Bioorganic & Medicinal Chemistry Letters 22 (2012) 2172–2174

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Radiosynthesis of [$^{11}$C]BBAC and [$^{11}$C]BBPC as potential PET tracers for orexin2 receptors

Fei Liu [a,c], Vattoly J. Majo [b,c], Jaya Prabhakaran [b,c], John Castrillion [a,c], J. John Mann [b,c,d,e], Diana Martinez [a,c], J.S. Dileep Kumar [b,c,d,*]

[a] Division of Substance Abuse, Columbia University Medical Center, United States
[b] Division of Molecular Imaging and Neuropathology, Columbia University Medical Center, United States
[c] Department of Psychiatry, Columbia University Medical Center, United States
[d] New York State Psychiatric Institute, Columbia University, New York, NY 10032, United States
[e] Department of Radiology, Columbia University, New York, NY 10032, United States

ARTICLE INFO

*Article history:*
Received 9 December 2011
Revised 25 January 2012
Accepted 30 January 2012
Available online 4 February 2012

*Keywords:*
Orexin
Hypocretin
Positron emission tomography
Radiotracer

ABSTRACT

Radiosynthesis of [*N*-methyl-$^{11}$C](*S*)-*N*-([1,1′-biphenyl]-2-yl)-1-(2-((1-methyl-1*H*-benzo[*d*]imidazol-2-yl)thio)acetyl)pyrrolidine-2-carboxamide ([$^{11}$C]BBAC or [$^{11}$C]**3**) and [*N*-methyl-$^{11}$C] (*S*)-*N*-([1,1′-biphenyl]-2-yl)-1-(3-(1-methyl-1*H*-benzo[*d*]imidazol-2-yl)propanoyl)pyrrolidine-2-carboxamide ([$^{11}$C]BBPC or [$^{11}$C]-**4**), two potential PET tracers for orexin2 receptors are described. Syntheses of non-radioactive standards **3**, **4** and corresponding desmethyl precursors **1**, **2** were achieved from common intermediate (*S*)-2-([1,1′-biphenyl]-2-yl)-1-(pyrrolidin-2-yl)ethanone. Methylation using [$^{11}$C]$CH_3$OTf in the presence of base in acetone afforded [$^{11}$C]**3** and [$^{11}$C]**4** in 30 ± 5% yield (EOS) with >99 % radiochemical purities with a specific activity ranged from 2.5 ± 0.5 Ci/μmol (EOB). The log*P* of [$^{11}$C]**3** and [$^{11}$C]**4** were determined as 3.4 and 2.8, respectively. The total synthesis time was 30 min from EOB. However, PET scans performed in a rhesus monkey did not show tracer retention or appropriate brain uptake. Hence [$^{11}$C]**3** and [$^{11}$C]**4** cannot be used as PET tracers for imaging orexin2 receptors.

