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Research Article

F-18 labelled *N,N-bis*-haloethylamino-phenylsulfoxides — a new class of compounds for the imaging of hypoxic tissue

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Summary

Two *N,N-bis*-chloroethylamino-phenylsulfoxides have been synthesized and radiolabelled with F-18 via halogen exchange. The radiolabelling of both compounds proceeds smoothly with K[^{18}F]F-kryptofix 2.2.2 complex at 100 °C in DMSO. Decay-corrected radiochemical yields were 25–35% for [^{18}F]**12** (SO201) and 35–45% for [^{18}F]**11** (SO101), with an average specific activity of 1.8 Ci/μmol. Both compounds remained 80% intact in plasma over a period of 2 h. *In vivo* binding in a stroke model indicates that both markers are selectively retained in hypoxic tissue. Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: hypoxia; PET; radiolabelling; stroke

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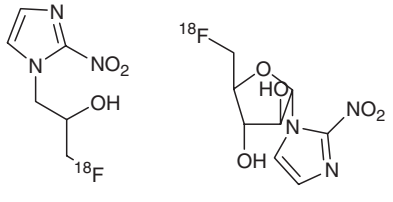
Introduction

Hypoxic tissue plays an important role in tumors as well as in ischemic stroke. In oncology, hypoxic tumors are more resistant to chemo- and radiotherapy[1,2] and may behave more aggressively than normoxic tumors.[3–5] In acute stroke, hypoxic tissue is functionally impaired but morphologically intact and at risk of infarction.[6] This tissue has the potential to recover completely if perfusion is restored. In order to better understand tissue recovery as well as assessing the efficiency of neuroprotective agents, imaging of hypoxic tissue is of great importance. Positron emission tomography (PET) is the perfect tool for such imaging studies due to its ability to quantify tissue uptake *in vivo*.[7] So far, most F-18-based compounds for PET imaging of hypoxic tissue in stroke are nitroimidazole derivatives such as [^{18}F]FMISO and [^{18}F]FAZA (Figure 1).[8–10]

These compounds are believed to undergo bioreduction at the nitro group and subsequently become trapped in the hypoxic cell by binding to intracellular molecules.[11] In normoxic cells the reduced intermediates are believed to be reoxidized to the original molecule, which can diffuse freely across the cell membrane and is therefore not taken up by the normoxic cell. Due to the lack of metabolism, no radiotracer uptake is observed in necrotic tissue. However, the optimum time for *in vivo* imaging with these nitroimidazole-based radiotracers is 2 h post tracer administration.[12] This time frame renders the routine use of these tracers in stroke difficult and justifies the need for the development of novel tracers.

In oncology, sulfoxide containing nitrogen mustards have shown great promise as targeted therapeutics for hypoxic tumors.[13,14] It is believed that this class of compounds undergoes bioreduction to the corresponding thioether in hypoxic cells, which activates the nitrogen mustard moiety.[15] This process results in the formation of an aziridinium ion intermediate, which binds in an irreversible manner to reductases in the cell and is responsible for the cytotoxic effect of these compounds. In normoxic tissue, the sulfoxide moiety is believed to be further oxidized to the sulfone which has no cytotoxic effect, can diffuse freely across the cell membrane, and therefore does not accumulate in the normoxic cell (Figure 2).[16]

Our aim was to radiolabel compounds **1** and **2** with the positron emitting isotope F-18 ($t_{\frac{1}{2}} = 110$ min) by halogen exchange, determine the stability of



[^{18}F]FMISO [^{18}F]FAZA

Figure 1. Chemical structures of [^{18}F]FMISO and [^{18}F]FAZA

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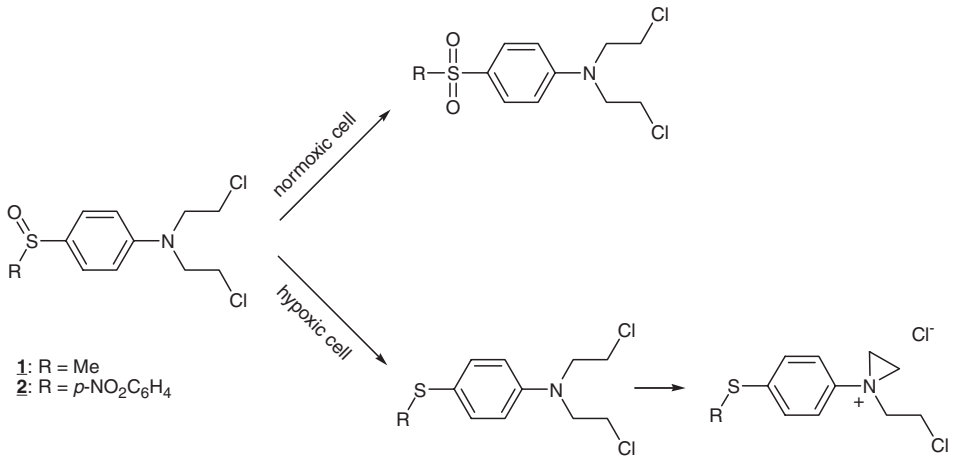

Figure 2. Mechanism of action of nitrogen mustards

these compounds in saline and human plasma, and determine whether they are selectively retained in hypoxic tissue *in vivo*.

Results and discussion

Chemistry

Synthesis of labelling precursors and F-19 standards

Reaction of 4 equivalents of 2-chloroethyltriflate with 1 equivalent of either 4-methylthioaniline (**3**) or 4-(*p*-nitrophenylthio) aniline (**4**) in the presence of potassium carbonate yielded the dialkylated products **5** and **6**. The dialkylated products **5** and **6** were oxidized with *in situ* generated trifluoroperacetic acid[12] to form the sulfoxide precursors **1** and **2** required for the labelling (Scheme 1).

