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Synthesis of carbon-11 labeled sulfonanilide analogues as new potential PET agents for imaging of aromatase in breast cancer

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Abstract—Aromatase is a particularly good target in the treatment of estrogen receptor positive breast cancer. Novel carbon-11 labeled sulfonanilide analogues, *N*-[^{11}C]methyl-*N*-(2-alkyloxy-4-nitrophenyl)-methanesulfonamides ([^{11}C]**3a–f**, alkyl = propyl, isopropyl, 1-ethyl-propyl, cyclopentyl, cyclohexyl, and cyclohexylethyl), were designed and synthesized as potential PET agents for imaging of aromatase in breast cancer.

Approximately 75% of breast cancers test positive for estrogen receptor (ER), the progesterone receptor (PgR), or both, and estrogen stimulation of these receptors is a significant factor in the development and growth of breast cancer.[1] Aromatase is a particularly attractive target for inhibition because it mediates the last in the series of steps in steroid biosynthesis and is rate-limiting for estrogen synthesis.[2] The inhibition of estrogen synthesis by aromatase inhibitors (AIs) and inhibition of estrogen action by compounds interacting with ERs (antiestrogens) are two strategies for the design of drugs that can be used to ameliorate the growth effects of estrogens on ER positive (ER^+) tumor cells.[2] AIs have been extensively studied as effective hormonal therapeutic drugs in the treatment of ER^+ breast cancer. They are equivalent or superior to antiestrogen tamoxifen in women with metastatic diseases. In addition, AIs provide an effective treatment in some patients relapsing from tamoxifen.[1,2] The enzyme aromatase also provides an attractive target for the development of enzyme-based breast cancer imaging agents for use in biomedical imaging technique positron emission tomography (PET). Recently, a novel series of sulfonanilide analogues derived from the cyclooxygenase-2 (COX-2) selective inhibitor NS-398 have been developed.[3] These compounds suppress aromatase expression and activity in breast cancer cells independent of COX-2 inhibition. COXs catalyze the key rate-limiting step in the conversion of arachidonic acid into prostaglandins and thromboxanes.[4] Aromatase levels in breast cancer cells are enhanced by prostaglandins and reduced by COX inhibitors. Therefore, COX selective inhibitors could serve as the first generation of selective aromatase expression regulators.[3] Sulfonanilide analogues labeled with a positron emitting radionuclide carbon-11 or fluorine-18 may enable non-invasive monitoring aromatase expression in breast cancer and breast cancer response to AIs therapy using PET imaging technique. We are interested in the development of PET breast cancer imaging agents. [^{18}F]fluoroestradiol (FES) has been used as a PET tracer to delineate the ER expression and evaluate the therapeutic efficacy of breast cancer treated with AIs.[5] Here we propose to directly use radiolabeled AIs to non-invasively image aromatase in breast cancer, which regulates the estrogen synthesis, and we report the design and synthesis of carbon-11 labeled sulfonanilide analogues as new potential PET agents for imaging of aromatase in breast cancer.

The synthesis of precursors *N*-(2-alkyloxy-4-nitrophenyl)methanesulfonamides (**2a–f**, alkyl = propyl, isopropyl, 1-ethyl-propyl, cyclopentyl, cyclohexyl, and cyclohexylethyl), and standard compounds *N*-methyl-*N*-(2-alkyloxy-4-nitrophenyl)-methanesulfonamides (**3a–f**, alkyl = propyl, isopropyl, 1-ethyl-propyl,

