Bioorganic & Medicinal Chemistry 17 (2009) 7971–7977

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Novel O-[$^{11}$C]methylated derivatives of candesartan as angiotensin II $AT_1$ receptor imaging ligands: Radiosynthesis and ex vivo evaluation in rats

Tayebeh Hadizad [a], Sheryn A. Kirkpatrick [a,b], Samantha Mason [a], Kevin Burns [b,c], Rob. S. Beanlands [a,b], Jean N. DaSilva [a,b,*]

[a] National Cardiac PET Centre, Division of Cardiology, University of Ottawa Heart Institute, 40 Ruskin Street, Ottawa, Canada ON K1Y 4W7
[b] Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada
[c] Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, Canada

ARTICLE INFO

*Article history:*
Received 7 July 2009
Revised 7 October 2009
Accepted 8 October 2009
Available online 13 October 2009

*Keywords:*
[$^{11}$C]Methyl-candesartan
[$^{11}$C]Desethyl-candesartan
PET imaging
[$^{11}$C]Radiolabeling

ABSTRACT

[$^{11}$C]Methyl-candesartan and its desethyl derivative ([$^{11}$C]TH4) were developed as potential radiotracers for imaging angiotensin II (Ang II) type 1 ($AT_1$) receptors. These compounds were synthesized via methylation of tetrazole-protected candesartan using [$^{11}$C]methyl iodide followed by deprotection through HCl hydrolysis at 65 °C to produce [$^{11}$C]methyl-candesartan, and 90 °C for [$^{11}$C]TH4. Ex vivo biodistribution and competition studies were carried out for both [$^{11}$C]methyl-candesartan and [$^{11}$C]TH4 to assess tissue retention time course and binding selectivity. Besides the liver, [$^{11}$C]methyl-candesartan and [$^{11}$C]TH4 displayed highest tissue retention in the $AT_1$ receptor-rich renal cortex and outer medulla. At tracer doses 15 min post-injection, [$^{11}$C]methyl-candesartan demonstrated higher specific binding proportion for $AT_1$ receptors, and selectivity for $AT_1$ over Ang II $AT_2$, Mas, β-adrenergic, and $\alpha_2$-adrenergic receptors in rat kidneys compared to [$^{11}$C]TH4. This study indicates that [$^{11}$C]methyl-candesartan has potential for in vivo imaging renal $AT_1$ receptors selectively using positron emission tomography.

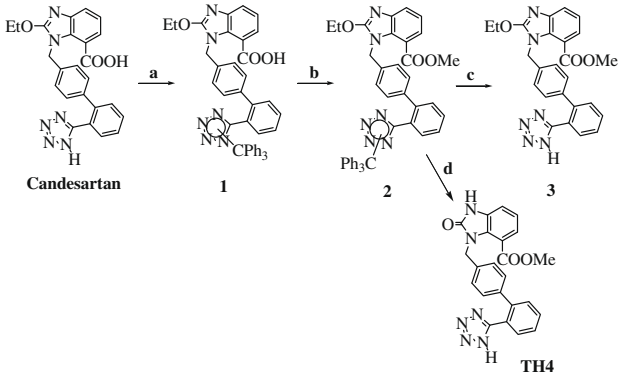


## 1. Introduction

Angiotensin II (Ang II) is a regulatory octapeptide that is involved in the control of salt and fluid balance, autonomic and cardiovascular functions as part of the renin-angiotensin system (RAS).[1] Dysfunction of the RAS plays a key role in the pathogenesis of ventricular remodeling, hypertension, diabetic nephropathy, and heart failure (HF).[2–6] Most of the physiological actions of Ang II are mediated via the G protein-coupled Ang II type 1 ($AT_1$) receptors. These receptors are expressed in many tissues including the brain, heart, blood vessels, lungs, aorta, adrenals, and kidneys.[7–9] $AT_1$ receptor stimulation leads to vasoconstriction, cell growth, catecholamine release, and increased aldosterone secretion. Prolonged stimulation of this system can lead to altered salt and water balance, cell loss, and fibrosis, all of which have a potential detrimental effect in cardiac hypertrophy, HF and diabetic nephropathy.[6,10–13]

RAS components have been shown to be altered in HF and diabetes in both animal models and patients.[14,15] Therapy with $AT_1$ receptor blockers (ARBs) or angiotensin converting enzyme inhibitors has been shown to be effective for treating ventricular remodeling post-myocardial infarction, improving outcomes in patients with HF[16,17] and decreasing glomerular hypertrophy and proteinuria in both diabetic patients[18] and animal models of diabetes.[19] The precise pathological mechanisms in the progression to diabetic nephropathy and the specific role of the RAS in these processes at present remain unclear.

Several $AT_1$ receptor radioligands have previously been synthesized for positron emission tomography (PET), however, in low




**Scheme 1.** Reagents and conditions: (a) TEA, trityl chloride, $CH_2Cl_2$; (b) MeI, $K_2CO_3$, DMF, room temperature, 15 min; (c) HCl (1 N), DMF, 65–70 °C, 1 h; (d) HCl (1 N), DMF, 90 °C, 1.5 h. For [$^{11}$C]methyl-candesartan: (b) [$^{11}$C]MeI, $K_2CO_3$, Kryptofix, DMF, 65–70 °C, 3 min; (c) HCl (1 N), DMF, 65–70 °C, 2 min. For [$^{11}$C]TH4: d) HCl (1 N), DMF, 90 °C, 2 min.

* Corresponding author. Tel.: +1 613 798 5555x19704; fax: +1 613 761 5406.
*E-mail address:* jdasilva@ottawaheart.ca (J.N. DaSilva).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.10.016