The monoalkylated intermediates **7** and **8** were synthesized by reacting either 4-methylthioaniline (**3**) or 4(*p*-nitrophenyl)-thioaniline (**4**) with 0.5 equivalent of 2-chloroethyltriflate in the presence of potassium carbonate to minimize the formation of the dialkylated products, which were difficult to separate. **7** and **8** were then reacted with fluoroethyltriflate using 2,6-lutidine as base to give the thioethers **9** and **10**. Oxidation of these compounds with *in situ* generated trifluoroperacetic acid gave the F-19 standards **11** and **12** (Scheme 2).

Radiochemistry

Radiolabelling of the sulfoxide precursors was achieved by halogen exchange using no-carrier added, dried K[^{18}F]F kryptofix 2.2.2 complex (Scheme 3).

Labelling efficiency at various temperatures and time points was measured and found to be optimum at 100°C for 15 min for both compounds. Optimized

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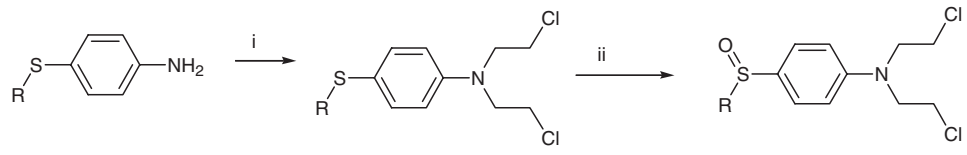


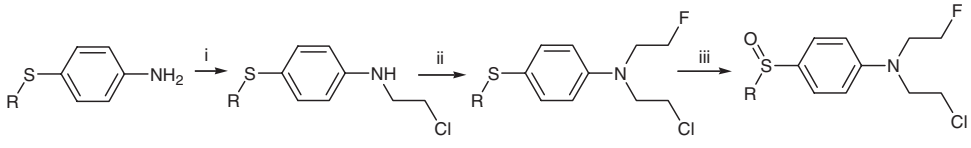
3: R = Me
4: R = *p*-$NO_2C_6H_4$

5: R = Me
6: R = *p*-$NO_2C_6H_4$

1: R = Me
2: R = *p*-$NO_2C_6H_4$

Reagents and conditions:(i): 2-chloroethyltriflate (4 equiv.), K_2CO_3 (1 equiv.), CH_2Cl_2, reflux, 18 h
(ii): H_2O_2 (1 equiv.), TFA, -15°C, 1 h

Scheme 1. Synthesis of labelling precursors



3: R = Me
4: R = *p*-$NO_2C_6H_4$

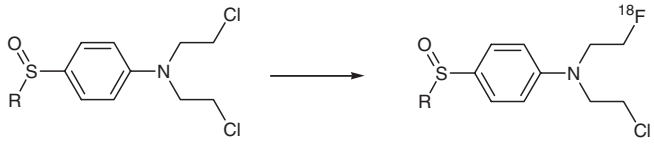
7: R = Me
8: R = *p*-$NO_2C_6H_4$

9: R = Me
10: R = *p*-$NO_2C_6H_4$

11: R = Me
12: R = *p*-$NO_2C_6H_4$

Reagents and conditions: (i): 2-chloroethyltriflate (0.5 equiv), K_2CO_3 (1 equiv.), CH_2Cl_2, reflux, 18 h
(ii): 2-fluoroethyltriflate (0.5 equiv), 2,6-lutidine (1 equiv.), CH_2Cl_2, reflux, 18 h
(iii): H_2O_2 (1 equiv), TFA, -15°C, 1 h

Scheme 2. Synthesis of labelling standards



1: R = Me
2: R = *p*-$NO_2C_6H_4$

[^{18}F]**11**: R = Me
[^{18}F]**12**: R = *p*-$NO_2C_6H_4$

Reagents and conditions: K[^{18}F]F kryptofix 2.2.2, 100°C, 15 min

Scheme 3. Synthesis of [^{18}F]11 and [^{18}F]12

yields for [^{18}F]**11** were 35–45%, and 25–35% for compound [^{18}F]**12**. Average specific activity was 1.8 Ci/μmol in both cases. The F-18 labelled compounds showed HPLC profiles that were identical to the F-19 standards. Separation of the radioactive products from their respective precursor molecules was achieved by reversed phase semi-preparative HPLC. After collection of the desired radioactive peak, the ligands were reformulated in 10% ethanol using the Sep Pak method.[17] The synthesis time including HPLC purification and reformulation was 1 h.