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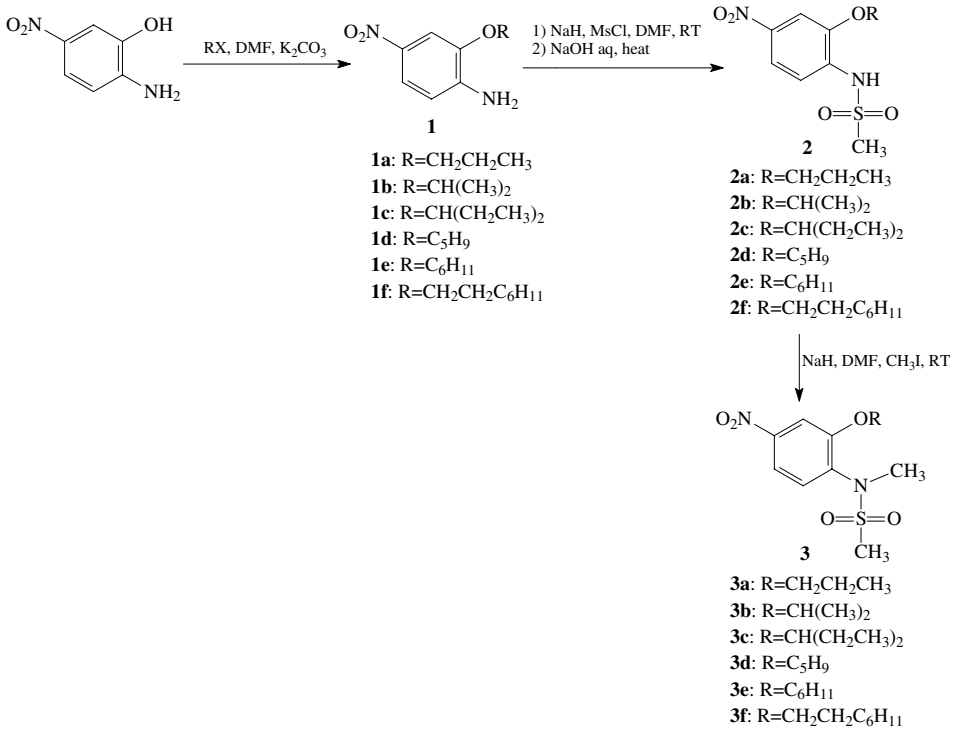
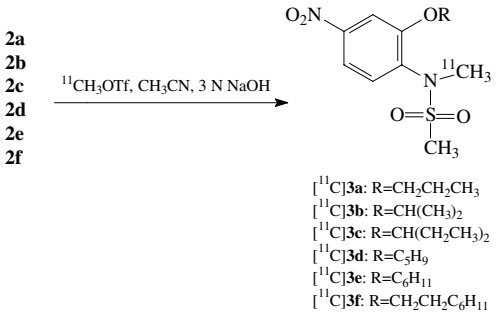


Scheme 1. Synthesis of sulfonanilide analogues.

cyclopentyl, cyclohexyl, and cyclohexylethyl) was achieved using a modification of the literature procedure.[3,6] The synthetic approach is outlined in Scheme 1. Commercially available starting material, 2-amino-5-nitrophenol, was reacted with alkyl halides (alkyl = propyl, isopropyl, 1-ethyl-propyl, cyclopentyl, cyclohexyl, and cyclohexylethyl) in the presence of K_2CO_3 in DMF to provide corresponding 2-alkyloxy-4-nitroanilines (**1a**–**f**, alkyl = propyl, isopropyl, 1-ethyl-propyl, cyclopentyl, cyclohexyl, and cyclohexylethyl) in 5–90% yield. Treatment of compounds **1a**–**f** with excess methanesulfonyl chloride was carried out to form *N*,*N*-bimethanesulfonimide intermediates, which were hydrolyzed by 50% KOH under reflux to give corresponding monomethanesulfonamide compounds **2a**–**f** as radiolabeling precursors, in 54–85% yield. Methylation of compounds **2a**–**f** with iodomethane in DMF was carried out to produce corresponding target compounds **3a**–**f** as reference standards, in 85–91% yield.



Scheme 2. Synthesis of carbon-11 labeled sulfonanilide analogues.

Synthesis of target radiotracers carbon-11 labeled sulfonanilide analogues is shown in Scheme 2. The precursors (**2a**–**f**) were labeled with [^{11}C]methyl triflate ($^{11}CH_3OTf$)[7] under basic conditions through *N*-[^{11}C]methylation and isolated by reversed-phase HPLC purification procedure[8] to give corresponding carbon-11 labeled sulfonanilide analogues, *N*-[^{11}C]methyl-*N*-(2-alkyloxy-4-nitrophenyl)-methanesulfonamides ([^{11}C]**3a**–**f**, alkyl = propyl, isopropyl, 1-ethyl-propyl, cyclopentyl, cyclohexyl, and cyclohexylethyl), in 30–35% radiochemical yields based on [^{11}C]CO_2, 20–25 min overall synthesis time from end of bombardment (EOB), >98% radiochemical purity, and 1.0–2.0 Ci/μmol specific activity at end of synthesis (EOS) measured by analytical HPLC method.[9]