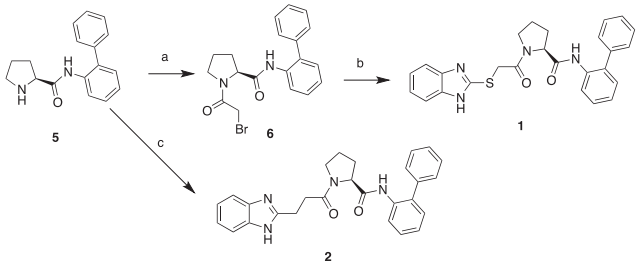


Orexin or hypocretin receptors are G-protein coupled receptors (GPCR) that mediate the central actions of the endogenous neurohormones orexin-A and -B (also known as hypocretins-1 and 2) produced in the lateral hypothalamus.[1,2] Pre-clinical and clinical studies have established that orexin pathway plays a critical role in motivation, arousal and sleep-wake regulation.[3,4] Abnormalities in orexin signaling have been implicated in sleep disorders such as human narcolepsy–cataplexy,[5] irregularities in central vestibular motor control,[6] feeding and gastrointestinal disorders[7,8] and addiction.[9] Based on the binding of endogenous ligands, the orexin receptors are divided into two major types: orexin1 ($OX_1R$, or $HCRT_1$), and orexin2 ($OX_2R$, or $HCRT_2R$) receptors.[10] Among these, $OX_1R$ have preferential affinity for orexin-A, whereas $OX_2R$ bind with equal affinities for both neuropeptides.[1] However, the in vivo selectivity, distribution and involvements of individual receptors in the pathophysiology of orexin-mediated disorders are not available. Positron emission tomography (PET) is an excellent tool for the in vivo quantification of biological processes. Currently, there are no PET tracers available for imaging orexin receptors. We have selected $OX_2R$ as an imaging target anticipating a predominant role for this receptor in the pathophysiology elicited by orexin A and B owing to its ability to bind to both endogenous ligands with equal affinity. Candidates belonging to different templates have been reported as selective $OX_2R$ antagonist ligands.[10–15] Among these *N*-methylated benzimidazole bis amides of proline as a lead structure had significantly reduced *para*-glycoprotein (P-gp) susceptibility, increased receptor selectivity thereby offering ligands with increased potency and good blood–brain barrier (BBB) penetration.[16,17] We have selected proline bis-amides **3** and **4** as candidate PET ligands for $OX_2R$ imaging owing to nanomolar affinity ($K_i$ = 0.2 nM for **3** and 0.8 nM for **4**), log*P* favorable to penetrate BBB (3.2 for **3** and 2.5 for **4**), and excellent pharmacokinetics, and brain/plasma ratios.[17] Moreover, compound **3** inhibit ADL-orexinB locomotion model in rats in a dose dependent manner.[17] Additionally, the availability of suitable sites for incorporating C-11 isotope encouraged us to test compounds **3** and **4** as potential PET imaging agents for $OX_2R$. Herein, we report the radiosynthesis and in vivo evaluation of two $OX_2R$ ligands **3**and **4** as potential PET imaging agents in rhesus monkey.

Syntheses of nonradioactive standards **3**, **4** and desmethyl precursors **1**, **2** were achieved in good yield starting from(*S*)-2-([1,1′-biphenyl]-2-yl)-1-(pyrrolidin-2-yl)ethanone (**5**), reported previously (Scheme 1).[17] Compound **5** was subsequently treated with 2-bromoacetyl bromide in presence of triethylamine and DMAP to

* Corresponding author. Fax: +1 212 543 1163.
*E-mail address:* dk2038@columbia.edu (J.S.D. Kumar).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.01.114

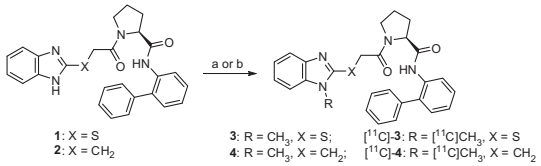


**Scheme 1.** Syntheses of compounds **1** and **2**. Reagents and conditions: (a) 2-bromoacetyl bromide, $Et_3N$, DMAP, $CH_2Cl_2$, 0 °C, 1 h, 72%; (b) 1*H*-benzo[*d*]imidazole-2-thiol, $Et_3N$, ethanol, 86%; (c) 3-(1*H*-benzo[*d*]imidazol-2-yl)propanoic acid, EDC, HOBT, $Et_3N$, DMF, 76%.

obtain the bromide **6** in 72% yield. The radiolabeling precursor **1** for [$^{11}$C]**3** was synthesized by coupling compound **6** with 1*H*-benzo[*d*]imidazole-2-thiol in 86% yield.[18] Similarly, condensation of **5** with 3-(1*H*-benzo[*d*]imidazol-2-yl)-propanoicacid afforded, compound **2** (76%), the radiolabeling precursor for [$^{11}$C]**4** (Scheme 1).[19]