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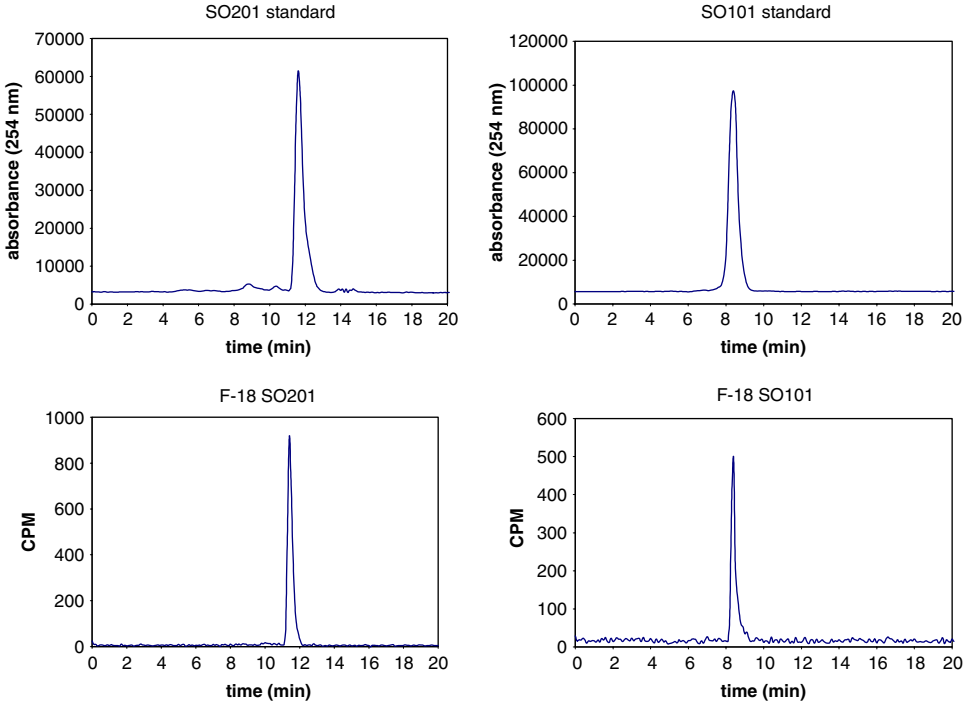

Figure 3. HPLC chromatograms of the independent quality control of SO101 and SO201

Independent quality control was performed on a Develosil UG (C-30, 5 μ, 10 × 250 mm, 20 μl injection loop) HPLC column at a flow rate of 2 ml/min with 0.1 M NH_4HCO_2/ ethanol : 40/60 (v/v) as mobile phase (Figure 3).

Stability in saline and plasma

The *in vitro* stability of compounds [^{18}F]**11** and [^{18}F]**12** was assessed by incubating 100 μCi of activity at 37 °C in 4 ml of saline or plasma over various time points. After the end of each timepoint, radioactivity was extracted from the plasma by addition of 1 ml of acetonitrile. The sample was spun down and the supernatant analyzed by radio thin layer chromatography (TLC) and HPLC. In both cases 80% of the tracer is still intact after 2 h. [^{18}F]**11** and [^{18}F]**12** were found to be stable in saline over a period of 2 h.

Uptake in ischemic tissue

For all experimental animals for each compound there was increased signal within the normal distribution of the occluded middle cerebral artery (MCA) (Figure 4). Average hypoxic:normoxic ratio was 2.07 ± 0.27 ([^{18}F]**11**) and

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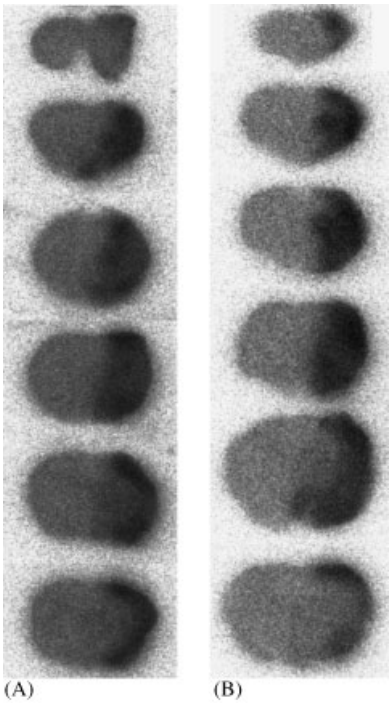

Figure 4. [^{18}F]11 (A) and [^{18}F]12 (B) autoradiographs of representative brains after 2 h focal ischemia. For each animal 6 × 2 mm coronal sections are shown. Increased radioactivity is seen within the normal distribution of the occluded middle cerebral artery in each case

2.26 ± 0.20 ([^{18}F]**12**). The area of increased binding was 479 ± 170 mm^3 ([^{18}F]**11**) and 526 ± 94 mm^3 ([^{18}F]**12**).

The results of the labelling studies have demonstrated that halogen exchange is a simple and convenient way to synthesize compounds [^{18}F]**11** and [^{18}F]**12**. The difference in HPLC retention time between the precursors **1** and **2** and their [^{18}F]labelled analogues [^{18}F]**11** and [^{18}F]**12** are 6.16 and 8 min, respectively, which is sufficiently large to ensure complete separation on a semi-preparative HPLC column. It is noteworthy that both compounds could be synthesized in good radiochemical yields, despite the need to displace the relatively weak leaving group Cl^-. These yields are sufficient for animal and potential human studies.

We decided to target compounds [^{18}F]**11** and [^{18}F]**12** because of the large difference in hypoxic selectivity between the parent molecules **1** and **2**. Clonogenic assays with V-79 cells have shown that under normoxic conditions the IC_{90} value of **2** is 3680 and 182 μM under hypoxic conditions.[14] The value for hypoxic selectivity, which is defined as the ratio between the normoxic and hypoxic IC_{90} values, is 20 for compound **2**. In the same set of experiments a

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hypoxic selectivity of 3 was measured for compound **1**. This difference in hypoxic selectivity of the parent molecules could result in higher uptake of [^{18}F]**12** in hypoxic tissue compared to [^{18}F]**11**.

Since uptake of these putative tracers depends on active metabolism, it can be expected that both [^{18}F]**11** and [^{18}F]**12** will not be taken up by necrotic cells.