Compound **2e** is the COX-2 inhibitor NS-398, *N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide. Compounds **2a**–**d** and **2f** are potential COX-2 inhibitors derived from NS-398. These nitro-compounds **2a**–**f** can be directly labeled with another positron emitting radionuclide fluorine-18 by a conventional nucleophilic

Table 1. IC_{50} values of the compounds **3a**–**f** in comparison with the parent compound NS-398 in SK-BR-3 breast cancer cells

Compound	IC_{50} (μM)
2e (NS-398)	0.72
3a	1.24
3b	1.79
3c	0.84
3d	1.09
3e	0.66
3f	2.54

substitution with $K^{18}F$/Kryptofix 2.2.2[10] to be new fluorine-18 PET probes to monitor enzyme COX expression in breast cancer and breast cancer response to COX inhibitor therapy. Compounds **3a**–**f** are potential aromatase inhibitors with no COX-2 inhibitory activity. Likewise, nitro-compounds **3a**–**f** can be labeled with fluorine-18 as new potential fluorine-18 PET agents for imaging of enzyme aromatase in breast cancer. However, the functional nitrogroup ($-NO_2$) might be critically important for the aromatase inhibition, replacing $-NO_2$ in the fluorine-18 labeling could abolish the desired imaging probe specificity. Therefore, the development of fluorine-18 labeled sulfonanilide analogues including synthesis and in vitro or in vivo validation remains to be further studied, and the results will be reported in due course.

The target compounds **3a**–**e** are the known compounds with moderate to excellent aromatase inhibitory activity in SK-BR-3 breast cancer cells,[3] and the target compound **3f** is a new compound. The in vitro validation of the compounds **3a**–**f** we synthesized was performed via a cytotoxicity assay. Cytotoxicity of the compounds **3a**–**f** in comparison with the parent compound NS-398 was assessed by a dose-response MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay[11] in SK-BR-3 breast cancer cells, and the resulting IC_{50} values of the compounds are listed in Table 1. The results show that the modified compound **3e** exhibits the strongest cytotoxicity activity in SK-BR-3 breast cancer cells greater than the parent compound NS-398, and the new compound **3f** is a potent aromatase inhibitor too albeit not as effective as the parent compound NS-398. Preliminary findings from in vitro biological assay of the unlabeled compounds **3a**–**f** encourage further in vivo evaluation of the radiolabeled compounds [^{11}C]**3a**–**f**.

The experimental details and characterization data for compounds **1a**–**f**, **2a**–**f**, **3a**–**f**, and new tracers [^{11}C]**3a**–**f**, and cytotoxicity assay of the compounds **3a**–**f** with NS-398 are given.[12]

In summary, an efficient and convenient chemical and radiochemical synthesis of the precursors, reference standards, and target tracers has been well developed. Preliminary findings from biological assay indicate the synthesized analogues have similar strong cytotoxicity activity in SK-BR-3 breast cancer cells in comparison with the parent compound NS-398. The chemistry result with in vitro IC_{50} data provide the foundation for further in vivo biological evaluation of carbon-11 labeled sulfonanilide analogues as new potential PET agents for imaging of aromatase in breast cancer.

