85 (s, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.03, 147.20, 147.10, 137.41, 136.13, 135.78, 122.53, 118.14, 115.06, 105.41, 95.85, 82.03 (d, *J* = 170.6 Hz), 68.84 (d, *J* = 21.0 Hz), 60.86, 56.25, 56.05, 38.38, 37.35.

^{19}F NMR (375 MHz, $CDCl_3$): δ = –223.71 to –224.46 (m).

HRMS (ESI): *m*/*z* calcd for $C_{21}H_{27}FO_6$ [M + Na]$^+$: 417.1689; found: 417.1688.

Fluoroethyl-Erianin, 2-(2-Fluoroethoxy)-5-(3,4,5-trimethoxyphenethyl)phenol (9, The Standard FEE)

A solution of compound **8** (100 mg, 253.5 μmol) in DMF (2 mL) was heated to 120 °C, then hydrochloric acid (6 M, 0.4 mL) was added. After stirring for 3 min, TLC analysis indicated that the reaction was complete. The mixture was poured into ice-water (20 mL), extracted with ethyl acetate (3 × 5 mL) and the combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel column chromatography to yield compound **9** (18.00 mg, yield 63%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.85 (d, *J* = 2.0 Hz, 1 H), 6.80 (d, *J* = 8.0 Hz, 1 H), 6.65 (dd, *J* = 8.0, 2.0 Hz, 1 H), 6.40 (s, 2 H), 5.80 (s, 1 H), 4.77 (ddd, *J* = 47.5, 4.0, 4.0 Hz, 2 H), 4.28 (ddd, *J* = 28.5, 8.0, 3.5 Hz, 2 H), 3.85 (d, *J* = 0.5 Hz, 9 H), 2.84 (s, 4 H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 153.04, 146.06, 143.62, 137.54, 136.15, 119.93, 115.27, 112.70, 105.41, 81.79 (d, *J* = 170.8 Hz), 68.72 (d, *J* = 20.2 Hz), 60.86, 56.06, 38.31, 37.31.

^{19}F NMR (375 MHz, $CDCl_3$): δ = –223.87 to –224.28 (m).

HRMS (ESI): *m*/*z* calcd for $C_{19}H_{23}FO_5$ [M + Na]$^+$: 373.1427; found: 373.1427.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Information

This research was supported by the National Natural Science Foundation of China (grant numbers 21172148, 216721521, 21771041).

Ethics Approval and Consent to Participate

All procedures involving animal research were carried out in accordance with the ethical standards in place at the institution or practice in which the study was performed.
All applicable international, national and/or other institutional directives regarding animal care and the use of animals were followed.

Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/a-1818-8330. Included are ^{1}H, ^{13}C and ^{19}F NMR spectra, HPLC elution conditions and traces.

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