We found that methylation of **1** and **2** with $CH_3I$ in the presence of $Cs_2CO_3$ in DMF at room temperature afforded compounds **3** and **4**, respectively, in good yield (Scheme 2).[20] Therefore, radiolabeling of [$^{11}$C]-**3** and [$^{11}$C]-**4** were attempted initially with [$^{11}$C]$CH_3I$ to optimize the reaction conditions. However, [$^{11}$C]methylation proceeded in better yield by using [$^{11}$C]$CH_3OTf$ in acetone at room temperature. Accordingly, radiolabeling reactions were performed using 0.5 mg of the respective precursors in 0.5 ml acetone in the presence of 5 M NaOH at rt using [$^{11}$C]$CH_3OTf$ to provide [$^{11}$C]**3** and [$^{11}$C]**4** in 30 ± 5% yield at the end of synthesis (EOS) (Scheme 2). The radioproducts were purified via semipreparative HPLC and solid phase extraction and formulated in saline containing 10% ethanol. The specific activity of the radioproducts were found to be in the range of 2–3 Ci/μmol ($n$ = 8) at the end of bombardment (EOB) with excellent chemical and radiochemical purities.[21] The total time required for the radiosyntheses were ~30 min. The partition coefficient for [$^{11}$C]**3** and [$^{11}$C]**4** obtained by standard shake flask method were 3.4 and 2.8, respectively.[22]

Subsequently we examined the BBB permeability and in vivo distribution of the radiotracers, by PET scans in an anesthetized female rhesus monkey. Anesthesia was performed with isoflurane (1–2%) and ketamine to induce general anesthesia and the animal was under constant cardiovascular monitoring. The radiotracers were injected as a bolus (dose: 2.03 mCi, specific activity: 1.43 Ci/μmol and injected mass: 1.5 μg for [$^{11}$C]**3** and dose: 1.94 mCi, specific activity: 1.15 Ci/μmol and injected mass: 1.7 μg for [$^{11}$C]**4**) and emission data was collected for 122 min. The images from each scan are shown in Figure 1. Although radioactivity is seen in the area of the pituitary (transaxial scan, Fig. 1, 3rd column), no brain region inside the BBB had detectable binding consistent with specific receptor binding to the radiotracers. The rational for this lack of specific uptake inside the brain is not clear.



**Scheme 2.** Synthesis and radiosynthesis of [$^{11}$C]**3** and [$^{11}$C]**4**. Reagents and conditions: (a) $Cs_2CO_3$, DMF, $CH_3I$; (b) (i) NaOH, acetone, [$^{11}$C]$CH_3OTf$, (ii) HPLC purification, yield = 30 ± 5% (EOS).

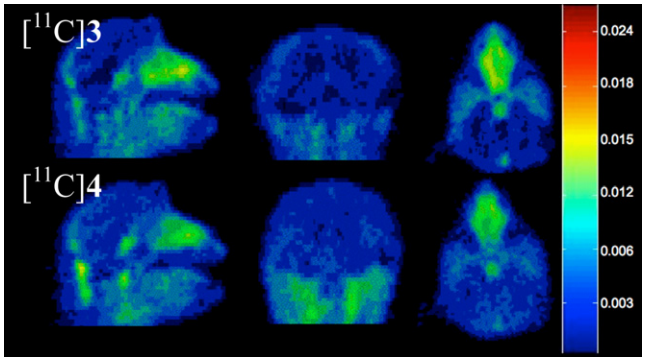



**Figure 1.** PET images of [$^{11}$C]**3** (first row) and [$^{11}$C]**4** (bottom row) in rhesus monkey. The images shown are sagittal, coronal, and transaxial PET scans (left to right) generated as the sum of all frames acquired over 120 min. The units of the color bar are MBq/cc.

One issue could be the affinity and permeability of the radiotracers. However, both the affinity and the log*P* suggested that these radiotracers were favorable for use as radiotracers. Another potential issue is that the radiotracers may be P-glycoprotein (P-GP) or multi drug resist (MDR) substrates or the density of orexin receptors in the rhesus monkey brain may not be high enough to permit brain PET imaging. Perhaps a high affinity radioligand with higher specific activity, and which is not a P-GP or MDR substrate may be required for the quantification of $OX_2R$ by PET.

