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Research Article

Synthesis and ^{124}I-labeling of *m*-iodophenylpyrrolomorphinan as a potential PET imaging agent for delta opioid (DOP) receptors

EYUP AKGÜN[1,*], PHILIP S. PORTOGHESE[1], MUNAWWAR SAJJAD[2] and HANI A. NABI[2]

[1]Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA
[2]Department of Nuclear Medicine, State University of New York, Buffalo, NY 14214, USA

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Abstract: Condensation of phenylazo-β-ketoamide **4** with oxymorphone **5** afforded an *m*-iodophenylpyrrolomorphinan (*m*-IPPM) **6** mediated by elemental zinc in acetic acid/sodium acetate buffer. *m*-IPPM **6** is a novel opioid receptor agonist ($K_i = 4.53$ nM for DOP) with high selectivity for DOP receptors. *m*-IPPM **6** was converted into the positron emitter *m*-[^{124}I]PPM **8** via the stannylated intermediate **7**. The final yield was 24.5 $\pm$ 1.9 % ($n = 6$) with a specific activity of 2.5 $\pm$ 1.2 Ci/μmol. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: *m*-IPPM; DOP receptor agonist; iodine-124; positron emission tomography

Introduction

The delta opioid (DOP) receptor is a member of the opioid (ORs) receptor family that comprises of MOP, DOP, KOP and NOP.[1–3] Opioid drugs which exert their effects predominantly via mu opioid (MOP) receptors[4] are the therapeutic of choice for treatment of acute and chronic pain. However, the long-term use of these drugs is limited due to severe side effects (tolerance, dependence, etc.). The development of kappa opioid (KOP) receptor agonists has waned because of dysphoric effects associated with KOP receptors.[5,6] Moreover, MOP and KOP receptor agonists have limited effectiveness in suppressing the allodynia and hyperalgesia that occurs during neuropathic pain in humans and in animal models. More recently, the focus has shifted to DOP receptors. Several literature reports suggest that stimulation of DOP receptors by selective agonists may be a promising way to treat pain in general and the neuropathic pain in particular.[7–9] In this regards DOP receptor agonists would have lower potential for abuse and fewer unwanted side effects associated in comparison with MOP and KOP receptor agonists. DOP receptors are present in the central nervous system and in the periphery. In the central nervous system DOP receptors are found in the neocortex, striatum, olfactory areas, substantia nigra, amygdala and the nucleus accumbens.[10] Activation of these receptors results in many physiological and behavioral effects ranging, from modulation of antinociception, mood, sensory system, motor integration and cognitive functions. In addition, DOP receptors are also present in several immunocompetent cells, which suggest they may play an important role in regulating the immune system.[11]

Pharmacological studies of DOP receptors have suggested the existence of at least two putative receptor subtypes (delta$_1$ and delta$_2$) which have not been substantiated by cloning.[12,13] These pharmacological results can be explained on the basis that DOP receptors form heterodimers[14–17] with other G protein-coupled receptors (GPCRs) and these heterodimers have different phenotypical behaviors.[18,19] The physiological and/or pathological consequence of DOP receptor heterodimerization *in vivo* remains to be explored. It is possible that these investigations can contribute to the development of new drugs with unique properties.[20] Since the relative expression of different GPCRs in various cell types differs, it may be possible to develop drugs that are devoid of abuse potential and

*Correspondence to: E. Akgün, Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA. E-mail: akgun001@umn.edu
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other side-effects. A second possibility for existence of putative subtypes of DOP receptors is that they represent different affinity states of the same receptors due to dimerization (heterodimerization/or oligomerization).[21]

