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Research Article

A novel method for the synthesis of carbon-14-labeled *N*-[3-(1-methyl-4-piperidinyl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl]propanamide and its use in quantitative whole-body autoradiography studies

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Dedicated to the memory of my friend and mentor, Winston S. Marshall (1938–2005)

Summary

Sumitriptan (**1**), a non-selective 5-$HT_{1B/1D}$ agonist is an effective therapeutic agent for the acute treatment of migraine, but it is contraindicated for use in patients with known heart disease. The first Selective Serotonin One F Receptor Agonist (SSOFRA), 5-(4′-fluorobenz-amido)-3-(*N*-methyl-piperidin-4-yl)-1H-indole (**2**) was demonstrated to be clinically useful in the treatment of migraine. Although **2** exhibited high affinity for the 5-HT_{1F} receptor as well as high selectivity for the 5-HT_{1F} receptor relative to 5-HT_{1B} and 5-HT_{1D} receptors, it demonstrated appreciable affinity for the 5-HT_{1A} receptor. Subsequently, a program was launched to discover SSOFRA's with improved selectivity over other 5-HT_1 receptor subtypes. As a result of these efforts, *N*-[3-(1-methyl-4-piperidinyl)-1*H*-pyrrolo[3,2-b]pyridin-5-yl]propanamide (**3**) was found to possess greater than 100-fold selectivity over 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors. Pursuant to a potential clinical investigation of **3**, its carbon-14-labeled isotopomer has been prepared by a circuitous route from unlabeled **3** and used in quantitative whole-body autoradiography studies in rats. The results of these efforts are reported herein. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: SSOFRA's; carbon-14; QWBA

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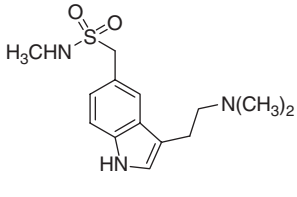
Introduction

Over the past several years, research into the molecular pharmacology of the receptors which are influenced by the neurotransmitter 5-hydroxytryptamine (serotonin, 5-HT), has provided evidence for several 5-HT receptor subtypes (e.g. 5-HT_1, 5-HT_2, 5-HT_3). Within individual subtypes, receptors have been identified which show preferential binding for various agonists and antagonists (e.g. 5-$HT_{1A–1F}$, 5-$HT_{2A–2C}$). Sumitriptan (**1**), a non-selective 5-$HT_{1B/1D}$ agonist is an effective acute therapy for the treatment of migraine.[1] Sumitriptan (**1**) and other agents in the triptan class are known to be potent vasoconstrictors which are believed to be responsible for their mechanism of action in the treatment of migraine as well as for some of their reported associated adverse events;[2] sumatriptan is contraindicated in patients with known heart disease.[3] The first Selective Serotonin One F Receptor Agonist (SSOFRA) (5-(4′-fluoro-benzamido)-3-(*N*-methyl-piperidin-4-yl)-1H-indole, **2**), was found to be useful clinically in the treatment of migraine without the cardiovascular adverse events often associated with non-selective 5-HT_1 receptor agonists.[4] In addition to high affinity for the 5-HT_{1F} receptor, **2** also exhibited high selectivity for the 5-HT_{1F} receptor relative to 5-HT_{1B} and 5-HT_{1D} receptors, although it demonstrated appreciable affinity for the 5-HT_{1A} receptor.[5] As the result of an effort to identify more selective SSOFRA's, *N*-[3-(1-methyl-4-piperidinyl)-1*H*-pyrrolo-[3,2-b]pyridin-5-yl]propanamide (**3**) was selected as a potentially useful agent for the treatment of migraine.[6] In the course of pre-clinical development, the C-14-labeled isotopomer of **1a** was required for use in drug metabolism and disposition studies. Preliminary discussions of this chemistry have been previously published.[7] In this paper, we will discuss the complete details of the preparation of *N*-[3-(1-methyl-4-piperidinyl)-1*H*-pyrrolo-[3,2-b]pyridin-5-yl]propanamide-[indole-2-^{14}C] succinate **(3-[^{14}C])** and its use in quantitative whole-body autoradiography (QWBA) studies. Hamilton *et al.* have presented this material in a preliminary report.[8]



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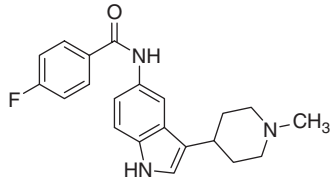