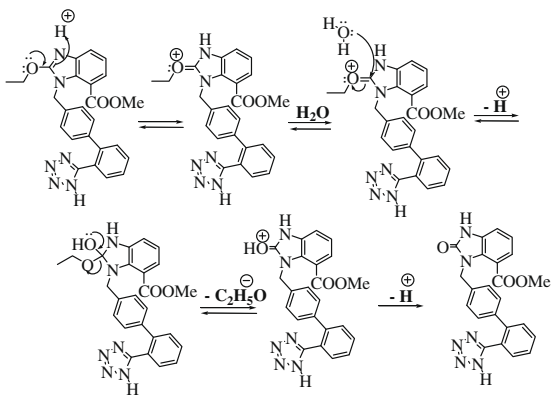
yields[20] and with no uptake in the brain.[21] With the goal to cross the blood–brain barrier (BBB) and bind to brain $AT_1$ receptors, we have selected a more lipophilic drug (candesartan, Scheme 1) for labeling as lead molecule. Based on previous in vivo and structure–activity relationship studies, the clinically used ARB candesartan crosses the BBB and produces blocking effects in the brain.[22] The unlabeled *O*-methylated analog of candesartan was previously prepared and exhibited similar binding affinities for $AT_1$ receptors ($IC_{50}$ methyl-candesartan 66 nM, candesartan 110 nM), and in vivo antagonistic activities.[23] Due to greater lipophilicity, this methyl-ester derivative of candesartan is expected to cross the BBB more readily than the more polar carboxylic acid parent molecule. The corresponding $^{11}C$-labeled derivative is hypothesized to display binding selectivity as a high affinity radioligand for $AT_1$ receptors. We describe here the synthesis and ex vivo evaluation in rats of [$^{11}C$]methyl-2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate ([$^{11}C$]methyl-candesartan) and its desethyl derivative [$^{11}C$]methyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2,3-dihydro-2-oxo-1*H*-benzimidazole-7-carboxylate ([$^{11}C$]TH4).

## 2. Results

### 2.1. Chemistry

Unlabeled methyl-candesartan and TH4 were synthesized by direct esterification of candesartan as outlined in Scheme 1. The tetrazole-protected candesartan (**1**) was prepared following an adaptation of a previous procedure,[24] by the addition of trityl chloride solution to an ice-cooled solution of candesartan in the presence of triethyl amine in a yield of 52.3%. The methyl-ester derivative (**2**) of the tetrazole-protected candesartan was synthesized by the addition of methyl iodide to a mixture of compound **1** and potassium carbonate in DMF. In the course of optimizing the reaction condition, the organic base tetrabutyl ammonium hydroxide and a range of reaction times and temperatures were evaluated. The *N*-trityl protective group was removed by heating a solution of compound **2** in HCl in quantitative yield. Methyl-candesartan was produced at 65 °C, while a reaction temperature of 90 °C resulted in a greater formation of TH4 in which the ethoxy group is converted to a cyclic urea derivative. A proposed mechanism for this conversion is presented in Scheme 2.

Methyl-candesartan and TH4 were characterized by $^1H$ NMR, IR, melting point and high-resolution mass spectrometry (HRMS). In the $^1H$ NMR spectrum of methyl-candesartan (**3**) and TH4, a singlet was observed at 3.80 ppm corresponding to the methyl-ester groups. The $^1H$ NMR spectrum of methyl-candesartan showed a quartet at 4.68 ppm (-$CH_2$–) and a triplet at 1.47 ppm (-$CH_3$) while these two signals were not observed in $^1H$ NMR spectrum of TH4. A singlet at 11.45 ppm in the $^1H$ NMR spectrum of TH4 (N–H of cyclic urea) confirms the conversion of methyl-candesartan to TH4. Infrared spectroscopy (IR) showed a characteristic C=O peak at 1717 $cm^{-1}$ for methyl-candesartan, while the IR spectrum of TH4 indicates two peaks, 1718 $cm^{-1}$ for the carbonyl of the ester group and 1693 $cm^{-1}$ for the carbonyl of cyclic urea. The melting point of TH4 was higher than the melting point of methyl-candesartan due to the hydrogen bonding in the cyclic urea group and the higher polarity of TH4.




**Scheme 2.** Proposed mechanism for the conversion of the ethoxy group in methyl-candesartan to cyclic urea in TH4.

### 2.2. Radiosynthesis of [$^{11}C$]methyl-candesartan and [$^{11}C$]TH4

[$^{11}C$]Methyl-candesartan and its desethyl derivative ([$^{11}C$]TH4) were radiosynthesized with slight modifications to the procedure described above (Scheme 1) in high yields (50–70% from [$^{11}C$]methyl iodide) in a total synthesis time of approximately 30 min plus 5 min for quality control analysis. Both compounds were obtained in high radiochemical (>99%) and chemical purities (>90%). The resulting specific activities were approximately 11.1–55.5 GBq/μmol (300–1500 Ci/mmol) at end-of-synthesis.

### 2.3. Ex vivo biodistribution in rats

#### 2.3.1. Time course

Ex vivo biodistribution time course studies of [$^{11}C$]methyl-candesartan or [$^{11}C$]TH4 are presented in Figure 1. The renal cortex and outer medulla retention was higher relative to other tissues but much less than the liver for both radioligands. Relative to renal cortex and outer medulla uptake, the uptake of remaining tissues was moderate to low with no uptake observed in the brain. High contrast between the tissue and blood radioactivity was observed at 15 min following radioligand injection so this time point was selected for tracer characterization studies. Ligand uptake in the liver was more than twofold higher than the remaining tissues at 5 min post-injection. The liver is not an organ of interest in the context of this research and as a site of metabolism and excretion, the liver was excluded from further figures for clarity purposes.

#### 2.3.2. Competition

Binding specificity and selectivity for $AT_1$ were assessed using the above ex vivo biodistribution protocols with minor modifications. Rats were treated with receptor antagonists, sacrificed at 15 min following radiotracer injection. Tracer retention was expressed as a tissue-to-blood ratio to normalize tracer delivery between treatment groups. Rats treated with all doses of ARBs candesartan and losartan displayed significant reduction of both tracer retention in the renal cortex and outer medulla, and [$^{11}C$]methyl-candesartan accumulation in the adrenal. In the renal inner medulla, only the highest dose of losartan reduced [$^{11}C$]TH4 retention significantly (Fig. 2). A dose-dependent effect was observed on the specific binding component (percent change in retention to $AT_1$ receptors) of [$^{11}C$]methyl-candesartan in the renal cortex (57–81% with candesartan, 89–90% losartan), renal outer medulla (74–80% candesartan, 87–90% losartan), and adrenals (64–80% with candesartan or losartan). Dose-dependent reductions were less for [$^{11}C$]TH4 in the renal cortex (47–71% with candesartan, 73–79% losartan) and outer medulla (62–77% candesartan, 77–83% losartan).

