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Synthesis of new carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides as potential PET agents for imaging of tubulin polymerization in cancers

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The tubulin polymerization is an attractive target for anticancer therapy and in the development of cancer imaging agents for use in biomedical imaging technique positron emission tomography (PET). 7-Aroyl-aminoindoline-1-sulfonamides are a novel class of potent antitublin agents. Carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides have been synthesized as new potential PET agents for imaging of tubulin polymerization in cancers. The target tracers were prepared by *O*-[^{11}C]methylation of their corresponding precursors using [^{11}C]CH_3OTf and isolated by a simplified solid-phase extraction purification procedure in 40–55% radiochemical yields based on [^{11}C]CO_2 and decay corrected to the end of bombardment (EOB), 15–20 min overall synthesis time from EOB, >98% radiochemical purity, and 74–111 GBq/μmol specific activity at the end of synthesis.

Keywords: carbon-11; 7-aroyl-aminoindoline-1-sulfonamides; PET; tubulin polymerization; cancer imaging

Introduction

Microtubules are one of the components of the cytoskeleton and are composed of α- and β-tubulin dimers, which serve as structural components within cells and are involved in many cellular processes including mitosis, cytokinesis, and vesicular transport.[1] Tubulin dimers constantly polymerize and depolymerize, and microtubules can undergo rapid cycles of assembly and disassembly.[2] Microtubules are recognized as an important target for anticancer therapy.[3] Recently, a novel series of 7-aroyl-aminoindoline-1-sulfonamides have been developed as potent inhibitors of tubulin polymerization, and four title compounds *N*-[1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-acetamide (**4a**), furan-2-carboxylic acid [1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-amide (**4b**), 4-fluoro-*N*-[1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-benzamide (**4c**), and *N*-[1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-isonicotinamide (**4d**) are all potent antitubulin agents with excellent biological activity, in which the lead compound **4d** inhibited the human cancer cell growth of KB, MKN45, H460, HT29, and TSGH, and one human-resistant cancer line of KB-vin 10 with nanomolar IC_{50} values (9.6, 8.8, 9.4, 8.6, 10.8, and 8.9 nM, respectively).[4] Tubulin polymerization and depolymerization also provide an attractive target for the *in vivo* biomedical imaging technique positron emission tomography (PET) to image cancers. 7-Aroyl-aminoindoline-1-sulfonamides labeled with a positron-emitting radionuclide such as carbon-11 may enable non-invasive monitoring of cancer tubulin polymerization and depolymerization and their response to tubulin polymerization inhibitor treatment using PET. To translate chemotherapeutic agent for diagnostic use, we are interested in the design and synthesis of medical probes for PET imaging of cancer. In our previous work, we have synthesized radiolabeled antimitotic agent T138067 analogues [^{11}C]T138067 and [^{18}F]T138067. However, animal PET imaging results with these tracers were disappointing.[5] The specific binding of the tracers to target microtubules in appropriate tumor models awaits further investigations. In this ongoing study, we report the design and synthesis of new carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides, *N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*indol-7-yl]-acetamide ([^{11}C]**4a**), furan-2-carboxylic acid [1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-amide ([^{11}C]**4b**), 4-fluoro-*N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-benzamide ([^{11}C]**4c**) and *N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-isonicotinamide ([^{11}C]**4d**), as potential PET agents for imaging of tubulin polymerization in cancers.

