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Research Article

Radiosynthesis of 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]FAHA) for PET imaging of histone deacetylase (HDAC)

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Summary

Radiosynthesis of a novel substrate for histone deacetylase (HDAC), 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]FAHA, [^{18}F]-**3**) is reported. For precursor synthesis, compound **1** (6-amino-1-hexanoicanilide) was prepared by the reaction of 6-amino hexanoic acid with thionyl chloride in dichloroethane followed by addition of aniline. Compound **1** was reacted with bromoacetic anhydride in tetrahydrofuran (THF) in the presence of triethylamine to produce the precursor compound 6-(bromoacetamido)-1-hexanoicanilide **2**. Fluorination reactions were performed using tetrabutylammonium fluoride in various solvents at 80°C to prepare the unlabeled reference compound **3**. Radiofluorinations were performed using either *n*-$Bu_4N^{18}F$ or $K^{18}F$/kryptofix, and the crude product was purified by high performance liquid chromatography (HPLC). The radiochemical yields were 9–13% decay corrected (d.c.) with an average of 11% using $K^{18}F$/kryptofix, and specific activity >2 GBq/μmol at the end of synthesis. The synthesis time was 67–75 min from the end of bombardment (EOB). Copyright © 2006 John Wiley & Sons, Ltd.

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Key Words: fluorine-18; histone deacetylase; HDAC; PET

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Introduction

The reversible acetylation of histones, mediated by histone acetyl transferases (HATs) and histone deacetylase (HDACs), plays an important role in remodeling chromatin architecture, and hence in regulation of gene expression.[1,2] Acetylation of cationic lysine tails in nucleosome-associated histones neutralizes charge and promotes relaxation of chromatin, leading to transcriptional activation. Conversely, deacetylation of these lysine residues promotes formation of condensed chromatin, and repression of transcription. In some tumor cells excessive hypocetylation of histones results in the underexpression of growth regulatory factors such as the cyclin-dependent kinase inhibitor $p21^{Wafl}$ and thus contributes to the development of cancer.[1–3] Histone hyperacetylation caused by HDAC inhibitors such as trichostatin (TSA), MS-275 and suberoylanilide hydroxamic acid (SAHA) can cause growth arrest in a wide range of transformed cells and can inhibit the growth for human tumor xenografts.[1–5] SAHA was in clinical trial and recently achieved orphan drug status for multiple myeloma.[6,7] Histone deacetylase is under intense investigation in molecular biology possibly due to its function in chromatin remodeling and effect on transcription regulation that leads to many groups interested in drug development targeting histone deacetylases.[8,9] Many HDAC inhibitors including SAHA have been developed, and shown to cause growth arrest, differentiation and apoptosis.[3] Some of these inhibitors suffer from lack of specificity among the various forms of HDAC including deacetylases that target non-histone proteins.[3,10] Most recently several structurally modified HDAC inhibitors have been reported with enhanced activity and selectivity.[3]

Like many bio-modulating agents, SAHA has little host toxicity, and it is difficult to evaluate its effectiveness as an anticancer agent, unless using invasive techniques to biopsy the tumor.[11] Therefore, interests remain in the development of a non-invasive technique to evaluate HDAC expression and activity in tumors *in vivo* before and during therapy. While much effort has been directed towards the development of HDAC inhibitors, to our knowledge, no substrate for HDAC, especially an imaging agent, has been reported yet. In this paper we report on synthesis and radiolabeling a substrate for HDAC, 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F)FAHA, [^{18}F]-**3**), which should allow for non-invasive whole body imaging of HDAC expression and activity in tumors (including other organs and tissues) with positron emission tomography (PET).

Results and discussion

Figure 1 represents the scheme for the synthesis of the HDAC substrate, 6-(fluoroacetamido)-1-hexanoicanilide (FAHA, **3**). Compound **1** was prepared from 6-amino hexanoic acid by treatment with thionyl chloride in