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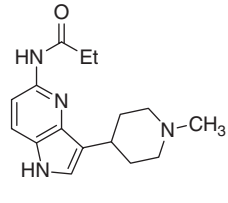
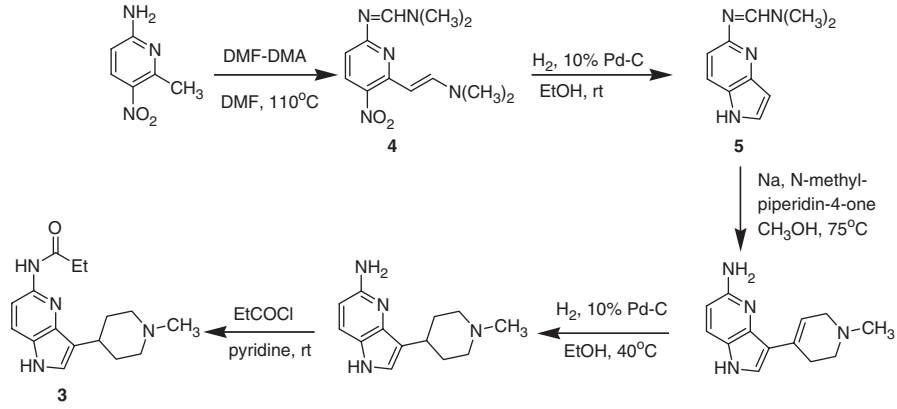


3

Discussion

Chemistry

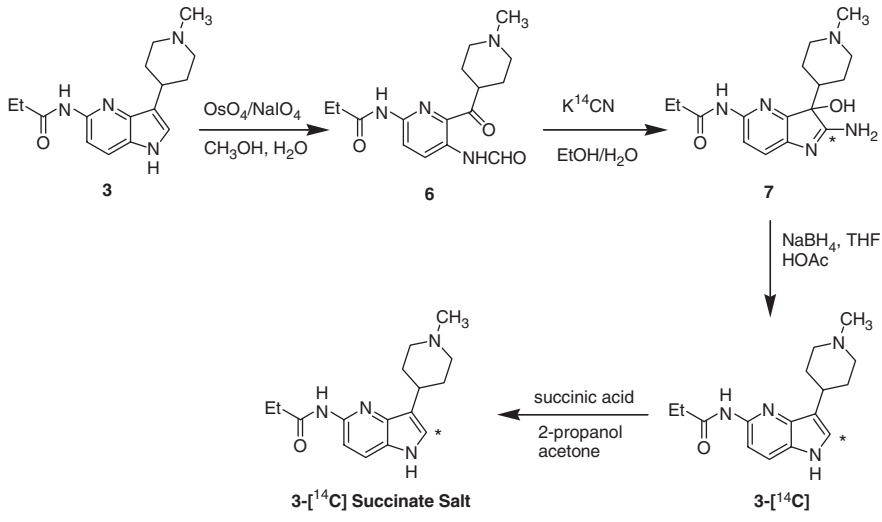
Filla *et al.* discussed the chemistry and SAR that led to the discovery of **3**. In the original synthesis of **3**, 6-methyl-5-nitropyridin-2-ylamine was reacted with



Scheme 1.

dimethylformamide-dimethyl acetal (DMF-DMA) using Leimgruber–Batcho chemistry (Scheme 1).[6] In this manner, the amine was protected as an amidine and the methyl was converted to the corresponding enamine **4**. Catalytic hydrogenation of the nitro group in **4** yielded the desired indole **5** starting material.

Based on the experience in the early synthesis of **2-[^{14}C]**,[9] certainly, Leimgruber–Batcho chemistry as described in Scheme 1, could be used in the preparation of C-14-labeled **3**. This would of course require installation of the C-14 label very early in the synthesis and protection of the amine with C-14-labeled DMF-DMA would waste one equivalent of the reagent. The use of *N*-(4-methyl-5-nitropyridin-2-yl)propionamide in the Leimgruber–Batcho reaction, rather than 4-methyl-5-nitropyridin-2-ylamine, would only save one step and would still require carrying the label through five steps including the salt formation; the synthesis of C-14-labeled DMF-DMA would require an additional two steps from DMF-[^{14}C] which is quite expensive. As an alternative, using an elegant method originally described by Waterhouse *et al.* and used in an earlier preparation of **2-[^{14}C]**,[10] *N*-[3-(1-methyl-4-piperidinyl)-1*H*-pyrrolo-[3,2-b]pyridin-5-yl]propanamide (**3**) was oxidized with OsO_4/$NaIO_4$ in CH_3OH/H_2O to yield *N*-[5-formylamino-6-(1-methylpiperidinine-4-carbonyl)pyridin-2-yl]acetamide (**6**) as a crystalline solid in 16% yield after chromatography. Reaction of **6** with an equimolar amount of KCN-[^{14}C] in $EtOH/H_2O$ yielded *N*-[2-amino-3-hydroxy-3-(1-methylpiperidin-4-yl)-3*H*-pyrrolo[3,2-b]pyridin-5-yl-2-[^{14}C]]-propionamide (**7**) which was isolated by concentration of the reaction mixture (Scheme 2). TLC (silica gel, EtOAc:2-propanol:NH_4OH:H_2O) (25:15:2:2) showed **7** was a single spot ($r_f = 0.25$) which contained 97.5% of the radioactivity. The residue was dissolved in 1:1 THF/HOAc, chilled to 0–5°C and treated portion/wise with an excess of



