JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 635–640.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1082

Research Article

Synthesis of [^{3}H]-labelled 4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]-2,3-[^{3}H]-butan-1-ol: a high affinity radioligand for the corticotropin-releasing hormone type 1 receptor

Anne-Cécile Hiebel[1], John S. Partilla[2], Richard B. Rothman[2], Arthur E. Jacobson[1] and Kenner C. Rice[1,*]

[1] *Laboratory of Medicinal Chemistry, Building 8, Room B1-23, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, 8 Center Drive, MSC 0815, Bethesda, MD 20892, USA*

[2] *Behavioral Neuroscience Branch, National Institute for Drug Abuse, Intramural Research Program, National Institutes of Health, DHHS, 5500 Nathan Shock Dr, Baltimore, MD 21224, USA*

Summary

[^{3}H]-Labelled 4-[ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]-2,3-[^{3}H]-butan-1-ol (**3b**) was prepared as a novel non-peptidic radiolabelled high affinity antagonist of the corticotropin-releasing hormone type 1 receptor ($CRHR_1$) that could be useful as a more stable and receptor-selective alternative to the radiolabelled peptides now used to label the $CRHR_1$ receptor for displacement studies in cell-based binding assays. The precursor (Z)-4-[ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]but-2-en-1-ol (**2**) was reduced with tritium gas using palladium as the catalyst. After HPLC purification **3b** was obtained with a specific activity of 35 Ci/mmol in high radiochemical purity (> 97%). Copyright © 2006 John Wiley & Sons, Ltd.

Received 11 April 2006; Revised 19 April 2006; Accepted 19 April 2006

Key Words: corticotropin-releasing hormone; $CRHR_1$ antagonist; radioligand; tritium-labelled; antalarmin

*Correspondence to: Kenner C. Rice, Laboratory of Medicinal Chemistry, Building 8, Room B1-23, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, DHHS, 8 Center Drive, MSC 0815, Bethesda, MD 20892, USA. E-mail: kr21f@nih.gov

Contract/grant sponsor: National Institute of Diabetes and Digestive and Kidney Diseases
Contract/grant sponsor: National Institute on Drug Abuse

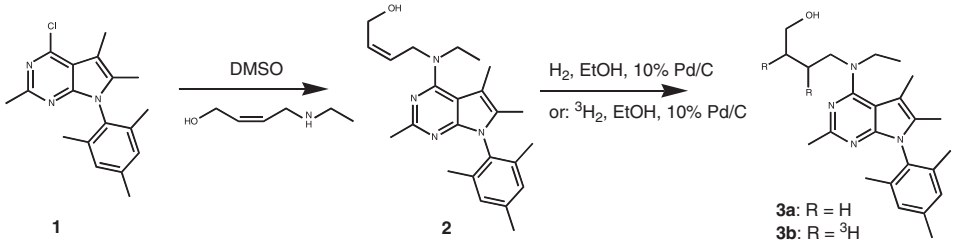
Introduction

Vale *et al.*, isolated corticotropin releasing hormone (CRH) from ovine hypothalamus extracts and characterized it as a 41 amino acid neuropeptide in 1981.[1] CRH has been found to act as a major regulator of the hypothalamic–pituitary–adrenal (HPA) axis, coordinating neuroendocrine, autonomic, immune, and behavioral responses to stress.[2,3] The CRH system is known to be composed of saturable, high affinity corticotropin-releasing hormone type 1 and type 2 receptors ($CRHR_1$ and $CRHR_2$) and their endogenous ligands. The characterized and cloned[4–9] receptors are located in anatomically well-defined regions of the CNS and periphery.[10] Increases in brain CRH have been associated with anxiety, depression, and substance abuse.[11–14] Excessive chronic activation of the CRH system is postulated to be involved in the pathogenesis of eating[15] and gastrointestinal disorders.[16] To further study this system, we have been engaged in the synthesis and evaluation of CRH ligands (e.g. antalarmin)[17–23] with the goal of finding those that act with high affinity and specificity to each of the known receptors. Antalarmin is being considered for clinical trials and is in the final phase of preclinical toxicology.

