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Research Article

Synthesis of [*O*-methyl-^{11}C]-4-(1,3-dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]pyrazolo-1,3,5-triazine ([^{11}C]DMP696): a potential PET ligand for CRF_1 receptors

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Summary

Synthesis of [*O*-methyl-^{11}C]-4-(1,3-dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]pyrazolo-1,3,5-triazine ([^{11}C]DMP696), a highly selective CRF_1 antagonist has been achieved. The total time required for the synthesis of [^{11}C]DMP696 is 30 min from EOB using [^{11}C]methyl triflate in THF, with a 16% yield (EOS) and >99% chemical and radiochemical purities along with a specific activity of >2000 Ci/mmol (EOS). Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: CRF antagonist; positron emission tomography; radiotracer; DMP696

Introduction

Corticotropin releasing factor (CRF) plays a critical role in modulating the endocrinal, autonomic, behavioral and immune responses to stress.[1] The functions of CRF are mainly mediated through two CRF receptor subtypes (CRF_1 and CRF_2) and a CRF binding protein (CRF-BP).[2] The CRF_1 receptors are the most abundant CRF receptor subtype found in rodent and primate pituitary and are widely distributed in the brain including cerebral cortex, hippocampus and amygdala.[3] The CRF_2 receptors are primarily distributed in lateral septum, choroid plexus, hypothalamus and sympathetic

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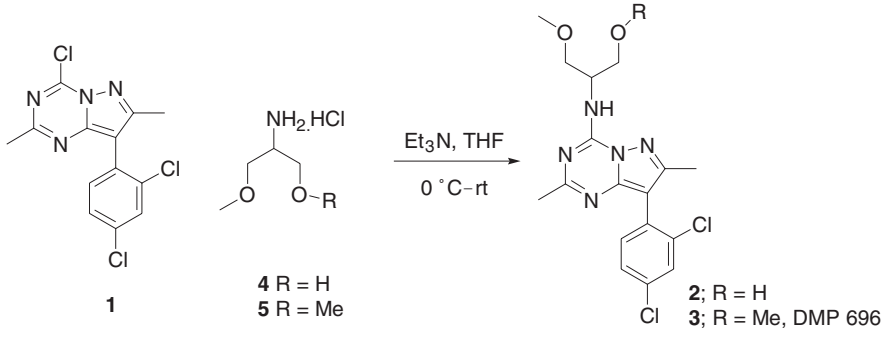
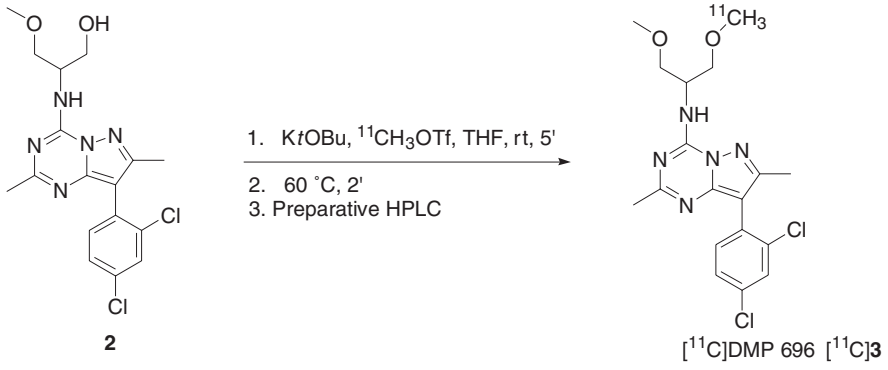
nuclei, whereas, CRF binding protein is widely distributed in the central nervous system (CNS) including hippocampus and amygdala and in human, but not in rodent, it is also found in plasma.[4] Abnormal expression of CRF_1, mostly over-expression, comprises part of the pathogenesis of a diverse range of neuropsychiatric disorders such as anxiety disorders, mood disorders, obsessive-compulsive disorders, posttraumatic stress disorders and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.[5–10] A number of non-peptide CRF_1 receptor antagonists that can specifically and selectively block the CRF_1 receptor subtype have recently been identified. To be effective; these molecules must have receptor subtype specificity (a high CRF_1/CRF_2 ratio for affinity), aqueous solubility, good oral bioavailability and rapid permeability across the blood brain barrier (BBB).[11] Development of a high-specific activity radiolabeled, selective CRF_1 receptor antagonists for PET would make it possible to quantify binding to CRF_1 receptors *in vivo*, repeatedly to study the pathophysiology of depression, anxiety and other neurodegenerative diseases. The radiosynthesis of a number of potential candidates for imaging the CRF_1 receptor have been recently developed.[12–15] Although these ligands have been evaluated *in vitro*/*in vivo* as potential PET/ SPECT probes, currently no published imaging agent is available for the *in vivo* measurement of CRF_1 receptor in human subjects. We have selected DMP696 as a potential PET ligand based on its high binding affinity ($Ki = 1.7$ nmol/l) and the available *in vivo* data of the unlabeled compound towards CRF_1 receptors.[16,17]