In vivo binding of both radiotracers indicates successful retention within hypoxic tissue, consistent with potential applications as hypoxic imaging agents. Further characterization of time-course of binding and comparison with established hypoxic tracers in stroke[18] and oncology[19] is being undertaken.

Experimental

General

No-carrier-added [^{18}F] fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction with a 10 MeV proton beam generated by the IBA Cyclone 10/5 cyclotron in a titanium target using [^{18}O]H_2O at Austin Health, Centre for PET. Typical irradiation parameters were 20 μA/h for 30 min, which produced 5.4–8.1 GBq (146–219 mCi) of [^{18}F]fluoride. Isolation of the [^{18}F]F^- ion from [^{18}O]H_2O was achieved by trapping on a QMA ion exchange column. Elution of the column with a solution containing 3.5 mg of potassium carbonate in 0.2 ml of water and 20 mg of kryptofix 2.2.2 in 0.4 ml of acetonitrile followed by repeated azeotropic distillation with acetonitrile then evaporation to dryness gave the anhydrous fluoride ion used in the labelling experiments.

All chemicals and solvents were purchased from Sigma-Aldrich and used as received. 2-Chloroethyltriflate and 2-fluoroethyltriflate were synthesized as described and used without further purification. Flash chromatography was carried out with silica gel 60 (0.040–0.063 mm, Merck). ^{1}H Nuclear magnetic resonance spectra were recorded at 400 MHz and ^{19}F spectra were recorded at 375 MHz using a JOEL GX-400 spectrometer. ^{13}C nuclear magnetic resonance spectra were recorded at 100 MHz using a JOEL GX-400 spectrometer.

Melting points were determined with a Gallenkamp Capillary melting point apparatus and are uncorrected.

The labelling procedure including semi-preparative HPLC purification, formulation and sterile filtration was carried out using a modified IBA automated synthesis module.

Semi-preparative HPLC was performed using a Shimadzu LC-10AS isocratic pump equipped with a 1.2 ml injection loop and a reversed phase column (Apollo C-18, 5 μ, 10 × 250 mm). For SO101 the mobile phase consisted of 0.1 M NH_4HCO_2/ acetonitrile : 40/60 (v/v) at a flow rate of 4 ml/min. 0.1 M NH_4HCO_2/ acetonitrile : 50/50 (v/v) at a flow rate of 4 ml/min was used as mobile phase for SO201. Detection of chemical compounds was

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achieved with a Shimadzu SPD-6AV UV detector (254 nm) and a Geiger-Müller tube as radiodetector.

Radioactive analytical TLC was carried out on aluminum backed silica gel 60 (0.25 mm plates, Alltech) with CH_3CN/H_2O : 95/5 (v/v) as mobile phase.

For both compounds, independent quality control was performed on a Develosil UG (C-30, 5 µ, 10 × 250 mm, 20 µl injection loop) HPLC column at a flow rate of 2 ml/min with 0.1 M NH_4HCO_2/ ethanol : 40/60 (v/v) as mobile phase. Detection of chemical compounds was achieved with a Shimadzu SPD-6AV UV detector (254 nm) and a Berthold LB506 C-1 monitor for radiochemical detection.

Chemistry

2-*chloroethyltriflate*

Trifluoromethanesulfonic anhydride (5 g, 3 ml, 17.7 mmol) was added to a stirred solution of 4-(polyvinyl) pyridine (5.70 g, 51.75 mmol) in dichloromethane (100 ml). 2-Chloroethanol (1.29 g, 1.08 ml, 16.02 mmol) was then added dropwise over 1 min. The reaction was left to stir at room temperature for 30 min, and then filtered under gravity. The 4-(polyvinyl) pyridinium precipitate was washed with 5 ml of dichloromethane. The organic phases were combined and washed with $NaHCO_3$ (3 × 50 ml) and water (3 × 50 ml) respectively, dried ($MgSO_4$), and concentrated under reduced pressure to give 2-chloroethyltriflate (2.105 g, 9.9 mmol, 61.8%) as colorless oil. This product was used without further purification.

^{1}H NMR ($CDCl_3$): 3.80 (t, 2H, CH_2-Cl), 4.65 (t, 2H CH_2-OTf)
^{13}C NMR ($CDCl_3$): 40.32 (CH_2-Cl), 75.07 (CH_2-OTf), 118.35 (q, $J = 319$ Hz)
IR: 1144.4, 1215.4 (O = S = O).

2-*fluoroethyltriflate*

Trifluoromethanesulfonic anhydride (8.57 g, 5.13 ml, 30.37 mmol) was added to a stirred solution of 4-(polyvinyl) pyridine (6.68 g, 60.78 mmol) in dichloromethane (100 ml). 2-Fluoroethanol (1.77 g, 1.62 ml, 27.63 mmol) was then added drop wise over 1 min. The reaction was left to stir at room temperature for 30 min, and then filtered under gravity. The 4-(polyvinyl) pyridinium precipitate was washed with 5 ml of dichloromethane. The organic phases were combined and washed with $NaHCO_3$ (3 × 50 ml) and water (3 × 50 ml) respectively, dried ($MgSO_4$), and concentrated under reduced pressure to give 2-fluoroethyltriflate (5.10 g, 26 mmol, 94.1%) as colorless oil. The product was used without further purification.

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^{1}H NMR ($CDCl_3$): 4.66 (m, 2H, CH_2-F), 4.65 (m, 2H CH_2-OTf)
^{13}C NMR ($CDCl_3$): 74.50 (d, CH_2-F, $J = 20$ Hz), 79.86 (d, CH_2-OTf, $J = 175$ Hz), 118.47 (q, CF_3, $J = 320$ Hz)
IR: 1156.3, 1217.8 (O = S = O).