Pretreatment with β- or $\alpha_2$-adrenoceptor antagonists propranolol and yohimbine did not reduce tissue retention of either radioligand (Fig. 3). At 5 mg/kg of the Ang II $AT_2$ receptor antagonist PD123,319, [$^{11}C$]methyl-candesartan displayed significant reduction in tracer retention in the renal cortex and outer medulla. At 2 mg/kg, a dose employed in other characterization studies of previously developed $AT_1$ radioligands,[21] no effect on [$^{11}C$]methyl-candesartan uptake was observed in any tissue. Mas

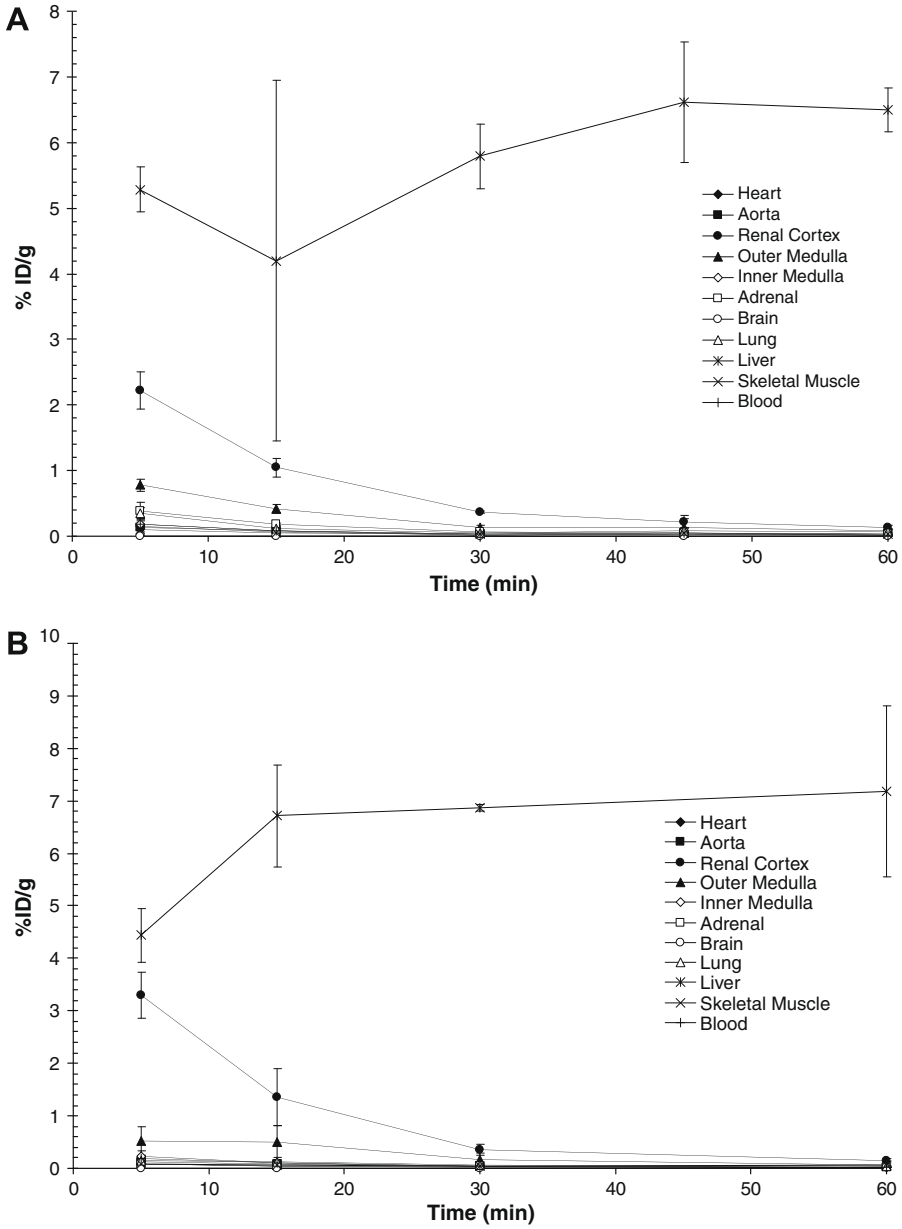



**Figure 1.** Time course of (A) [$^{11}$C]methyl-candesartan and (B) [$^{11}$C]TH4 in male Sprague-Dawley rats in sampled tissues. Data ($n$ = 3 at all time points) are expressed as mean percent injected dose per gram of tissue (%ID/g) ± standard deviation.

blocker A-779 pretreatment significantly reduced only [$^{11}$C]TH4 retention in the renal outer medulla.

## 3. Discussion

The synthesis of methyl-candesartan was reported by Kubo et al.[23] with a multi-step synthetic route (seven steps) while the ethyl ester derivative of candesartan was synthesized by Naka et al.[25] using a similar approach. Due to the short half-life of the $^{11}$C isotope this method was not appropriate to synthesize [$^{11}$C]methyl-candesartan and [$^{11}$C]TH4. The same group had also reported a shorter synthetic route (three steps) for double ester derivatives of candesartan.[24] This direct esterification of candesartan was therefore chosen for the synthesis of unlabeled and labeled methyl-candesartan and TH4 with slight modifications.

Since candesartan has three possible positions for methylation (the carboxyl group and two positions on the tetrazole ring),[24] protection of the tetrazole group was required to prevent the formation of undesired by-products. Trityl chloride was selected as a protecting agent and tetrazole-protected candesartan (**1**) was purified by flash column chromatography. The product was identified by spectroscopic means and the results are in agreement with the literature.[24] The methyl-ester derivative (**2**) of the tetrazole-protected candesartan was synthesized using the previously published procedure.[24] The NMR studies agreed with the chemical structure and a singlet was observed corresponding to the methyl-ester group. Due to the poor solubility of $K_2CO_3$ in organic solvents, attempts were made to increase the yield using tetrabutyl ammonium hydroxide. However, results were unsuccessful to generate the corresponding intermediate even with a large excess amount of the organic base, or an increase in reaction time and/or temperature. Due to the presence of the ethoxy group in compound **2**, the deprotection reaction was highly sensitive to time and temperature. A lower reaction temperature was needed to pre-