Scheme 2.

$NaBH_4$. After stirring and subsequent work-up, **3-[^{14}C]** was recovered as a crude product in 60% yield. Chromatographic purification on silica gel yielded pure **3-[^{14}C]** in 47% yield. A mixture of **3-[^{14}C]** and succinic acid was heated in acetone/2-propanol until dissolved. The mixture was allowed to cool to room temperature; after seeding with **3** succinate salt, a white crystalline solid was formed to yield **3-[^{14}C]** succinate salt in 67%. The specific activity was adjusted by re-crystallization of a mixture of **3/3-[^{14}C]** succinate salt from acetone/2-propanol to yield **3-[^{14}C]** succinate salt in 71% yield. Thus, in three radiochemical steps, **3-[^{14}C]** succinate salt has been prepared by a circuitous route from **3** in 22.3% radiochemical yield.

Tissue distribution evaluated by QWBA in rats

During the course of development of a new chemical entity (NCE) for marketing as a pharmaceutical, many countries require radiolabeled studies in humans. As required by Institutional Review Boards and regulatory agencies, preclinical tissue distribution studies are used to estimate the potential human organ exposures that support dosimetry calculations prior to the administration of a radiolabeled compound to humans. QWBA studies have become the method of choice for the determination of tissue distribution in small animals. In this instance, male Long Evans rats, approximately 6 weeks old, each received a single oral 5-mg/kg dose (approximately 30 μCi/animal) of **3-[^{14}C]**. One animal per time point was anesthetized by isoflurane and exsanguinated via cardiac puncture at 1, 3, 6, 8, 12, 24, 48, 72, and 96 h postdose and

processed for whole-body autoradiography as described in detail by Ullberg.[11] Following euthanasia, animals were rapidly frozen in hexane cooled with dry ice and stored frozen at −20°C. Frozen carcasses were embedded in 2% carboxymethylcellulose (CMC) which when frozen, supported the carcass for sectioning on a cryomicrotome. Radiolabeled liver homogenate was used as an internal standard and added to the CMC blocks prior to sample collection. Sagittal whole-body sections of approximately 20 μm thickness were collected to include internal standards and major organs/tissues, and then freeze dried for at least 48 h. Selected tissue sections were mounted and exposed, along with tissue calibrated polymethacrylate commercial standards and internal standards, to phosphor imaging plates for approximately 7 days. Before exposure, background was erased by exposing the imaging plates to bright visible light. After exposure, the imaging plates were scanned with a helium–neon laser. Autoradiographic images were recorded and quantitated using phosphor imaging technology as described by Johnston *et al.*[12] Quantitation of tissue concentrations were conducted using tissue calibrated commercial standards and internal standards to correct for section thickness variation as described by Chay and Pohland.[13] Single samples were taken on multiple sections for each tissue from each animal. Standard curves associated with individual scans were fit with a least-squares regression line from which the concentrations of radiocarbon were interpolated. The reported lower limit of detection was based on the mean standard curve concentrations. Phosphor images were also visually evaluated and representative images were reproduced. Regions of high and low radiocarbon concentrations were observed in the adrenal gland, kidney, liver and salivary gland. This is due to the ability of whole-body autoradiography to show heterogeneous disposition of radioactivity within these tissues. The values for these tissues are referred to as high and low concentrations in Table 1.