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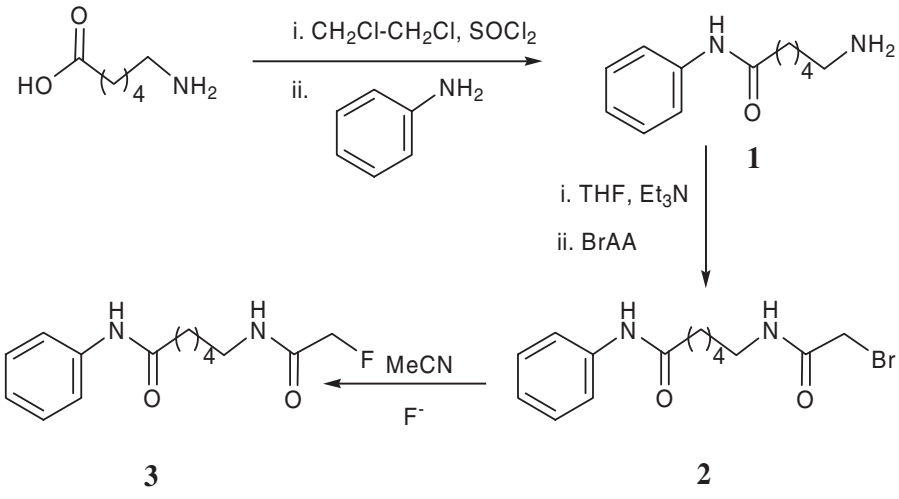

Figure 1. Synthetic scheme of 6-(fluoroacetamido)-1-hexanoicanilide (FAHA)

dichloroethane followed by addition of aniline. In this step the reaction was straight forward, which provided 80% yield of the desired product. The product was characterized by ^{1}H NMR spectroscopy and mass spectrometry. Compound **1** was then reacted with bromoacetic anhydride in tetrahydrofuran (THF) in the presence of triethylamine to produce the precursor compound **2** in 62% yield. Compound **2** was characterized by ^{1}H NMR spectroscopy and high-resolution mass spectrometry. When the NMR spectrum was run in $CDCl_3$ the amido protons were not observed due to fast exchange with the solvent, however, in DMSO-D_6 all amido protons were observed and could be accounted in the spectrum.

Fluorination reactions were performed using tetrabutylammonium fluoride (Bu_4NF) in various solvents at 80°C. The precursor compound **2** was sparingly soluble in MeCN, relatively more soluble in THF and much more soluble in N,N-dimethylsulfoxide (DMSO). Fluorination in THF produced the desired product in 50–54% yield. Most of the starting material disappeared in 15–20 min as observed by high performance liquid chromatography (HPLC). Reactions in MeCN and DMSO produced identical results (HPLC). Purification of the crude product on normal phase silica gel column produced 50–54% yield of the cold compound **3**. The unlabeled reference compound **3** was fully characterized by ^{1}H and ^{19}F NMR spectroscopy and high-resolution mass spectrometry. ^{1}H NMR spectrum showed a doublet of the fluoromethyl protons at 4.80 ppm with a coupling constant 47.4 Hz, characteristic coupling between fluorine and the geminal protons. Interestingly, ^{19}F NMR spectrum showed a peak at −224.7 ppm as a doublet of triplate with coupling constants 47.4 and 3.0 Hz, which are due to the geminal coupling between fluorine and

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proton, and long range coupling between fluorine and the C_6-amido proton, respectively.

Radiofluorination of compound **2** was performed using either $Bu_4N^{18}F$ or $K^{18}F$/kryptofix. The ^{18}F-fluoride was obtained from the cyclotron in the form of either aqueous $Bu_4N^{18}F$ or $K^{18}F$/kryptofix, which was evaporated to dryness by azeotropic removal of water with MeCN. The reactions were performed at 80°C for 20 min with $Bu_4N^{18}F$, and 115°C for 25 min with $K^{18}F$/kryptofix. Un-reacted fluoride was removed from the reaction mixture by passing the crude reaction mixture through a silica Sep-Pak cartridge, which was then eluted with 30% MeOH in CH_2Cl_2. The eluate was then injected into the semi-preparative HPLC system. The crude product was purified by HPLC on a semi-prep column. Figure 2 shows a representative semi-preparative HPLC chromatogram of the crude reaction mixture. The desired product fraction containing [^{18}F]-**3** was collected at a retention time of about 12 min.

