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Research Article

Synthesis of [phenyl-U-^{14}C]aryl and [8-^{14}C]carboxy labeled tracers of vorinostat

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Summary

In support of a program to develop a treatment for advanced, refractory cutaneous T-cell lymphoma, two differentially [^{14}C]-labeled forms of vorinostat, a histone deacetylase inhibitor, were synthesized for use in metabolism studies. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: CTCL; HDAC; SAHA; suberoylanilide hydroxamic acid; vorinostat

Introduction

The reversible acetylation of the side-chain amino group of specific lysines, within histones, plays a crucial role in the transcription of DNA.[1] Recent studies have shown that inhibition of histone deacetylases (HDACs), the enzymes controlling the acetylation state of histones, evokes anticancer effects in several types of tumor cells by inhibition of cell growth and induction of cell differentiation.[2] Work to support this assumption has shown that HDAC inhibition triggers growth arrest, differentiation, and apoptosis in tumor cells through the transcriptional activation of a small set of genes that regulate cell proliferation and cell cycle progression. As a result, the development of HDAC inhibitors is a rapidly growing and promising area for cancer treatment.[3]

Cutaneous T-cell lymphoma (CTCL), a subset of post-thymic lymphomas, accounts for approximately half of all lymphomas that arise in the skin.[4] Although patients with advanced stage CTCL respond to various forms of cytotoxic or biological therapies, these responses are generally short lived.[5] Case reports have been presented that suggest HDAC inhibitors may be effective in the treatment of T-cell lymphomas.[6] In addition to others,

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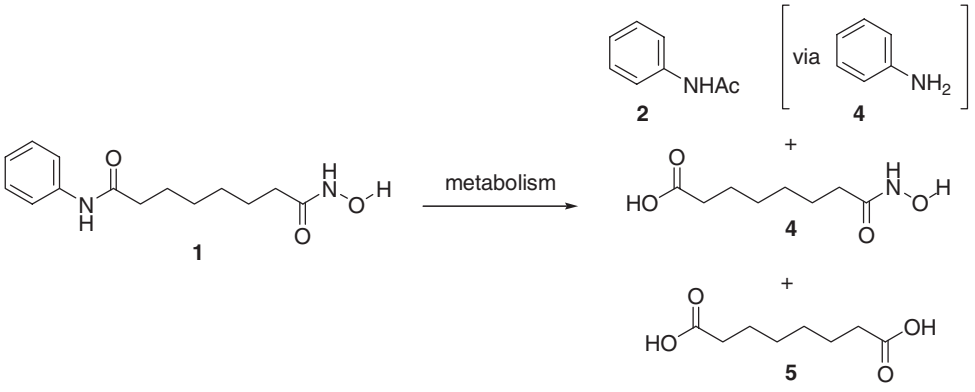


Scheme 1.

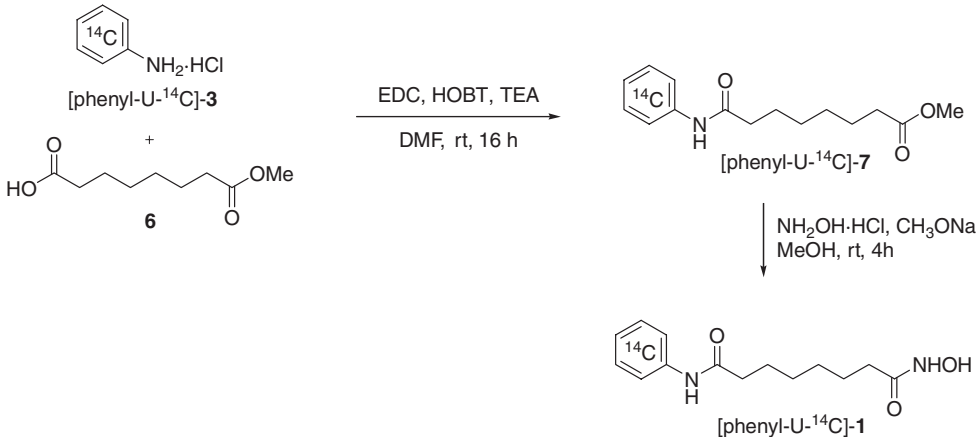
vorinostat (suberoyl anilide hydroxamic acid; SAHA, **1**) has been studied for this purpose in cancer cell lines and tumor animal models.[7]