By 1 h postdose, low-to-moderate levels of radioactivity associated with **3-[^{14}C]** were distributed to most major tissues including adrenal gland, blood, bone marrow, brown fat, gastrointestinal contents and wall, Harderian gland, kidney, liver, lung, muscle, myocardium, pancreas, pituitary gland, salivary gland, skin, spleen, thymus and thyroid gland (Figure 1). Peak radiocarbon tissue concentrations were reached at various time points, but were reached between 3 and 6 h in most major tissues. Highest levels of radioactivity associated with **3-[^{14}C]** and/or its metabolites were quantitated in the gastrointestinal contents, ranging from 16.52 to 150.73 μg-eq/g in the small intestinal contents at 1 h and the large intestinal contents at 8 h, respectively. Highest tissue concentrations were 7.53 μg-eq/g in the kidney at 3 h, 6.71 μg-eq/g in the uveal tract of the eye at 12 h and 3.93 μg-eq/g in the liver (high) at 1 h. By 96 h postdose, all radiocarbon concentrations had declined to below the limit of quantitation (mean 0.50 ± 0.17 μg-eq/g) with the exception of

Table 1. Mean tissue concentrations of radiocarbon (μg-eq/g) in male Long Evans rats following a single oral 5-mg/kg dose of 3-[^{14}C] administered as the succinate salt

	Time after dose (h)								
Tissue	1	3	6	8	12	24	48	72	96
Adrenal gland (H)	1.09	1.22	1.80	NS	1.68	1.98[a]	1.61[a]	1.29[a]	1.42[a]
Adrenal gland (L)	NA	NA	1.07	1.04	1.00	1.04	0.98	NA	NA
Blood	0.99	1.11	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Bone marrow	1.06	1.11	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Brown fat	1.24	1.75	1.74	1.99[a]	1.61[a]	1.1[a]	1.03[a]	BLQ	BLQ
Cecal contents	1.17	47.45	81.61	80.67	117.31[a]	2.92[a]	1.13[a]	BLQ	BLQ
Cecal wall	1.13	1.69[a]	1.54[a]	1.48[a]	1.38[a]	1.39[a]	BLQ	BLQ	BLQ
Epididymis	BLQ	1.14	TNS	TNS	1.04	BLQ	BLQ	BLQ	BLQ
Eye	BLQ	3.52	3.45	2.93	6.71	3.38	2.64	2.34	2.96
Harderian gland	1.04	1.30	1.28	1.15	1.24	BLQ	BLQ	BLQ	BLQ
Kidney (H)	4.14	7.53	2.71	1.51	2.23	1.11	1.75	BLQ	BLQ
Kidney (L)	1.06	1.10	1.15	1.11	1.11	1.03	0.99	NA	NA
Large int. cont.	BLQ	76.75	NS	150.73[a]	38.39[a]	NS	1.35[a]	BLQ	BLQ
Large int. wall	1.18	1.85	1.77[a]	1.53[a]	1.33[a]	1.14[a]	BLQ	BLQ	BLQ
Liver (H)	3.93	2.76	2.53	1.64	1.35[a]	1.16[a]	1.13[a]	1.16[a]	1.06[a]
Liver (L)	2.53	2.18	1.70	1.31	NA	NA	NA	NA	NA
Lung	1.08	1.21	1.25[a]	1.16[a]	1.16[a]	BLQ	BLQ	BLQ	BLQ
Muscle	0.98	0.98	1.08	1.11	1.04	BLQ	BLQ	BLQ	BLQ
Myocardium	1.12	1.47[a]	1.45[a]	1.25[a]	BLQ	BLQ	BLQ	BLQ	BLQ
Pancreas	1.05	1.32	1.35[a]	1.19[a]	1.06[a]	BLQ	BLQ	BLQ	BLQ
Pituitary gland	1.12	1.48	1.44	1.28	1.46[a]	1.19[a]	1.09[a]	BLQ	BLQ
Preputial gland	BLQ	1.32	1.89[a]	1.17[a]	1.21[a]	BLQ	BLQ	BLQ	BLQ
Salivary gland (H)	1.12	1.82	2.03	1.59[a]	1.52[a]	1.29[a]	BLQ	BLQ	BLQ
Salivary gland (L)	1.15	1.36	1.32	1.17	1.05	1.02	BLQ	NA	NA
Skin	1.00	1.07	1.20	1.23	1.28	1.13	1.16	1.21	1.14
Small int. cont.	16.52	25.18	78.70	3.27	1.92[a]	1.32[a]	1.19[a]	BLQ	BLQ
Small int. wall	2.68	1.35	1.63	1.30	1.22	1.33	BLQ	BLQ	BLQ
Spleen	1.11	1.23	1.25[a]	1.18[a]	1.11[a]	BLQ	BLQ	BLQ	BLQ
Stomach contents	5.57	27.54	1.38	TNS	1.07	1.77	BLQ	BLQ	BLQ
Stomach wall	1.01	1.65[a]	1.49[a]	1.25[a]	1.14[a]	BLQ	BLQ	BLQ	BLQ
Testes	BLQ	1.08	1.05	1.02	1.08	1.06	1.09	1.08	1.11
Thymus	1.01	1.15[a]	1.06[a]	1.07[a]	BLQ	BLQ	BLQ	BLQ	BLQ
Thyroid gland	1.12	TNS	1.33[a]	1.24[a]	1.2[a]	1.11[a]	BLQ	BLQ	BLQ