Results and discussion

The synthesis of 2-[8-(2,4-dichlorophenyl)-2,7-dimethylpyrazolo[1,5-a][1,3,5] triazin-4-ylamino]-3-methoxypropan-1-ol (**2**), the precursor molecule for the radiolabeling was achieved by the reaction of 4-chloro-8-(2,4-dichlorophenyl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazine (**1**) and 2-amino-3-methoxypropan-1-ol hydrochloride (**4**) in THF using triethylamine as base (Scheme 1). The



Scheme 1. Synthesis of DMP696 and radiolabeling precursor 2



Scheme 2. Radiosynthesis of DMP696

unlabeled DMP696 (**3**) was synthesized by the reaction of **1** with 2-methoxy-1-methoxymethylethylamine hydrochloride (**5**) in presence of triethylamine (Scheme 1). The methylaminoalcohol **4** was obtained by the treatment of serine methyl ester with ethyl benzimidate followed by reduction and cleavage of the corresponding oxazoline by adopting Meyer's protocol.[18] The dimethoxyamine was synthesized in three steps from tritylated dimethylester of aminomalonic acid by following a reported procedure.[19] The precursor **2** has two vulnerable sites for radiomethylation, the secondary amine (4th position of the triazine ring) and the hydroxyl group at the side chain, respectively. Therefore, we have designed the labeling reaction using a bulky base to minimize the deprotonation of the sterically hindered amino group in the precursor **2**. The labeling reaction was initially modeled with non-radioactive methyl triflate and the reaction condition was optimized using potassium *t*-butoxide as the base in THF. Under this condition, DMP696 was obtained in 93% yield.

Subsequently, our labeling experiments of **2** with [^{11}C]MeOTf in THF using K*t*OBu provided [^{11}C]-**3**. The radiolabeled product was separated from the reaction mixture by RP-HPLC with an average yield of 16% (EOS, Scheme 2). The chemical identity of [^{11}C]-**3** was confirmed by co-injection with an authentic sample of **3** on analytical reverse-phase HPLC. The specific activity was calculated based on a standard mass curve using HPLC technique. The chemical and radiochemical purity of [^{11}C]-**3** was found to be $>99\%$ with a specific activity >2000 Ci/mmol (EOS). The average time required for the [^{11}C]labeling was 30 min from EOB.

Conclusions

In summary, a simple and convenient method for the synthesis of [*O*-methyl-^{11}C]-4-(1,3-dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl) [1,5-a]pyrazolo-1,3,5-triazine ([^{11}C] DMP696) is achieved. The total time required for the synthesis of [^{11}C]DMP696 was 30 min from EOB by using

[^{11}C]-methyl triflate in THF. The chemical and radiochemical purity was >99% with a specific activity >2000 Ci/mmol (EOS). This ligand will be evaluated for its *in vitro* and *in vivo* abilities to bind CRF_1 receptors.