There are two radioligands used for the *in vitro* determination of the affinity of $CRHR_1$ and $CRHR_2$ ligands. One of these is a commercially available peptide, [^{125}I]Tyr^0-sauvagine.[24] In general, peptide radioligands used for competitive displacement studies *in vitro* are large, 'sticky' (e.g. non-selective), ligands and may be more unstable than non-peptides. The [^{125}I]Tyr^0-sauvagine, although very useful, presents these drawbacks. More recently, Zhang *et al.*,[25] synthesized a tritiated non-peptide, ($\pm$)-*N*-[2-methyl-4-methoxyphenyl]-1-(1-(methoxymethyl)propyl)-6-methyl-1*H*-1,2,3-triazolo[4,5-c] pyridin-4-amine ([^{3}H]SN003) for these purposes. That compound, however, is not commercially available. Both non-peptide and peptide radioligands would be useful tools, since there is evidence that they bind differently at $CRHR_1$.[25]

We sought a high affinity non-peptide ligand that would be easily available and that could be tritiated for use as a radioligand. We now report the preparation of 4-[ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*] pyrimidin-4-yl]amino]-2,3-[^{3}H]-butan-1-ol] (**3b**), a novel, high affinity antagonist at CRHR1, and a radioligand that could be useful for *in vitro* studies in future cell-based binding assays. We previously reported that the unlabelled pyrrolopyrimidine compound **3a** (Scheme 1) was among the highest affinity ligands for $CRHR_1$ ($Ki = 0.68$ nM), as determined in displacement assays using [^{125}I]Tyr^0-sauvagine.[10,26] In order to provide a non-peptidic and hopefully more stable and selective radioligand, pyrrolopyrimidine **2** was tritiated to afford **3b**.

DOI: 10.1002/jlcr



Scheme 1.

Results and discussion

The unsaturated pyrrolopyrimidine compound **2** (Z)-4-[ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]but-2-en-1-ol,[26] appeared ideal as the precursor since it had a double bond that could be tritiated (Scheme 1). It was obtained by reaction of the known 4-chloro-3,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidine (**1**) (in about 5 steps, as reported by Chen),[17] and (Z)-4-ethylaminobut-2-en-1-ol. The synthesis of **2** was improved in our laboratory, for our use in the synthesis of radiolabelled fluorinated antalarmin.[10] In an initial experiment, we found that **2** was easily reduced with a balloon pressure of hydrogen over 10% palladium on charcoal. Compound **3b** was then prepared by RC TRITEC Ltd., 9053 Teufen, Switzerland, through tritiation of **2** using $^3H_2$ and 10% Pd/C in absolute ethanol, and purified by reverse phase high performance liquid chromatography. Compound **3b** was obtained with high radiochemical purity ($>97\%$) and a specific activity of 35 Ci/mmol. The radioligand was repurified for pharmacological evaluation, and the pharmacological data will be reported elsewhere when completed.

Experimental

^{1}H and ^{13}C NMR spectra were recorded using a Varian Gemini XL-300 spectrometer. Chemical shifts are reported in parts per million with tetramethylsilane (0.0 ppm) used as the internal standard for ^{1}H NMR spectra and the $CDCl_3$ absorption (77.23 ppm) for ^{13}C NMR. The tritiation was carried out by RC TRITEC Ltd., 9053 Teufen, Switzerland.

