SUPPLEMENTAL DATA

Methods and Material

$AmBF_3$-conjugated alkyne **3** (*1*), $AmBF_3$-conjugated alkyne **4** (*2*), and 2-chloro-N-(4-sulfamoyl-phenyl)-acetamide (*3*) were prepared according to published procedures. Other chemicals were obtained from commercial sources, and used without further purification. ^{18}F-fluoride Trap & Release columns were purchased from ORTG Inc. (Oakdale, TN). C18 light Sep-Pak cartridges (1cc, 50 mg) were obtained from Waters (Milford, MA). Balb/c mouse plasma was obtained from Innovative Research (Novi, MI). Mass analyses were performed using a Bruker (Billerica, MA) Esquire-LC/MS system with ESI ion source. Purification and quality control of cold and ^{18}F-labeled sulfonamides were performed on an Agilent HPLC system equipped with a model 1200 quaternary pump, a model 1200 UV detector, and a Bioscan (Washington, DC) NaI scintillation detector. The radio-detector was connected to a Bioscan B-FC-1000 Flow-count system, and the output from the Bioscan Flow-count system was fed into an Agilent 35900E interface which converted the analog signal to digital signal. Operation of the Agilent HPLC system was controlled using the Agilent ChemStation software. Radioactivity of ^{18}F-labeled tracers was measured using a Capintec (Ramsey, NJ) CRC®-25R/W dose calibrator, and the radioactivity of mouse tissues collected from biodistribution studies were counted using a Packard (Meriden, CT) Cobra II 5000 Series auto-gamma counter.

HPLC analysis

The following table lists the different HPLC conditions used during purification, QC and plasma stability assays.

Condition	Column	Solvent System (containing 0.1% TFA)	Flow Rate (mL/min)
A	Phenomenex Luna C18, 5 µm, 10 × 250 mm semi-prep	11% MeCN in water	4.5
B	Phenomenex Luna C18, 5 µm, 10 × 250 mm semi-prep	10% MeCN in water	4.5
C	Agilent Eclipse XDB-C18 5 µm 9.2 × 250 mm semi-prep	0 to 5 min, 5 to 25% MeCN in water; 5 to 15 min, 25 to 45% MeCN in water	3.0
D	Agilent Eclipse XDB-C18 5 µm 9.2 × 250 mm semi-prep	0 to 5 min, 10 to 20% MeCN in water;5 to 15 min, 20 to 60% MeCN in water	3.0
E	Phenomenex Luna C18, 5 µm, 4.6 × 250 mm analytical	13% MeCN in water	2.0
F	Phenomenex Luna C18, 5 µm, 4.6 × 250 mm analytical	11% MeCN in water	2.0
G	Phenomenex Jupiter 10 µm C18 4.6 × 250 mm analytical	0 to 2 min, 5% MeCN in water; 2 to 7 min, 5 to 20% MeCN in water; 7 to 15 min, 20 % MeCN in water to 100% MeCN	2.0
H	Phenomenex Luna C18, 5 µm, 10 × 250 mm semi-prep	14% MeCN in water	4.5
I	Phenomenex Luna C18, 5 µm, 10 × 250 mm semi-prep	12% MeCN in water	4.5

Synthesis of azidoacetyl-AEBS (1)

A solution of azidoacetic acid (101 mg, 1.0 mmol), 1,3-dicyclohexylcarbodiimide (248 mg, 1.2 mmol) and N-hydroxysuccinimide (138 mg, 1.2 mmol) in DMF (2 mL) was stirred at room temperature for 1 h. After filtration, AEBS (220 mg, 1.1 mmol) in DMF (1 mL) was added. The resulting solution was stirred at room temperature overnight, diluted with water (25 mL), and extracted with DCM (125 mL). The organic layer was dried with anhydrous $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica gel using 9:1 ethyl acetate/hexane to obtain the desired product as a white solid (210 mg, 74%). ESI-MS: calculated for azidoacetyl-AEBS **1** $C_{10}H_{13}N_5O_3S$ 283.07, found $[M+H]^+$ 284.07. ^{1}H-NMR (CD_3CN-D_2O): δ

2.85 (t, 3.7 Hz, 2H), 3.47 (m, 4.4 Hz, 2H), 5.67 (s, 2H), 7.43 (d, 7.5 Hz, 2H), 7.78 (d, 7.5 Hz, 2H).