During the course of clinical studies, acetanilide (**2**), 8-hydroxyamino-8-oxooctanoic acid (**4**), and suberic acid (**5**) were identified as the major first pass metabolites of vorinostat (**1**) found in human urine, bile and serum (Scheme 1).[8] In order to fully understand the metabolic fate of each fragment in man and animals, it was necessary to synthesize two differentially labeled [^{14}C]-tracers of vorinostat (**1**). In this article, we report on the efficient preparation of both the aryl- and alkyl-labeled tracers of vorinostat (**1**).

Results and discussion

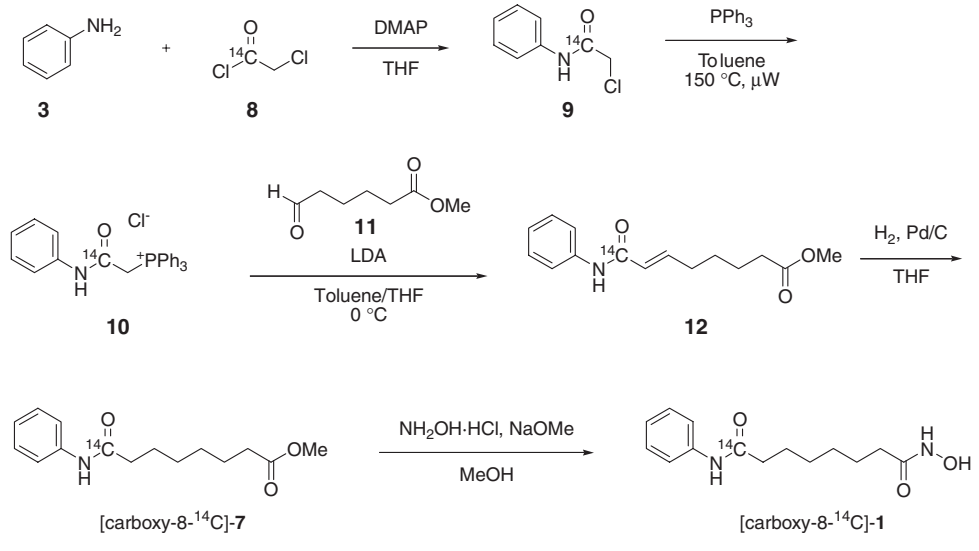
Previous reports for the synthesis of vorinostat (**1**)[9a–c] suffer from low yields, formation of byproducts that are difficult to remove, lengthy reaction times, and harsh conditions. In a similar manner to the Geydia group,[9e] we felt that subjecting [^{14}C]aniline and the commercial availability monomethyl ester of suberic acid, (**6**), to peptide coupling conditions would be an efficient beginning for the [^{14}C]ring-labeled tracer (Scheme 2). In this fashion, acid (**6**) was coupled with [^{14}C]aniline · HCl ([phenyl-U-^{14}C]-**3**) in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, 1-hydroxybenzotriazole, and triethylamine to afford anilide [phenyl-U-^{14}C]-**7** in 80% yield after preparative HPLC purification. The resulting ester, [phenyl-U-^{14}C]-**7**, was treated with methanolic hydroxylamine and sodium methoxide to afford [^{14}C]vorinostat ([phenyl-U-^{14}C]-**1**) in 85% yield (68% overall yield) after preparative HPLC purification.