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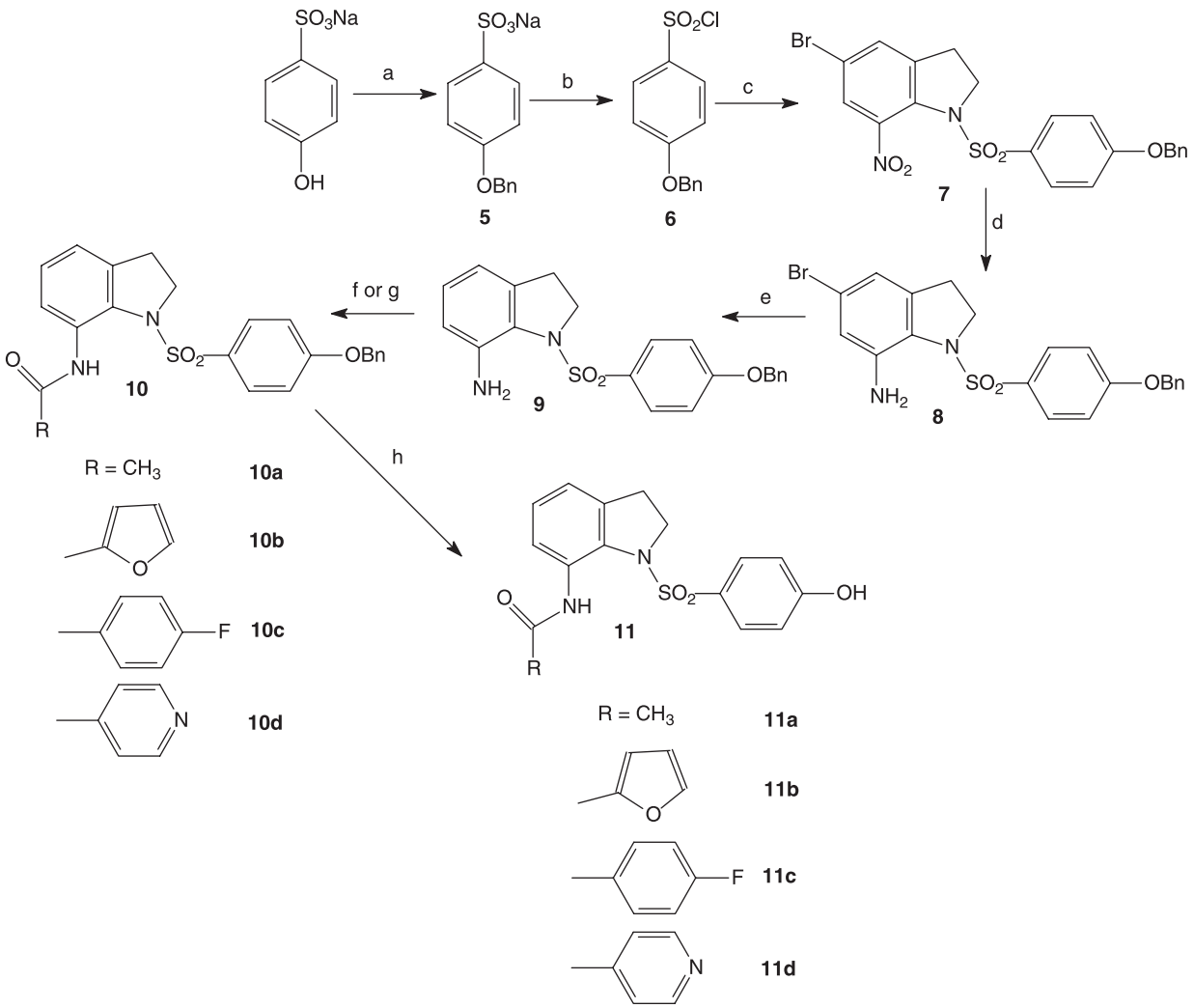
Results and discussion

Chemistry

Synthesis of reference standards was accomplished using a modification of the previously reported procedures.[4] Coupling of commercially available 5-bromo-7-nitroindoline with 4-methoxybenzenesulfonyl chloride in pyridine afforded the corresponding sulfonamide derivative 5-bromo-1-(4-methoxy-benzenesulfonyl)-7-nitro-2,3-dihydro-1*H*-indole (**1**) in 90% yield, which was then reduced with Fe/NH_4Cl in isopropanol to give 5-bromo-1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-ylamine (**2**) in 92% yield. Free radical-mediated debromination reaction was performed with Bu_3SnH in the presence of 2,2′-azobisisobutyronitrile (AIBN),[6,7] and the resulting amine derivative **3** (71% yield) was coupled with acyl chloride or acetic anhydride to produce the reference standards, 7-aroyl-aminoindoline-1-sulfonamides (**4a–d**) in moderate to excellent yields (66–83% yields).

In order to prepare the demethylated precursors, we envisioned that precursor compounds **11a–d** could be prepared by *O*-demethylation of target compounds **4a–d**. A variety of protocols were screened for this purpose including protic acid (HBr),[8] Lewis acids (BBr_3, $AlCl_3$/EtSH),[9,10] and base (EtSNa).[11] However, in all cases, an intractable red solid that could not be characterized or a demethylated product with very low yield was obtained. As a result of these unsuccessful attempts, we chose to explore an alternative strategy, which is outlined in Scheme 1. Benzylation of sodium 4-hydroxybenzenesulfonate dihydrate was achieved by the protection of phenolic hydroxyl group to give the corresponding ether **5** in 89% yield, which was converted into sulfonyl chloride derivative **6** by treatment with thionyl chloride in 95% yield.[12] Coupling of compound **6** with 5-bromo-7-nitroindoline produced 5-bromo-1-(4-benzyloxy-benzenesulfonyl)-7-nitro-2,3-dihydro-1*H*-indole (**7**) in 70% yield. Reduction of nitro group to amine group was achieved with $SnCl_2$ in ethanol to give 5-bromo-1-(4-benzyloxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl-amine (**8**) in 81% yield,[13] which was debrominated using Bu_3SnH and AIBN to afford 1-(4-benzyloxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl-amine (**9**) in 80% yield. Coupling compound **9** with acyl chloride or acetic anhydride to provide compounds **10a–d** in moderate to excellent yields, which were hydrogenated to remove benzyl group to give the precursors **11a–d** in 41–98% yields.[14]