Experimental

Melting points were determined on a Fisher Melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker PPX 300 and 400 MHz spectrometer. Spectra are recorded in $CDCl_3$ and chemical shifts are reported in ppm relative to TMS as internal standards. The mass spectra were recorded on JKS-HX 11UHF/HX110 HF Tandem Mass Spectrometer in the FAB+ mode. The HPLC analyses were performed using Waters 1525 HPLC system (column: Phenomenex, Prodigy ODS 4.6 × 250 mm, 5 μ). Flash column chromatography was performed on silica gel (Fisher 200–400 mesh) using the solvent system indicated. [^{11}C]-Methyl triflate was synthesized in the radioligand laboratory of Columbia University by transferring [^{11}C]-methyl iodide through a glass column containing AgOTf at 200°C.[20] The radiochemical and chemical purities were analyzed by RP-HPLC with UV detector at 280 nm wavelength and NaI detectors. The compound **1** was synthesized based on a reported procedure.[16]

4-(1-Hydroxy-3-methoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]pyrazolo-1,3,5-triazine (2)

To a mixture of 4-chloro-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]-2-aminopropane **1** (200 mg, 0.6105 mmol) and 2-amino-3-methoxypropan-1-ol hydrochloride **4** (110 mg, 0.7767 mmol) in THF (5 ml), triethylamine (130 μl, 0.92 mmol) was added dropwise at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for further 30 min. The reaction mixture was quenched with water, extracted with ethyl acetate and the combined organic layer was dried over magnesium sulfate, filtered and concentrated under vacuum and column chromatographed (50% ethyl acetate in hexane) to give the product as a colorless solid (104 mg, 43%). Mp. 171°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (s, 3 H), 2.47 (s, 3 H), 3.44 (s, 3 H), 3.52–3.54 (m, 1 H), 3.71–3.77 (m, 2 H), 3.86–3.91 (m, 1 H), 3.96–4.00 (m, 1 H), 4.41–4.46 (m, 1 H,), 7.04 (d, 1 H, J = 7.3 Hz), 7.28–7.33 (m, 2 H), 7.51 (d, 1 H, J = 1.6 Hz); HRMS (FAB^+) calculated for $C_{17}H_{19}Cl_2N_5O_2$: 396.0994, found 396.1008 (MH^+).

4-(1,3-Dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]pyrazolo-1,3,5-triazine (3)

To a mixture of 4-chloro-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-a]-2-aminopropane **1** (200 mg, 0.6105 mmol) and 1,3-dimethoxypropyl-2-aminopropane hydrochloride **5** (133 mg, 0.8547 mmol) in THF (5 ml), triethylamine (130 μl,

0.92 mmol) was added dropwise at 0°C. The reaction mixture was allowed to warm to room temperature and stirred for further 30 min. The reaction mixture was quenched with water, extracted with ethyl acetate and the combined organic layer was dried over magnesium sulfate, filtered and concentrated under vacuum and column chromatographed (15% ethyl acetate in hexane) to give the product as a colorless solid (135 mg, 54%). Mp.126°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.33 (s, 3 H), 2.48 (s, 3 H), 3.43 (s, 6 H), 3.59 (m, 2 H), 3.68 (m, 2 H), 4.64 (septet, 1 H, J = 4.8 Hz), 6.73 (d, 1 H, J = 9 Hz), 7. 29–7.32 (m, 2 H), 7.51 (d, 1 H, J = 1.8 Hz); HRMS (FAB^+) calculated for $C_{18}H_{21}Cl_2N_5O_2$: 410.1151, found 410.1162 (MH^+).

Radiosynthesis of 4-(1,3-dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-[1,5-a]pyrazolo-1,3,5-triazine ([^{11}C]DMP696)