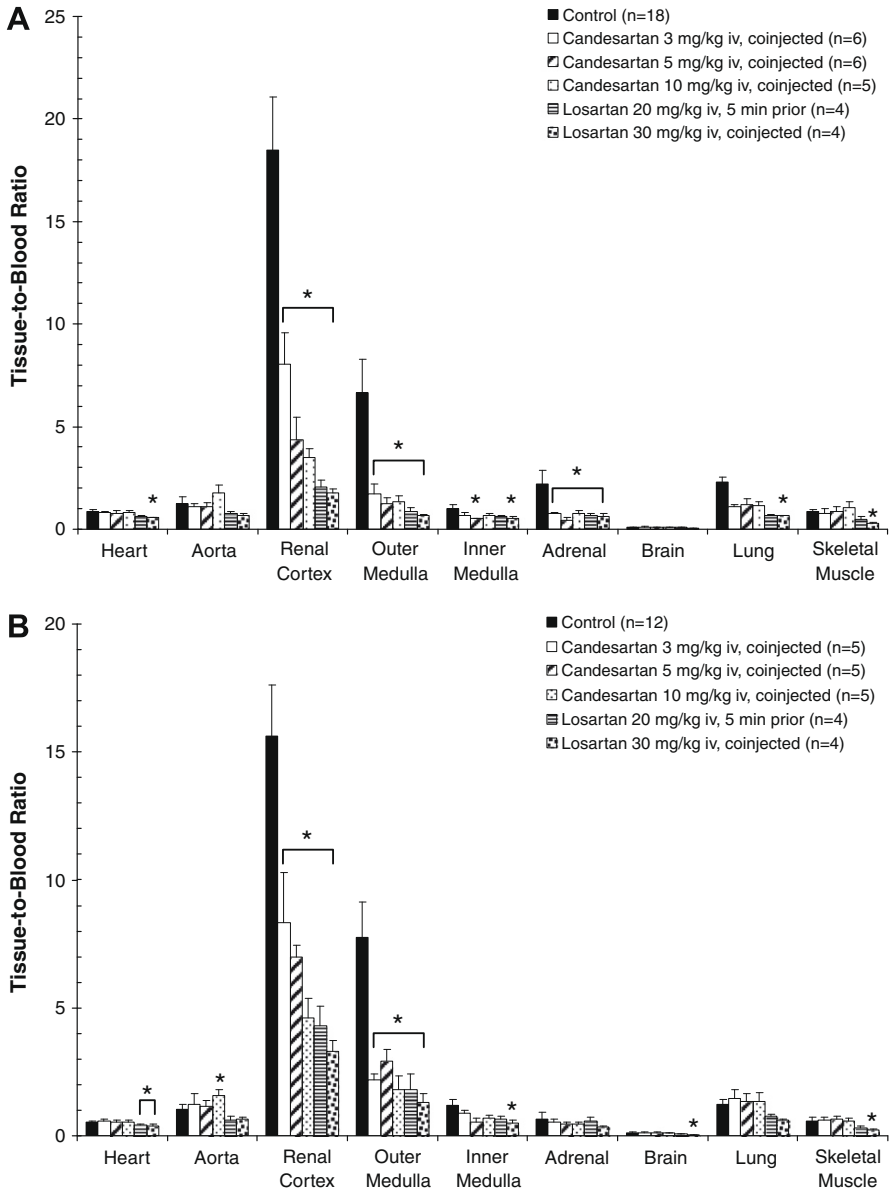



**Figure 2.** Effect of $AT_1$, receptor blockade on (A) [$^{11}$C]methyl-candesartan and (B) [$^{11}$C]TH4 tissue retention. Data are expressed as mean tissue-to-blood ratio ± standard deviation. (*) indicates $p$ <0.05 compared to controls using one-way ANOVA and Bonferroni post-hoc analysis.

vent the cleavage of the ethoxy group, while an increase in temperature resulted in the formation of TH4. The same result was achieved when the reaction time was increased. In order to prevent the conversion of methyl-candesartan to TH4, different acids were also tested. The deprotection failed using acetic acid with a reaction time of 2 min at room temperature. This failure was due to insufficient activation of the acid. Similar results were obtained with trifluoroacetic acid as de-protecting agent.[26] Hydrochloric acid (1 N) was the most suitable reagent for this step.

[$^{11}$C]Methyl-candesartan and [$^{11}$C]TH4 were produced using similar conditions as the unlabeled analogs. We found the use of kryptofix essential to increase the reactivity of the base, as large amounts of un-reacted starting materials were detected in the absence of kryptofix. Higher temperatures (up to 120 °C), an increased reaction time (5 min) or a larger amount of $K_2CO_3$ did not increase the resulting yield when kryptofix was not present. The optimal time and temperature of the deprotection step for the synthesis of [$^{11}$C]methyl-candesartan was 2 min and 65–70 °C, respectively. Higher reaction temperature (>70 °C), higher HCl concentration (12 N) or a reaction time of more than 2 min resulted in the formation of [$^{11}$C]TH4. The identity of these novel tracers as [$^{11}$C]methyl-candesartan and [$^{11}$C]TH4 was determined by co-injection of authentic standards using analytical HPLC.

[$^{11}$C]Methyl-candesartan and [$^{11}$C]TH4 retention was high in the kidneys with low to moderate accumulation in the other $AT_1$-rich tissues, such as the adrenals and aorta,[27] suggesting a possible dependence of the tracer uptake on blood flow, or some other tissue-specific mechanism. In tracer doses, no uptake was observed in the brain indicating that neither radioligands crossed the BBB contrary to what was hypothesized for methyl-ester derivatives of candesartan. Previous work reported inhibition of central effects following peripheral administration of a greater dose of candesartan (1–10 mg/kg).[22] These doses are significantly higher than the ones used here (2.4–10.8 μg/kg) for methyl-candesartan. Receptor saturation with the unlabeled compound present in radioligand formulations may lower the specific binding proportion of the total