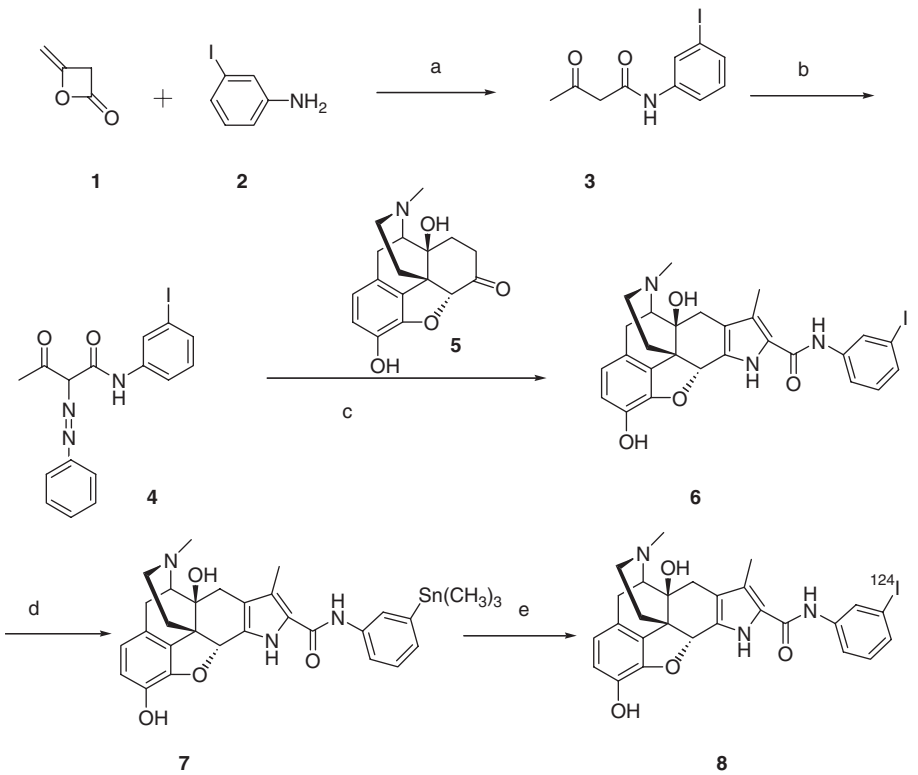
The characterization of DOP receptors will help to understand DOP receptors role at the functional level. One useful tool for this is positron emission tomography (PET) which is a non-invasive technique.[22] PET utilizes a pharmacophore labeled with positron emitters. We were interested in developing a DOP receptor agonist ligand labeled with a positron emitter of relatively long half-life such as ^{124}I ($t_{1/2} = 4.2$ days). For DOP receptors studies, the DOP receptor agonist **6** ([8R-(4bS, 8α, 8áβ, 12bβ)-7,10-dimethyl-5,6,7,8,12, 12b,-hexahydro-4,8-methanobenzofuro [3,2-e] pyrrolo [2,3q] isoquinoline-1,8^{a}-dihydroxy-11-(3-iodo-phenyl)-carboxamide)[23–26] has been prepared and evaluated *in vitro*. The synthesis of the corresponding positron emitting ligand **8** has been prepared in two steps from the pyrrolomorphinan **6** as it is illustrated in Scheme 1. The radioligand **8** ($t_{1/2} = 4.2$ days) complements existing PET imaging agents such as *N1′*-([^{11}C] methyl) naltrindole[27,28] ($t_{1/2} = 20$ min) and *N1′*-([^{18}F] fluoroethyl) naltrindole[29] ($t_{1/2} = 110$ min). The present report summarizes our results.

Results and discussion

Reaction of diketene **1** with *m*-iodoaniline **2** in anhydrous benzene proceeded smoothly at 0°C affording β-ketone **3** in nearly quantitative yield. The phenyl diazonium chloride was prepared *in situ* and reacted with β-ketone **3** mediated by pyridine (Scheme 1).

The β-keto phenyl diazonium amide **4** was obtained in 41% yield after the gravity column (SiO_2) separation using a solvent mixture of dichloromethane(D)/methanol(M)/ammonium hydroxide(A). The condensation of **4** with oxymorphone **5** was effected by elemental zinc in glacial acetic acid affording the pyrrolomorphinan **6**. The morphinan **6** was separated on a gravity column (SiO_2) using solvent mixture of dichloromethane/methanol/ammonium hydroxide. The average yield was ~40%. The proton NMR and mass spectra confirmed the formation of the product **6**. In its biological evaluation, the *in vitro* characteristic of **6** showed good affinity for DOP receptor ($K_i = 4.53$ nM for DOP). The binding assay (inhibition of ^{3}H-diprenorphine in HEK 293 cells) showed K_i selectivity of $\mu/\delta =$ 24 and $\kappa/\delta = 98$.