In order to monitor the fate of the alkyl fragment of vorinostat (**1**), we prepared a tracer with the label in the carbonyl group of the anilide side of the chain. The synthesis of the side-chain labeled [^{14}C]-tracer of vorinostat was initiated by the acylation of aniline with 2-chloro[1-^{14}C]acetyl chloride (**8**) in the presence of dimethylaminopyridine to afford chloride **9** (Scheme 3).



Scheme 2.

The crude reaction material was then dissolved in toluene followed by the addition of triphenylphosphine then heated at 150°C for 5 min in a microwave reactor. Upon completion, the insoluble product was filtered to afford phosphonium salt **10**. The resulting phosphonium salt (**10**) was olefinated with methyl 6-oxohexanoate (**11**) in the presence of lithium diisopropylamide, employing standard Wittig conditions, to afford olefin **12**. After flash chromatography purification, olefin **12** was reduced via catalytic hydrogenation with 10% Pd/C under a balloon of hydrogen. The resulting ester, [carboxy-8-^{14}C] **7**, was converted to vorinostat [carboxy-8-^{14}C]-**1** as previously described (*vide supra*) in 90% yield (52% overall yield) after preparative HPLC purification.



Scheme 3.

In summary, we have reported the synthesis of differentially labeled tracers of [^{14}C]vorinostat, which served as important tools for obtaining detailed, late-stage, drug metabolism data. Both syntheses are efficient from readily available labeled precursors.

Experimental

All reagents were of commercial quality unless otherwise stated. Anhydrous solvents were purchased from Aldrich and dried over 4 Å molecular sieves for at least 24 h prior to use. The labeled reagents, [^{14}C]aniline · HCl ([phenyl-U-^{14}C]-**3**, Amersham Biosciences) and chloro[1-^{14}C]acetyl chloride (**8**, American Radiolabeled Chemicals, Inc.) were used without further purification. Radioactive measurements were carried out using a Packard Tri-carb 1000 TR liquid scintillation spectrometer and Packard Ultima-flo Gold™ as scintillant. Analytical high-performance liquid chromatography (HPLC) analyses were conducted using a Phenomenex C18(2) column (250 × 4.6 mm, 5 μm) on an Agilent 1100 Series HPLC with quaternary pumps, degasser, auto-injector, temperature controller, multi wavelength detector, and Packard Radiomatic™ 500TR flow scintillation analyzer with Packard Ultima-flo M™ scintillation cocktail. Mass spectra were recorded on an Agilent 1100 LCMSD instrument in API-ES positive ionization mode. Preparative HPLCs were performed using Rainin HPXL pumps, eluting 0.1% TFA in water (A) and acetonitrile (B) through a Phenomenex C18(2) column (250 × 21.2 mm, 5 μm). Flash chromatography was performed using an Argonaut FlashMaster Solo system eluting hexanes and ethyl acetate through the indicated packaged column. NMR (^{1}H, ^{13}C) spectra were recorded on a Varian U-400 spectrometer using the indicated solvent. Chemical shifts are reported in parts per million (δ), and coupling constants (J values) are given in hertz. Microwave reactions were performed in a Personal Chemistry Emrys Creator using the indicated reaction vessel, temperature and time.

*[^{14}C]Methyl 8-anilino-8-oxooctanoate ([phenyl-U-^{14}C]-**7**)*

A solution of [^{14}C]aniline · HCl ([phenyl-U-^{14}C]-**3**, 116.8 mg, 0.88 mmol, 100 mCi, 114 mCi/mmol) in anhydrous DMF (3 ml) was treated with suberic acid monomethyl ester (170 mg, 0.9 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide · HCl (253 mg, 1.32 mmol), 1-hydroxybenzotriazole · H_2O (202 mg, 1.32 mmol) and triethylamine (368 μl, 2.69 mmol). The resulting heterogeneous mixture was aged overnight (16 h) at ambient temperature. The slurry was diluted with EtOAc (30 ml) and washed with 2 M HCl (10 ml), water (10 ml) and brine (10 ml). The organic layer was dried (Na_2SO_4), filtered, and evaporated to afford a brown oil. The oil was purified by preparative HPLC eluting with 30% B for 10 min then 30–60% B over 30 min. Reaction afforded 186 mg (80 mCi, 80%, > 99% radiochemical purity) of ester [phenyl-U-^{14}C]-**3**