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References and notes

1. Altundag, K.; Ibrahim, N. K. *Oncologist* **2006**, *11*, 553.
2. Brodie, A. *Trends Endocrinol. Metab.* **2002**, *13*, 61.
3. Su, B.; Diaz-Cruz, E. S.; Landini, S.; Brueggemeier, R. W. *J. Med. Chem.* **2006**, *49*, 1413.
4. Kuge, Y.; Katada, Y.; Shimonaka, S.; Temma, T.; Kimura, H.; Kiyono, Y.; Yokota, C.; Minematsu, K.; Seki, K.-i.; Tamaki, N.; Ohkura, K.; Saji, H. *Nucl. Med. Biol.* **2006**, *33*, 21.
5. Linden, H. M.; Stekhova, S. A.; Link, J. M.; Gralow, J. R.; Livingston, R. B.; Ellis, G. K.; Petra, P. H.; Peterson, L. M.; Schubert, E. K.; Dunnwald, L. K.; Krohn, K. A.; Mankoff, D. A. *J. Clin. Oncol.* **2006**, *24*, 2793.
6. Julemont, F.; de Leval, X.; Michaux, C.; Damas, J.; Charlier, C.; Durant, F.; Pirotte, B.; Dogne, J.-M. *J. Med. Chem.* **2002**, *45*, 5182.
7. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.
8. Fei, X.; Wang, J.-Q.; Miller, K. D.; Sledge, G. W.; Hutchins, G. D.; Zheng, Q.-H. *Nucl. Med. Biol.* **2004**, *31*, 1033.
9. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.
10. Wang, J.-Q.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4380.
11. Alley, M. C.; Scudieron, D. A.; monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
12. Experimental details and characterization data. (a) General: All commercial reagents and solvents were used without further purification unless otherwise specified. The $^{11}CH_3OTf$ was made according to a literature procedure.[7] Melting points were determined on a MEL-TEMP II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 FT NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) are reported in hertz (Hz). The low resolution mass spectra (LRMS) were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high resolution mass spectra (HRMS) were obtained using a Kratos MS80 mass spectrometer. Chromatographic solvent proportions are expressed on a volume: volume basis. Thin layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C_{18} column, 4.6×250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ (buffer solution) mobile phase, flow rate 1.5 mL/min, and UV (270 nm) and γ-ray (NaI) flow detectors. Semi-preparative HPLC was performed using a YMC-Pack ODS-A, S-5 μm, 12 nm, 10×250 mm i.d. (Waters) C-18 column; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ mobile

phase, 5.0 mL/min flow rate, UV (270 nm) and γ-ray (NaI) flow detectors. Sterile vented Millex-GS 0.22 μm filter unit was obtained from Millipore Corporation, Bedford, MA.

(b) General procedure for synthesis of compounds **1a–f**. Anhydrous K_2CO_3 (6.1 g, 44.0 mmol) and alkyl halide (53.0 mmol, 1.2 equiv) were added to a solution of 2-amino-5-nitrophenol (6.8 g, 44.0 mmol) in DMF (40 mL). The mixture was heated at reflux for 4 h to 5 days. After cooling, the reaction mixture was pour into water and extracted with CH_2Cl_2 (3× 200 mL). The organic layer was washed with saturated aqueous Na_2CO_3 solution and water, dried over anhydrous $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography (1:5 EtOAc/hexanes) to give desired compounds.

2-Propyloxy-4-nitroaniline (**1a**).1-Iodopropane was used and it was refluxed for 5 h. Yellow solid, 87% yield. Mp: 58–59 °C (lit.[3] 59–61 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (dd, J = 2.2, 8.9 Hz, 1H, H-5), 7.65 (d, J = 1.5 Hz, 1H, H-3), 6.64 (d, J = 8.8 Hz, 1H, H-6), 4.55 (br, 2H, NH_2), 4.04 (t, J = 6.8 Hz, 2H, $OCH_2CH_2CH_3$), 1.91–1.84 (m, 2H, $OCH_2CH_2CH_3$), 1.07 (t, J = 7.3 Hz, 3H, $OCH_2CH_2CH_3$).

2-Isopropyloxy-4-nitroaniline (**1b**). 2-Iodopropane was used and it was refluxed for 30 h. Yellow oil, 64% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (dd, J = 2.2, 8.8 Hz, 1H, H-5), 7.65 (d, J = 2.2 Hz, 1H, H-3), 6.67 (d, J = 8.9 Hz, 1H, H-6), 4.68–4.60 (m, 3H, NH_2, $OCH(CH_3)_2$), 1.39 (s, 3H, $OCH(CH_3)CH_3$), 1.38 (s, 3H, $OCH(CH_3)CH_3$).