Scheme 1

Radiochemistry

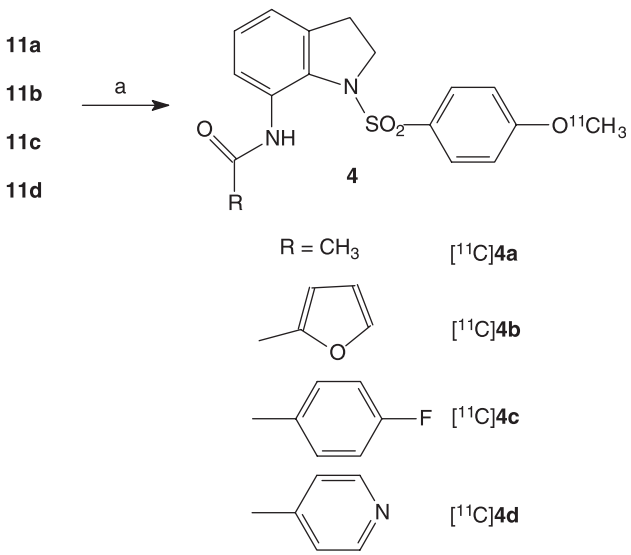
Synthesis of the target tracers, carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides [^{11}C]**4a–d**, is outlined in Scheme 2. The precursor **11a**, **11b**, **11c**, or **11d** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[15,16] through *O*-[^{11}C]methylation[17] and isolated by a simplified solid-phase extraction (SPE) purification[18] to produce corresponding pure target radiolabeled compound [^{11}C]**4a**, [^{11}C]**4b**, [^{11}C]**4c**, or [^{11}C]**4d** with 40–55% radiochemical yields, based on [^{11}C]CO_2, decay corrected to end of bombardment (EOB). The large polarity difference between the phenolic hydroxyl precursor and the labeled *O*-methylated product permitted the use of SPE technique for purification of labeled product from radiolabeling reaction mixture. Either a light C-18 Sep-Pak cartridge or a semi-prep C-18 guard cartridge column was used in SPE purification technique. The reaction mixture was loaded onto the cartridge by gas pressure. The cartridge was washed with water to remove non-reacted [^{11}C]CH_3OTf, precursor and reaction solvent, and then the final labeled product was eluted with ethanol. Overall synthesis time was 10–15 min from EOB. SPE technique is fast, efficient, and convenient and works very well for the *O*-methylated tracer production. The radiosynthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis.[19,20] The specific activity was in a range of 74–111 GBq/μmol at the end of synthesis (EOS). Chemical purity and radiochemical purity were determined by analytical high-pressure liquid chromatography (HPLC) method.[21] The chemical purity of precursors and reference standards was >95%. The radiochemical purity of target tracers was >98% as determined by radio-HPLC through γ-ray (NaI) flow detector, and the chemical purity of target tracers was >93% as determined by reversed-phase HPLC through UV flow detector.

Experimental

General

All commercial reagents and solvents were used without further purification. [^{11}C]CH_3OTf was made according to a literature procedure.[15] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 2000 200 MHz FT-NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (*J*) were reported in hertz (Hz). The low-resolution mass spectra were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high-resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are expressed on a volume:volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates (5 × 10 cm^2). Plates were visualized using 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 (254 nm) and γ-ray (NaI) flow detectors. Light C-18 Sep-Pak cartridges were obtained from Waters Corporate Headquarters, Milford, MA. Semi-prep C-18 guard cartridge column 1 × 1 cm was obtained from E. S. Industries, Berlin, NJ, and part number 300121-C18-BD 10 μ. Sterile Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.



Scheme 2

Chemistry

5-Bromo-1-(4-methoxy-benzenesulfonyl)-7-nitro-2,3-dihydro-1H-indole (**1**): Compound **1** was prepared using a modification of the literature procedure[4] as a yellow solid in 90% yield, m.p. 132–133°C. ^{1}H NMR ($CDCl_3$) δ: 2.69 (t, *J* = 7.7 Hz, 2H, PhC*H*$_2$CH$_2$N), 3.87 (s, 3H, OCH_3), 4.06 (t, *J* = 7.7 Hz, 2H, PhCH$_2$C*H*$_2$N), 6.93–6.97 (m, 2H, ArH), 7.50 (d, *J* = 1.5 Hz, 1H, ArH), 7.64–7.68 (m, 2H, ArH), 7.91 (d, *J* = 1.5 Hz, 1H, ArH).

5-Bromo-1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-ylamine (**2**): Compound **2** was prepared using a modification of the literature procedure[4] as a white solid in 92% yield, m.p. 173°C (dec.). ^{1}H NMR ($CDCl_3$) δ: 2.14 (t, *J* = 7.4 Hz, 2H, PhC*H*$_2$CH$_2$N), 3.85 (s, 3H, OCH_3), 3.96 (*t*, *J* = 7.5 Hz, 2H, PhCH$_2$C*H*$_2$N), 6.54 (d, *J* = 2.0 Hz, 1H, ArH), 6.74 (d, *J* = *2.0* Hz, 1H, ArH), 6.85–6.89 (m, 2H, ArH), 7.54–7.58 (m, 2H, ArH).