General procedure for the alkylation of anilines (procedure A)

The aniline and 1 equivalent of the respective base were added to a stirred solution of the crude alkylating agent in dichloromethane (100 ml). The reaction was refluxed (80 °C) overnight, then cooled and washed with $NaHCO_3$ (3 × 50 ml) and water (3 × 50 ml), respectively. The organic layers were combined, dried ($MgSO_4$) and evaporated under reduced pressure. The crude material was then purified by flash column chromatography on silica gel.

N,N-bis-(2-chloroethyl)-(4-methylthio) aniline **5**

Compound **5** was synthesized following procedure A starting from 4-(methylthio) aniline (**3**) (0.38 g, 2.73 mmol), anhydrous potassium carbonate as base and 2-chloroethyltriflate (2.32 g, 10.91 mmol) as alkylating agent. Purification by flash column chromatography (eluent 5% ether in hexane) gave 0.48 g (1.82 mmol, 66.7%) of product as brown oil. TLC R_f0.76 in ether/hexane (1:1).

^{1}H NMR ($CDCl_3$): 2.43 (s, 3H), 3.62 (t, 4H, CH_2, $J = 7.2$ Hz), 3.74 (t, 4H, CH_2, $J = 7.2$ Hz), 6.64 (d, 2H, $J = 8.4$ Hz), 7.28 (d, 2H, $J = 8.4$ Hz)
^{13}C NMR ($CDCl_3$): 18.83 (CH_3), 43.30 (CH_2-Cl), 45.38 (CH_2-N), 113.79 (CH-Ar), 125.34 (C-S, Ar), 131.24 (CH-Ar), 145.78 (C-N, Ar).

N-(2-chloroethyl)-(4-methylthio) aniline **7**

Compound **7** was obtained following procedure A, starting from starting from 4-(methylthio) aniline (**3**) (8.89 g, 63.85 mmol), anhydrous potassium carbonate as base and 2-chloroethyltriflate (6.79 g, 31.93 mmol) as alkylating agent. Purification by flash column chromatography (eluent 5% ether in hexane) gave 2.23 g (11.05 mmol, 34.6%) of product as brown oil. TLC R_f 0.61 in ether/hexane (1:1)

^{1}H NMR ($CDCl_3$): 2.43 (s, 3H), 3.48 (t, 2H, CH_2, $J = 4.8$ Hz), 3.69 (t, 2H, CH_2, $J = 4.8$ Hz), 6.58 (d, 2H, CH, $J = 6.4$ Hz), 7.24 (d, 2H, CH, $J = 6.4$ Hz).
^{13}C NMR ($CDCl_3$): 18.79 (CH_3), 43.23 (CH_2-Cl), 45.43 (CH_2-N), 113.86 (CH-Ar), 125.46 (C-S, Ar), 131.16 (CH-Ar), 145.61 (C-N, Ar).
Accurate mass (ESI): calculated (M + H) 202.0457. Found 202.0455.

N-(2-chloroethyl)-N-(2-fluoroethyl)-(4-methylthio) aniline **9**

Compound **9** was obtained from *N*-(2-chloroethyl)-(4-methylthio) aniline (**7**) (8.89 g, 44.07 mmol), 2,6-lutidine as base and fluoroethyltriflate (4.32 g,

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22.03 mmol) as alkylating agent following procedure A. The crude material was purified by flash column chromatography (eluent 5% ether in hexane) to give 2.23 g (9.0 mmol, 40.9%) of product as brown oil. TLC R_f 0.61 in ether/hexane (1:1).

^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 3.59–3.69 (m, 6H), 4.58 (dt, 2H, F-CH_2, $J = 47.5$, 5.0 Hz), 6.63 (d, 2H, CH, $J = 8.0$ Hz), 7.27 (d, 2H, CH, $J = 8.0$ Hz).
^{13}C NMR ($CDCl_3$): 18.79 (CH_3), 40.02 (CH_2-Cl), 51.72 (d, F-CH_2-CH_2-N, $J = 21.5$ Hz), 53.53 (CH_2-N), 81.38 (d, F-CH_2-CH_2-N, $J = 169.7$ Hz), 112.70 (CH-Ar), 124.79 (CH-Ar), 131.39 (C-S, Ar), 145.20 (C-N, Ar).
Accurate mass (ESI): calculated (M + H) 248.0676. Found 248.0673.

N,N-bis-(2-chloroethyl)-[4-(4-nitrophenylthio)] aniline **6**

Compound **6** was synthesized from (4-nitrophenylthio) aniline (**4**) (0.87 g, 3.53 mmol), anhydrous potassium carbonate as base and 2-chloroethyltriflate (3.00 g, 14.13 mmol) as alkylating agent, following procedure A. The crude material was purified by flash column chromatography (eluent 5% ether in hexane) to give 0.57 g (1.54 mmol, 43.5%) of **6** as yellow solid. TLC R_f 0.60 in ether/hexane (1:1).