Synthesis of azidoacetyl-ABS (2)

A mixture of 2-chloro-N-(4-sulfamoyl-phenyl)-acetamide (353 mg, 1.4 mmol) and sodium azide (102 mg, 1.6 mmol) in DMSO (5 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water (12.5 mL) and extracted with ether (75 mL). The organic layer was dried with anhydrous $MgSO_4$, concentrated under reduced pressure, and recrystallized with water to obtain the desired product as a white solid (313 mg, 60%). ESI-MS: calculated for azidoacetyl-ABS **2** $C_8H_9N_5O_3S$ 255.04, found $[M+H]^+$ 256.04. ^{1}H-NMR (DMSO-d_6): δ 4.07 (s, 2H), 7.25 (s, 2H), 7.75 (m, 4H), 10.5 (s, 1H).

Synthesis of $AmBF_3$-AEBS

A 1.5-mL Eppendorf tube was loaded with $AmBF_3$-conjugated alkyne **3** (10 mg, 61 μmol), azidoacetyl-AEBS **1** (10 mg, 35 μmol), aqueous $CuSO_4$ (1.0 M, 56.3 μL), sodium ascorbate (1.0 M, 140.7 μL) and DMF (150 μL). This mixture was allowed to react at 45 °C for 2 h and then purified by HPLC (Condition **A**, T_R = 22.3 min) to obtain $AmBF_3$-AEBS in 41% yield. ESI-MS: calculated for $AmBF_3$-AEBS $C_{16}H_{24}BF_3N_6O_3S$ 448.2, found $[M+Na]^+$ 471.2. ^{1}H-NMR (DMSO-d_6): δ 2.23 (m, 2H), 2.50 (m, 2H), 2.82 (t, 7.1 Hz, 2H), 2.93 (s, 6H), 4.53 (s, 2H), 5.16 (s, 2H), 7.31 (s, 2H), 7.41 (d, 8.3 Hz, 2H), 7.75 (d, 8.3 Hz, 2H), 8.30 (s, 1H), 8.48 (t, 5.5 Hz, 1H).

Synthesis of $AmBF_3$-ABS

A 1.5-mL Eppendorf tube was loaded with $AmBF_3$-conjugated alkyne **3** (10 mg, 61 μmol), azidoacetyl-ABS **2** (10 mg, 39 μmol), aqueous $CuSO_4$ (1.0 M, 62.7 μL), sodium ascorbate (1.0 M, 156.8 μL) and DMF (150 μL). This mixture was allowed to react at 45 °C for 2 h and then purified by HPLC (Condition **B**, T_R = 22.1 min) to obtain $AmBF_3$-ABS in 40% yield. ESI-MS:

calculated for $AmBF_3$-ABS $C_{14}H_{20}BF_3N_6O_3S$ 420.1, found $[M+Na]^+$ 443.2. ^{1}H-NMR (DMSO-d_6): δ 2.24 (m, 2H), 2.95 (s, 6H), 4.57 (s, 2H), 5.47 (s, 2H), 7.27 (s, 2H), 7.76 (q, 8.0 Hz, 9.0 Hz, 4H), 8.41 (s, 1H), 10.86 (s, 1H).

Synthesis of $AmBF_3$-$(AEBS)_3$

A 1.5-mL Eppendorf tube was loaded with $AmBF_3$-conjugated alkyne **4** (5 mg, 12.3 μmol), azidoacetyl-AEBS **1** (12.1 mg, 44 μmol), aqueous $CuSO_4$ (1.0 M, 5 μL), sodium ascorbate (1.0 M, 12.5 μL) and 5% NH_4OH (in 1:1 MeCN/H_2O, 50 μL). This mixture was allowed to react at 45 °C for 2 h and then purified by HPLC (Condition **C**, T_R = 12.8 min) to obtain $AmBF_3$-$(AEBS)_3$ in 47% yield. ESI-MS: calculated for $AmBF_3$-$(AEBS)_3$ $C_{49}H_{68}BF_3N_{16}O_{13}S_3$ 1252.44, found $[M+H]^+$ 1253.49. ^{1}H-NMR (CD_3CN-D_2O): δ 2.71 (s, 2H), 2.88 (m, 8H), 3.17 (s, 6H), 3.47 (q, 4.4 Hz, 6H), 3.63 (t, 2.7 Hz, 2H), 3.80 (s, 8H), 4.19 (s, 6H), 5.67 (s, 6H), 7.43 (d, 7.4 Hz, 2H), 7.78 (d, 7.4 Hz, 2H).