1-(4-Methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-ylamine (**3**): Compound **3** was prepared using a modification of the literature procedure[4] as a straw-colored solid in 71% yield, m.p. 104–106°C. ^{1}H NMR ($CDCl_3$) δ: 2.15 (t, *J* = 7.3 Hz, 2H, PhC*H*$_2$CH$_2$N), 3.83 (s, 3H, OCH_3), 3.96 (t, *J* = 7.3 Hz, 2H, PhCH$_2$C*H*$_2$N), 6.42 (d, *J* = 7.1 Hz, 1H, ArH), 6.60 (d, *J* = 7.8 Hz, 1H, ArH), 6.81–6.95 (m, 3H, ArH), 7.51–7.56 (m, 2H, ArH).

N-[1-(4-Methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-acetamide (**4a**): Compound **4a** was prepared using a modification of the literature procedure[4] as a white solid in 75% yield, m.p. 153–154°C (lit.[4] 154–155°C). ^{1}H NMR ($CDCl_3$) δ: 2.21–2.24 (t and s, *J* = 7.4 Hz, 5H, PhC*H*$_2$CH$_2$N and CH_3CO), 3.83 (s, 3H, OCH_3), 4.00 (t, *J* = 7.4 Hz, 2H, PhCH$_2$C*H*$_2$N), 6.77–6.85 (m, 3H, ArH), 7.13 (t, *J* = 7.8 Hz, 1H, ArH), 7.44 (d, *J* = 8.7 Hz, 2H, ArH), 8.10 (d, *J* = 8.3 Hz, 1H, ArH), 9.34 (s, 1H, CONH).

Furan-2-carboxylic acid [*1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl*]-*amide* (**4b**): Compound **4b** was prepared using a modification of the literature procedure[4] as a white solid in 83% yield, m.p. 170–172°C (lit.[4] 163–164°C). ^{1}H NMR ($CDCl_3$) δ: 2.25 (t, $J = 7.3$ Hz, 2H, PhCH_2CH$_2$N), 3.83 (s, 3H, OCH_3), 4.03 (t, $J = 7.3$ Hz, 2H, PhCH$_2$CH_2N), 6.53–6.55 (m, 1H, ArH), 6.82–6.86 (m, 3H, ArH), 7.14–7.26 (m, 2H, ArH), 7.51(d, $J = 8.8$ Hz, 2H, ArH), 7.60 (s, 1H, ArH), 8.23 (d, $J = 8.1$ Hz, 1H, ArH), 10.2 (s, 1H, CONH).

4-Fluoro-N-[*1-(4-methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl*]-*benzamide* (**4c**): Compound **4c** was prepared using a modification of the literature procedure[4] as a white solid in 75% yield, m.p. 161–162°C (lit.[4] 184–184°C). ^{1}H NMR ($CDCl_3$) δ: 2.26 (t, $J = 7.3$ Hz, 2H, PhCH_2CH$_2$N), 3.84 (s, 3H, OCH_3), 4.03 (t, $J = 7.4$ Hz, 2H, PhCH$_2$CH_2N), 6.83–6.87 (m, 3H, ArH), 7.14–7.24 (m, 3H, ArH), 7.48–7.53 (m, 2H, ArH), 8.06–8.13 (m, 2H, ArH), 8.23–8.27 (m, 1H, ArH), 10.2 (s, 1H, CONH).

N-[*1-(4-Methoxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl*]-*4-isonicotinamide* (**4d**): Compound **4d** was prepared using a modification of the literature procedure[4] as a white solid in 66% yield, m.p. 119–120°C (lit.[4] 219–220°C). ^{1}H NMR ($CDCl_3$) δ: 2.28 (t, $J = 7.4$ Hz, 2H, PhCH_2CH$_2$N), 3.84 (s, 3H, OCH_3), 4.04 (t, $J = 7.4$ Hz, 2H, PhCH$_2$CH_2N), 6.84–6.91 (m, 3H, ArH), 7.18–7.26 (m, 1H, ArH), 7.50 (d, $J = 9.1$ Hz, 2H, ArH), 7.90–7.93 (m, 2H, ArH), 8.26 (d, $J = 8.3$ Hz, 1H, ArH), 8.82 (d, $J = 6.8$ Hz, 2H, ArH), 10.4 (s, 1H, CONH).

Sodium 4-benzyloxybenzenesulfonate (**5**): A solution of sodium 4-hydroxybenzenesulfonate dihydrate (20 g, 86.1 mmol) in anhydrous dimethyl formamide (DMF) (200 mL) was cooled to 0°C. NaH (60% dispersion in mineral oil, 4.13 g, 103.4 mmol) was added under nitrogen. After being stirred for 20 min at 0°C, benzyl bromide (12.3 mL, 103.4 mmol) was added and the reaction mixture was stirred overnight at room temperature. A small amount of EtOAc was added to quench the reaction. The mixture was filtered and the residue was washed with EtOAc to afford compound **5** as a white solid (24.6 g, 89%). ^{1}H NMR (DMSO-d_6) δ: 5.11 (s, 2H, PhCH$_2$O), 6.90–6.97 (m, 2H, ArH), 7.31–7.48 (m, 5H, ArH), 7.48–7.56 (m, 2H, ArH).