^{1}H NMR ($CDCl_3$): 3.68 (t, 4H, CH_2, $J = 7.2$ Hz), 3.80 (t, 4H, CH_2, $J = 7.2$ Hz), 6.74 (d, 2H, $J = 8.4$ Hz), 7.09 (d, 2H, $J = 8.4$ Hz), 7.43 (d, 2H, $J = 8.4$ Hz), 8.04 (d, 2H, $J = 8.4$ Hz).
^{13}C NMR ($CDCl_3$): 40.12 (CH_2-Cl), 53.30 (CH_2-N), 112.96 (CH-Ar), 115.75 (C-S, Ar), 123.92 (CH-Ar), 125.29 (CH-Ar), 137.43 (CH-Ar), 144.84 (C-S, Ar), 147.47 (C-N, Ar), 150.64 (C-N, Ar).
MP 113.6–114.9°C.

N-(2-chloroethyl)[4-(4-nitrophenylthio)] aniline **8**

Compound **8** was obtained from (4-nitrophenylthio) aniline (**4**) (0.56 g, 2.27 mmol), anhydrous potassium carbonate as base and 2-chloroethyltriflate (0.24 g, 1.13 mmol) as alkylating agent following procedure A. The crude material was purified by flash column chromatography (eluent 5% ether in hexane) to give 0.32 g (1.04 mmol, 92.0%) of product as yellow solid. TLC R_f 0.61 in ether/hexane (1:1).

^{1}H NMR ($CDCl_3$): 3.57 (t, 2H, CH_2, $J = 5.9$ Hz), 3.76 (t, 2H, CH_2, $J = 5.9$ Hz), 6.70 (d, 2H, $J = 8.7$ Hz), 7.09 (d, 2H, $J = 8.7$ Hz), 7.37 (d, 2H, J = 8.7 Hz), 8.03 (d, 2H, $J = 8.7$ Hz).
^{13}C NMR ($CDCl_3$): 43.10 (CH_2-Cl), 44.92 (CH_2-N), 114.06 (CH-Ar), 115.83 (C-S, Ar), 123.89 (CH-Ar), 125.15 (CH-Ar), 137.27 (CH-Ar), 144.72 (C-S, Ar), 148.64 (C-N, Ar), 151.01 (C-N, Ar).

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Accurate mass (ESI): calculated (M + Na) 331.0284. Found 331.0278.
MP 102.5–104°C.

N-(2-chloroethyl)-N-(2-fluoroethyl)-[4-(4-nitrophenylthio)] aniline **10**

Compound **10** was prepared from *N*-(2-chloroethyl)-[4-(4-nitrophenylthio)] aniline (**8**) (0.19 g, 0.62 mmol), 2,6-lutidine as base and 2-fluoroethyltriflate (0.06 g, 0.31 mmol) as alkylating agent. The crude material was purified by flash column chromatography (eluent 5% ether in hexane) to give 62.5 mg (0.18 mmol, 56.8%) of product as a yellow solid. TLC Rf 0.50 in ether/hexane (1:1).

^{1}H NMR ($CDCl_3$): 3.70 (t, 2H, CH_2-Cl, $J = 7.3$ Hz), 3.76–3.83 (m, 4H), 4.64 (dt, 2H, F-CH_2, J = 47.8, 4.7 Hz), 6.75 (d, 2H, $J = 8.8$ Hz), 7.09 (d, 2H, $J = 8.8$ Hz), 7.42 (d, 2H, $J = 8.8$ Hz), 8.64 (d, 2H, $J = 8.8$ Hz).
^{13}C NMR ($CDCl_3$): 39.79 (CH_2-Cl), 51.61 (d, F-CH_2-CH_2-N, $J = 21.4$ Hz), 53.34 (CH_2-N), 81.23 (d, F-CH_2-CH_2-N, $J = 169.4$ Hz), 112.98 (CH-Ar), 115.40 (C-S, Ar), 123.91 (CH-Ar), 125.26 (CH-Ar), 137.36 (CH-Ar), 144.70 (C-S, Ar), 147.88 (C-N, Ar), 150.76 (C-N, Ar).
^{19}F NMR (CDCl3): −146.4 (m).
Accurate mass (ESI): calculated (M + Na) 377.0503. Found 377.0502.
MP 116.3–117.8°C.

General procedure for the oxidation of thioethers to sulfoxides (procedure B)

A solution of 30% H_2O_2 (0.033 g, 0.030 ml, 0.29 mmol) in TFA (0.970 ml) was added to a solution of 0.29 mmol of the respective thioether in TFA (5 ml) while stirring at −15°C. The reaction was allowed to proceed for 1 h at −15°C. The dry ice bath was removed and the reaction was left to warm to room temperature. TFA was evaporated and the residual product dissolved in dichloromethane (30 ml) and washed with $NaHCO_3$ (3 × 30 ml) and water (3 × 30 ml) respectively. The organic layers were combined, dried ($MgSO_4$) and evaporated under reduced pressure and the crude material was purified by flash column chromatography on silica gel.

N,N-bis-(2-chloroethyl)-(4-methylsulfinyl) aniline **1**

This compound was prepared from *N,N-bis*-(2-chloroethyl)-(4-methylthio) aniline (**5**) (76 mg, 0.29 mmol) following procedure B. The crude material was purified by flash column chromatography (eluent 80% ether in hexane) giving 58 mg (0.21 mmol, 71%) of **1** as a brown solid. TLC R_f 0.31 in ether hexane (7:1).

^{1}H NMR ($CDCl_3$): 2.68 (s, 3H), 3.64 (t, 4H, CH_2, $J = 6.9$ Hz), 3.78 (t, 4H, CH_2, $J = 6.9$ Hz), 6.77 (d, 2H, CH, $J = 7.2$ Hz), 7.54 (d, 2H, CH, $J = 7.2$ Hz).