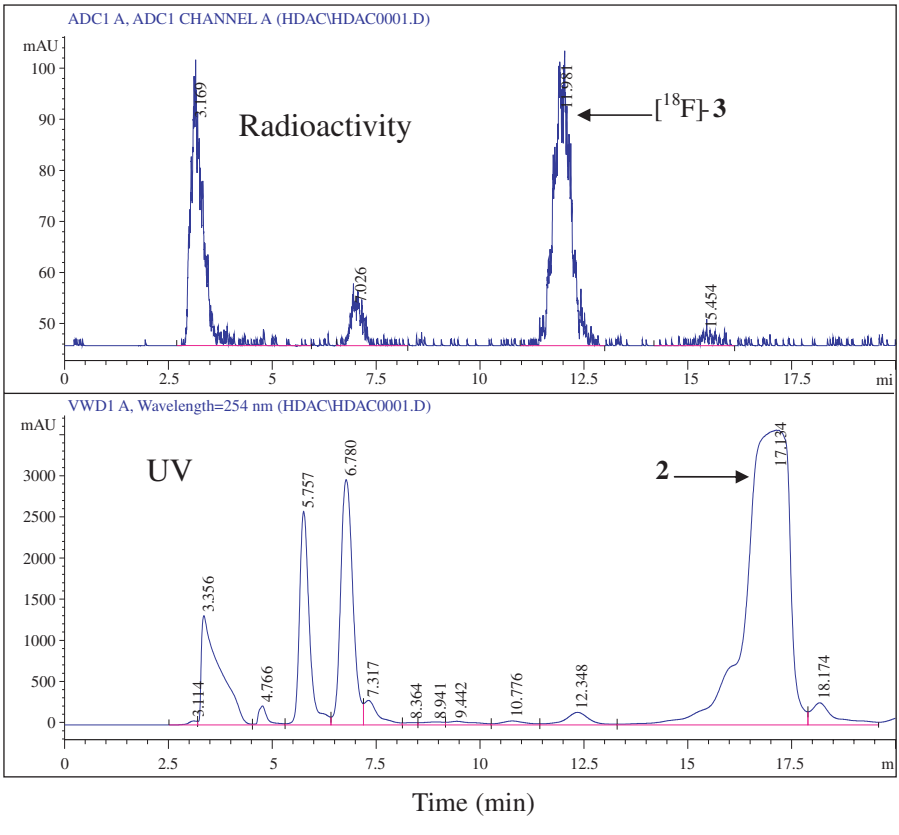

Figure 2. HPLC purification of crude [^{18}F]FAHA, prepared by radiofluorination with $Bu_4N^{18}F$: Semi-prep C_{18} column; 35% MeCN and 65% sodium phosphate (50 mM) in H_2O; flow 4.0 ml/min

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The radiochemical purity of the purified [^{18}F]-**3**, which co-eluted on analytical HPLC with an authentic sample of unlabeled **3**, was > 99% (Figure 3).

The cold fluorination with Bu_4NF and normal phase purification using organic solvents produced >50% yield of the desired product, however, the radiochemical yields using the described approach was only 1.0–3.8% (d.c.) with an average of 1.8%. Therefore, a carrier added synthesis was performed, but did not provide any significant improvement in yields.

Radiofluorination reactions with $Bu_4N^{18}F$ were also carried out in several solvents such as THF, MeCN, DMSO and DMSO/MeCN mixture to optimize the radiochemical yields. However, no significant improvement in yields was obtained. When the reactions were carried out with $K^{18}F$/kryptofix at 115°C in MeCN, the radiochemical yields were significantly increased from 3 to 13%. Although, we have been using $Bu_4N^{18}F$ as a preferred fluorinating agent in many syntheses,[12–15] the approach using $K^{18}F$/kryptofix appears to be superior to that using $Bu_4N^{18}F$ for radiofluorination of **2**.

The crude yields in the $Bu_4N^{18}F$ reactions were in the range of 4–14%, however, purified yields were much less (Table 1). There are several reasons for an overall low radiochemical yields. In addition to the low labeling efficiency,