In summary, we have successfully synthesized [$^{11}$C]**3** and [$^{11}$C]-**4**, as potential PET tracer agents for $OX_2R$. The total time required for the radiosynthesis were 30 min from EOB using [$^{11}$C]$CH_3OTf$ in acetone. Radioproducts were obtained in 30 + 5% yield (EOS) with excellent purities and specific activity in the formulation. However, in vivo PET studies in rhesus monkeys did not show tracer uptake in brain.

## References and notes


1. Sakurai, T.; Amemiya, A.; Ishii, M.; Matsuzaki, I.; Chemelli, R. M.; Tanaka, H.; Williams, S. C.; Richardson, J. A.; Kozlowski, G. P.; Wilson, S.; Arch, J. R.; Buckingham, R. E.; Haynes, A. C.; Carr, S. A.; Annan, R. S.; McNulty, D. E.; Liu, W. S.; Terrett, J. A.; Elshourbagy, N. A.; Bergsma, D. J.; Yanagisawa, M. *Cell* **1998**, *92*, 573.
2. van den Pol, A. N.; Gao, X. B.; Obrietan, K.; Kilduff, T. S.; Belousov, A. B. *J. Neurosci.* **1998**, *18*, 7962.
3. Brisbare-Roch, C.; Dingemanse, J.; Koberstein, R.; Hoever, P.; Aissaoui, H.; Flores, S.; Mueller, C.; Nayler, O.; van Gerven, J.; de Haas, S. L.; Hess, P.; Qiu, C.; Buchmann, S.; Scherz, M.; Weller, T.; Fischli, W.; Clozel, M.; Jenck, F. *Nat. Med.* **2007**, *13*, 150.
4. Thompson, J. L.; Borgland, S. L. *Behav. Brain Res.* **2011**, *217*, 446.
5. Nishino, S. *Handb. Clin. Neurol.* **2011**, *99*, 765.
6. Zhang, J.; Li, B.; Yu, L.; He, Y. C.; Li, H. Z.; Zhu, J. N.; Wang, J. J. *Neuron* **2011**, *69*, 793.
7. Okumura, T.; Nozu, T. *J Gastroenterol. Hepatol.* **2011**, *26*, 61.
8. Sakurai, T. *Regul. Pept.* **1999**, *85*, 25.
9. Sharf, R.; Sarhan, M.; Dileone, R. *J. Brain Res.* **2010**, *130*, 1314.
10. Boss, C.; Brisbare-Roch, C.; Jenck, F. *J. Med. Chem.* **2009**, *52*, 891.
11. Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Boss, C. *Chem. Med. Chem.* **2010**, *5*, 1197.
12. Hirose, M.; Egashira, S.; Goto, Y.; Hashihayata, T.; Ohtake, N.; Iwaasa, H.; Hata, M.; Fukami, T.; Kanatani, A.; Yamada, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4497.
13. McAtee, L. C.; Sutton, S. W.; Rudolph, D. A.; Li, X.; Aluisio, L. E.; Phuong, V. K.; Dvorak, C. A.; Lovenberg, T. W.; Carruthers, N. I.; Jones, T. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4225.
14. Aissaoui, H.; Koberstein, R.; Zumbrunn, C.; Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Treiber, A.; Boss, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5729.
15. Micheli, F.; Antolini, M.; Di Fabio, R.; Pellacani, A.; Pozzan, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6405.
16. Porter, R. A.; Chan, W. N.; Coulton, S.; Johns, A.; Hadley, M. S.; Widdowson, K.; Jerman, J. C.; Brough, S. J.; Coldwell, M.; Smart, D.; Jewitt, F.; Jeffrey, P.; Austin, N. *Bioorg. Med. Chem. Lett.* **1907**, *2001*, 11.
17. Bergman, J. M.; Roecker, A. J.; Mercer, S. P.; Bednar, R. A.; Reiss, D. R.; Ransom, R. W.; Meacham Harrell, C.; Pettibone, D. J.; Lemaire, W.; Murphy, K. L.; Li, C.; Prueksaritanont, T.; Winrow, C. J.; Renger, J. J.; Koblan, K. S.; Hartman, G. D.; Coleman, P. J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1425.
18. Synthesis of (*S*)-1-(2-((1*H*-benzo[*d*]imidazol-2-yl)thio) acetyl)-*N*-([1,1′-biphenyl]-2-yl)pyrrolidine-2-carboxamide (**1**): 2-Mercaptobenimidazole

