Published in final edited form as:

Nucl Med Biol. 2010 November ; 37(8): 989–996. doi:10.1016/j.nucmedbio.2010.06.002.

Synthesis and In Vivo Brain Distribution of Carbon-11 Labeled δ -Opioid Receptor Agonists¹

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Abstract

Three new radiolabeled compounds, [¹¹C]SNC80 ((+)-4-[(α R)- α -(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl]-3-[(¹¹C)methoxybenzyl-N,N-diethylbenzamide), *N,N*-diethyl-4-[3-methoxyphenyl-1-[(¹¹C)methylpiperidin-4-ylidenemethyl]benzamide, and *N,N*-diethyl-4-[(1-[(¹¹C)methylpiperidin-4-ylidene)phenylmethyl]benzamide, were prepared as potential in vivo radiotracers for the δ opioid receptor. Each compound was synthesized by alkylation of the appropriate desmethyl compounds using [¹¹C]methyl triflate. In vivo biodistribution studies in mice showed very low initial brain uptake of all three compounds, and no regional specific binding for [¹¹C]SNC80. A monkey PET study of [¹¹C]SNC80 confirmed low brain permeability and uniform regional distribution of this class of opioid agonists in a higher species. Opioid receptor ligands of this structural class are thus unlikely to succeed as in vivo radiotracers, likely due to efficient exclusion from the brain by the *P*-glycoprotein efflux transporter.

Keywords

Opioid; Tomography, emission computed; SNC80 carbon radioisotopes

Introduction

Opioid receptors are widely distributed throughout the central nervous system. Opioid receptors are implicated in Alzheimer's disease, chronic pain states, movement of disorders, and psychiatric illnesses [1]. Each of the three opioid receptors, μ , δ and κ , possesses a unique pharmacological profile. μ Receptors have high affinity for morphine and related compounds and δ receptors have highest affinity for the enkephalins. κ Opiate receptors are distinguished by their high affinity for dynorphins and certain benzomorphan synthetic

¹Presented this work at the 222nd American Chemical Society National Meeting, Chicago, IL August 26-30, 2001.

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opioids. From the data of in vitro autoradiographic studies the μ receptors are concentrated in the thalamus and grey matter; δ receptors are localized in the basal ganglia, and κ receptors in the cerebral cortex and amygdale. But the level of non-specific binding cannot be predicted from the in vitro data. Peptides are known to have limited permeability in blood brain barrier and poor oral bioavailability. For a long period of time, there were only peptides available for δ receptors. This difficulty was finally overcome by the discoveries of non peptide δ receptors antagonists such as naltrindole in 1988 and agonists BW373U86, a methyl ether derivative of (+) BW373U86 and which was known as SNC 80. Agonists at the δ opioid receptor are antinociceptive in animal models of pain, yet lack the undesirable side effects associated with μ opioid analgesics [2,3]. Naltrindole is a highly potent, highly selective δ opioid receptor antagonist. [^{11}C]Methylnaltrindole is the first radioligand which binds to δ -opioid receptors in vivo in mice, and showed promise for PET studies of δ -opioid sites in human brain [4]. The δ opioid antagonist naltrindole has been reported to suppress the development of morphine tolerance and physical dependence and to block some of the behavioral effects of cocaine [5,6]. Naltrindole has also exhibited immunosuppressant properties and is thus of potential utility in organ transplantation [7,8]. The nonpeptide benzhydrylpiperazine BW373U86 is selective for δ -opioid receptors. This ligand BW373U86 binds to small cell lung cancer cells [9]. In appropriately radiolabeled forms both δ receptor agonists and antagonists would have applications in imaging studies of pain mechanisms and the involvement of opioid receptor types in drug abuse and therapy.

Although a number of opioid receptor ligands have been radiolabeled with gamma- or positron-emitting radionuclides [10,11] and [^{11}C]carfentanil is used as a μ -selective radioligand in human positron emission tomography (PET) studies, there has been limited success at developing type-specific radioligands for the other δ and κ opioid receptors types. In rat brain, carfentanil is 90 and 250 times more selective for μ opiate receptors compared with δ and κ opiate receptors, respectively. With [^{11}C]carfentanil, high concentrations of radioactivity were observed in the basal ganglia and thalamus and low concentration in the cerebellum and occipital cortex. Previous attempts to provide δ selective PET ligands have centered on methylnaltrindole [12,13] and indolic-N substituted analogues [14]. While displaying good selectivity for the δ receptor in vitro, ligands based on the naltrindole structure have not found widespread use due to poor pharmacokinetics and high non-specific binding.