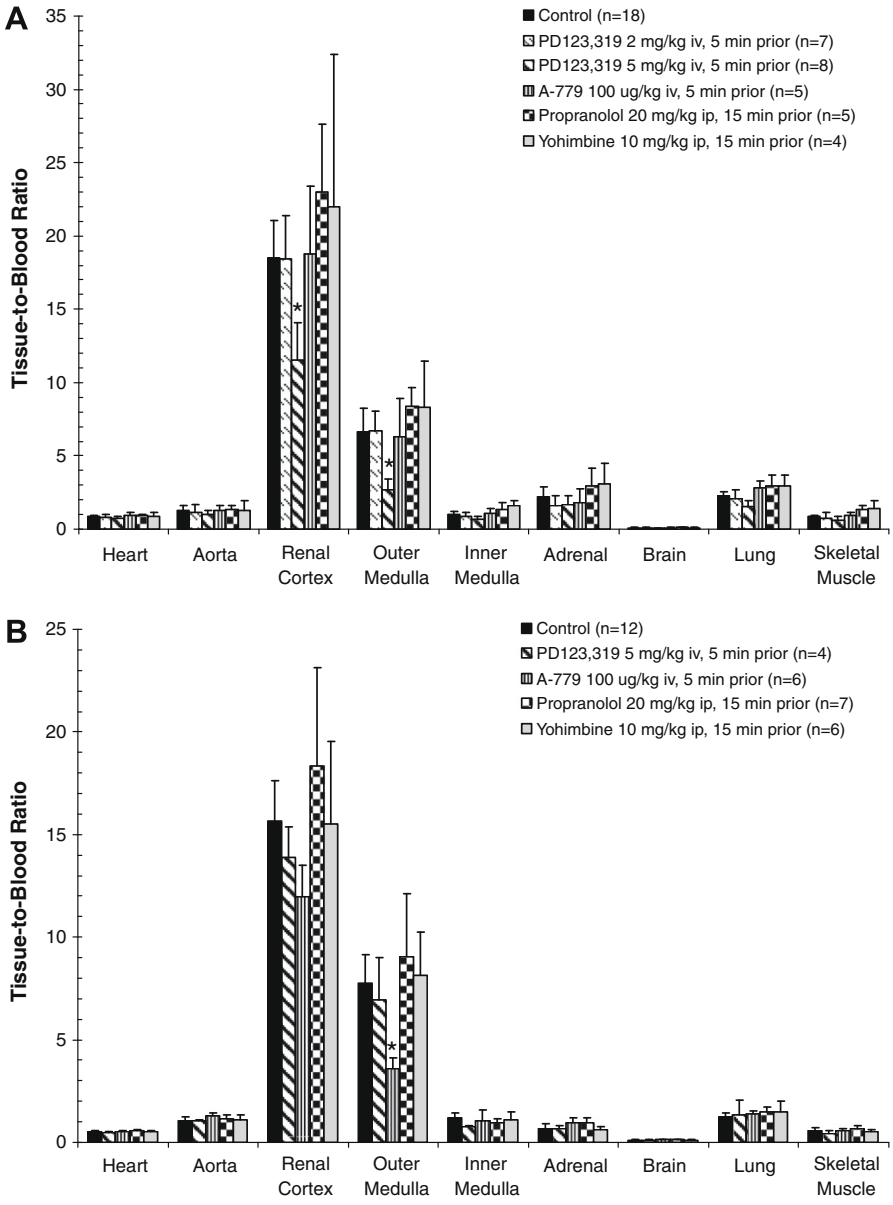



**Figure 3.** Effect of $AT_2$, Mas, β-adrenergic and $\alpha_2$-adrenergic receptor blockade on (A) [$^{11}$C]methyl-candesartan and (B) [$^{11}$C]TH4 tissue retention. Data are expressed as mean tissue-to-blood ratio ± standard deviation. (*) indicates $p$ <0.05 compared to controls using one-way ANOVA and Bonferroni post-hoc analysis.

tracer retention in tissues expressing low amounts of $AT_1$ receptor such as the heart.[27] Cardiac uptake may therefore increase with improved specific activity (less mass) of the tracer formulations.

At 15 min post-injection, a higher proportion of $AT_1$ specific binding was observed for [$^{11}$C]methyl-candesartan than for [$^{11}$C]TH4. [$^{11}$C]Methyl-candesartan binding selectivity for $AT_1$ over $AT_2$, Mas, β-adrenergic and $\alpha_2$-adrenergic receptors was demonstrated at tracer doses (2.4–10.8 μg/kg). Although significant reductions of [$^{11}$C]methyl-candesartan retention occurred in the renal cortex and outer medulla regions at a higher dose (5 mg/kg) of PD123,319, at 2 mg/kg this effect was not observed. The 2 mg/kg dose of PD123,319 treatment was previously used for characterization of other $AT_1$ radioligands in the literature.[21] At tracer doses, [$^{11}$C]methyl-candesartan is not expected to bind to $AT_2$ thus selectivity is not expected to be impacted. [$^{11}$C]TH4 displayed binding selectivity at 15 min post-injection for $AT_1$ over $AT_2$, β-adrenergic and $\alpha_2$-adrenergic receptors. Selectivity was not demonstrated over Mas receptors, as significant reductions in [$^{11}$C]TH4 retention were observed in the renal outer medulla. Moreover, this reduction occurred at a dose of A-779 (100 μg/kg) that approaches the tracer dose range tested for [$^{11}$C]TH4 (2.9–8.5 μg/kg). This effect may be an indication of specific binding of the tracer to this receptor. It has been reported that $AT_1$ and Mas receptors can hetero-oligomerize[28] and the reduction may be occurring as a result of partial displacement of [$^{11}$C]TH4 by A-779 from this hetero-oligomer complex.

## 4. Conclusion

Two new radiotracers were synthesized and evaluated for in vivo $AT_1$ receptor measurement using PET. [$^{11}$C]Methyl-candesartan and [$^{11}$C]TH4 were produced in high yields (50–70% from [$^{11}$C]MeI) with a total synthesis and purification time of approximately 30 min plus 5 min for quality control (from the end of the beam). Both compounds were obtained in high radiochemical (>99%) and chemical (>90%) purities. The resulting specific activi-

ties of [$^{11}$C]methyl-candesartan and [$^{11}$C]TH4 were 11.1–55.5 GBq/μmol (300–1500 mCi/μmol) at end-of-synthesis.

Tracer accumulation was observed to the highest degree in tissues rich in $AT_1$ receptors for both radioligands. Neither [$^{11}$C]methyl-candesartan nor [$^{11}$C]TH4 crossed the BBB. Compared to [$^{11}$C]TH4, [$^{11}$C]methyl-candesartan displayed a higher specific binding proportion for $AT_1$ receptors in rat kidneys. At tracer doses, [$^{11}$C]methyl-candesartan but not [$^{11}$C]TH4 binds selectively to $AT_1$ over Ang II $AT_2$, Mas, β-adrenergic or $\alpha_2$-adrenergic receptors thus [$^{11}$C]methyl-candesartan shows better potential for imaging renal $AT_1$ receptors using PET.