The product **6** was converted into stannylated derivative **7** via replacement of iodine using hexamethydistannane mediated by bis-(triphenylphosphine)-



Scheme 1 (a) Benzene 0°C; (b) phenylhydrozonium chloride, 0°C, then pyridine, rt; (c) Zn, aceticacid/sodium acetate, 60°C, then reflux. (d) hexamethyldistannane, bis-(triphenylphosphine)-palladium (II)-dichloride; (e) [^{124}I]I_2.

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palladium (II)-dichloride catalysis. ^{1}H-NMR of the product **7** has the characteristic trimethylstannine peak at 0.25 ppm with Sn-satellite peaks. The electrophilic aromatic iodination of the *m*-trimethylstannyl morphinan **7** with Na^{124}I in 5% acetic acid in methanol in the presence of an iodogen bead afforded ^{124}I-labelled *m*-[^{124}I]IPPM **8**. Representative semi-preparative HPLC traces for the purification [^{124}I]IPPM **8** are shown in Figure 1. The radioligands were separated easily. The overall radiochemical yield was 24.5 ± 1.9%(n = 6). The radiochemical purity was greater than 98% and the specific activity was 2.5 ± 1.2 Ci/μmol (n = 6). The product **8** was dissolved in saline containing 10% ethanol and the product was stable *in vitro*. Figure 2 illustrates HPLC trace of the purified product **8**. Figure 3 shows that the purified product **8** and the cold compound **6** eluted at the same time.

In summary, *m*-IPPM **5** was prepared and converted into *m*-^{124}IPPM **8** via stannylated precursor **7** that will be used to study DOP receptors *in vivo* in mouse brain. In the future, the mouse brain will be imaged using micro PET system to map DOP receptors.

Experimental

All solvents and reagents used in the syntheses were of the highest quality commercially available and when required were purified and dried by standard methods. Melting points were determined with Mel-Temp Laboratory Devices and uncorrected. ^{1}H-NMR (300.007 MHz) and ^{13}C-NMR (75.462 MHz) were obtained with a Varian Gemini-300 instrument. Chemical shifts are reported in ppm (δ) relative to internal Me_4Si in $CDCl_3$. High-resolution fast bombardment mass

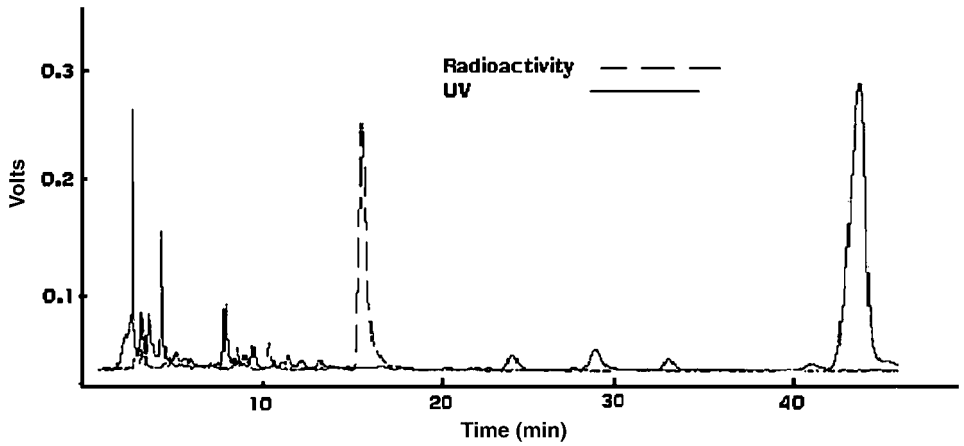

Figure 1 HPLC trace of the purification of *m*-[^{124}I]IPPM **8**. The peak eluted between 15 and 18 min was **8** and the major UV peak at 43–45 min was trimethyl tin precursor **7**.