As peptide ligands for the δ receptor, even if exhibiting high affinity and selectivity, are unlikely to be successful as in vivo radiopharmaceuticals. Peptide δ agonists have limited access to central targets. BBB is the most important barrier involved in the regulation of molecules accessing the brain. Peptides may not cross the blood-brain barrier (BBB) for many reasons, the main ones being rapid degradation, large molecules, protein binding, and tight adhesion to the endothelial cells of the BBB. The brain is highly vascularised, containing a very intricate network of capillaries. The endothelial cells that form the brain capillaries are sealed together by junctions, and have no openings. Our synthetic efforts have centered on a series of non-peptide compounds reported to have high affinity and good selectivity for the δ opioid receptor [15,16,17,18]. *N*-benzhydrylpiperazines and 4-(diarylmethylene)piperidines, the best known and characterized of which is SNC80 [18], are agonists for the δ opioid receptor with a wide range of affinities (K_i values from 0.1 to >1000 nM), and generally with good to excellent selectivity over the μ and κ receptor binding sites (K_i values 200 to >10000 nM). We report here the synthesis and initial in vivo evaluation of carbon-11 labeled SNC80 and two structurally related compounds as potential ligands for the δ receptor (Fig-1).

2. Materials and Methods

2.1. General

SNC80 was obtained from Tocris Chemical, Bristol, UK. All other chemicals and solvents were purchased from Aldrich Chem. Co. or Fisher Chem. Co. and were used without further purification. Melting points are reported but uncorrected. ¹H NMR spectra were recorded on Bruker 500 NMR instrument, and low resolution mass spectra and high resolution fast atom bombardment mass spectra (FAB-MS) were obtained on a VG 70-250-S spectrometer. Column chromatography was done on silica gel, Merck grade 230-400 mesh, 60 Å.

2.2. Synthesis

2.2.1. (+)-4-[(αR)-α-((2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl)-3-hydroxybenzyl]-N,N-diethylbenzamide [(+)-4,(+)-BW373U86] 4—The compound **4** obtained according to a modified literature procedure [15]. To a cooled (−50° C) solution of **1** (1.0 g, 2.32 mmol) in CH₂Cl₂ (30 mL) was added dropwise a 1.0 M solution of BBr₃ in CH₂Cl₂ (10.0 mL, 9.28 mmol) under nitrogen. After the addition was completed, the reaction temperature was brought into 0° C. After stirring at 0° for 4 h, the reaction mixture was quenched by dropwise addition of absolute methanol (30 mL) at −78° C. The volatile materials were removed under reduced pressure. The residue was treated with 15% NH₄OH solution followed by addition of 1M HCl until the pH of the solution was adjusted to 9. The mixture was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layers were dried (MgSO₄) and concentrated under reduced pressure to give a residue which was purified by preparative TLC to furnish **4** (0.34 g 34%), ¹H NMR (CDCl₃) δ 7.38 (d, J = 8.0 Hz, 2H, ArH), 7.22 (d, J = 8.0 Hz, 2H, ArH), 7.05-7.18 (m, 1H, ArH), 6.95 (m, 2H, ArH), 6.68 (m, 1H, ArH), 5.77-5.94 (m, 1H, C=CH), 5.11-5.26 (m, 2H, C=CH₂), 4.9 (s, 1H, CHN), 3.23-3.62 (m, 5H), 2.54-2.95 (m, 5H), 2.12 (brt, 1H), 1.92 (brt, 1H), 1.16-1.30 (m, 9H), 1.12-1.21 (m, 3H).

2.2.2. Synthesis of 4-(4-N',N'-Diethylaminocarbonylbenzoyl)-N-tert-butoxycarbonylpiperidine (5)—This compound was obtained by reaction of 4-iodo-*N,N*-diethylbenzamide, *tert*-butyllithium and *N-tert*-butoxycarbonyl-*N'*-methyl-*N'*-methoxyisonepicotamide following the procedure of Wei et al [17] with minor modifications. Compound **5** (4 g, 34%) was isolated as yellow oil: ¹H NMR (CDCl₃) δ 7.96 (d, J = 8 Hz, 2H, ArH), 7.46 (d, J = 8 Hz, 2H, ArH), 4.17 (brs, 2H), 3.57 (brs, 2H), 3.38 (m, 1H), 3.23 (brs, 2H), 2.90 (m, 2H), 1.83 (m, 2H), 1.68 (m, 2H), 1.47 (s, 9H), 1.26 (brs, 3H, amide-Me), 1.11 (brs, 3H, amide-Me).