Synthesis of $AmBF_3$-$(ABS)_3$

A 1.5-mL Eppendorf tube was loaded with $AmBF_3$-conjugated alkyne **4** (5 mg, 12.3 μmol), azidoacetyl-ABS **2** (11.0 mg, 44 μmol), aqueous $CuSO_4$ (1.0 M, 5 μL), sodium ascorbate (1.0 M, 12.5 μL) and 5% NH_4OH (in 1:1 MeCN/H_2O, 50 μL). The mixture was allowed to react at 45 °C for 2 h, and then purified by HPLC (Condition **D**, T_R = 13.1 min) to obtain $AmBF_3$-$(ABS)_3$ in 55% yield. ESI-MS: calculated for $AmBF_3$-$(ABS)_3$ $C_{43}H_{56}BF_3N_{16}O_{13}S_3$ 1168.34, found $[M+H]^+$ 1169.40 ^{1}H-NMR (CD_3CN-D_2O): δ 4.16 (s, 6H), 4.30 (s, 2H), 4.61 (m, 4H), 4.72 (s, 8H), 5.77 (s, 6H), 6.54 (s, 6H), 8.94 (d, 9.0 Hz, 6H), 9.03 (d, 9.0 Hz, 6H), 9.16 (s, 3H).

Binding affinity measurements

Inhibition constants (K_i) of $AmBF_3$-AEBS, $AmBF_3$-ABS, and $AmBF_3$-$(AEBS)_3$ and $AmBF_3$-$(ABS)_3$ for CA-I, -II, -IX and -XII were determined using the CA catalyzed CO_2 hydration stopped-flow assays following published procedures (*3*).

Radiolabeling

100-150 nmol of ^{19}F-$AmBF_3$-AEBS, ^{19}F-$AmBF_3$-ABS, ^{19}F-$AmBF_3$-$(AEBS)_3$ or ^{19}F-$AmBF_3$-$(ABS)_3$ was resuspended with aqueous pyridazine-HCl buffer (15-20 μL, 1M, pH = 2) and DMF (15-20 μL) in a polypropylene tube. No carrier-added ^{18}F-fluoride was obtained by bombardment of $H_2{}^{18}O$ with 18 MeV protons, followed by trapping on an anion exchange column (9 mg, QMA, chloride form). The ^{18}F-fluoride was eluted off with saline (100 μL) into the reaction vial. The reaction mixture was heated at 80 °C for 20 min, and quenched with 5% aqueous NH_4OH (2 mL). The quenched solution was loaded onto a C18 light Sep-Pak cartridge. ^{18}F-Fluoride was removed by washing the cartridge with DI water (5 mL × 2). ^{18}F-$AmBF_3$-AEBS, ^{18}F-$AmBF_3$-ABS, ^{18}F-$AmBF_3$-$(AEBS)_3$ or ^{18}F-$AmBF_3$-$(ABS)_3$ was eluted off the cartridge with 0.5 mL 4:1 ethanol/saline and diluted with saline (5 mL) for stability and biodistribution/imaging studies. Samples were removed for QC analysis by HPLC using Condition **E** for ^{18}F-$AmBF_3$-AEBS (T_R = 8.0 min), Condition **F** for ^{18}F-$AmBF_3$-ABS (T_R = 7.9 min), or Condition **G** for ^{18}F-$AmBF_3$-$(AEBS)_3$ (T_R = 12.4 min) and ^{18}F-$AmBF_3$-$(ABS)_3$ (T_R = 11.9 min).

In vitro stability

20 μL of ^{18}F-$AmBF_3$-AEBS, ^{18}F-$AmBF_3$-ABS, ^{18}F-$AmBF_3$-$(AEBS)_3$ or ^{18}F-$AmBF_3$-$(ABS)_3$ was added to mouse plasma (500 uL) and incubated at 37 °C for 2 h. The reaction was quenched by adding 1 mL MeCN to the plasma solution. The quenched solution was centrifuged, and the supernatant was collected, filtered and analyzed by HPLC using Condition **H** for ^{18}F-$AmBF_3$-

AEBS (T_R = 13.0 min), Condition **I** for ^{18}F-AmBF$_3$-ABS (T_R = 15.4 min), or Condition **G** for ^{18}F-AmBF$_3$-(AEBS)$_3$ (T_R = 12.4 min) and ^{18}F-AmBF$_3$-(ABS)$_3$ (T_R = 11.9 min).