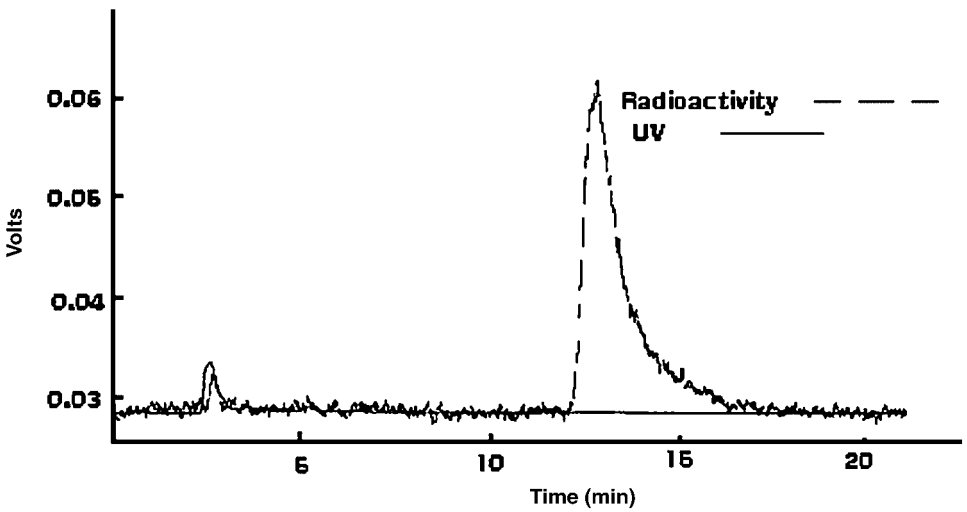

Figure 2 HPLC analysis of the purified *m*-[^{124}I]IPPM **8**.

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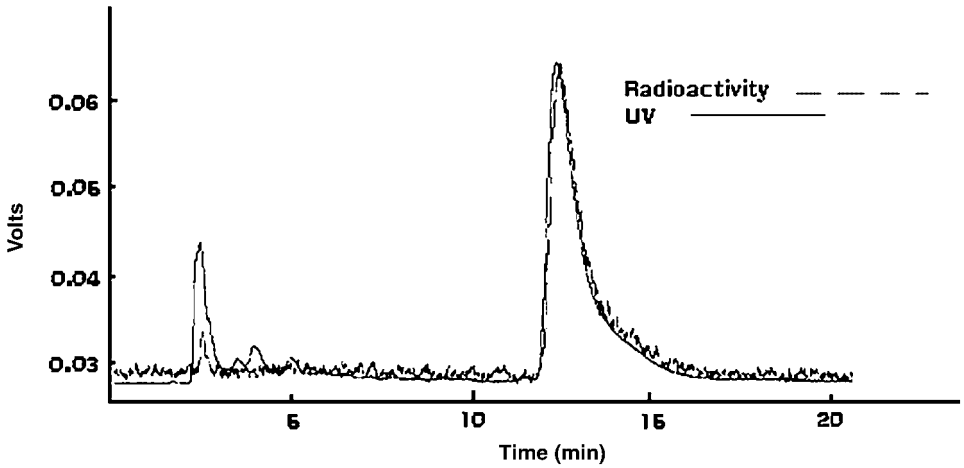

Figure 3 HPLC profile of the mixture of purified *m*-[^{124}I]IPPM **8** and *m*-IPPM **6**. The labeled **8** and the authentic cold compound *m*-IPPM **6** are eluted simultaneously.

spectroscopy (HRFABMS) was done at the University of Minnesota Mass Spectroscopy Facility. Analytical TLC was done on Baker-flex, silica gel IB2-F plates.