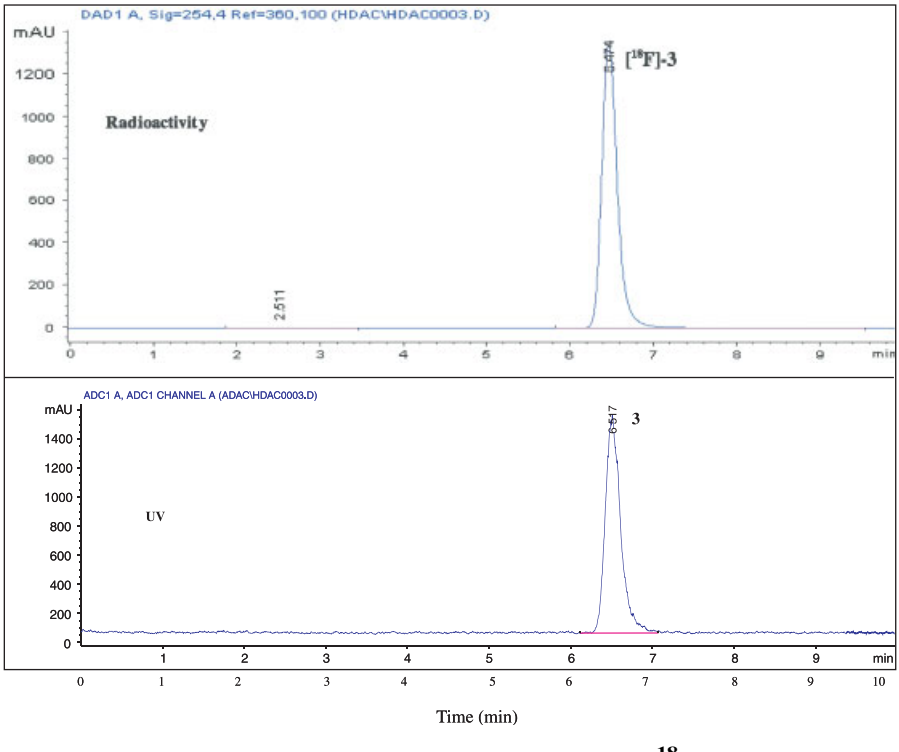

Figure 3. Analytical HPLC chromatogram of purified [^{18}F]FAHA, co-injected with standard FAHA: analytical C_{18} column; 35% MeCN and 65% sodium phosphate (50 mM) in H_2O; flow 1.0 ml/min

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Table 1. Radiochemical yields of [^{18}F]FAHA with two different [^{18}F]-fluoride salts in various solvent systems under different reaction conditions

No. of Expt.	Fluoride	Solvent	Temperature (°C)	Time (min.)	%Yield crude (d.c.)	%Yield pure (d.c.)
1	$Bu_4N^{18}F$	MeCN	80	20	12.83	1.0
2	$Bu_4N^{18}F$	MeCN	80	20	14.83	1.8
3	$Bu_4N^{18}F$	THF	80	20	6.5	1.92
4	$Bu_4N^{18}F$	THF	80	20	3.8	2.7
5	$Bu_4N^{18}F$	THF	80	20	4.0	1.8
6	$Bu_4N^{18}F$	DMSO	80	20	7.1	3.03
7	$Bu_4N^{18}F$	DMSO/MeCN	80	20	11.44	3.87
8	$Bu_4N^{18}F$	DMSO/MeCN	80	20	2.75	1.76
9	$Bu_4N^{18}F$	DMSO/MeCN	80	20	3.69	2.03
10	$K^{18}F$/Kryptofix	MeCN	115	25	30.9	12.5
11	$K^{18}F$/Kryptofix	MeCN	115	25	22.5	9.1
12	$K^{18}F$/Kryptofix	MeCN	115	25	27.3	11.5

the major loss of the product was during the HPLC purification step; only 40–50% of the total radioactivity was eluted from the column, while 50–60% radioactivity, including by-products, was retained on the column. The loss of the product during HPLC purification is due to poor solubility of the product in aqueous solvents. When the crude product was mixed with the HPLC solvent (water/MeCN), the compound had partially precipitated out of the solution, and therefore, could not be analyzed by HPLC. The use of an aqueous buffer (50 mM Na_2HPO_4) instead of water in MeCN as a HPLC solvent, had improved the HPLC peak shape and to some extent, the yield due to enhanced solubility of the product in the buffer.

Also, we have explored the feasibility of product purification using organic solvent systems. In one preparation, we purified the crude product on a small silica gel column using 30% acetone in hexane as eluent with manual fraction collection. The radiochemical yield was increased from 13 to 15% in this normal phase purification approach suggesting that the compound was not retained on the column. However, the product had a small amount of chemical impurities. The later suggests that use of HPLC with a normal phase column may be suitable for purification of [^{18}F]FAHA to improve recovery, however, this HPLC method has to be further optimized.

In the current work, the method of choice for radiosynthesis and purification of [^{18}F]FAHA involved $K^{18}F$/kryptofix as the fluorinating agent, and aqueous buffer on a reverse phase HPLC column. The radiochemical purity in this synthesis was $>99\%$ with specific activity >74 GBq/μmol. The specific activity in this no carrier added synthesis was very high and comparable with that of [^{18}F]FDG. The synthesis time was 67–75 min from the end of bombardment (EOB). In a representative synthesis, 2.5 mCi (92.5 MBq) of labeled product **3** could be obtained starting from 25 mCi (925 MBq) of [^{18}F]fluoride.