[a] Data point used to determine half-life ($t_{1/2}$).
BLQ, below limit of quantitation (mean 0.50 ± 0.17 μg-eq/g; range 0.17–0.82 μg-eq/g); H, high tissue concentrations within an organ; L, low tissue concentrations within an organ; NA, not applicable; NS, no sample, TNS, tissue not sampled due to quality or size.

adrenal gland, eye, liver (high), skin and testes which continued to have low concentrations of radioactivity. Throughout the time course of this study, the uveal tract in the eye contained approximately 3 μg-eq/g, which continued through the 96 h time point. Qualitative evaluation determined that urine within the urinary bladder contained high levels of radioactivity at 1–12 h postdose.

Half-lives and $AUC_{0-\infty}$ in several tissues were not calculated due to the shape of the concentration–time curve, which made these calculations

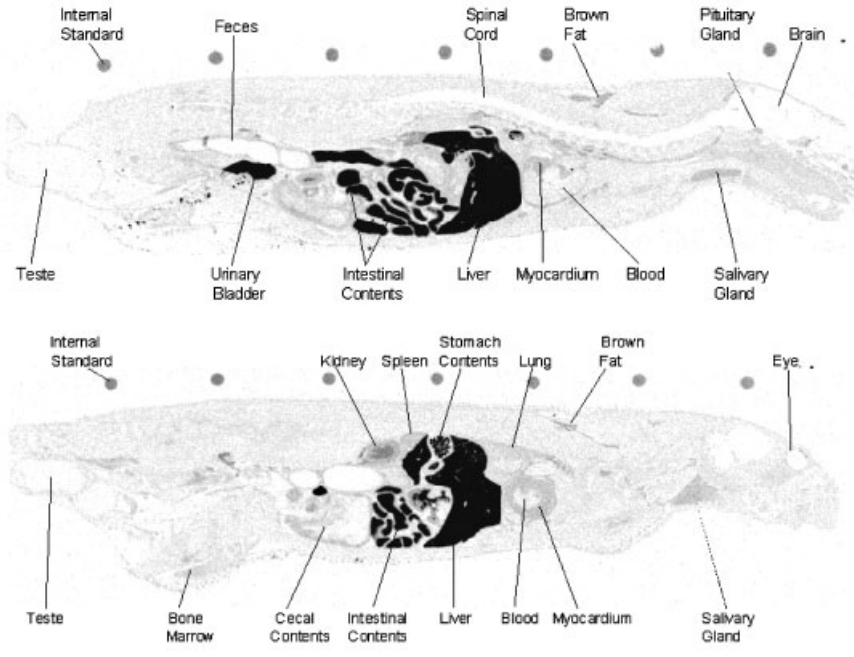


Figure 1. Autoradiogram 1 h after oral administration of 5 mg/kg of 3-[^{14}C]

either incalculable or inaccurate. Pharmacokinetic parameters indicated that radiocarbon concentrations in tissues had estimated terminal (β-phase) half-lives ranging from approximately 6 h in cecal contents to approximately 332 h in the liver (Table 2). AUC concentrations were greatest in cecal and large intestinal contents. The highest projected effective doses were 0.19, 0.19 and 1.67 mRem for thymus, pituitary gland and liver, respectively (Table 3). The total effective dose was approximately 2.6 mRem.

Results

We have described the synthesis of the C-14-labeled isotopomer of **3** (**3-[^{14}C]**) in 22.3% radiochemical yield by a circuitous four step sequence (three radioactive steps) from **3**. The specific activity was 20.61 μCi/mg; the radiochemical purity (RCP) was 99.3% by TLC/autoradiography on silica gel, (CH_2Cl_2/CH_3OH/NH_4OH, 80:20:1) and 97.6% by radio-HPLC (no distinct impurities were detected; the actual RCP is probably higher as there was significant tailing observed) using the following gradient HPLC system on a Inertsil ODS-3 column (4.6 × 250 mm):

Solvent A: 50 mM K_2HPO_4/CH_3OH/CH_3CN (90:5:5).

Solvent B: 50 mM K_2HPO_4/CH_3OH/CH_3CN (50:25:25).