2.2.3. N,N-Diethyl-4-[(N-tert-butoxycarbonylpiperidin-4-yl)-3-methoxyphenylhydroxymethyl]benzamide (6)—This compound was obtained by reaction of 3-bromoanisole, *tert*-butyl lithium and 4-(4'-*N,N'*-diethylaminocarbonylbenzoyl)-*N-tert*-butoxycarbonylpiperidine **5**. Purification by silica gel column chromatography yielded pure compound **6** (0.56 g, 58% yield); mp 100°C (lit^[17]. 95-103° C); ¹H NMR (CDCl₃) δ 7.49 (d, J = 6.32 Hz, 2H, ArH), 7.31 (d, J = 6.76 Hz, 2H, ArH), 7.23 (d, J = 7.82 Hz, 1H, ArH), 7.05 (s, 1H, ArH), 7.01 (d, J = 6.85 Hz, 1H, ArH), 6.74 (m, 1H, ArH), 4.13 (brs, 2H, piperidine CH-), 3.78 (s, 3H, OCH₃), 3.52 (brs, 2H, CH₃-CH₂-N), 3.24 (brs, 2H, CH₃CH₂-N-), 2.69 (brs, 2H, piperidine CH-), 2.51 (m, 1H, piperidine CH), 1.43 (s, 9H, CH₃C), 1.30 (m, 4H, piperidine CH), 1.22 (brs, 3H, CH₃-CH₂-N-), 1.10 (brs, 3H, CH₃-CH₂N).

2.2.4. N,N-Diethyl-4-(3-methoxyphenylpiperidin-4-ylidenemethyl)benzamide (7)—The product obtained from reaction of **6** and trifluoroacetic acid according to the procedure of Wei et al [17] with minor modifications. Compound **7** (0.31 g, 81%) isolated as

a glass which failed to crystallize (lit^[18] 7.HCl, lyophilized, mp 83-85). ¹H NMR (CDCl₃) δ 7.26-7.31 (m, 4H, ArH), 7.14 (m, 1H, ArH), 6.64-6.78 (m, 3H, ArH), 3.77 (s, 3H, OMe), 3.48 (bs, 2H, CONCH₂), 3.21 (bs, 2H, CONCH₂), 2.99 (m, 4H, CH₂-N), 2.42 (m, 4H, CH₂-C=C), 1.99 (bd s, NH), 1.12-1.23 (m, 6H, 2CH₃, amide-Me). HR-FAB-MS (m/e) calcd. for C₂₄H₃₁N₂O₂ (M+H) 379.2385. Found 379.2386.

2.2.5. 4-(4'-N',N'-diethylaminocarbonylbenzoyl)piperidine (8)—A mixture of 4-(4-N,N'-diethylaminocarbonylbenzoyl)-N-tert-butoxycarbonylpiperidine **5** (2.55 g, 6.57 mmol), trifluoroacetic acid (40 mL) and dry CH₂Cl₂ (30 mL) was stirred at room temperature under nitrogen for overnight. The reaction was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (100 mL). The solution was washed with 50% NaOH (10 mL), dried over MgSO₄ and concentrated. The desired compound **8** (0.9 gr, 50%) was obtained as an amber oil and used for next step without further purification. ¹H NMR (CDCl₃) δ 7.96 (d, J = 10 Hz, 2H, ArH), 7.46 (d, J = 10 Hz, 2H, ArH), 3.70 (brs, 2H), 3.55 (brs, 2H), 3.40 (brs, 1H), 3.18 (brs, 2H), 2.72 (m, 2H), 1.88 (m, 2H), 1.71 (m, 2H), 1.26 (brs, 3H, amide-Me), 1.11 (brs, 3H, amide-Me).

2.2.6. 4-(4'-N',N'-diethylaminocarbonylbenzoyl)-N-methylpiperidine (9)—A solution of amine **8** (0.90 g, 3.12 mmol) in formic acid (4 mL) was treated with formaldehyde aqueous solution (37%, 6 mL) and stirred at 85° C for 16 hours. The reaction mixture was cooled to 0°C, made basic with aqueous 50% NaOH, and extracted with dichloromethane. The combined organic layers were dried over MgSO₄ and concentrated. The crude product was filtered through a short silica gel column, eluting with CHCl₃:MeOH:NH₄OH (100:10:1) to give amber oil **9** (0.65 g, 70%). ¹H NMR (CDCl₃) δ 7.95 (d, J = 10 Hz, 2H, ArH), 7.46 (d, J = 10 Hz, 2H, ArH), 3.56 (brs, 2H, CH₂-amide), 3.21 (brs, 2H, CH₂-amide), 3.20 (s, 1H, CH-CH₂), 2.95 (m, 2H), 2.31 (s, 3H, N-Me), 2.08 (m, 2H), 1.84-1.88 (m, 4H), 1.26 (brs, 3H, CH₃-amide), 1.10 (brs, 3H, CH₃-amide). HR-FAB-MS (m/e) calcd. for C₁₈H₂₆N₂O₂ (M+) 302.1994. Found 302.2000.