4-Benzyloxybenzenesulfonyl chloride (**6**): To a suspension of compound **5** (21 g, 73.4 mmol) in DMF (150 mL) was added thionyl chloride (6.62 mL, 90.9 mmol). The reaction mixture was stirred for 5 min at room temperature, poured into ice–water and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated to give compound 6 as a white solid (19.8 g, 95%), m.p. 93–94°C (lit.[12] 92–94°C). ^{1}H NMR ($CDCl_3$) δ: 5.18 (s, 2H, PhCH$_2$O), 7.08–7.16 (m, 2H, ArH), 7.35–7.45 (m, 5H, ArH), 7.94–8.02 (m, 2H, ArH).

5-Bromo-1-(4-benzyloxy-benzenesulfonyl)-7-nitro-2,3-dihydro-1H-indole (**7**): To a stirred solution of 5-bromo-7-nitroindoline (10 g, 41.1 mmol) in pyridine (20 mL) was added compound **6** (15.1 g, 53.5 mmol). The resulting solution was heated under reflux for 39 h, poured into ice–water and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by gradient column chromatography with EtOAc–hexanes (3:1) to give compound **7** as a yellow solid (14.0 g, 70%), m.p. 182–183°C. ^{1}H NMR ($CDCl_3$) δ: 2.68 (t, $J = 7.7$ Hz, 2H, PhCH_2CH$_2$N), 4.05 (t, $J = 7.7$ Hz, 2H, PhCH$_2$CH_2N), δ 5.13 (s, 2H, PhCH$_2$O), 6.98–7.06 (m, 2H, ArH), 7.35–7.43 (m, 5H, ArH), 7.49 (d, $J = 1.8$ Hz, 1H, ArH), 7.62–7.70 (m, 2H, ArH), 7.92 (d, $J = 1.8$ Hz, 1H, ArH). MS (CI, *m/z*): 488 (M^+, 93%), 490 ($M^+ + 2$H, 100%). HRMS (CI, *m/z*): calcd. for $C_{21}H_{17}O_5N_2BrS$ 488.0036 (M^+), found 488.0019.

5-Bromo-1-(4-benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl-amine (**8**): A mixture of compound **7** (10.3 g, 21.1 mmol) and tin(II) chloride (19.4 g, 105.5 mmol) in EtOH (100 mL) was refluxed and stirred under nitrogen for 4 h. The solvent was evaporated and the residue was extracted with EtOAc. The combined organic fraction was shaken with 2 M NaOH and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The crude product was purified by column chromatography using EtOAc–hexanes (2:3) to afford compound **8** as a white solid (7.8 g, 81%), m.p. 86–87°C. ^{1}H NMR ($CDCl_3$) δ: 2.13 (t, $J = 7.5$ Hz, 2H, PhCH_2CH$_2$N), 3.95 (t, $J = 7.5$ Hz, 2H, PhCH$_2$CH_2N), δ 5.09 (s, 2H, PhCH$_2$O), 6.54 (d, $J = 1.5$ Hz, 1H, ArH), 6.74 (d, $J = 1.5$ Hz, 1H, ArH), 6.91–6.98 (m, 2H, ArH), 7.35–7.42 (m, 5H, ArH), 7.53–7.60 (m, 2H, ArH). MS (CI, *m/z*): 132 (100%), 458 (M^+, 55%). HRMS (CI, *m/z*): calcd. for $C_{21}H_{19}O_3N_2BrS$ 458.0294 (M^+), found 458.0298.

1-(4-Benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl-amine (**9**): A mixture of compound **8** (6.82 g, 14.9 mmol), Bu_3SnH (11.8 mL, 44.6 mmol), and AIBN (0.25 g, 1.52 mmol) in toluene (100 mL) was stirred and heated to reflux for 25 h. The solvent was evaporated and the residue was extracted with CH_2Cl_2. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The crude product was purified by column chromatography using EtOAc–hexanes (1:4) to afford compound **9** as a white solid (4.5 g, 80%), m.p. 83–84°C. ^{1}H NMR ($CDCl_3$) δ: 2.16 (t, $J = 7.3$ Hz, 2H, PhCH_2CH$_2$N), 3.96 (t, $J = 7.4$ Hz, 2H, PhCH$_2$CH_2N), δ 5.07 (s, 2H, PhCH$_2$O), 6.43 (d, $J = 7.3$ Hz, 1H, ArH), 6.60 (d, $J = 8.0$ Hz, 1H, ArH), 6.89–6.95 (m, 3H, ArH), 7.38 (s, 5H, ArH), 7.54 (d, $J = 8.8$ Hz, 2H, ArH). MS (CI, *m/z*): 133 (100%), 380 (M^+, 14%). HRMS (CI, *m/z*): calcd. for $C_{21}H_{20}O_3N_2S$ 380.1189 (M^+), found 380.1202.