2-(1-Ethyl-propyloxy)-4-nitroaniline (**1c**). 3-Bromopentane was used and it was refluxed for 4 days. Yellow solid, 41% yield. Mp: 59–61 °C (lit.[3] 62–63 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (dd, J = 2.3, 8.9 Hz, 1H, H-5), 7.65 (d, J = 1.6 Hz, 1H, H-3), 6.64 (d, J = 8.1 Hz, 1H, H-6), 4.56 (br, 2H, NH_2), 4.30–4.26 (m, 1H, $OCH(CH_2CH_3)_2$), 1.78–1.69 (m, 4H, $OCH(CH_2CH_3)_2$), 0.97 (t, J = 7.1 Hz, 6H, $OCH(CH_2CH_3)_2$).

2-Cyclopentyloxy-4-nitroaniline (**1d**). Cyclopentyl iodide was used and it was refluxed for 23 h. Red oil, 40% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (dd, J = 2.2, 8.1 Hz, 1H, H-5), 7.63 (d, J = 2.2 Hz, 1H, H-3), 6.62 (d, J = 8.9 Hz, 1H, H-6), 4.87-4.83 (m, 1H, cyclopentyl CH), 4.59 (br, 2H, NH_2), 2.00–1.62 (m, 8H, cyclopentyl).

2-Cyclohexyloxy-4-nitroaniline (**1e**). Cyclohexyl iodide was used and it was refluxed for 5 days. Yellow oil, 5% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (dd, J = 2.2, 8.8 Hz, 1H, H-5), 7.65 (d, J = 2.9 Hz, 1H, H-3), 6.63 (d, J = 8.8 Hz, 1H, H-6), 4.61 (br, 2H, NH_2), 4.39–4.32 (m, 1H, cyclohexyl CH), 2.04–1.98 (m, 2H, cyclohexyl), 1.81–1.76 (m, 2H, cyclohexyl), 1.62-1.33 (m, 6H, cyclohexyl).

2-Cyclohexylethyloxy-4-nitroaniline (**1f**). 1-Bromo-2-cyclohexylethane was used and it was refluxed for 6 h. Yellow solid, 90% yield. Mp: 60–62 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.79 (dd, J = 2.2, 8.9 Hz, 1H, H-5), 7.63 (d, J = 2.2 Hz, 1H, H-3), 6.64 (d, J = 8.8 Hz, 1H, H-6), 4.67 (br, 2H, NH_2), 4.09 (t, J = 6.6 Hz, 2H, OCH_2CH_2), 1.78–1.65 (m, 8H, cyclohexyl), 1.28–1.46 (m, 3H, cyclohexyl), 1.05–0.97 (m, 2H, OCH_2CH_2).

(c) General procedure for synthesis of compounds **2a–f**. 2-Alkyloxy-4-nitroanilin **1a–f** (5.0 mmol) was dissolved in anhydrous DMF (15 mL) and NaH (420.0 mg, 17.5 mmol, 3.5 equiv) was added. The suspension was stirred for 20 min, and MsCl (1.7 g, 15.0 mmol, 3 equiv) was added. The reaction mixture was stirred at room temperature overnight. Water was added and the reaction mixture was neutralized with 5 N HCl until pH 1–2. The intermediate precipitate was filtered and washed with water, which was used in the next reaction. Then the intermediate was suspended in a 50% KOH solution and heated at reflux for 2 h. After cooling, it was neutralized with 5 N HCl until pH 6–7. The precipitated solid was collected and washed with water. The product was purified by flash column chromatography (1:7 EtOAc/hexanes) to afford desired compounds.

N-(2-Propyloxy-4-nitrophenyl)methanesulfonamide (**2a**). Yellow solid, 68% yield. Mp: 116–117 °C (lit.[3] 117–119 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.92 (dd, J = 2.2, 8.9 Hz, 1H, H-5), 7.78 (d, J = 2.9 Hz, 1H, H-3), 7.66 (d, J = 8.9 Hz, 1H, H-6), 7.23 (br, 1H, NH), 4.12 (t, J = 8.6 Hz, 2H, $OCH_2CH_2CH_3$), 3.11 (s, 3H, SO_2CH_3), 1.95–1.88 (m, 2H, $OCH_2CH_2CH_3$), 1.09 (t, J = 7.3 Hz, 3H, $OCH_2CH_2CH_3$).