Table 2. Estimated pharmacokinetic parameter from selected tissues in male Long Evans rats following a single oral dose of 5 mg/kg of 3-[^{14}C] administered as the succinate salt

	Pharmacokinetic parameter (units of measure)				
Tissue	AUC_{0-t} (μg-eq · h/g)	$AUC_{0-\infty}$ (μg-eq · h/g)	C_{max} (μg-eq/g)	T_{max} (h)	Half-life (h)
Adrenal gland[a]	149.64	404.57	1.98	24	136.48
Blood	2.10	NC	1.11	3	NC
Bone marrow	2.17	NC	1.11	3	NC
Brown fat	60.98	123.36	1.99	8	45.75
Cecal contents	1570.43	1576.57	117.31	12	5.97
Cecal wall	33.03	201.22	1.69	3	86.96
Epididymis	9.81	NC	1.14	3	NC
Eye	292.26	NC	6.71	12	NC
Harderian gland	13.42	NC	1.30	3	NC
Kidney[a]	93.09	NC	7.53	3	NC
Large int. cont.	1662.26	1674.16	150.73	8	6.42
Large int. wall	32.30	82.97	1.85	3	31.72
Liver[a]	121.44	629.52	3.93	1	331.61
Lung	13.03	120.54	1.25	6	64.93
Muscle	11.54	NC	1.11	8	NC
Myocardium	9.67	52.41	1.47	3	22.93
Pancreas	13.42	40.55	1.35	6	17.92
Pituitary gland	58.44	200.84	1.48	3	92.65
Preputial gland	12.64	30.67	1.89	6	11.09
Salivary Gland[a]	35.42	133.19	2.03	6	52.48
Skin	111.51	NC	1.28	12	NC
Small int. cont.	339.43	434.07	78.70	6	57.73
Small int. cont.	31.77	NC	2.68	1	NC
Spleen	13.07	70.80	1.25	6	36.19
Stomach contents	100.88	NC	27.54	3	NC
Stomach wall	14.89	40.85	1.65	3	16.13
Testes	100.43	NC	1.11	96	NC
Thymus	7.61	75.93	1.15	3	44.93
Thyroid gland	27.44	152.73	1.33	6	78.92

[a] High tissue concentration value from Table 1 used for calculations.
NC, not calculated.

Table 3. Projected effective dose in man following a single oral dose of 100 μCi of 3-[^{14}C] administered as the succinate salt

Tissue	Effective dose (mRem)
Adrenal gland	0.10
Liver	1.67
Lung	0.14
Pancreas	0.04
Pituitary gland	0.19
Salivary gland	0.10
Stomach wall	0.13
Thymus	0.19
Thyroid gland	0.06
Total effective dose (mRem)	2.61

Time (min)	Solvent A	Solvent B
0	100	0
10	100	0
40	0	100
50	0	100
52	100	0
65	100	0
70	100	0

This method was ideally suited for a potential preparation of material for use in a human C-14 study. Because **3** was used as the starting material, it would have been possible to launch the c-GMP synthesis of **3-[^{14}C]** from readily available c-GMP-qualified starting material.

We have also discussed the use of **3-[^{14}C]** in the determination of its tissue distribution after oral dosage in Long Evans rats using QWBA. Based on the autoradiograms (Figure 1), it was evident that following a single oral dose of 5 mg/kg, radiocarbon associated with **3-[^{14}C]** and/or its metabolites was widely distributed to tissues 1 h after dosing. The phosphor images and radiocarbon concentrations indicated that radioactivity was in highest concentrations in the gastrointestinal contents and urine throughout most of the time course and declined over time. Peak radiocarbon concentrations in most tissues occurred between 3 and 6 h postdose and decreased over time with levels no longer detectable in most tissues by 24 h postdose. The total effective dose of 2.61 mRem was approximately 138-fold below the average whole-body radiation exposure of 360 mRem per year that a person in the US receives from natural and man-made sources.[14] Radiation dosimetry calculations indicated that a single oral 100 μCi dose of **3-[^{14}C]** to man would not be expected to represent a significant radiation exposure risk.