(90 mg, 0.6 mmol) in 2 ml ethanol was added to a solution of 0.1 ml of triethylamine and 210 mg (0.54 mmol) of (*S*)-2-([1,1′-biphenyl]-2-yl)-1-(1-(2-bromo-acetyl)pyrrolidin-2-yl)ethanone (**6**) in 2 ml ethanol. The solution was stirred for 1 h at room temperature. The solvent was removed and 10 ml of the water was added to the residue. the organic layers were collected, dried over $MgSO_4$, filtered and concentrated under vacuum. The result residue was purified by the chromatographed on silica gel using a mixture of 2:1 ethyl acetate with hexane and 9**:1 ethyl acetate with methanol as eluents to obtain the desmethyl compound **1** (214 mg, 86%) as a brown solid. $^1$H NMR ($CDCl_3$, 400 MHz): 2.1 (3H, m, $CH_2$), 2.4 (1H, m, $CH_2$), 3.6 (2H, m, $CH_2$), 3.7 (2H, t, CH), 4.2 (1H, m, CH),4.5 (1H, t, CH),7.3(4H, m), 7.4 (4H, m),7.5 (4H, m), 8.1 (1H, s, NH), 8.2 (1H, dd), APCI+ calculated for $C_{26}H_{25}N_4O_2S$ (MH+): 457.17; Found: 456.86.