High-performance liquid chromatography was carried out on a system comprised of a Chrom Tech Iso-2000 pump, Hitachi L-4000 UV detector and a radiation detector. These detectors are in series and are also connected to a computer with HP Chemstation software via HP35900E interface. Iodine-124 was produced by the ^{124}Te (p, n) ^{124}I reaction.[30] $^{124}TeO_2$ (tellurium oxide) target was irradiated by 14.1 MeV protons at 24 μA current for about 4 h in a cyclotron. The radioiodine was separated by the dry distillation method. The distilled iodine-124 was trapped in 0.1 N NaOH.

Synthesis of β-ketoamide 3[31]

m-Iodoaniline (5 g, 22.8 mmol) and diketene (3 ml, excess) were mixed in anhydrous benzene at 0°C and stirred overnight. The following day, the white precipitate was filtered and washed with *n*-hexanes and the solid was dried *in vacuo*. R_f: 0.8 in D:M:A (97:2.5:0.5; v/v/v).

Yield: 6.91 g, White powder, 97.6%; mp 110–112°C.; ^{1}H-NMR (d_6-DMSO):δ = 10.1 (s, 1H), 7.5–7.0 (m, 4H), 3.5 (s, 2H), 2.1 (s, 3H); ^{13}C (d_6-DMSO): δ = 203.1(C=O), 165.9 (C=O), 140.8, 132.5, 131.4, 127.8, 118.8, 95.3, 53.0, 31.0.

Synthesis of phenylazo-β-ketoamide 4[32]

Aniline (214 mg, 2.3 mmol) was dissolved in 10 ml HCl (5 N) and cooled with methanol–ice. Sodium nitrite (159 mg, 2.3 mmol) dissolved in water was dripped into an aniline solution at a rate so that the temperature did not reach 0°C. The entire solution was then transferred via a double needle to a solution containing amide **3** (2.3 mmol) in pyridine/water (10 ml, 1:1, v/v) cooled with ice–water. The product precipitated as viscous oil. The solvent was decanted and the material was dissolved in methylene chloride. After drying over sodium sulfate, the solvent was evaporated. The residue was separated via flash chromatography (SiO_2, D: M: A (97:2.5:0.5, v/v/v)). Yield: 41%; mp >130°C Decomposed; R_f 0.54 (SiO_2, D:M:A, 97:2.5:0.5, v/v/v); ^{1}H-NMR (d_6-DMSO):δ = 11.1 (s, 1H), 8.2 (s, 1H), 7.5–7.1 (m, 9H); ^{13}C (d_6-DMSO): δ = 198.6, (C = O), 162.3 (C = O), 142.4, 139.3, 130.2, 128.9, 127.8, 126.6, 95.5; MS (M+Na): 430.0025; 430.0030 calculated for $C_{16}H_{14}IN_3O_2Na$.

(8R-(4bS,8α,8áβ,12bβ)-7,10-Dimethyl-5,6,7,8,12,12b,-hexahydro-4,8-methanobenzofuro (3,2-e)pyrrolo (2,3q) isoquinoline-1,8^{Q}-dihydroxy-11-(3-iodo-phenyl)-carboxamide 6

To diazoamide **4** (0.63 mmol) dissolved in 2 ml glacial acetic acid oxymorphone **5** (177.8 mg, 0.52 mmol, 1.2 eq) was added, followed by sodium acetate (52 mg, 0.63 mmol, 1.2 eq). The mixture was warmed to 60°C under N_2-flow. At this temperature, elemental zinc (156 mg, 2.38 mmol, 4.4 eq) was added in small portion to the mixture. After completion of zinc addition, the mixture was refluxed for 2 h. Then it was cooled to room temperature and decanted into ice–water. The reaction flask was rinsed twice, each with 2 ml glacial acetic acid. The combined acetic acid solution was adjusted to pH ~ 9 with 20% NaOH solution. The pyrrolomorphinan

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was extracted with EtOAc (4×50 ml). After drying over sodium sulfate, the solvent was evaporated. The final purification using flash chromatography (SiO_2, D: M: A (95:4.5:0.5, v/v/v)). Yield: 34.5%; colorless powder, mp 213–216°C.; R_f 0.6 (SiO_2, D:M:A, 97:2.5:0.5, v/v/v).; ^{1}H-NMR (d_6-DMSO):$\delta = 9.4$ (s, 1H), 8.1 (s, 1H), 8.6–7.05 (m, 4H), 6.5 (AB, $J_{AB} = 7.3$ Hz, 2H), 5.29 (s, 1H), 3.1–1.4 (m, 8H), 2.4 (s, 3H), 2.2 (s, 3H); MS: (M+1): 584.1064; 584.1048, calculated for $C_{28}H_{26}IN_3O_4$.