N-(2-Isopropyloxy-4-nitrophenyl)methanesulfonamide (**2b**). Yellow solid, 80% yield. Mp: 131–132 °C (lit.[3] 128–131 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (dd, J = 2.3, 8.9 Hz, 1H, H-5), 7.77 (d, J = 2.2 Hz, 1H, H-3), 7.65 (d, J = 8.8 Hz, 1H, H-6), 7.25 (br, 1H, NH), 4.81-4.73 (m, 1H, $OCH(CH_3)_2$), 3.10 (s, 3H, SO_2CH_3), 1.45 (s, 3H, $OCH(CH_3)CH_3$), 1.43 (s, 3H, $OCH(CH_3)CH_3$).

N-[2-(1-Ethyl-propyloxy-4-nitrophenyl)]methanesulfonamide (**2c**). Yellow solid, 85% yield. Mp: 104–105 °C(lit.[3] 100–102 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (dd, J = 2.3, 8.9 Hz, 1H, H-5), 7.76 (d, J = 2.2 Hz, 1H, H-3), 7.66 (d, J = 8.8 Hz, 1H, H-6), 7.27 (br, 1H, NH), 4.41–4.33 (m, 1H, $OCH(CH_2CH_3)_2$), 3.10 (s, 3H, SO_2CH_3), 1.82–1.72 (m, 4H, $OCH(CH_2CH_3)_2$), 0.98 (t, J = 7.4 Hz, 6H, $OCH(CH_2CH_3)_2$).

N-(2-Cyclopentyloxy-4-nitrophenyl)methanesulfonamide (**2d**). Yellow solid, 84% yield. Mp: 149–151 °C (lit.[3] 139–140 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (dd, J = 2.2, 8.8 Hz, 1H, H-5), 7.77 (d, J = 2.9 Hz, 1H, H-3), 7.64 (d, J = 8.9 Hz, 1H, H-6), 7.16 (br, 1H, NH), 4.97–4.92 (m, 1H, cyclopentyl CH), 3.10 (s, 3H, SO_2CH_3), 2.08–2.03 (m, 2H, cyclopentyl), 1.93–1.71 (m, 6H, cyclopentyl).

N-(2-Cyclohexyloxy-4-nitrophenyl)methanesulfonamide (**2e**). Yellow solid, 54% yield. Mp: 134–135°C (lit.[3] 124–126 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (dd, J = 2.2, 8.8 Hz, 1H, H-5), 7.77 (d, J = 2.2 Hz, 1H, H-3), 7.65 (d, J = 8.8 Hz, 1H, H-6), 7.23 (br, 1H, NH), 4.50–4.41 (m, 1H, cyclohexyl CH), 3.10 (s, 3H, SO_2CH_3), 2.10–2.05 (m, 2H, cyclohexyl), 1.87–1.80 (m, 2H, cyclohexyl), 1.68–1.31 (m, 6H, cyclohexyl).

N-(2-Cyclohexylethyloxy-4-nitrophenyl)methanesulfonamide (**2f**). Pale yellow solid, 76% yield. Mp: 109–111 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.91 (dd, J = 2.9, 8.8 Hz, 1H, H-5), 7.77 (d, J = 2.2 Hz, 1H, H-3), 7.65 (d, J = 8.8 Hz, 1H, H-6), 7.22 (br, 1H, NH), 4.12 (t, J = 7.4 Hz, 2H, OCH_2CH_2), 3.10 (s, 3H, SO_2CH_3), 1.81–1.60 (m, 8H, cyclohexyl), 1.30–1.19 (m, 3H, cyclohexyl), 1.07–1.00 (m, 2H, OCH_2CH_2). LRMS (CI, *m*/*z*): 343 ($[M+H]^+$, 100%). HRMS (CI, *m*/*z*): calcd for $C_{15}H_{23}O_5N_2S$ 343.1322 $[M+H]^+$; found 343.1316.

(d) General procedure for synthesis of compounds **3a–f**. *N*-(2-Alkyloxy-4-nitrophenyl)methanesulfonamide **2a–f** (1.5 mmol) was dissolved in anhydrous DMF (9 mL) and NaH (43.0 mg, 1.8 mmol, 1.2 equiv) was added. The suspension was stirred at room temperature for 10 min and iodomethane (255.5 mg, 1.8 mmol, 1.2 equiv) was added. The stirring was continued for 2 h. Then the mixture was taken up with water (14 mL) and saturated aqueous Na_2CO_3 solution (4 mL). The solid was

collected by filtration and washed with water. The product was purified by flash column chromatography (1:6 EtOAc/hexanes) to afford desired compounds.