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Experimental

Reagents and instrumentation

All reagents and solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), and used without further purification. Solid phase extraction cartridges (silica gel, 900 mg) were purchased from Alltech Associates (Deerfield, IL).

Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F254 (Merck) glass plates. Proton and ^{19}F NMR spectra were recorded at the University of Texas MD Anderson Cancer Center on a Brucker 300 MHz spectrometer using tetramethylsilane as an internal reference and hexafluorobenzene as an external reference, respectively. Low-resolution mass spectral analysis was performed in house on HPLC-mass spectrometer (Applied Biosystem Q-Trap LC/MS/MS). High-resolution mass spectra were obtained on a Waters Micromass Q-TOF Ultima mass spectrometer at the University of Texas MD Anderson Cancer Center using electrospray ionization (ESI) technique.

HPLC was performed on a 1100 series pump (Agilent, Germany), with built in UV detector operated at 254 nm, and a radioactivity detector with single-channel analyzer (Bioscan, Washington DC) using a semi-preparative C_{18} reverse phase column (Alltech, Econosil, 10×250 mm, Deerfield, IL) and an analytical C_{18} column (Rainin, Microsorb-MV, 4.6×250 mm, Emeryville, CA). A mixture of acetonitrile and sodium phosphate solution (35% MeCN and 65% 50 mM Na_2HPO_4 in H_2O) solvent system was used for purification of the radiolabeled compound, and for quality control analysis on analytical HPLC.

Preparation of 6-amino-1-hexanoicanilide: **1**

To a solution of 6-amino hexanoic acid (2.5 g, 19 mmol) in dichloroethane (25 ml) thionyl chloride (2.5 g, 21 mmol in 25 ml dichloroethane) was added and the reaction mixture stirred for 2 h at room temperature (r.t.). Aniline (1.3 g, 14.3 mmol) was added to the reaction mixture, which was first stirred at r.t. for 0.5 h and then refluxed for 1.5 h. The reaction mixture was then cooled to 0°C, which resulted in the formation of a precipitate. The residue was filtered washed with dichloromethane and dried under vacuum. The product was obtained in 80% yield and used for the next step without further purification. ^{1}H NMR (DMSO-D_6) δ: 9.96 (s, 1H, NH), 7.86 (bs, 2H, NH_2), 7.62 (d, 2H, $J = 7.5$ Hz, aromatic), 7.30 (t, 2H, $J = 7.8$ Hz, aromatic), 6.98 (t, 1H, $J = 7.5$ Hz, aromatic), 2.80–2.74 (m, 2H, C_6H), 2.32 (t, 2H, $J = 7.2$ Hz, C_2H), 1.64–1.48 (m, 4H, $C_{3,5}H$), 1.39–1.29 (m, 2H, C_4H). MS: M + 1, 207.5

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Preparation of 6-(bromoacetamido)-1-hexanoicanilide: **2**

Crude compound **1** (1.0 g, 4.8 mmol) was dissolved in THF (20 ml) under argon and triethylamine (0.77 g, 7.6 mmol) was added. The reaction mixture was cooled to −10°C, and bromoacetic anhydride (2.0 g, 7.7 mmol) in 20 ml THF was added in 2 min. The reaction mixture was stirred for 20 min in the cold and subsequently at r.t. until TLC analysis (40% acetone in hexane) showed that no starting material (s.m.) remained (R_f; s.m. = 0.00, product = 0.38). The reaction mixture was quenched with cold water (10 ml) and extracted with 50% EtOAc/hexane (3 × 50 ml). The organic extract was dried ($MgSO_4$) and evaporated to dryness. The crude product was recrystallized from dichloroethane and hexane mixture. Pure compound (1.0 g) was obtained in 62% yield. ^{1}H NMR ($CDCl_3$) δ: 7.54 (d, 2H, J = 9.0 Hz, aromatic), 7.34 (t, 2H, J = 9.0 Hz, aromatic), 7.12 (t, 1H, J = 6.0 Hz, aromatic), 3.90 (s, 2H, CH_2-Br), 3.36 (q, 2H, J = 6.00 Hz, C_6H), 2.40 (t, 2H, J = 6.0 Hz, C_2H), 1.79 (q, 2H, J = 6.9 Hz, C_5H), 1.61 (q, 2H, J = 7.0 Hz, C_3H), 1.45 (q, 2H, J = 6.6 Hz, C_4H). ^{1}H NMR (DMSO-D_6) δ: 9.85 (s, 1H, NH), 8.26 (s, 1H, NH), 7.58 (d, 2H, J = 7.5 Hz, aromatic), 7.28 (t, 2H, J = 6.6 Hz, aromatic), 7.01 (t, 1H, J = 7.5 Hz, aromatic), 3.8 (s, 2H, CH_2-Br), 3.07 (q, 2H, J = 6.9 Hz, C_6H), 2.29 (t, 2H, J = 7.5 Hz, C_2H), 1.59 (q, 2H, J = 7.5 Hz, C_5H), 1.42 (q, 2H, J = 7.0 Hz, C_3H), 1.30 (q, 2H, J = 4.5 Hz, C_4H). High resolution MS: M + Na, Calculated 349.0528; found 349.0249.