The precursor alcohol **2** (1.0 mg) was dissolved in 400 µl of freshly distilled anhydrous THF in a capped 5 ml V-vial. KO*t*Bu (1 mg) was added to the solution and the reaction mixture was vortexed for 10 s and then allowed to stand for 4 min. [^{11}C]-Methyl triflate was transported by a stream of argon (20–30 ml/min) into the vial for 5 min at room temperature. At the end of the trapping, the reaction mixture was heated on a waterbath at 60°C for 2 min and then directly injected into a semi preparative RP-HPLC (Phenomenex C18, 10 × 250 mm, 10 µl) and eluted with acetonitrile: 0.1 M ammonium formate solution (65:35) at a flow rate of 10 ml/min. The precursor appeared at 5–6 min during the HPLC analysis. The product fraction with a retention time of 9–10 min based on γ-detector was collected, diluted with 100 ml of deionized water, and passed through a classic C-18 Sep-Pak cartridge. Reconstitution of the product in 1 ml of absolute ethanol afforded [^{11}C]DMP696 (16% yield; EOS). A portion of the ethanol solution was analyzed by analytical HPLC (Phenomenex C18; mobile phase: acetonitrile/0.1 M ammonium formate solution 70:30, flow rate: 2 ml/min, retention time: 6.9 min) to determine the specific activity and radiochemical purity. Then the final solution of the [^{11}C]DMP696 in 10% ethanol-saline (10 ml) was analyzed to confirm the chemical and radiochemical purities.

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References

1. Owens MJ, Nemeroff CB. *Pharmacol Rev* 1991; **43**(4): 425–473.
2. Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. *Trends Pharmacol Sci* 1996; **17**(4): 166–172.

3. Gilligan PJ, Robertson DW, Zaczek R. *J Med Chem* 2000; **43**(9): 1641–1660.
4. De Souza EB. *Psychoneuroendocrinology* 1995; **20**(8): 789–819.
5. O'Brien D, Skelton KH, Owens MJ, Nemeroff CB. *Hum Psychopharmacol Clin Exp* 2001; **16**(1): 81–87.
6. Skutella T, Probst JC, Renner U, Holsboer F, Behl C. *Neuroscience* 1998; **85**(3): 795–805.
7. Mitchell AJ. *Neurosci Biobehav Rev* 1998; **22**(5): 635–651.
8. Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A. *Behav Brain Res* 1999; **100**(1–2): 207–215.
9. Behan DP, Grigoriadis DE, Lovenberg T, Chalmers D, Heinrichs S, Liaw C, De Souza EB. *Mol Psychiatry* 1996; **1**(4): 265–277.
10. De Souza EB, Whitehouse PJ, Kuhar MJ, Price DL, Vale WW. *Nature* 1986; **319**(6054): 593–595.
11. Holsboer F. *CNS Spectrums* 2001; **6**(7): 590–594.
12. Jagoda E, Contoreggi C, Lee MJ, Kao CH, Szajek LP, Listwak S, Gold P, Chrousos G, Greiner E, Kim BM, Jacobson AE, Rice KC, Eckelman W. *J Med Chem* 2003; **46**: 3559–3562.
13. Tian X, Hsin LW, Webster EL, Contoreggi C, Chrousos GP, Gold PW, Habib K, Ayala A, Eckelman WC, Jacobson AE, Rice KC. *Bioorg Med Chem Lett* 2001; **11**: 331–333.
14. Martarello L, Kilts CD, Ely T, Owens MJ, Nemeroff CB, Camp M, Goodman MM. *Nucl Med Biol* 2001; **28**: 187–195.
15. Kumar JSD, Majo VJ, Prabhakaran J, Simpson NR, Van Heertum RL, Mann JJ. *J Label Compd Radiopharm* 2003; **46**: 1055–1065.
16. Hodge CN, Aldrich PE, Wasserman ZR, Fernandez CH, Nemeth GA, Arvanitis A, Cheeseman RS, Chorvat RJ, Ciganek E, Christos TE, Gilligan PJ, Krenitsky P, Scholfield E, Strucely P. *J Med Chem* 1999; **42**(5): 819–832.
17. Li Y-W, Hill G, Wong H, Kelly N, Ward K, Pierdomenico M, Ren Shelly GP, Grossman S, Trainor G, Taub R, McElroy J, Zazcek R. *J Pharm Exp Therap* 2003; **305**: 86–96.
18. Meyers AI, Schmidt W, McKennon MJ. *Synthesis* 1993; **2**: 250–262.
19. Bakthavatchalam R, Arvantis AG, Beck JP, Cain GA, Chorvat RJ, Gilligan PJ, Olsen RE. *PCT Int Appl* 1997; WO 9735539.
20. Jewett DM. *Nucl Med Biol* 2001; **28**(6): 733–734.