19. Synthesis of (*S*)-1-(3-(1*H*-benzo[*d*]imidazol-2-yl)propanoyl)-*N*-([1,1′-biphenyl]-2-yl)pyrrolidine-2-carboxamide (**2**): To a solution of (*S*)-2-([1,1′-biphenyl]-2-yl)-1-(pyrrolidin-2-yl)ethanone (5, 200 mg, 0.75 mmol) and 2-benzimidazole propionic acid (143 mg, 0.75 mmol) in DMF (3 ml) was added HOBT (102 mg, 0.75 mmol), EDC (144 mg, 0.75 mmol), and triethylamine (76 mg, 1.5 mmol). The reaction mixture was heated to 100 °C for 1 h. After removal of DMF under high vacuum, the residue was extracted with ethyl acetate (3 × 10 ml) and water (3 × 10 ml). The combined ethyl acetate extracts was dried over anhydrous $MgSO_4$, filtered and evaporated to provide crude product. This was chromatographed over silica gel using a mixture of 2:1 ethyl acetate–hexane to afford desmethyl compound **2** (250 mg, 76%) as a white solid.$^1$H NMR ($CDCl_3$, 400 MHz): 1.9 (2H, m, $CH_2$), 2.1 (1H, m, $CH_2$), 2.3 (1H, m, $CH_2$), 2.5 (1H, m, $CH_2$), 2.8(1H, m, $CH_2$), 3.0 (1H, m, CH), 3.2 (1H, m, CH), 3.3 (1H, m, CH), 4.2 (1H, m, CH), 4.6 (1H, m, CH), 7.3 (2H, m), 7.4 (10H, m),8.2 (1H, m), 8.4 (1H, s); APCI+ calculated for $C_{27}H_{27}N_4O_2$ (MH+): 439.21; Found: 439.12.
20. Synthesis of **3** and **4**: To a solution of **1** or **2** (0.45 mmol) in dry DMF (1.5 ml) a room temperature was added $Cs_2CO_3$ (186 mg, 0.5 mmol) and iodomethane (30 ml, 0.45 mmol). The reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with water (5 ml), extracted with ethyl acetate (3 × 10 ml) and the combined extract were dried over anhydrous $MgSO_4$ and evaporated. The crude products were chromatographed over silica gel using a mixture of 95:5 ethyl acetate–hexane to afford 65 mg of **3** (32%) and 90 mg **4** (45%) as light brown solids which were identified by the $^1$H NMR and mass spectrum. $^1$H NMR of **3** ($CDCl_3$, 400 MHz): 2.1 (3H, m, $CH_2$), 2.4 (1H, m, $CH_2$), 3.7 (2H, m, $CH_2$), 3.7 (3H, s, $CH_3$), 4.0 (1H, m, CH),4.4 (1H, m, CH), 4.6 (1H, m, CH), 7.3 (6H, m), 7.4 (6H, m), 8.1 (1H, dd), 8.4 (1H, s, NH), APCI+ calculated for $C_{27}H_{27}N_4O_2S$ (MH+): 472.19; Found: d472.46. $^1$H NMR of **4** ($CDCl_3$, 400 MHz): 1.9 (3H, m, $CH_2$), 2.1 (1H, m, $CH_2$), 2.4 (1H, m, $CH_2$), 2.8 (1H, m, $CH_2$), 3.0 (2H, m, $CH_2$), 3.5 (2H, m, CH), 3.8 (3H, s, $CH_3$), 4.6 (1H, m, CH),7.2 (4H, m), 7.3 (4H, m),7.5 (3H, m), 7.6 (1H, dd), 8.1(1H, dd), 8.5 (1H, s, NH); HRMS calculated for $C_{28}H_{29}N_4O_2$ (MH+): 453.2291; Found: 453.2294.
21. Radiosynthesis of [$^{11}$C]**3** and [$^{11}$C]**4**: Precursor **1** or **2** (0.6 mg) was dissolved in 400 μL of acetone in a capped 1 ml reactivial. Aqueous NaOH (5 μL, 5 N) was then added to the solution and the reaction mixture was vortexed. [$_{11}$C]-methyl triflate was transported by a stream of argon (20–30 ml/min) into the reactivial over a period of 5 min at room temperature. At the end of the trapping, the reaction mixture was diluted with water and directly injected into a semipreparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 μm) and eluted with acetonitrile: 0.1 M ammonium formate (50:50) at a flow rate of 10 ml/min. The product fractions of [$^{11}$C]- and [$^{11}$C]**4** with a retention time of 6–7 and 4–5 min based on γ-detector was collected, diluted with 100 ml of deionized water, and passed through a classic C-18 Sep-pak® cartridge, respectively. Reconstitution of the product in 1 ml of absolute ethanol afforded [$^{11}$C]**3** in 30 ± 5% yield and [$^{11}$C]**4** in 25 ± 5% (EOS, $n$ = 6). A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex, Prodigy ODS (3) 4.6 × 250 mm, 5 μm mobile phase: acetonitrile/0.1 M ammonium formate 50:50, flow rate of: 2 ml/min, retention time: 7.1 min for [$^{11}$C]**3** and 4.8 min for [$^{11}$C]**4**, respectively) to determine the specific activity and radiochemical purity. The chemical and radiochemical purities of [$^{11}$C]**3** and [$^{11}$C]**4** were reconfirmed by RP-HPLC (Waters μBondapak 4.6 × 300 mm, 10 μm, mobile phase, 50:50 acetonitrile/0.1 M ammonium formate flow rate: 2 ml/min, retention time = 5.8 min). The ethanol solution was then diluted with 9 ml of saline and passed through a sterile filter.
22. Wilson, A. A.; Jin, L.; Garcia, A.; DaSilva, J. N.; Houle, S. *Appl. Rad. Isotop.* **2001**, *54*, 203.