Synthesis of (8R-(4bS,8α,8αβ,12bβ)-7,10-dimethyl-5,6,7,8,12,12b,-hexahydro-4,8-methanobenzofuro (3,2-e) pyrrolo(2,3q) isoquinoline-1,8^a-dihydroxy-11-(3-trimethylstannyl-phenyl)-carboxamide 7[33]

To a double neck flask a solution of pyrrolomorphinan **6** (100 mg, 0.171 mmol) in degassed 1,4-dioxane (5 ml) were added 100 µl hexamethyldistannane (0.41 mmol) and (1.7 mg, 2.4×10^{-6} mmol) bis-(triphenylphosphine)-palladium (II)-dichloride. The solution was stirred at 60°C for 90 min. After removal of solvent the crude mixture was separated by gravity column (SiO_2) using chloroform/methanol in a ratio of 97:3 (v: v). ^{1}H-NMR (d_6-DMSO): $\delta = 11.3$ (s, 1H), 9.3 (s, 1H), 8.9(s, 1H), 7.8–7.05 (m, 4H), 6.4 (AB, $J_{AB} = 7.2$ Hz, 2H), 5.3 (s, 1H), 4.6 (s, 1H), 3.2–1.4 (m, unresolved), 0.25 (s, with Sn satellites, 9H).

Biological Studies

Receptor binding assays

The receptor binding assays were conducted exactly same in the same manner as described previously.[34]

Radiolabeling

The trimethyltin compound **7** (40 µg) was dissolved in 50 µl of 50% acetic acid in methanol and 100 µl of 5.0% acetic acid in methanol was added to Na^{124}I in 20 µl of 0.1 N NaOH. The two solutions were mixed and an IODOGEN bead was added. The reaction mixture was incubated at room temperature for 15 min. The bead was removed from the reaction mixture and washed with water. $K_2S_2O_5$ (100 µl of 0.1 M) was added and the solution was mixed. Two hundred microliters of saturated solution of $NaHCO_3$ was added to neutralize the acid. The labeled product was extracted with ethyl acetate (3×0.2 ml) and dried by passing through a small Na_2SO_4 column. The solvent was evaporated under a stream of nitrogen gas at 110°C. The residue was dissolved in 150 µl of the HPLC eluant. The resulting solution was injected on to a HPLC column (Luna 5µ C18(2), 4.6×250 mm, Phenomenex), which was eluted with $CH_3OH/H_2O/NH_4OH$ (70:30:0.1) at a flow rate of 1 ml/min. The UV detector was set at 254 nm wavelength. The desired product **8** (*m*-^{124}IPPM) eluted at 12–14 min (Figure 1) was collected and the solvent was evaporated to dryness azeotropically with CH_3CN (2×1 ml) at 110°C. The product was dissolved in saline containing 10% ethanol. A standard curve was obtained between peak area versus mass by injecting known mass of carrier **6** (Scheme 1) onto the column. The mass associated with the labeled product was calculated by relating the peak area of UV absorbance peak of **6** in the labeled product to the standard curve. The specific activity was obtained by dividing the activity of the labeled product collected by the calculated mass in micromoles.

The product was analyzed for radiochemical purity on HPLC column (Maxsil 5 C18, 4.6×250 mm, Phenomenex), which was eluted with CH_3CN/0.01 M $(NH_4)_2HPO_4$ (70:30) at a flow rate of 1 ml/min. Figure 2 shows the chromatogram of the final product. Carrier **6** was added to the labeled product and was analyzed. Figure 3 shows the chromatogram showing the labeled and cold compound eluting at the same time.

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