N-[*1-(4-Benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl*]-*acetamide* (**10a**): To a stirred solution of compound **9** (400 mg, 1.05 mmol) in pyridine (4 mL) was added acetic anhydride (0.13 mL, 1.37 mmol). The resulting solution was heated at 50°C for 6 h, poured into ice–water and extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by column chromatography using $CHCl_3$–MeOH (100:1) to afford compound **10a** as a white solid (300 mg, 68%), m.p. 147–148°C. ^{1}H NMR ($CDCl_3$) δ: 2.18–2.23 (t and s, $J = 7.4$ Hz, 5H, PhCH_2CH$_2$N and CH_3CO), 4.00 (t, $J = 7.4$ Hz, 2H, PhCH$_2$CH_2N), δ 5.07 (s, 2H, PhCH$_2$O), 6.79 (d, $J = 7.1$ Hz, 1H, ArH), 6.91 (d, $J = 9.1$ Hz, 2H, ArH), 7.13 (t, $J = 7.8$ Hz, 1H, ArH), 7.38 (s, 5H, ArH), 7.46 (d, $J = 9.0$ Hz, 2H, ArH), 8.10 (d, $J = 8.0$ Hz, 1H, ArH), 9.33 (s, 1H, CONH). MS (CI, *m/z*): 175 (100%), 422 (M^+, 51%). HRMS (CI, *m/z*): calcd. for $C_{23}H_{22}O_4N_2S$ 422.1295 (M^+), found 422.1295.

Furan-2-carboxylic acid [*1-(4-benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl*]-*amide* (**10b**): To a stirred solution of compound **9** (350 mg, 0.92 mmol) in pyridine (3.5 mL) was added 2-furoyl chloride (0.14 mL, 1.39 mmol). The resulting solution was heated at 50°C for 1.5 h, poured into ice–water and extracted with EtOAc. The combined layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by column chromatography using $CHCl_3$–MeOH (250:3) to afford compound **10b** as a white solid (360 mg, 75%), m.p. 185–186°C. ^{1}H NMR ($CDCl_3$) δ: 2.25 (t, $J = 7.3$ Hz, 2H, PhCH_2CH$_2$N), 4.03 (t, $J = 7.3$ Hz, 2H, PhCH$_2$CH_2N), δ 5.08 (s, 2H, PhCH$_2$O), 6.51–6.54 (m, 1H, ArH), 6.83 (d, $J = 7.3$ Hz, 1H, ArH), 6.92 (d, $J = 8.5$ Hz, 2H, ArH), 7.16 (d, $J = 7.8$ Hz, 1H, ArH), 7.22–7.26 (m, 1H, ArH), 7.38 (s, 5H, ArH), 7.51 (d, $J = 8.8$ Hz, 2H, ArH), 7.59 (s, 1H, ArH), 8.23 (d, $J = 8.3$ Hz, 1H, ArH), 10.2

(s, 1H, CONH). MS (CI, *m/z*): 227 (100%), 474 (M^+, 23%). HRMS (CI, *m/z*): calcd. for $C_{26}H_{22}O_5N_2S$ 474.1244 (M^+), found 474.1238.

4-Fluoro-N-[1-(4-benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-benzamide (**10c**): To a stirred solution of compound **9** (400 mg, 1.05 mmol) in pyridine (4 mL) was added 4-fluorobenzoyl chloride (0.19 mL, 1.58 mmol). The resulting solution was heated at 71°C for 1 h, poured into ice–water and extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by column chromatography using $CHCl_3$–MeOH (100:1) to afford compound **10c** as a white solid (322 mg, 55%), m.p. 170–172°C. ^{1}H NMR ($CDCl_3$) δ: 2.25 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 4.03 (t, J = 7.3 Hz, 2H, PhCH$_2$CH_2N), 5.09 (s, 2H, PhCH$_2$O), 6.84 (d, J = 7.4 Hz,1H, ArH), 6.93 (d, J = 8.8 Hz, 2H, ArH), 7.13–7.26 (m, 3H, ArH), 7.39 (s, 5H, ArH), 7.51 (d, J = 8.8 Hz, 2H, ArH), 8.05–8.10 (m, 2H, ArH), 8.25 (d, J = 8.3 Hz, 1H, ArH), 10.2 (s, 1H, CONH). MS (CI, *m/z*): 55 (100%), 502 (M^+, 32%). HRMS (CI, *m/z*): calcd. for $C_{28}H_{23}O_4N_2FS$ 502.1357 (M^+), found 502.1357.