N-Methyl-*N*-(2-propyloxy-4-nitrophenyl)methanesulfonamide (**3a**). Pale yellow solid, 82% yield. Mp: 63–64 °C (lit.[3] 66–69 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87–7.81 (m, 2H, H-5, H-3), 7.54 (d, J = 8.1 Hz, 1H, H-6), 4.12 (t, J = 6.6 Hz, 2H, $OCH_2CH_2CH_3$), 3.30 (s, 3H, SO_2CH_3), 2.97 (s, 3H, NCH_3), 1.93 (m, 2H, $OCH_2CH_2CH_3$), 1.12 (t, J = 7.3 Hz, 3H, $OCH_2CH_2CH_3$).

N-Methyl-*N*-(2-isopropyloxy-4-nitrophenyl)methanesulfonamide (**3b**). Pale yellow solid, 85% yield. Mp: 124–125 °C (lit.[3] 99–100 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.79 (m, 2H, H-5, H-3), 7.54 (d, J = 8.1 Hz, 1H, H-6), 4.84-4.76 (m, 1H, $OCH(CH_3)_2$), 3.28 (s, 3H, SO_2CH_3), 2.97 (s, 3H, NCH_3), 1.47 (s, 3H, $OCH(CH_3)CH_3$), 1.45 (s, 3H, $OCH(CH_3)CH_3$).

N-Methyl-*N*-[2-(1-ethyl-propyloxy-4-nitrophenyl)]methanesulfonamide (**3c**). Pale yellow solid, 90% yield. Mp: 89–90 °C (lit.[3] 89–90 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82–7.77 (m, 2H, H-5, H-3), 7.53 (d, J = 8.0 Hz, 1H, H-6), 4.45-4.38 (m, 1H, $OCH(CH_2CH_3)_2$), 3.29 (s, 3H, SO_2CH_3), 2.97 (s, 3H, NCH_3), 1.83-1.74 (m, 4H, $OCH(CH_2CH_3)_2$), 1.02 (t, J = 7.4 Hz, 6H, $OCH(CH_2CH_3)_2$).

N-Methyl-*N*-(2-cyclopentyloxy-4-nitrophenyl)methanesulfonamide (**3d**). Pale yellow solid, 91% yield. Mp: 110-112 °C (lit.[3] 102–104 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.84–7.81 (m, 2H, H-5, H-3), 7.53 (d, J = 8.1 Hz, 1H, H-6), 4.99–4.95 (m, 1H, cyclopentyl CH), 3.27 (s, 3H, SO_2CH_3), 2.95 (s, 3H, NCH_3), 2.12–2.06 (m, 2H, cyclopentyl), 1.92–1.77 (m, 6H, cyclopentyl).

N-Methyl-*N*-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide (**3e**). Pale yellow solid, 86% yield. Mp: 131–133 °C (lit.[3] 129–132 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83–7.78 (m, 2H, H-5, H-3), 7.53 (d, J = 8.0 Hz, 1H, H-6), 4.53–4.47 (m, 1H, cyclohexyl CH), 3.30 (s, 3H, SO_2CH_3), 2.97 (s, 3H, NCH_3), 2.13–2.08 (m, 2H, cyclohexyl), 1.86–1.82 (m, 2H, cyclohexyl), 1.71–1.33 (m, 6H, cyclohexyl).

N-Methyl-*N*-(2-cyclohexylethyloxy-4-nitrophenyl)methanesulfonamide (**3f**). Pale yellow solid, 89% yield. Mp: 95-97 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.86–7.81 (m, 2H, H-5, H-3), 7.54 (d, J = 8.8 Hz, 1H, H-6), 4.18 (t, J = 6.6 Hz, 2H, OCH_2CH_2), 3.29 (s, 3H, SO_2CH_3), 2.97 (s, 3H, NCH_3), 1.81–1.61 (m, 8H, cyclohexyl), 1.28–1.20 (m, 3H, cyclohexyl), 1.07–1.00 (m, 2H, OCH_2CH_2). LRMS (CI, *m*/*z*): 357 ($[M+H]^+$, 100%). HRMS (CI, *m*/*z*): calcd for $C_{16}H_{25}O_5N_2S$ 357.1479 $[M+H]^+$; found 357.1477.