as a white solid. Physical and spectroscopic properties of [phenyl-U-^{14}C]-**3** were in agreement with previously reported values.[9a]

*[^{14}C]N-Hydroxy-N′-phenyloctanediamide (vorinostat [phenyl-U-^{14}C]-**1**)*

Hydroxylamine · HCl (25 mg, 0.35 mmol) was dissolved in 500 µl of methanol followed by ~0.5 mg of phenolphthalein. Sodium methoxide (25 wt%) was added dropwise to make the solution pink (~100 µl). A precipitate of NaCl appeared. A solution of [^{14}C]ester [phenyl-U-^{14}C]-**3** (46 mg, 20 mCi) in methanol (500 µl) was added to the pink reaction mixture. Additional sodium methoxide (25 wt%, 30 µl) was added and the mixture was stirred for 4 h. The reaction was diluted with water (1 ml) to make it homogeneous. The resulting solution was purified via preparative HPLC eluting with 15% B for 10 min then 15–70% B over 55 min. Reaction afforded 41 mg (17 mCi, 85%, >99% radiochemical purity) of vorinostat [phenyl-U-^{14}C]-**1** as a white solid. Physical and spectroscopic properties of [phenyl-U-^{14}C]-**1** were in agreement with previously reported values.[9a]

[^{14}C]2-Chloro-N-phenylacetamide (**9**)

2-Chloro[1-^{14}C]acetyl chloride (**8**, 100 mCi, 1.92 mmol) was transferred from a break seal ampoule to a dry flask. The ampoule was rinsed with THF (1.6 ml) and the residue was transferred to the flask. The solution was cooled to 0°C and a solution of aniline (**3**, 146 µl, 1.6 mmol) and 4-dimethylaminopyridine (98 mg, 0.8 mmol) in THF (2 ml) was added dropwise. The homogeneous solution was stirred at 0°C for 1 h, diluted with water (5 ml) and warmed to ambient temperature. The solution was extracted with $CHCl_3$ (3 ×), the organic layers were combined, dried (Na_2SO_4) and evaporated to afford [^{14}C]2-Chloro-*N*-phenylacetamide (**9**, 73 mCi, 73% radiochemical yield, 95% rcp) as a pale yellow solid. Physical and spectroscopic properties were identical to commercially available (Aldrich) material. LCMS: 172.2 ($[M + H]^+$).

[^{14}C](2-Anilino- 2-oxoethyl)(triphenyl)phosphonium chloride (**10**)

To a 2–5 ml microwave reaction vessel was added triphenylphosphine (419 mg, 1.6 mmol), [^{14}C]2-Chloro-*N*-phenylacetamide (**9**, 83 mCi, 1.6 mmol), degassed toluene (3 ml), and a stir bar. The vial was capped and heated at 150°C for 5 min in the microwave. The resulting precipitate was filtered, rinsed with cold toluene, and dried under vacuum. The reaction afforded 541 mg (65 mCi, 78%) of [^{14}C](2-anilino-2-oxoethyl)(triphenyl)phosphonium chloride (**10**) as a tan solid with >98% radiochemical purity. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.25 (bs, 1H), 7.87–7.81 (m, 6H), 7.77–7.73 (m, 3H), 7.65–7.59 (m, 8H), 7.19 (t, $J = 7.8$ Hz, 2H), 7.02 (t, $J = 7.4$ Hz, 1H), 5.26 (d, $J = 14.4$ Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 160.6, 137.7, 135.0, 134.1 (d, $J = 10.3$ Hz), 130.1

(d, $J = 13.0$ Hz), 128.5, 124.6, 120.2, 118.1 (d, $J = 89.0$ Hz), 33.3 (d, $J = 55.0$); LCMS: 398 ($[M + H]^+$).