N-[1-(4-Benzyloxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-4-isonicotinamide (**10d**): A mixture of compound **9** (450 mg, 1.18 mmol), isonicotinoyl chloride hydrochloride (254 mg, 1.42 mmol,) and cesium carbonate (925 mg, 2.84 mmol) in acetonitrile (30 mL) was stirred and heated at 85°C for 3 h. The solvent was evaporated and the residue was added to water and extracted with CH_2Cl_2. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated. The crude product was purified by column chromatography using $CHCl_3$–MeOH (100:1) to afford compound **10d** as a white solid (370 mg, 64%), m.p. 185–186°C. ^{1}H NMR ($CDCl_3$) δ: 2.27 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 4.04 (t, J = 7.4 Hz, 2H, PhCH$_2$CH_2N), 5.09 (s, 2H, PhCH$_2$O), 6.86–6.95 (m, 3H, ArH), 7.24 (d, J = 7.8 Hz, 1H, ArH), 7.38 (s, 5H, ArH), 7.51 (d, J = 8.8 Hz, 2H, ArH), 7.91 (d, J = 4.4 Hz, 2H, ArH), 8.26 (d, J = 8.0 Hz, 1H, ArH), 8.83 (s, 2H, ArH), 10.4 (s, 1H, CONH). MS (CI, *m/z*): 485 (M^+, 77%), 486 (M^++1, 100%). HRMS (CI, *m/z*): calcd for $C_{27}H_{24}O_4N_3S$ 486.1482 (M^++H), found 486.1491.

N-[1-(4-Hydroxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-acetamide (**11a**): A solution of compound **10a** (250 mg, 0.59 mmol) in tetrahydrofuran (THF) (7 mL) and MeOH (3 mL) was hydrogenated at atmospheric pressure over 10% Pd–C (40 mg) for 6 h. The catalyst was removed by filtration, and the solution was evaporated to afford compound **11a** as a white solid (186 mg, 95%), m.p. 232°C (dec.). ^{1}H NMR (DMSO-d_6) δ: 2.10 (s, 3H, CH_3CO), 2.22 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 3.97 (t, J = 7.4 Hz, 2H, PhCH$_2$CH_2N), 6.78 (d, J = 8.7 Hz, 2H, ArH), 6.87 (d, J = 7.3 Hz, 1H, ArH), 7.11 (t, J = 7.9 Hz, 1H, ArH), 7.34 (d, J = 8.8 Hz, 2H, ArH), 7.82 (d, J = 7.7 Hz, 1H, ArH), 9.37 (s, 1H, CONH), 10.6 (s, 1H, OH). MS (CI, *m/z*): 332 (M^+, 100%). HRMS (CI, *m/z*): calcd. for $C_{16}H_{16}O_4N_2S$ 332.0825 (M^+), found 332.0819.

Furan-2-carboxylic acid [1-(4-hydroxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-amide (**11b**): A solution of compound **10b** (250 mg, 0.53 mmol) in THF (10 mL) and MeOH (3 mL) was hydrogenated at atmospheric pressure over 10% Pd–C (30 mg) overnight. The catalyst was removed by filtration, and the solution was evaporated. The product was purified by column chromatography using $CHCl_3$–MeOH (250:6) to afford compound **11b** as a white solid (150 mg, 74%), m.p. 158–159°C. ^{1}H NMR ($CDCl_3$) δ: 2.27 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 4.01 (t, J = 7.4 Hz, 2H, PhCH$_2$CH_2N), 6.53–6.56 (m, 1H, ArH), 6.80–6.87 (m, 3H, ArH), 7.14 (t, J = 7.8 Hz, 1H, ArH), 7.27 (s, 1H, ArH), 7.41 (d, J = 8.6 Hz, 2H, ArH), 7.60 (s, 1H, ArH), 7.77 (s, 1H, OH), 8.12 (d, J = 8.3 Hz, 1H, ArH), 10.3 (s, 1H, CONH). MS (CI, *m/z*): 227 (100%), 384 (M^+, 33%). HRMS (CI, *m/z*): calcd. for $C_{19}H_{16}O_5N_2S$ 384.0774 (M^+), found 384.0779.

4-Fluoro-N-[1-(4-hydroxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-benzamide (**11c**): A solution of compound **10c** (250 mg, 0.50 mmol) in THF (7 mL) and MeOH (3 mL) was hydrogenated at atmospheric pressure over 10% Pd–C (40 mg) for 6 h. The catalyst was removed by filtration, and the solution was evaporated to afford compound **11c** as a white solid (205 mg, 98%), m.p. 203–205°C. ^{1}H NMR ($CDCl_3$) δ: 2.27 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 4.01 (t, J = 7.3 Hz, 2H, PhCH$_2$CH_2N), 6.78–6.87 (m, 3H, ArH), 7.11–7.22 (m, 3H, ArH), 7.38–7.43 (m, 2H, ArH), 8.03–8.16 (m, 3H, ArH), 10.3 (s, 1H, CONH). MS (CI, *m/z*): 255 (100%), 412 (M^+, 33%). HRMS (CI, *m/z*): calcd for $C_{21}H_{17}O_4N_2FS$ 412.0888 (M^+), found 412.0881.