## 5. Experimental section

### 5.1. Materials and methods

Candesartan was purchased from Toronto Research Chemicals Inc. PD123,319, propranolol and yohimbine were purchased from Sigmal Aldrich. A-779 was purchased from Bachem Bioscience. Losartan was a gift from Dr. F. H. H. Leenen. All other reagents and solvents were purchased from Aldrich Chemical and were used as received without further purification. $^1$H and $^{13}$C NMR were recorded on Varian Inova 500 MHz spectrophotometer. All chemical shifts were reported as parts per million (ppm) and coupling constants were given in hertz (Hz). The high-resolution mass spectra (HRMS) were obtained on a Micromass Q-TOF mass spectrometer. IR spectra were recorded on a ABB Bomem spectrometer (MB 100). Melting points were determined on a Fisher Scientific apparatus. HPLC analysis was performed with a Waters series with inline radiation detector and Waters 486 UV detector at 254 nm. [$^{11}$C]$CO_2$ was produced by our CTI-RDS111 cyclotron.

#### 5.1.1. 2-Ethoxy-1-[[2′-[*N*-(triphenylmethyl)-1*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid (tetrazole-protected candesartan, 1)

Candesartan (100 mg, 0.23 mmol) was dissolved in methylene chloride ($CH_2Cl_2$, 1 mL) in the presence of triethylamine (25 mg, 0.25 mmol). The solution was ice-cooled and a solution of trityl chloride (75 mg, 0.27 mmol) in methylene chloride ($CH_2Cl_2$, 0.3 mL) was added dropwise. The resulting mixture (cloudy) was stirred at 0–4 °C for two hours and then at room temperature for 17 h. The progress of the reaction was monitored by analytical HPLC (acetonitrile/0.1 M ammonium formate solution (25/75), 2 mL/min, Luna $C_{18}$, 250 × 4.6 mm). The reaction mixture was washed with water, dried over sodium sulfate ($Na_2SO_4$) and the solvent was evaporated. The tetrazole-protected candesartan was purified by flash column chromatography (silica, $CH_2Cl_2$/MeOH = 40/1, v/v) to provide 81 mg (52.3%) of product as colorless powder. Mp: 163–165 °C; $^1$H NMR ($CDCl_3$, 500 MHz) $\delta_H$ 7.73–7.71 (m, 2H), 7.57 (d, 1H, *J* = 7.8 Hz), 7.33–7.11 (m, 12H), 7.04 (t, 1H, *J* = 7.8 Hz), 6.88–6.81 (m, 8H), 6.75 (d, 2H, *J* = 8.0 Hz), 5.58 (s, 2H), 4.48 (q, 2H, *J* = 7.1 Hz), 1.28 (t, 3H, *J* = 7.1 Hz). $^{13}$C NMR ($CDCl_3$, 500 MHz) $\delta_C$ 170.25, 164.16, 158.41, 141.75, 141.41, 141.14, 139.59, 136.54, 131.31, 130.61, 130.35, 130.15, 129.92, 129.40, 129.26, 128.26, 128.21, 127.90, 127.78, 127.62, 127.58, 127.33, 126.20, 126.18, 123.99, 120.90, 120.83, 82.86, 66.50, 53.42, 46.94, 44.83, 14.58.

#### 5.1.2. Methyl-2-ethoxy-1-[[2′-[*N*-(triphenylmethyl)-1*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate (2)

The solution of tetrazole-protected candesartan, **1** (30 mg, $4.40 \times 10^{-2}$ mmol) in DMF (0.7 mL) was added to potassium carbonate ($K_2CO_3$, 120 mg, 0.87 mmol) and the reaction mixture was stirred in an ice bath for 10 min. Methyl iodide (340 mg, 2.4 mmol) was added dropwise and the reaction mixture was stirred vigorously at room temperature for 15 min. Water was added to the light yellow reaction mixture and the product was extracted with ethyl acetate. The organic phase was washed with more water, dried over $Na_2SO_4$ and all the solvent was evaporated to give 27 mg of crude product. Since the compound was to be used for the subsequent step immediately, no further purification was applied. In $^1$H NMR spectrum (THF-$d_8$) of the crude product, a singlet was observed at 3.80 ppm (3H) corresponding to the protons of methyl-ester group, while HPLC analysis revealed that all starting materials were converted to more lipophilic compounds.

#### 5.1.3. Methyl-2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl]-1*H*-benzimidazole-7-carboxylate (methyl-candesartan, 3)

Hydrochloric acid (1 N, 0.85 mL) was added to a solution of compound **2** (13 mg, 0.019 mmol) in DMF (2 mL) and the reaction solution was heated at 65–70 °C for 1 h. The progress of the reaction was monitored by HPLC. The reaction was then quenched by the addition of $CH_2Cl_2$ (3 mL) and immediately purified by semi-preparative HPLC (Luna $C_{18}$, 10μ, 250 × 10 mm, acetonitrile/0.1 M ammonium formate solution = 35/65). After evaporation of the solvent, the residue was washed with water, extracted with ethyl acetate and dried over $Na_2SO_4$. All the ethyl acetate was evaporated to give 5.5 mg of pure product (64.7%). Mp: 190–192 °C; $^1$H NMR (DMF-d7, 500 MHz) $\delta_H$ 7.70–7.35 (m, 6H), 7.20 (t, 1H, *J* = 7.8 Hz), 7.13 (d, 2H, *J* = 8.1 Hz), 6.87 (d, 2H, *J* = 8.1 Hz), 5.54 (s, 2H), 4.68 (q, 2H, *J* = 7.0 Hz), 3.80 (s, 3H), 1.47 (t, 3H, *J* = 7.0 Hz). IR (Nujol, $cm^{-1}$): 1717 (C=O, carbonyl of ester). HRMS calcd for $C_{25}H_{22}O_3N_6$: 454.1753, found: 454.1748.

#### 5.1.4. Methyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2,3-dihydro-2-oxo-1*H*-benzimidazole-7-carboxylate (TH4)

The compound was synthesized using the same procedure as outlined for the synthesis of methyl-candesartan but at a different reaction temperature. Starting with compound **2** (35 mg, 0.051 mmol), HCl (1 N, 2 mL) and DMF (3 mL), the reaction solution was heated at 90 °C for 1.5 h. The reaction was quenched by the addition of $CH_2Cl_2$ (3 mL) and immediately purified by semi-preparative HPLC (Luna $C_{18}$, 10μ, 250 × 10 mm, acetonitrile/0.1 M ammonium formate solution = 35/65). Using acetonitrile/0.1 M ammonium formate solution (35/65, Luna $C_{18}$ 10μ, flow rate: 2 mL/min), TH4 was eluted at 2.68 min, revealing that this compound is more polar than candesartan ($t_R$ = 3.1 min). The retention time of methyl-candesartan was 5.58 min under these conditions. Similar results were obtained with the Prodigy 10 μm and Partisil SCX 10 μm columns using the same conditions. The pure TH4 was 16.7 mg (78%). Mp: 272–274 °C; $^1$H NMR (DMF-d7, 500 MHz) $\delta_H$ 11.45 (s, 1H), 7.70–7.35 (m, 6H), 7.20 (t, 1H, *J* = 7.8 Hz), 7.13 (d, 2H, *J* = 8.1 Hz), 6.87 (d, 2H, *J* = 8.1 Hz), 5.38 (s, 2H), 3.70 (s, 3H). IR (Nujol, $cm^{-1}$): 1718 (C=O, carbonyl of ester) and 1693 (C=O, carbonyl of cyclic urea group). HRMS calcd for $C_{23}H_{18}O_3N_6$: 426.1440, found: 426.1421.