Preparation of 6-(fluoroacetamido)-1-hexanoicanilide (FAHA): **3**

To a solution of 6-(bromoacetamido)-1-hexanoicanilide **2** (50 mg, 0.15 mmol) in dry THF (2.5 ml) was added tetrabutylammonium fluoride solution in THF (1 M Bu_4NF, 0.30 ml). The resulting mixture was heated at 80°C for 20 min when TLC analysis (40% acetone in hexane) showed that no starting material remained (R_f; s.m. = 0.38, product = 0.29). The solvent was evaporated and the crude product was purified by flash chromatography on a silica gel column using 30% acetone/hexane solvent system. The appropriate fractions were combined and evaporated to dryness. Pure compound **3** (22 mg) was obtained in 54% yield. ^{1}H NMR ($CDCl_3$) δ: 7.54 (d, 2H, J = 7.8 Hz, aromatic), 7.34 (t, 2H, J = 7.5 Hz, aromatic), 7.12 (t, 1H, J = 7.5 Hz, aromatic), 4.80 (d, 2H, J = 47.4 Hz, CH_2-F), 3.36 (q, 2H, J = 6.6 Hz, C_6H), 2.40 (t, 2H, J = 7.5 Hz, C_2H), 1.80 (q, 2H, J = 7.2 Hz, C_5H), 1.60 (q, 2H, J = 7.2 Hz, C_3H), 1.46 (q, 2H, J = 7.1 Hz, C_4H). ^{19}F NMR: ($CDCl_3$) δ: −224.9 (dt, J_{gem} = 47.4 Hz and $J_{long\ range}$ = 3.0 Hz).

High resolution MS: M + Na, Calculated 289.1328; found 289.1324.

Preparation of 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]AHA):

The aqueous [^{18}F]fluoride produced from the cyclotron by the reaction ^{18}O(p, n)^{18}F was trapped on anion exchange cartridge (ABX, Germany) and

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eluted with an aqueous solution of *n*-Bu_4NHCO_3 (400 µl, 1% by wt) or K_2CO_3/kryptofix 2.2.2 solution (1.2 ml) into a V-vial. The K_2CO_3/kryptofix 2.2.2 solution was prepared by mixing a solution of kryptofix 2.2.2 (12 mg/ml in MeCN) and K_2CO_3 (2.75 mg/ml in water) in the ratio of 80:20, respectively. The solution was evaporated azeotropically with acetonitrile (1.0 ml) to dryness at 79–80°C ($Bu_4N^{18}F$) or 115°C ($K^{18}F$/kryptofix) under a stream of argon. To the dried *n*-$Bu_4N^{18}F$, a solution of **2** (3–4 mg, 9–12 µmol) in anhydrous MeCN (0.4 ml) was added, and the mixture was heated at 79–80°C for 20 min. The reaction with $K^{18}F$/kryptofix was performed at 115°C for 25 min. The reaction mixture was cooled, passed through a silica gel cartridge (Alltech), and eluted with 30% methanol in dichloromethane (2.5 ml). After evaporation of the solvent with a stream of argon at 80°C, the residue was diluted with HPLC solvent (0.5 ml) and purified by HPLC. The desired product was isolated and radioactivity was measured in a dose calibrator (Capintec, Ramsey, NJ). The solvent was evaporated on a high vacuum pump, the product was re-dissolved in saline and filtered through a Millipore filter. The product was co-injected with an authentic unlabeled sample onto an analytical column to confirm its identity.

Conclusion

Cold synthesis and radiolabeling of a novel substrate for HDAC, 6-([^{18}F]fluoroacetamido)-1-hexanoicanilide ([^{18}F]FAHA) has been accomplished. This new compound may be useful in measuring the level of HDAC expression and activity in cancer patients using PET imaging.

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