N-[1-(4-Hydroxy-benzenesulfonyl)-2,3-dihydro-1H-indol-7-yl]-4-isonicotinamide (**11d**): A solution of compound **10d** (240 mg, 0.49 mmol) in THF (7 mL) and MeOH (3 mL) was hydrogenated at atmospheric pressure over 10% Pd–C (40 mg) overnight. The catalyst was removed by filtration, and the solution was evaporated. The product was purified by column chromatography using $CHCl_3$–MeOH–$NH_3 \cdot H_2O$ (200:10:1) to afford compound **11d** as a white solid (80 mg, 41%), m.p. 210°C (dec.). ^{1}H NMR (DMSO-d_6) δ: 2.29 (t, J = 7.3 Hz, 2H, PhCH_2CH$_2$N), 4.03 (t, J = 7.4 Hz, 2H, PhCH$_2$CH_2N), 6.82 (d, J = 8.7 Hz, 2H, ArH), 7.00 (d, J = 7.8 Hz, 1H, ArH), 7.20 (d, J = 7.7 Hz, 1H, ArH), 7.42 (d, J = 8.4 Hz, 2H, ArH), 7.85–7.92 (m, 3H, ArH), 8.84 (d, J = 5.9 Hz, 2H, ArH), 10.3 (s, 1H, OH), 10.7 (s, 1H, CONH). MS (CI, *m/z*): 238 (100%), 395 (M^+, 27%). HRMS (CI, *m/z*): calcd for $C_{20}H_{17}O_4N_3S$ 395.0934 (M^+), found 395.0920.

Radiochemistry

General method for the preparation of carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides, *N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-acetamide ([^{11}C]**4a**), furan-2-carboxylic acid [1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-amide ([^{11}C]**4b**), 4-fluoro-*N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-benzamide ([^{11}C]**4c**) and *N*-[1-(4-[^{11}C]methoxy-benzenesulfonyl)-2,3-dihydro-1*H*-indol-7-yl]-isonicotinamide ([^{11}C]**4d**).

[^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). The phenolic hydroxyl precursor **11a**, **11b**, **11c**, or **11d** (0.1–0.3 mg, 0.3–0.7 μM) was dissolved in CH_3CN (300 μL). To this solution was added 3 N NaOH (2 μL, 6 μM). The mixture was transferred to a small reaction vial. No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[15] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial, which was cooled to 0°C, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80°C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (1 mL, 0.1 M). The reaction tube was connected to either a light C-18 Sep-Pak cartridge or a semi-prep C-18 guard cartridge column. The labeled product mixture solution was passed onto the cartridge for SPE purification by gas pressure. The cartridge was washed with H_2O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the

column with EtOH (2 × 3 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation under vacuum. The labeled product [^{11}C]**4a**, [^{11}C]**4b**, [^{11}C]**4c**, or [^{11}C]**4d** was formulated with saline, whose volume was dependent upon the use of the labeled product in tissue biodistribution studies (~6 mL, 3 × 2 mL) or in PET imaging studies (1–3 mL). Total radioactivity was assayed and the total volume (1.0–6.0 mL) was noted. The overall synthesis time including SPE purification and formulation was 10–15 min. The radiochemical yields were 40–55% decay corrected to EOB from [^{11}C]CO_2 and 28–39% at EOS, respectively. Retention times in the analytical HPLC system were t_R **11a** = 2.27 min, t_R **4a** = 2.79 min, t_R [^{11}C]**4a** = 2.79 min; t_R **11b** = 2.59 min, t_R **4b** = 3.26 min, t_R [^{11}C]**4b** = 3.26 min; t_R **11c** = 3.25 min, t_R **4c** = 4.37 min, t_R [^{11}C]**4c** = 4.37 min; and t_R **11d** = 1.94 min, t_R **4d** = 3.46 min, t_R [^{11}C]**4d** = 3.46 min.

Conclusion

An efficient and convenient chemical and radiochemical synthesis of phenolic hydroxyl precursors, reference standards 7-aroyl-aminoindoline-1-sulfonamides, and target tracers carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides has been well developed. The synthetic methodology employed classical organic chemistry such as the free radical-mediated debromination in the presence of AIBN and Bu_3SnH and deprotecting hydrogenation of benzyl group. Carbon-11 labeling at oxygen position through *O*-[^{11}C]methylation was incorporated efficiently using [^{11}C]CH_3OTf, a signature reaction of carbon-11 radiochemistry from our laboratory. Radiosynthesis produced new probes in amounts and purity suitable for the preclinical application in animal studies using PET. Labeled product suitable for injection, with the higher specific radioactivities in a range of 74–111 GBq/μmol at EOS, can be obtained within 15 min from EOB including fast SPE purification and formulation. These chemistry results combined with the reported *in vitro* biological data encourage further *in vivo* biological evaluation of carbon-11-labeled 7-aroyl-aminoindoline-1-sulfonamides as new potential PET agents for imaging of cancer tubulin polymerization.

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