2.2.7. N,N-Diethyl-4-[3-methoxyphenyl-1-methylpiperidin-4-ylidenemethyl]benzamide (2)—n-BuLi (2.5 M, 0.36 mL, 0.91 mmol) was added to 3-bromoanisole (0.14 g, 0.78 mmol) in THF (3 mL) at -76° C with stirring under nitrogen. After 1 min, the ketone **9** (0.22 g, 0.73 mmol) in THF (3 mL) was added at the same temperature. The reaction mixture was stirred at room temperature overnight. The reaction mixture was poured into saturated ammonium chloride and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO₄ and concentrated. The crude product N,N-diethyl-4-[1-methylpiperidiyl]-3-methoxyphenylhydroxymethylbenzamide **13** was stirred overnight in CH₂Cl₂ (8 mL) and trifluoroacetic acid (4 mL) at room temperature. The reaction mixture was then concentrated, the residue dissolved in CH₂Cl₂, washed with 1 N NaOH and brine, dried over MgSO₄ and concentrated. The residue was purified silicagel flash column chromatography (CHCl₃:MeOH, NH₄OH, 100:8:1) to provide cold standard **2** as colorless oil. ¹H NMR (CDCl₃) δ 7.14-7.30 (m, 5H, ArH), 6.66-6.77 (m, 3H, ArH), 3.77 (s, 3H, OMe), 3.53 (brs, 2H, CH₂-NCO), 3.28 (brs, 2H, CH₂NCO), 2.39-2.44 (m, 8H, CH₂-N, piperidine-H), 2.29 (s, 3H, N-Me), 1.25 (s, 3H, amide-Me), 1.12 (s, 3H, amide-Me). HR-FAB-MS (m/e) calcd. for C₂₅H₃₃N₂O₂ (M+H) 393.2542. Found 393.2542.

2.2.8. 4-Benzoyl-N-tert-butoxycarbonylpiperidine (10)—This compound was prepared according to the reported procedure with minor modifications [17]. The compound was prepared (9.05 gr, 98%) from readily available 4-benzoylpiperidine hydrochloride (6.80 g, 30 mmol) and di-tert-butyl dicarbonate (7.21 g) and used without further purification.

2.2.9. 4-(α -Hydroxy- α -(4-*N*-tert-butoxycarbonylpiperidinyl)benzyl)-*N,N*-diethylbenzamide (11**)**—This compound was prepared from **10** by an improvement of the reported procedure [17]. Obtained from reaction of *n*-butyllithium, 4-iodo-*N,N*-diethylbenzamide and 4-benzoyl-*N*-tert-butoxycarbonylpiperidine **10** to give crystalline **11** (0.645 g, 36%), mp 102 °C (lit^[17] 103°C).

2.2.10. *N,N*-Diethyl-4-(phenylpiperidin-4-ylidene-methyl)benzamide (12**)**—Reaction of 4-(α -hydroxy- α -(4-*N*-tert-butoxycarbonylpiperidinyl)benzyl)-*N,N*-diethylbenzamide **11** with trifluoroacetic acid gave **12** by following the procedure of Wei et al [17]. The compound **12** (0.15 g, 55%) which was treated with HCl in ether to furnish hydrochloride salt, mp 114° C (lit^[17] 110-120° C).

2.2.11. *N,N*-Diethyl-4-[(1-methylpiperidin-4-ylidene)phenylmethyl]benzamide (3**)**—To a solution of **12** (17 mg, 0.04 mmol) in formic acid (500 μ L) was added aqueous (37%) formaldehyde solution (80 μ L) at 0° C. The reaction mixture was stirred at 80 C for 16 hours. The reaction mixture was cooled to 0° C, made basic with 50% aqueous NaOH solution, and extracted with dichloromethane. The combined organic layers were dried over MgSO₄ and concentrated. The crude product was filtered through a short silica gel column, eluting with CHCl₃:MeOH:NH₄OH (100:10:1) to give brown oil **3** (12 mg, 82%). Compound **3** (17 mg) which was converted into HCl salt by treating with HCl in ether, mp 112 °C (lit^[17] 110° C dec). ¹H NMR (CDCl₃) δ 7.11-7.30 (m, 9H, ArH), 3.53 brs, 2H, CH₂-amide), 3.28 (brs, CH₂-amide), 2.43 (m, 8H, piperidine-H), 2.29 (s, 3H, N-Me), 1.12-1.25 (m, 6H, amide-CH₃). HR-FAB-MS (m/e) calcd. for C₂₄H₃₀N₂O (M⁺) 362.2358. Found 362.2347.