#### 5.1.5. [$^{11}$C]Methyl-2-ethoxy-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate ([$^{11}$C]methyl-candesartan)

Approximately 5 min before the end of the beam, tetrazole-protected candesartan **1** (0.5 mg, $0.733 \times 10^{-3}$ mmol) was dissolved in 0.18 mL of dry DMF and in a separate vial, 0.25 mg ($0.66 \times 10^{-3}$ mmol) of kryptofix (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane) was dissolved in 0.02 mL DMF. Both solutions were transferred to a 1-mL V-vial containing 2 mg ($14.5 \times 10^{-3}$ mmol) of $K_2CO_3$, vortexed well and purged with argon. [$^{11}$C]$CO_2$ was prepared by proton bombardment of a nitrogen gas target [$^{14}$N(P,α)$^{11}$C] and converted to [$^{11}$C]methyl iodide using the wet method (lithium aluminum hydride reduction/hydroiodic acid).

[$^{11}$C]Methyl iodide was distilled through a $P_2O_5$ trap to the reaction vial with helium by a flow rate of 30 mL/min. Once maximum [$^{11}$C]methyl iodide is trapped, the reaction vial was sealed and heated at 65–70 °C for 3 min. The reaction mixture was then cooled to −20 °C and 1 N HCl (0.2 mL) was added. The reaction temperature was raised to 65–70 °C and heated at this temperature for 2 min. The reaction mixture was then cooled to −40 °C, quenched with HPLC buffer (acetonitrile/0.1 M ammonium formate solution = 35/65, 0.3 mL) and injected into the semi-preparative HPLC column (Luna $C_{18}$, 10μ, 250 × 10 mm, 8 mL/min). [$^{11}$C]Methyl-candesartan was eluted at 8.5 min. The fraction containing the product was collected in a rotary evaporator, evaporated to dryness and the final product was dissolved in a saline/sterile water/8.4% sodium bicarbonate (5/4.4/0.66 v/v/v) mixture. Quality control analyses were determined by injection of a formulation sample onto Luna $C_{18}$ (250 × 4.6 mm, 2 mL/min) using the same solvent as for semi-preparative HPLC.

#### 5.1.6. [$^{11}$C]-Methyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-2,3-dihydro-2-oxo-1*H*-benzimidazole-7-carboxylate ([$^{11}$C]TH4)

[$^{11}$C]TH4 was obtained using similar procedure as outlined for [$^{11}$C]methyl-candesartan at 90 °C in both the methylation and deprotection steps. [$^{11}$C]TH4 was eluted at 4.2 min in semi-preparative HPLC.

#### 5.1.7. Ex vivo bio-testing

**5.1.7.1. Time course.** Radiotracer retention time course was investigated using published methods for C-11 radioligands.[29,30] In brief, male Sprague-Dawley rats (200–275 g, Charles River, Montreal, Canada) were intravenously injected with 55.5–62.9 MBq (1.5–1.7 mCi) of [$^{11}$C]methyl-candesartan or [$^{11}$C]TH4 at a specific activity of 11.5–37 GBq/μmol of the respective tracer (311–1000 mCi/μmol at time of first injection; 2.4–10.8 μg/kg mass injected). Rats were sacrificed by decapitation without anesthesia 5, 15, 30, 45 or 60 min after [$^{11}$C]methyl-candesartan injection, and 5, 15, 30, or 60 min after [$^{11}$C]TH4 injection. Tissues were quickly dissected out and collected into pre-weighed gamma tubes: heart, kidney (renal cortex, outer medulla and inner medulla separately), adrenal, brain, lung, liver, and skeletal muscle (quadriceps). Trunk blood was collected in heparinized tubes, from which 1 mL was removed and transferred to pre-weighed gamma tubes. Blood and tissues were counted (decay-corrected) in a gamma-counter (Packard) along with injected standard solutions. Data were expressed as percent of injected dose per gram of tissue (%ID/g).

**5.1.7.2. Competition.** Radiotracers were evaluated for in vivo binding selectivity for the $AT_1$ receptor subtype using the published procedures[29,30] described above. $AT_1$ receptor blockers candesartan (3, 5 or 10 mg/kg)[31] or losartan (20 or 30 mg/kg)[32] were co-administered iv with the tracer or intravenously injected 5 min prior to radioligand injection. The $AT_2$ antagonist PD123,319 (2 or 5 mg/kg for [$^{11}$C]methyl-candesartan; 5 mg/kg for [$^{11}$C]TH4),[21,33] and a Mas receptor (Ang 1–7) antagonist A-779 (100 μg/kg)[34] were injected through the tail vein, 5 min prior to radioligand injection. Interperitoneal injections of the β-adrenergic receptor blocker propranolol (20 mg/kg)[35] or the $\alpha_2$-adrenergic receptor blocker yohimbine (10 mg/kg)[36] were given 15 min prior to the radioligand tail vein injection.

Rats (200–275 g) were sacrificed by decapitation without anesthesia 15 min following radioligand injection. Trunk blood and tissue samples or whole organ samples were collected and counted as in the time course studies. Data were expressed as a ratio of the %ID/g of tissue to that of blood to normalize for any differences in tracer delivery between treatment groups. Group means were compared using one-way ANOVA and Bonferroni post-hoc analysis with $p < 0.05$ as significant.

## Acknowledgements

We thank Jeffrey Collins and Paul Coletta for assistance in radiochemistry, Stephanie Thorn, Miran Kenk and James Thackeray for biodistribution studies. We are also grateful to the Canadian Institutes of Health Research MOP-80203, and the Heart and Stroke Foundation Molecular Function and Imaging program Grant # 6242 for funding this work.