LogD$_{7.4}$ measurements

An aliquot of ^{18}F-AmBF$_3$-AEBS, ^{18}F-AmBF$_3$-ABS, ^{18}F-AmBF$_3$-(AEBS)$_3$ or ^{18}F-AmBF$_3$-(ABS)$_3$ was added to a Falcon tube containing 4 mL of octanol and 1.5 mL of phosphate buffer (0.1 M, pH = 7.4). The mixture was vortexed for 2 min and centrifuged at 5,000 rpm for 2 min. Samples of the octanol (3.5 mL) and buffer (1 mL) layers were taken and counted. LogD$_{7.4}$ was calculated using the following equation: LogD$_{7.4}$ = log$_{10}$[(counts in octanol phase/3.5)/(counts in buffer phase)]. The major portion of the phosphate buffer layer (1 mL) was diluted with 0.5 mL of phosphate buffer and mixed with octanol (4 mL). The equilibration procedure described above was repeated until a constant value of LogD$_{7.4}$ was obtained.

Cell Line and Animal Model

HT-29 human colorectal cancer cells were obtained as a gift from Dr. Donald Yapp (BC Cancer Research Centre, Vancouver, Canada). HT-29 cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum 100 U/mL penicillin-streptomycin, and non-essential amino acids. Male NODSCID IL2RKO mice bred in-house at the Animal Research Centre, BC Cancer Research Centre were used. Mice were subcutaneously inoculated with 5 × 10^6 HT-29 cells in the right dorsal flank. Biodistribution studies and PET/CT imaging were performed when tumors reached 7-9 mm in diameter.

Biodistribution Studies

Tumor bearing mice were injected with ~ 0.37 MBq of ^{18}F-labeled tracer (100 – 200 μL in saline, i.v.). For blocking experiments, mice were intravenously pre-injected with 10 mg/kg acetazolamide 1 h (100 – 200 μL in saline, i.v.) before administering the radiotracer. After an

uptake period of 0.5, 1, or 2 h, mice were euthanized by CO_2 asphyxiation. Blood was promptly withdrawn, and organs/tissues of interest were harvested, rinsed with saline, blotted dry and weighed. Radioactivity in collected tissues was counted, normalized to the injected dose and expressed as the percentage of the injected dose per gram of tissue (%ID/g).

PET/CT Imaging

Imaging experiments were performed using a Siemens (Erlangen, Germany) Inveon micro PET/CT scanner. Tumor bearing mice were injected with ~ 3.7 MBq of ^{18}F-labeled tracer (100 – 200 μL in saline, i.v.). For blocking experiments, mice were intravenously pre-injected with 10 mg/kg acetazolamide 1 h (100 – 200 μL in saline, i.v.) before administering the radiotracer. At 0.5, 1, or 2 h p.i., a 10-min PET acquisition scan was performed, which was preceded by a 10-min CT scan. Body temperature of mice was maintained at 37 °C with the use of thermal pads. PET data were acquired in list mode acquisition, reconstructed using the 3d-OSEM-MAP algorithm with CT-based attenuation correction, and co-registered for alignment. At the conclusion of the imaging study, mice were euthanized and processed for biodistribution as described above.

Data Analysis

All statistics were performed using Prism 6 software (GraphPad). *P* values for the difference of tracer uptake in mouse tissues between unblocked and blocked groups were calculated using a Student's *t*-test (unpaired, one-tailed) and values < 0.05 were considered statistically significant.

REFERENCES

1. Liu Z, Radtke MA, Wong MQ, Lin KS, Yapp DT, Perrin DM. Dual mode fluorescent ^{18}F-PET tracers: Efficient modular synthesis of rhodamine-[cRGD]-[^{18}F]-organotrifluoroborate, rapid, and high yielding one-step ^{18}F-labeling at high specific activity, and correlated in vivo PET imaging and ex vivo fluorescence. *Bioconjug Chem.* 2014;25:1951-1962.

2. Liu Z, Pourghiasian M, Benard F, Pan J, Lin KS, Perrin DM. Preclinical evaluation of a high-affinity ^{18}F-trifluoroborate octreotate derivative for somatostatin receptor imaging. *J Nucl Med.* 2014;55:1499-1505.

3. Sarathi Addy P, Saha B, Pradeep Singh ND, et al. 1,3,5-Trisubstituted benzenes as fluorescent photoaffinity probes for human carbonic anhydrase II capture. *Chem Commun (Camb).* 2013;49:1930-1932.

4. Pacchiano F, Carta F, McDonald PC, et al. Ureido-substituted benzenesulfonamides potently inhibit carbonic anhydrase IX and show antimetastatic activity in a model of breast cancer metastasis. *J Med Chem.* 2011;54:1896-1902.