2.3. Radiochemistry

The following was the general procedure for carbon-11 labeling of compounds **1**, **2** and **3**. [¹¹C]Carbon dioxide was produced by the ¹⁴N(p, α)¹¹C reaction in a nitrogen target and converted to [¹¹C]methyl triflate by the method of Jewett [19]. The [¹¹C]methyl triflate was distilled via a stream of nitrogen gas 30 mL/minute into a solution of 1 mg of the appropriate precursor (**4**, **7**, **12**) dissolved in 0.1 mL DMSO. When transfer was complete the reaction mixture was diluted with 4 mL NH₄OH solution (1 mL conc. NH₄OH in 100 mL water). This solution was passed through an Empore SDBXC extraction disk. The disk was washed 2 times with 4 mL of the above NH₄OH solution and then with 2 mL water, and then dried briefly using the nitrogen flow. The products were eluted from the disks with 0.7 mL ethanol and then diluted with water to a volume of 10 mL.

Quality control analyses were done using HPLC and the following system: BetaBasic-8, Keystone Scientific, dimensions = 150 $\times$ 4.6 mm, 5 μ m, particle size 150 Å; solvent water: acetonitrile 600:400 (containing 0.1% of ammonium acetate, 1.0 gram); flow rate 1.5 mL/min. Retention times (r.t.) for the desired products and precursors were as follows: compound **1** (SNC80) r.t. = 7.36 min, precursor = 5.8 min; compound **2** = 6.14 min, precursor = 4.24 min; compound **3** = 6.24 min, precursor = 4.15 min). Specific activities of all isolated products exceeded 500 Ci/mmol at time of delivery for animal studies. The reported log P and Log D of the compound **1** is 4.7 at 25° C and 3.28 at pH 7. The reported Log P and Log D of the desmethyl precursor compound **7** is 4.25 and 1.33 at pH 7. The reported log P and log D of the compound **3** is 4.66 at 25° C and 2.77 at pH 7 (Source: SciFinder Scholar-compound properties).

2.4. Mouse Brain Distribution Study

All studies using vertebrate animals were approved by the University of Michigan Committee on the Use and Care of Animals (IACUC). Studies were done in CD1 mice (25

g: Charles River). Groups of four mice were anesthetized with diethyl ether and injected with ~ 200 μ Ci of **1**, **2** or **3** through the tail vein. At 2, 20 or 40 min after injection, animals were killed, a blood sample obtained, and the brain removed by blunt dissection. For compound **1** the brains were further dissected into samples of striatum, cortex, cerebellum, hippocampus, hypothalamic region and thalamus. Blood and brain tissue samples were weighed and radioactivity measured with a Packard autogamma scintillation counter. Data was calculated as % injected dose/g tissue and % injected dose/brain.

2.5. Monkey PET study

A single PET study was done in an adult m. nemistrina monkey (6.5 kg). The animal was anesthetized using ketamine (10 mg/kg) and xylazine (0.5 mg/kg) and maintained under 1% -1.5% isoflurane during the entire scanning session. Positioning in the PET scanner (TCC 4600A; in-plane resolution 7.5 mm FWHM, axial 9.5 mm FWHM after custom collimators installed) was aided by first imaging the brain following an intravenous bolus injection of oxygen-15 labeled water (2-5 mCi). The [^{15}O] water images were reconstructed and used as an aid to correctly position the animal within the field of view of the scanner. Carbon-11 labeled **1** was administered as an intravenous bolus (12 mCi in 1 mL, administered over 30 seconds) and the animal imaged for 60 minutes using short early scan times (6 frames of 30 seconds) progressing to 5 minute frames from 40-60 minutes.