(e) Typical experimental procedure for the radiosynthesis of [^{11}C]**3a**–**f**. The precursor (**2a**, **2b**, **2c**, **2d**, **2e**, or **2f**) (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added 3 N NaOH (2 μL). The mixture was transferred to a small volume, three-neck reaction tube. $^{11}CH_3OTf$ was passed into the air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until radioactivity reached a maximum (~3 min), then the reaction tube was heated at 70–80 °C for 1 min. The contents of the reaction tube were diluted with $NaHCO_3$ (1 mL, 0.1 M) and HPLC mobile phase 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ (0.5 mL), and injected onto the semi-preparative HPLC column with 2 mL injection loop. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product [^{11}C]**3a**, [^{11}C]**3b**, [^{11}C]**3c**, [^{11}C]**3d**, [^{11}C]**3e** or [^{11}C]**3f** was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis, purification, and formulation time was 20–25 min from EOB. Retention times in the analytical HPLC system were: t_R**2a** = 2.78 min, t_R**3a** = 2.91 min, t_R[^{11}C]**3a** = 2.91 min; t_R**2b** = 2.69 min, t_R**3b** = 2.80 min, t_R[^{11}C]**3b** = 2.80 min; t_R**2c** = 3.38 min, t_R**3c** = 3.52 min, t_R[^{11}C]**3c** = 3.52 min; t_R**2d** = 3.17 min, t_R**3d** = 3.35 min, t_R[^{11}C]**3d** = 3.35 min; t_R**2e** = 3.53 min, t_R**3e** = 3.84 min, t_R[^{11}C]**3e** = 3.84 min; and t_R**2f** = 5.52 min, t_R**3f** = 6.16 min, t_R [^{11}C]**3f** = 6.16 min. Retention times in the semi-preparative HPLC system were: t_R**2a** = 3.47 min, t_R**3a** = 4.38 min, t_R[^{11}C]**3a** = 4.38 min; t_R**2b** = 3.85 min, t_R**3b** = 4.43 min, t_R[^{11}C]**3b** = 4.43 min; t_R**2c** = 4.21 min, t_R**3c** = 4.96 min, t_R[^{11}C]**3c** = 4.96 min; t_R**2d** = 4.16 min, t_R**3d** = 4.77 min, t_R[^{11}C]**3d** = 4.77 min; t_R**2e** = 4.65 min, t_R**3e** = 5.31 min, t_R[^{11}C]**3e** = 5.31 min; and t_R**2f** = 6.84 min, t_R**3f** = 7.55 min, t_R [^{11}C]**3f** = 7.55 min. The radiochemical yield of [^{11}C]**3a**–**f** was 30–35%. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method. The chemical purities of precursors **2a**–**f**, and standard samples **3a**–**f** were >95%, the radiochemical purity of target radiotracers [^{11}C]**3a**–**f** was >98%, and the chemical purity of radiotracers [^{11}C]**3a**–**f** was >93%.

(f) Cytotoxicity assay. Cytotoxicity of the compounds **3a**–**f** with NS-398 was assessed by MTT assay to evaluate growth inhibition of SK-BR-3 breast cancer cells. SK-BR-3 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum, L-glutamine (2 mM), streptomycin (100 μg/mL), and penicillin (100 U/mL) at 37 °C in a humidified incubator containing 5% CO_2. Freshly trypsinized cell suspension was seeded in 96-well microtiter plates at a density of 4×10^3 cells per well. After 24 h of incubation, 100 μL aliquot of medium containing the test compounds was added to each well. The plates were incubated for an additional 48 h prior to the addition of 50 μL aliquot of MTT (1 mg/mL). After 4 h of incubation, 200 μL of 0.04 N HCl-isopropanol was added to each well to dissolve the black formazan precipitate, and the absorbance at 540 nm was measured on a 96-well microplate reader. All assays were done in triplicate. The IC_{50} value (the drug concentration producing 50% inhibition of cell growth) was then determined for each compound and calculated using an Excel spreadsheet program.