Experimental

NMR spectra were obtained on a General Electric QE-300 or a Varian 500 MHz nuclear magnetic resonance spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Mass spectra were recorded on a Nermag R30-10 triple stage quadrapole mass spectrometer (DCI), a VG Analytical VG-ZAB3F mass spectrometer (FAB), a Varian Associates MAT 731 mass spectrometer (FD) or a Waters Micromass ZQ single quadrupole mass spectrometer (ES-MS). Flash chromatography was performed on silica gel. RCP was assessed by HPLC with radiochemical detection on a Packard Flow Scintillation Analyzer. RCP was further assessed by TLC-autoradiography. The radioactivity was detected on Kodak X-ray

film BB-5; the lanes of the TLC were cut, mixed with methanol and scintillation fluid and counted. Microanalyses were conducted in the Physical Chemistry Department of the Lilly Research Laboratories. The carboxymethylcellulose used in the QWBA studies was purchased from Sigma. The cryomicrotome used to section the frozen carcasses was a Leica Model CM3600. The commercial radiocarbon standards were from American Radiolabeled Chemicals. The phosphor plates were from Molecular Dynamics as were the model 410A Image Eraser and the model Storm 860 PhosphorImager. Scanner operations, data display and analysis were performed using ImageQuant (Molecular Dynamics) and Microsoft Excel software.

Synthesis of N-[[2-[(1-methyl-4-piperidinyl)-carbonyl-3-formylamino]]-pyridine]propionamide (**6**)

A solution of sodium *meta*-periodate (8.98 g, 42 mmol) in water (120 ml) was added dropwise to a solution of **3** (3 g, 10.5 mmol) in methanol (120 ml) at room temperature. After the addition was complete, stirring was continued for 63 h. The resulting mixture was poured into water/$NaHCO_3$ and extracted with ethyl acetate (3×200 ml). The combined EtOAc extracts were washed with saturated brine (2×50 ml), dried (anhydrous $MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (60×150 mm), eluting with 10 ml fractions of $CH_2Cl_2/CH_3OH/NH_4OH$ (80:20:1). Fractions 30–40 were combined and concentrated *in vacuo* to yield *N*-[[2-[(1-methyl-4-piperidinyl)-carbonyl-3-formylamino]]-pyridine]propionamide (**6**) as a yellow foam (0.451 g). Fractions 41–50 were combined and further purified as above to yield an additional 0.450 g of **6** in fractions 34–56. After mixing, these two purified lots of **6** were re-chromatographed as above to yield **6** (0.570 g, 16%) as an amber oil which crystallized upon standing: NMR (DMSO/d_6) δ 1.01 (3H, t, CH_3), 1.52 (2H, dq, 3′,5′-di-axial H), 1.71 (2H, bd, 3′,5′-di-equatorial H), 1.92 (2H, bt, 2′,6′-di-axial H), 2.11 (3H, s, *N*-CH3), 2.37 (2H, q, CH_2CO), 2.75 (2H, bd, 2′,6′-di-equitorial H), 3.72 (1H, tt, 4′-H), 8.22 (1H, d, H-5), 8.37 (1H, s, CHO), 8.76 (1H, d, H′-6), 10.35 (1H, s, amide NH, exchanges with D_2O), and 10.88 (1H, s, amide NH, exchanges with D_2O); ES-MS $[M + H]^+$, $m/z = 319$.

HR-FAB-MS: $[M + H]^+$; Analysis calculated for: 319.1756. Found: 319.0623.

Synthesis of N-[2-amino-3-hydroxy-3-(1-methylpiperidin-4-yl)-3H-pyrrolo[3,2-b]pyridin-5-yl-[2-^{14}C]]propionamide (7-[^{14}C])

A 19:1 ethanol/water (4 ml) solution of **6** (0.286 g, 0.9 mmol) was treated by the dropwise addition of potassium cyanide-[^{14}C] (50 mCi, 55 mCi/mmol, 0.9 mmol) in 2 ml of 19:1 ethanol/water to which 0.25 ml of water was added to

aid in dissolution. Stirring at room temperature was continued. After 3 h, TLC (silica gel, $CH_2Cl_2/CH_3OH/NH_4OH$, 80:20:1) showed that most of the starting material was consumed. TLC (silica gel, EtOAc/2-propanol/NH_4OH/H_2O, 25:15:2:2) showed a single spot ($r_f = 0.25$), which contained 97.5% of the radioactivity. This material is presumeably the intermediate amidine **7**. Stirring was continued for an additional 1 h and then concentrated *in vacuo* to yield *N*-[2-amino-3-hydroxy-3-(1-methylpiperidin-4-yl)-3*H*-pyrrolo[3,2-b]pyridin-5-yl-[2-^{14}C]]propionamide **(7-[^{14}C])** as a dark yellow foam. This material was used in the next step on an 'as is' basis.