3. Results and Discussion

3.1 Chemical syntheses

The syntheses of the precursors to compounds [^{11}C]**2** and [^{11}C]**3**, as well as authentic standards of these two compounds, followed the general synthetic route reported by Wei et al [17], with minor modifications. The synthesis of the precursor to compound [^{11}C]**2** is shown in Fig-3 and involved the reaction of 4-(4-*N*, *N*-diethylaminocarbonylbenzoyl)-*N*-*tert*-butoxycarbonylpiperidine **5** with 3-methoxyphenyllithium (generated immediately prior from 3-bromoanisoole and *tert*-butyllithium). The intermediate tertiary alcohol **6** was then dehydrated to the desired precursor **7** (Fig-3).

Synthesis of the authentic standard of **2**, which has not been previously reported, required first the deprotection of the t-BOC protected ketone, reductive alkylation with formaldehyde/formic acid to provide 4-(4-*N*,*N*-diethylaminocarbonylbenzoyl)-*N*-methylpiperidine **9**, followed by reaction with 3-methoxyphenyllithium and acid catalyzed dehydration (Fig-4).

Synthesis of the precursor to compound [^{11}C]**3** was slightly different. As shown in Fig-5, 4-benzoyl-*N*-*tert*-butoxycarbonylpiperidine was reacted with the organolithium reagent derived from 4-iodo-*N*,*N*-diethylbenzamide, followed by acid catalyzed dehydration of the intermediate tertiary alcohol **11** to furnish the precursor **12**. An authentic sample of **3** was then prepared by reductive methylation of the piperidine (Fig-6); this compound had been previously prepared [17] by alkylation of **12** with methyl iodide.

3.2. Radiochemical syntheses

The radiochemical synthesis of [^{11}C] SNC80, [^{11}C]**2** and [^{11}C]**3** utilized a general procedure of alkylation of the O- or N-desmethyl precursor with no-carrier-added [^{11}C] methyl triflate (Fig-7).

SDBXC is copolymer that was used as a reversed phase sorbent for solid phase extraction. This is not bonded to silica but it is 100% copolymeric particle that is spherical, porous and cross-linked for reversed-phase extractions. SDB-XC was used for the separation of

precursors from C-11 methylated products. The SDBXC provides for unique selectivity, especially in the retention of moderately polar compounds. Then change the pH of the eluting solvent so that only one of them ionized and eluted. Final products were obtained in excellent radiochemical and chemical purities using an Empore extraction disk. Specific activities of all products exceeded 500 Ci/mmol at time of delivery for animal studies. The yields of the [^{11}C]1, [^{11}C]2 and [^{11}C]3 were 15-20% (decay uncorrected).

3.3. Biological studies

3.3.1. Mouse brain distribution of [^{11}C] SNC80 (1)—Following i.v. injection of [^{11}C] SNC80, there was a low and uniform distribution of radioactivity throughout the mouse brain at 2 min after injection (Table 1). At 20 min regional brain concentrations were slightly (about 20%) lower, indicating some washout of radioactivity, but there continued to be a uniform regional distribution. At both times (2 and 20 min) brain concentrations were significantly lower than blood levels of radioactivity.

3.3.2. Mouse brain uptake of compounds [^{11}C] 2 and [^{11}C] 3—The whole brain uptake and retention of radioactivity for compounds [^{11}C] 2 and [^{11}C] 3 are shown in Table 2. Both radioligands showed low initial brain uptake. For both compounds brain concentrations at 2 and 20 or 40 minutes were much lower than radioactivity concentrations in the blood. Regional brain dissections were not performed.

3.3.3. Monkey brain PET imaging of [^{11}C] SNC80—Following intravenous injection of [^{11}C]SNC80, PET images of the brain showed a low initial uptake of radioactivity and a homogenous pattern that did not change throughout the 60 min scan session (data not shown).

The search for selective, non-peptide agonists for the δ opioid receptor led to the initial discovery of the diaryl piperazine (+/-)-BW373U86 and the morphinan (-)-TAN-67, followed shortly by synthesis of the potent and highly selective agonist SNC80 [15]. The complexity of the SNC80 structure, which has three asymmetric carbons and thus multiple potential stereoisomers, has led investigators to synthesize several series of more simplified compounds including *N,N*-diethyl(α -piperazinylbenzyl)benzamides, *N,N*-diethyl(α -piperidinylbenzyl)benzamides, and *N,N*-diethyl(α -piperidinylidenebenzyl)-benzamides [16,17,18]. Of the large number of such compounds already reported, many of which could be potentially labeled with carbon-11 or fluorine-18 for evaluation as in vivo radioligands for the δ opioid receptor, the *N,N*-diethyl (α -piperidinylidenebenzyl) benzamides are particularly appealing due to their simplicity and lack of chiral centers. Numerous compounds in the reported series have in vitro binding affinities in the low nanomolar or even sub-nanomolar range, and excellent selectivity (>100-fold) for δ receptors over μ and κ opioid sites [16,17].