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^{13}C NMR ($CDCl_3$): 40.04 (CH_2-Cl), 43.70 (CH_3), 53.26 (CH_2CH_2N), 112.06 (CH-Ar), 125.91 (CH-Ar), 132.52 (C-S, Ar), 148.50 (C-N, Ar).

N-(2-chloroethyl)-N-(2-fluoroethyl)-(4-methylsulfinyl) aniline **11**

This compound was prepared from *N*-(2-chloroethyl)-*N*-(2-fluoroethyl)-(4-methylthio) aniline (**9**) (72 mg, 0.29 mmol) following procedure B. The crude material was purified by flash column chromatography (eluent 80% ether in hexane) to give 65 mg (0.25 mmol, 85%) of pure product as a brown oil. TLC R_f 0.31 in ether hexane (7:1).

^{1}H NMR ($CDCl_3$): 2.73 (s, 3H), 3.61–3.82 (m, 6H), 4.58 (dt, 2H, F-CH_2, J = 47.1, 4.8 Hz), 6.77 (d, 2H, CH, J = 9.0 Hz), 7.54 (d, 2H, CH, J = 9.0 Hz).
^{13}C NMR ($CDCl_3$): 39.71 (CH_3), 43.63 (CH_2-Cl), 51.63 (d, F-CH_2-CH_2-N, J = 21 Hz), 53.32 (CH_2-N), 81.15 (d, F-CH_2-CH_2-N, J = 169.4 Hz), 112.09 (CH-Ar), 125.93 (CH-Ar), 132.08 (C-S, Ar), 148.96 (C-N, Ar).
Accurate MASS (ESI): calculated (M + Na) 286.0445. Found 286.0443.

N,N-bis-(2-chloroethyl)-[4-(4-nitrophenylsulfinyl)] aniline **2**

This compound was prepared from *N,N-bis*-(2-chloroethyl)-[4-(4-nitrophenylthio)] aniline (**6**) (107 mg, 0.29 mmol) following procedure B. The crude material was purified by flash column chromatography (eluent 80% ether in hexane) to give 110 mg (0.28 mmol, 98%) of pure product as a yellow solid. TLC R_f 0.30 in ether hexane (7:1).

^{1}H NMR ($CDCl_3$): 3.62 (t, 4H, CH_2, J = 6.8 Hz), 3.77 (t, 4H, CH_2, J = 6.8 Hz), 6.71 (d, 2H, J = 9.0 Hz), 7.50 (d, 2H, J = 9.0 Hz), 7.78 (d, 2H, J = 8.8 Hz), 8.30 (d, 2H, J = 8.8 Hz).
^{13}C NMR ($CDCl_3$): 39.89 (CH_2-Cl), 53.22 (CH_2N), 112.26 (CH-Ar), 124.22 (CH-Ar), 125.27 (CH-Ar), 128.27 (CH-Ar), 131.29 (C-S, Ar), 148.97 (C-S, Ar), 149.26 (C-N, Ar), 153.37 (C-N, Ar).
MP 106-107.5°C.

N-(2-chloroethyl)-N-(2-fluoroethyl)-[4-(4-nitrophenylsulfinyl)] aniline **12**

This compound was prepared from *N*-(2-chloroethyl)-*N*-(2-fluoroethyl)-[4-(4-nitrophenylthio)] aniline (**9**) (103 mg, 0.29 mmol) following procedure B. The crude material was purified by flash column chromatography (eluent 80% ether in hexane) to give 91 mg (0.25 mmol, 85%) of pure product as a yellow oil. TLC R_f 0.35 in ether hexane (7:1).

^{1}H NMR ($CDCl_3$): 3.73 (t, 2H, CH_2CH_2-Cl, J = 7.2 Hz), 3.70–3.78 (m, 4H), 4.57 (dt, 2H, F-CH_2, J = 47.2, 4.8 Hz), 6.69 (d, 2H, J = 8.8 Hz), 7.47 (d, 2H, J = 8.8 Hz), 7.76 (d, 2H, J = 8.8 Hz), 8.28 (d, 2H, J = 8.8 Hz).

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^{13}C NMR ($CDCl_3$): 39.51 (CH_2-Cl), 51.50 (d, F-CH_2-CH_2-N, J = 21.4 Hz), 53.14 (CH_2-N), 81.00 (d, F-CH_2-CH_2-N, J = 170.2 Hz), 112.15 (CH-Ar), 124.14 (C-S, Ar), 125.24 (CH-Ar), 128.20 (CH-Ar), 130.75 (CH-Ar), 148.86 (C-S, Ar), 149.64 (C-N, Ar), 153.36 (C-N, Ar).
Accurate mass (ESI): calculated (M + Na) 393.0452. Found 393.0450.

*General procedure for the radiosynthesis of [^{18}F]**11** and [^{18}F]**12***

The precursor (2 mg) in DMSO (1 ml) was added and to the dried K[^{18}F]F kryptofix complex and heated at 100°C for 15 min. After the labelling, 10 ml of water was added to the reaction vessel and the crude reaction mixture was subsequently pushed through a C-18 Sep Pak for trapping of the crude product. The Sep Pak was rinsed with 10 ml of water before elution with 1 ml of ethanol. The ethanol fraction was injected into the semi-preparative HPLC (Apollo C-18, 5 μ, 10 × 250 mm) using conditions as described below.

HPLC conditions for SO101

0.1 M NH_4HCO_2/ acetonitrile : 40/60 (v/v) at a flow rate of 4 ml/min was used as mobile phase. The radioactive peak at 12.16 min was collected and reformulated in 10% ethanol using the Sep Pak method. Retention time of the precursor was 18.32 min under these conditions.