[^{14}C]Methyl (6E)-8-anilino-8-oxooct-6-enoate (**12**)

To a solution of [^{14}C]phosphonium chloride (**10**, 227 mg, 0.52 mmol, 27 mCi) in anhydrous THF (5 ml) at 0°C was added lithium diisopropylamide (1.8 M, 455 µl, 0.82 mmol) and aged at 0°C for 1 h. After 1 h, a solution of aldehyde **11** (62 µl, 0.43 mmol) in toluene (2.5 ml) was added dropwise. Once addition was complete, the reaction was warmed to ambient temperature and aged for 18 h. The reaction was quenched with saturated NH_4Cl (4 ml) and extracted with EtOAc (3×4 ml). The organics were combined, washed with brine, dried (Na_2SO_4) and evaporated to afford a yellow oil. The oil was purified by preparative HPLC eluting with 35% B for 10 min then 35–55% B over 20 min. The reaction afforded 95 mg (20 mCi, 85%, >99% radiochemical purity) of alkene **12** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, $J = 7.8$, 2H), 7.32 (t, $J = 7.8$, 2H), 7.17 (bs, 1H), 7.11 (t, $J = 7.4$, 1H), 3.66 (s, 3H), 6.12 (ddd, $J = 11.4$, 7.4, 3.5, 1H), 5.84 (dt, $J = 11.4$, 1.0, 1H), 2.77 (dq, $J = 7.4$, 1.0, 2H), 2.34 (t, $J = 7.5$, 2H), 1.69 (p, $J = 7.5$, 2H), 1.51 (p, $J = 7.5$, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 174.6, 173.7, 135.6, 129.0, 124.6, 122.6, 119.7, 51.5, 33.8, 28.6, 28.3, 24.5; LCMS: 264.0 ($[M + H]^+$).

*[^{14}C]Methyl 8-anilino-8-oxooctanoate ([carboxy-8-^{14}C]-***7***)*

To a solution of [^{14}C]alkene **12** (41 mg, 0.15 mmol, 8 mCi) in anhydrous THF (3 ml) was added 10% Pd/C (25 mg). The reaction flask was evacuated and backfilled with H_2 ($+3\times$) and stirred overnight (15 h) under a balloon of H_2. The reaction was filtered, rinsed with THF (5 ml), and evaporated to afford a yellow oil. The oil was used without further purification. LCMS: 266.0 ($[M + H]^+$).

*[^{14}C]N-Hydroxy-N′-phenyloctanediamide (vorinostat [carboxy-8-^{14}C]-***1***)*

Hydroxylamine · HCl (21 mg, 0.30 mmol) was dissolved in 200 µl of methanol followed by ~0.5 mg of phenolphthalein. Sodium methoxide (25 wt%) was added dropwise to make the solution pink (~75 µl). A precipitate of NaCl appeared. A solution of ester [1-^{14}C]-**7** (41 mg, 0.15 mmol, 8 mCi) in methanol (400 µl) was added to the pink reaction mixture. Additional sodium methoxide (25 wt%, 30 µl) was added and the mixture was stirred for 4 h. The reaction was diluted with water (1 ml) to make homogeneous. The resulting solution was purified via preparative HPLC eluting with 15% B for 10 min then 15–70% B over 55 min. The reaction afforded 35 mg (7.3 mCi, 90%, >99% radiochemical purity) of oxamic acid [carboxy-8-^{14}C]-**1** as a white

solid. Physical and spectroscopic properties of [carboxy-8-^{14}C]-**1** were in agreement with previously reported values.[9a]

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