As an initial study we synthesized [^{11}C]SNC80 as it exhibits both high affinity (δ receptor IC_{50} 1.31 nM) and highly selectivity (μ receptor IC_{50} = 320 nM, κ receptor IC_{50} = 2480 nM: [17]). The radiolabeled form was simply prepared by first removing the phenolic methyl group, purifying the demethylated product by preparative TLC then O-alkylation using [^{11}C] methyl triflate. Injection of this radioligand into the mouse produced a very low initial brain uptake of radioactivity, followed by some washout from the brain, and no regional variance in distribution of radioactivity at the early or later time point. We have utilized the 1 or 2 minute time point as a reasonable estimate of the blood brain permeability of new radiotracers, and have such data for more than fifty compounds of widely varying chemical structures, although not any resembling the structure of SNC80. The low uptake of [^{11}C] SNC80 is very poor compared to a well extracted radiotracer, such as 4-*N*-[^{11}C]

methylpiperidinol propionate [20], where initial brain extraction into the striatum is 9% ID/g at 2 minutes; uptake of [^{11}C] SNC80 is also poor compared to a highly lipophilic but still moderately well extracted radiotracer such as [^{11}C] rotenone (6% ID/g at 2 min) [21]. The initial brain uptake is only marginally better than that observed for a very polar and permanently ionized molecule, [^{11}C]paraquat, which showed an initial concentration of 0.2-0.4% ID/g in various brain regions at 1 minute after i.v. injection (Jewett and Kilbourn, unpublished observations).

The low brain uptake of [^{11}C] SNC80 was discouraging but not totally unexpected. Prior in vivo experiments with [^3H] SNC121, the tritiated derivative of SNC80 where the alkene bond had been converted to a propyl group by reductive tritiation, had also shown very low levels of radioactivity in the brain [22]. Furthermore, the radioactivity in both brain and other tissues was clearly a mixture of [^3H]SNC121 and the metabolic product resulting from O-demethylation. The identification of radiolabelled compounds was supported by HPLC. This would suggest that in the rodent there is very rapid metabolism of [^{11}C]SNC80, which if primarily by O-demethylation would result in rapid loss of the radiolabel into the one-carbon metabolic pool and low circulating levels of remaining authentic radioligand, which might potentially explain the low initial brain uptake. As there are always the possibilities of species differences in the rate and route of metabolism of drugs, and the chance one might miss a radiotracer that would be useful in man, a single study was completed using i.v. injection of [^{11}C] SNC80 into a monkey and brain imaging for one hour using PET. This also holds for compounds **2** and **3** nevertheless no monkey study was done with these compounds. Initial uptake of radioactivity into the monkey brain was very low compared to numerous other radiotracers we have examined in the past (e.g., 4-*N*-[^{11}C]methylpiperidinol propionate, [^{11}C]tetra benzine, [^{11}C]rotenone, [^{11}C]flumazenil, and others), just as was observed in the mouse, and a low and uniform brain distribution of radioactivity persisted for the full hour of PET imaging. Thus, the rodent data for [^{11}C] SNC80 appears predictive of what would be found in higher species.

The *N,N*-diethyl-4-phenylpiperidine-4-ylidenemethyl)benzamides, such as compounds **2** and **3**, can be viewed as simplified versions of SNC80, without the multiple asymmetric centers, exhibiting lower molecular weights and lower lipophilicities, and for compound **3** even further simplification by removal of the methoxy substituent as a potential site of metabolism. Compound **3** also is a high affinity (δ receptor $K_i = 11.7$ nM) and selective (μ receptor $K_i = 10000$ nM: [16]) δ receptor agonist. If the affinity of the drug for its target is moderate or low (i.e., $K_d > 10$ nM), the ratio B_{max}/K_d (receptor density divided by drug affinity) will also be low and interaction of the drug with its target will not be visible in PET studies. Although biological data had not been previously reported for compound **2**, the methoxy substituent is well tolerated in that position of the aromatic ring, as indicated by the relative affinities of compounds **12** (IC_{50} values: δ 0.87 nM, μ 3800 nM, κ 7470 nM) and **7** (IC_{50} values: δ 1.56 nM, μ 5250 nM, $\kappa > 10000$ nM) [17] which differ only by that aryl methoxy substituent. The carbon-11 labeled forms of **2** and **3** were prepared by following the published routes to synthesis of the required desmethyl derivatives, and *N*-alkylation of the piperidine nitrogens using [^{11}C] methyl triflate. Similarly, both radiotracers also showed very low initial brain uptake in the mouse following i.v. injection, equal or less than that observed for [^{11}C] SNC80. The low brain uptake discouraged us from performing extensive regional brain distributions, as it was felt the results would likely be meaningless.