## References and notes

1. Ardaillou, R. *J. Am. Soc. Nephrol.* **1999**, *10*, S30.
2. Harada, K.; Sugaya, T.; Murakami, K.; Yazaki, Y.; Komuro, I. *Circulation* **1999**, *100*, 2093.
3. Yousef, Z.; Redwood, S.; Marber, M. *Heart* **2000**, *83*, 76.
4. Wolf, G.; Ziyadeh, F. *Am. J. Kidney Dis.* **1997**, *29*, 153.
5. Admiraal, P.; Danser, A.; Jong, M.; Pieterman, H.; Derkx, F.; Schalekamp, M. *Hypertension* **1993**, *21*, 173.
6. Opie, L. H.; Sack, M. *Circ. Res.* **2001**, *88*, 654.
7. Phillips, M. I.; Speakman, E. A.; Kimura, B. *Regul. Pept.* **1993**, *43*, 1.
8. Allen, A. M.; Zhuo, J.; Mendelsohn, F. A. *J. Am. Soc. Nephrol.* **1999**, *10*, S23.
9. Zhang, H.; Sun, G. Y. *J. Surg. Res.* **2006**, *134*, 190.
10. Dostal, D.; Baker, K. *Circ. Res.* **1999**, *85*, 643.
11. Wagenaar, L.; Voors, A.; Buikema, H.; Gilst, W. *Can. J. Cardiol.* **2002**, *18*, 1331.
12. Goodfriend, T.; Elliott, M.; Catt, K. *N. Eng. J. Med.* **1996**, *334*, 1649.
13. Dronavalli, S.; Duka, I.; Bakris, G. L. *Nat. Clin. Pract. Endocrinol. Metab.* **2008**, *4*, 444.
14. Allen, T. J.; Cao, Z.; Youssef, S.; Hulthen, U. L.; Cooper, M. E. *Diabetes* **1997**, *46*, 1612.
15. Anderson, S.; Jung, F.; Ingelfinger, J. *Am. J. Physiol.* **1993**, *265*, F477.
16. Pfeffer, M.; Swedberg, K.; Granger, C.; Held, P.; McMurray, J.; Michelson, E.; Olofsson, B.; Ostergren, J.; Yusuf, S.; Pocock, S. *Lancet* **2003**, *362*, 759.
17. Solomon, S. D.; Skali, H.; Anavekar, N. S.; Bourgoun, M.; Barvik, S.; Ghali, J. K.; Warnica, J. W.; Khrakovskaya, M.; Arnold, J. M.; Schwartz, Y.; Velazquez, E. J.; Califf, R. M.; McMurray, J. V.; Pfeffer, M. A. *Circulation* **2005**, *111*, 3411.
18. Brenner, B.; Cooper, M.; de Zeeuw, D.; Keane, W.; Mitch, W.; Parving, H.; Remuzzi, G.; Snapinn, S.; Zhang, Z.; Shahinfar, S. *N. Eng. J. Med.* **2001**, *345*, 861.
19. Remuzzi, A.; Perico, N.; Amuchastegui, C. S.; Malanchini, B.; Mazerska, M.; Battaglia, C.; Bertani, T.; Remuzzi, G. *J. Am. Soc. Nephrol.* **1993**, *4*, 40.
20. Mathews, W. B.; Burns, H. D.; Dannals, R. F.; Ravert, H. T.; Naylor, E. M. *J. Labelled Compd. Radiopharm.* **1995**, *36*, 729.
21. Zober, T. G.; Mathews, W. B.; Seckin, E.; Yoo, S. E.; Hilton, J.; Xia, J.; Sandberg, K.; Ravert, H. T.; Dannals, R. F.; Szabo, Z. *Nucl. Med. Biol.* **2006**, *33*, 5.
22. Unger, T. *Blood Press* **2001**, *10*, 12.
23. Kubo, K.; Kohara, Y.; Imamiya, E.; Sugiura, Y.; Inada, Y.; Furukawa, Y.; Nishikawa, K.; Naka, T. *J. Med. Chem.* **1993**, *36*, 2182.
24. Kubo, K.; Kohara, Y.; Yoshimura, Y.; Inada, Y.; Shibouta, Y.; Furukawa, Y.; Kato, T.; Nishikawa, K.; Naka, T. *J. Med. Chem.* **1993**, *36*, 2343.
25. Naka, T.; Nishikawa, K.; Kato, T. U.S. Patent 5,196,444, 1993.
26. Duncia, J. V.; Pierce, M. E.; Santella, J. B., III *J. Org. Chem.* **1991**, *56*, 2395.
27. Chang, R. S.; Lotti, V. J. *Life Sci.* **1991**, *49*, 1485.
28. Kostenis, E.; Milligan, G.; Christopoulos, A.; Sanchez-Ferrer, C. F.; Heringer-Walther, S.; Sexton, P. M.; Gembardt, F.; Kellett, E.; Martini, L.; Vanderheyden, P.; Schultheiss, H. P.; Walther, T. *Circulation* **2005**, *111*, 1806.
29. Lourenco, C. M.; Houle, S.; Wilson, A. A.; DaSilva, J. N. *Nucl. Med. Biol.* **2001**, *28*, 347.
30. Kenk, M.; Greene, M.; Thackeray, J.; Dekemp, R. A.; Lortie, M.; Thorn, S.; Beanlands, R. S.; Dasilva, J. N. *Nucl. Med. Biol.* **2007**, *34*, 71.
31. Nossaman, B. D.; Baber, S. R.; Nazim, M. M.; Detrolio, J. D.; Kadowitz, P. J. *Can. J. Physiol. Pharmacol.* **2007**, *85*, 360.
32. Sun, C. K.; Chang, L. T.; Sheu, J. J.; Wang, C. Y.; Youssef, A. A.; Wu, C. J.; Chua, S.; Yip, H. K. *Int. Heart J.* **2007**, *48*, 533.
33. Naruse, M.; Tanabe, A.; Sato, A.; Takagi, S.; Tsuchiya, K.; Imaki, T.; Takano, K. *Hypertension* **2002**, *40*, 28.
34. Bayorh, M. A.; Eatman, D.; Walton, M.; Socci, R. R.; Thierry-Palmer, M.; Emmett, N. *Peptides* **2002**, *23*, 57.
35. Friedman, E. M.; Irwin, M. *Brain Behav. Immun.* **2001**, *15*, 65.
36. Munzar, P.; Goldberg, S. R. *Psychopharmacology (Berl)* **1999**, *143*, 293.