Independent quality control (Develosil UG C-30, 5 μ, 10 × 250 mm, 20 μl injection loop) gave a single radioactive peak at 8.5 min, which was identical to the F-19 standard.

HPLC conditions for SO201

0.1 M NH_4HCO_2/ acetonitrile : 50/50 (v/v) at a flow rate of 4 ml/min was used as mobile phase. The radioactive peak at 11.5 min was collected and reformulated in 10% ethanol using the Sep Pak method. Retention time of the precursor was at 19.5 min under these conditions.

Independent quality control (Develosil UG C-30, 5 μ, 10 × 250 mm, 20 μl injection loop) gave a single radioactive peak at 11.7 min, which was identical to the F-19 standard.

In vivo binding

Animal experimentation was approved by the Austin Health animal ethics committee and was performed in accordance to guidelines of the NHMRC. Focal cerebral ischemia was induced in Sprague-Dawley rats ($n = 8$).[20] The middle cerebral artery was permanently occluded with a silicone-coated nylon monofilament. 150 μCi of the radiotracer was injected into the tail vein 10 min after induction of ischemia, and animals were decapitated 2 h later ($n = 4$ each compound). Brains were removed and immediately cut into 2 mm coronal

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sections using a rat brain template. These sections were covered with cling-film, then placed on phosphor-imaging plates for 20 min in the dark. Plates were read in a BAS-5000 plate reader (Fuji, Japan). Image analysis was performed using the MCID program (Imaging Research, Canada). Images were smoothed using a 15×15 smoothing kernel. Area of increased uptake was defined by thresholding at mean + 3 SD. above the contralateral hemisphere optical density.

Conclusion

In conclusion, we have been able to radiolabel two sulfoxide containing *bis*-haloethyl anilines (SO101 and SO201) with F-18 via halogen exchange. The optimum decay corrected labelling yields were 35–45% for [^{18}F]**11** (SO101) and 25–35% for [^{18}F]**12** (SO201) at 100°C for 15 min for both compounds. The putative tracers [^{18}F]**11** and [^{18}F]**12** were found to be stable in saline and 80% intact in plasma over a period of 2 h. Uptake by hypoxic tissues in animals suggest that further investigation of [^{18}F]**11** and [^{18}F]**12** as PET imaging agents for hypoxic tissue are warranted.

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References

1. Brizel DM, Dodge RK, Clough RW, Dewhirst MW. *Radiother Oncol* 1999; **53**: 113–117.
2. Gatenby RA, Kessler HB, Rosenblum JS, Coia LR, Moldofsky PJ, Harts WH, Broder GJ. *Int J Radiat Oncol Biol Phys* 1988; **14**: 831–838.
3. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, Giaccia AJ. *Nature* 1996; **379**: 88–91.
4. Lawrentschuk N, Poon AMT, Foo SS, Putra LGJ, Murone C, Davis ID, Bolton DM, Scott AM. *BJU Int* 2005; **96**(4): 540–546.
5. Foo SS, Abbott DF, Lawrentschuk N, Scott AM. *Mol Imaging Biol* 2004; **6**(5): 291–305.
6. Hossman KA. *Ann Neurol* 1994; **36**: 557–565.
7. Lewis JS, Welch MJ. *Q J Nucl Med* 2001; **45**: 138–188.
8. Markus R, Donnan GA, Kazui S, Read S, Hirano T, Scott AM, O'Keefe GJ, Tochon-Danguy HJ, Sachinidis JI, Reutens DC. *Neuroimage* 2002; **16**: 425–433.
9. Brady F, Luthra KS, Brown GD, Osman S, Aboagye E, Saleem A, Price PM. *Curr Pharm Des* 2001; **7**(18): 1863–1892.
10. Nunn A, Lindner K, Strauss HW. *Eur J Nucl Med* 1995; **22**: 265–280.
11. Franko AJ. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1195–1202.

DOI: 10.1002/jlcr

12. Read SJ, Hirano T, Abbott DF, Markus R, Sachinidis JI, Tochon-Danguy HJ, Chan JG, Egan GF, Scott AM, Bladin CF, McKay WJ, Donnan GA. *Ann Neurol* 2000; **48**: 228–235.
13. Kwon CH, Blanco DR, Baturay N. *J Med Chem* 1992; **35**: 2137–2139.
14. Sun ZY, Botros E, Su AD, Kim Y, Wang E, Baturay NZ, Kwon CH. *J Med Chem* 2000; **43**: 4160–4168.
15. Davis PJ, Guenthner LE. *Xenobiotica* 1985; **15**: 845–847.
16. Duggan DE, Hooke KF, Noll RM, Hucker HB, Van Arman CC. *Biochem Pharmacol* 1978; **27**: 2311–2320.
17. Lemaire C, Plenevaux A, Del Fiore G, Aerts J, Luxen A. *J Label Compd Radiopharm* 1995; **37**: 723.
18. Saita K, Chen M, Spratt NJ, Porritt MJ, Libratore GT, Read SJ, Levi CR, Donnan GA, Ackermann U, Tochon-Danguy HJ, Sachinidis JI, Howells DW. *Stroke* 2004; **35**: 975–980.
19. Tochon-Danguy HJ, Sachinidis JI, Chan F, Chan JG, Hall C, Cher L, Stylli S, Hill J, Kaye A, Scott AM. *Nucl Med Biol* 2000; **29**: 191–197.
20. Schmid-Elsaesser R, Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ, Garcia JH. *Stroke* 1998; **29**(10): 2162–2170.

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