The poor brain uptake of the fully simplified molecule, [^{11}C] **3**, indicates that the demethylation of a phenolic methoxy group was not the limitation on the brain uptake of [^{11}C] SNC80. Poor brain uptake appears a characteristic of this structural class of compounds. The reasons for the low brain uptake of [^{11}C] SNC80, [^{11}C] **2** and [^{11}C] **3** are not fully understood, but may apparently be due to the very efficient interaction of these

compounds with the brain *P*-glycoprotein efflux transporter (*Pgp*). *P*-glycoprotein, and which can restrict the entry of these three radioligands into the brain, resulting in a low occupancy of the brain. Subsequent to completion of our studies, Dagenais et al [23] reported that δ opioid agonists of the structure class investigated here, including [^3H]SNC121, are efficiently transported by the *P*-glycoprotein system, and brain uptake of such compounds greatly increased in *Pgp*-deficient (*mdr 1a*(-/-)) mice. The effects of the *Pgp* system on the in vivo effectiveness of other opioids are well known [24,25,26]. It would appear that the diarylalkylpiperidine structure, as exemplified in the radiolabeled compounds prepared here, unfortunately results in particularly good substrates for the brain *Pgp* system. Lipophilicity should be moderate (log *P* at pH 7.4 between +0.5 and +3.0, optimally +2.0) to ensure good cerebral uptake and low non-specific binding. Although SNC80 and related chemical structures can be easily prepared in carbon-11 form, this series of non-peptide opioid receptor agonists do not appear to be a viable option for the preparation of new in vivo imaging radiotracers.

Acknowledgments

This work was supported by grants from the National Institutes of Health (DA00254, MH47611), NIH training grant T32-CA09015 (to R.P.), and the Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG02-87ER60561.

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Figure 1.
Chemical Structure of delta-Opioid Receptor Ligands **1**, **2** and **3**

Figure 2.
Synthesis of labeling precursor **4** and reference compound **1**; (a) BBr₃

Figure 3.

Synthesis of labeling precursor 7: (b) $3\text{-CH}_3\text{OC}_6\text{H}_4\text{Li}$; (c) CF_3COOH

Figure 4. Synthesis of reference compound **2**: (c) CF_3COOH ; (d) HCOOH and HCHO ; (b) $3\text{-CH}_3\text{-OC}_6\text{H}_4\text{Li}$

Figure 5.
Synthesis of labeling precursor **12**: (e) 4-IC₆H₄CONEt₂ and BuLi; (c) CF₃COOH

Figure 6.
Synthesis of reference compound **3**: (d) HCOOH and HCHO

(f) $[^{11}\text{C}]$ -methyl triflate, DMSO

Figure 7.
Radiolabelling of precursors **4**, **7** and **12** with $[^{11}\text{C}]$ -methyl triflate, DMSO

Table 1Regional mouse brain distribution of [11C]SNC80 (**1**) following iv injection

	% ID/g		
	2 min	20 min	40 min
Striatum	0.98±0.08	0.81±0.11	0.73±0.09
Cortex	1.05±0.12	0.93±0.07	0.88±0.15
Cerebellum	1.18±0.15	1.00±0.13	0.80±0.12
Hippocampus	0.97±0.12	0.87±0.06	0.73±0.11
Hypothalamus	1.03±0.09	0.86±0.07	0.69±0.11
Thalamus	1.10±0.19	0.81±0.22	0.65±0.16
Pons/medulla	1.23±0.18	0.86±0.10	0.72±0.15
Blood	6.34±1.04	1.39±0.08	1.42±0.12

Data are given as mean SD for $n=4$.

Table 2

Whole brain and blood concentrations following iv injection of compounds [^{11}C]2 and [^{11}C]3. Data are given as mean $\pm$ S.D. for $N=4$.

	% ID/g	
	Brain	Blood
Compound 2		
2 min	0.42 $\pm$ 0.05	2.02 $\pm$ 0.18
40 min	0.22 $\pm$ 0.05	0.57 $\pm$ 0.09
Compound 3		
2 min	0.85 $\pm$ 0.10	1.66 $\pm$ 0.20
20 min	0.58 $\pm$ 0.08	0.79 $\pm$ 0.09