Synthesis of N-[3-methylpiperidin-4-yl-2-[^{14}C])-1H-pyrrolo[3.2-b]pyridin-5-yl] proprionamide (3-[^{14}C])

A THF/HOAc (1:1, 8 ml) of **7-[^{14}C]** was chilled to 0–5°C and treated portionwise with $NaBH_4$ (0.129 g, 3.4 mmol). After the addition was complete, stirring was continued for 2 h and the reaction was then quenched by the careful addition of ca. 2 N HCl (4.25 ml). Stirring in the cold was continued (2 h) and then the reaction mixture was made basic by the addition of 5 N NaOH. The mixture was then poured into saturated aqueous Na_2CO_3 and extracted with EtOAc (4×25 ml). The combined extracts were washed with saturated aqueous brine, dried (anhydrous $MgSO_4$), and concentrated *in vacuo* (0.155 g, 60%). This material was purified by flash chromatography on silica gel (10×150 mm) eluting with 10 ml fractions of $CH_2Cl_2/EtOH/NH_4OH$ (80:20:1). Fractions 4–7 were combined and concentrated *in vacuo* to yield 3-[(*N*-methyl)-1,2,3,4,5,6-hexahydropyrid-4-yl]-5-[(*N*-propionyl)amino]-pyrrolo[3,2-*b*]pyridine-[2-^{14}C] (**3-[^{14}C]**, 0.121 g, 47%).

Synthesis of N-[3-methylpiperidin-4-yl-2-[^{14}C])-1H-pyrrolo[3.2-b]pyridin-5-yl] proprionamide, succinate salt (3-[^{14}C], succinate salt)

A mixture of **3-[^{14}C]** (0.121 g, 0.42 mmol) and succinic acid (0.063 g, 0.53 mmol) was suspended in a mixture of acetone (0.83 ml) and 2-propanol (0.36 ml) and the mixture was warmed to reflux with stirring for 30 min. The mixture was allowed to cool to room temperature and then seeded with a seed crystal of **3** succinic acid salt and allowed to crystallize. Stirring was continued for 1.5 h. The resulting white solid was collected by filtration, washed with fresh acetone and dried *in vacuo* for 24 h to yield *N*-[3-methylpiperidin-4-yl-2-[^{14}C])-1*H*-pyrrolo[3.2-b]pyridin-5-yl]proprionamide, succinate salt (**3-[^{14}C]** succinate salt, 0.122 g, 67%). A mixture of **3-[^{14}C]** succinate salt (0.122 g) and **3** succinate salt (0.122 g) was suspended in a mixture of acetone (1.69 ml) and 2-propanol (0.73 ml). The mixture was heated to reflux and acetone/2-propanol (2:1) was added dropwise until all of the solid was in solution. The solution was allowed to cool to room temperature and seeded, whereupon the material crystallized. After stirring for 2 h, the white solid was collected

by filtration, washed with acetone and dried *in vacuo* for 24 h to yield *N*-[3-methylpiperidin-4-yl-2-[^{14}C])-1*H*-pyrrolo[3.2-b]pyridin-5-yl]proprionamide, succinate salt (**3-[^{14}C]** succinate salt, 0.173 g, 71%); specific activity = 59.67 μCi/mg; RCP (by TLC/autoradiography on silica gel, CH_2Cl_2/EtOH/NH_4OH, 80 : 20 : 1) = 99.5%.

In order to complete dilution to the desired specific activity, the above material (0.173 g, 10 333 μCi) was mixed with **3** succinate salt (0.343 g) and dissolved in methanol. This solution was concentrated, redissolved in 2-propanol (1.54 ml)/acetone (3.58 ml), and warmed to reflux with stirring. The stirred solution was allowed to slowly cool to room temperature as the desired material began to crystallize. The resulting mixture was stored in the freezer overnight and then collected by filtration. The white solid was washed with chilled acetone, and dried *in vacuo* to yield *N*-[3-methylpiperidin-4-yl-2-[^{14}C])-1*H*-pyrrolo[3.2-b]pyridin-5-yl]proprionamide, succinate salt (**3-[^{14}C]** succinate salt, 0.416 g, 81%); specific activity = 20.61 μCi/mg; RCP (by TLC/autoradiography on silica gel, CH_2Cl_2/CH_3OH/NH_4OH, 80 : 20 : 1) = 99.3%; RCP (by radio-HPLC) = 97.6% (no distinct impurities were detected; the actual RCP is probably higher as there was significant tailing observed) by the following gradient HPLC system on a Inertsil ODS-3 column (4.6 × 250 mm):

Solvent A: 50 mM K_2HPO_4/CH_3OH/CH_3CN (90:5:5).

Solvent B: 50 mM K_2HPO_4/CH_3OH/CH_3CN (50:25:25).

Time (min)	Solvent A	Solvent B
0	100	0
10	100	0
40	0	100
50	0	100
52	100	0
65	100	0
70	100	0

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