*4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-d]pyrimidin-4-yl]amino]butan-1-ol (***3a***)*

The starting oil **2**[10] (10 mg, 0.025 mmol) was placed in a round bottomed flask purged with argon. Absolute ethanol (0.4 ml) and 10% Pd/C (1 mg) were added and the mixture was degassed with argon for 20 min. Hydrogen gas was then introduced in the reaction mixture via a balloon. The reaction was stirred for 12 h at room temperature. The crude mixture was filtered (syringe filter,

DOI: 10.1002/jlcr

Titan HPLC, pore size 0.5 μm, membrane type: PTFE) and the crude product was purified by Radial PLC (gradient 2% MeOH/CH_2Cl_2 to 5% MeOH/CH_2Cl_2). The product **3a** was isolated as a clear oil (8 mg, 80%). The spectral data was identical to that reported in the literature.[26]

*Radiosynthesis of 4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-d]pyrimidin-4-yl]amino]-2,3-[^{3}H]-butan-1-ol (**3b**)*

The synthesis, HPLC purification and determination of specific activity of **3b** were accomplished by RC TRITEC Ltd., 9053 Teufen, Switzerland as follows: 80 mg of the oily unsaturated starting material[10] **2** were dissolved in 3.0 ml absolute ethanol. From this stock solution, 0.113 ml (3.0 mg of **2**, 7.6 μmol) was added to 1.73 mg of the catalyst (10% Pd/C) and diluted with 0.5 ml of absolute ethanol. The suspension was degassed four times at the stainless steel vacuum manifold (RC TRITEC Ltd.) and stirred under an atmosphere of tritium gas (12.4 Ci) for 3.5 h. The solvent was removed *in vacuo*, and labile tritium was exchanged by adding 1 ml methanol, stirring the solution, and removing the solvent again *in vacuo*. This process was repeated three times. Finally, the well-dried solid was extracted with 5 ml ethanol and the suspension filtered through a nylon membrane (0.2 μm), obtaining a clear and colorless solution with an activity of 513 mCi. The crude product (radiochemical purity 88%) was purified on a Waters XTerra RP8 (5 μm, 7.8 × 150 mm) column under isocratic conditions (A: water, 0.1% TFA; B: acetonitrile 0.1% TFA; 40% B). The compound decomposes slowly under acidic conditions, thus the compound was isolated by solid phase extraction (Waters Oasis®, HLB (6 cm^3, 200 mg)). The HPLC solvent mixture was first diluted with water containing 1% ammonia to adjust the acetonitrile content to 20%. After loading on the cartridge and an additional washing step with water (containing 1% ammonia), the product was eluted with ethanol. Finally, 10 mCi of purified product **3b** with a radiochemical purity of > 97% (HPLC) were obtained. The specific activity of approximately 35 Ci/mmol was calculated from the activity concentration (determined by LSC) and the chemical concentration (determined by UV peak area method) of the active sample.

Conclusion

4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]-2,3-[^{3}H]-butan-1-ol (**3b**) was prepared as a novel non-peptidic radiolabelled potent antagonist of the corticotropin-releasing hormone type 1 receptor ($CRHR_1$). This compound will be examined for its stability and its ability to label $CRHR_1$ for displacement studies in cell-based binding studies.

DOI: 10.1002/jlcr

Acknowledgements

This research was supported by the NIH Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the National Institute on Drug Abuse (NIDA). Anne-Cécile Hiebel thanks NIDDK for an IRTA fellowship. The authors (LMC, NIDDK) also thank NIDA for partial support of this work.

References

1. Vale W, Spiess J, Rivier C, Rivier J. *Science* 1981; **213**: 1394–1397.
2. Rivier J, Gulyas J, Corrigan A, Martinez V, Craig A-G, Taché Y, Vale W, Rivier C. *J Med Chem* 1998; **41**: 5012–5019.
3. Chrousos GP. *N Engl J Med* 1995; **332**: 1351–1362.
4. Chen R, Lewis KA, Perrin MH, Vale WW. *Proc Natl Acad Sci USA* 1993; **90**: 8967–8971.
5. Perrin M, Donaldson C, Chen R, Blount A, Berggren T, Bilezikjian L, Sawchenko P, Vale W. *Proc Natl Acad Sci USA* 1995; **92**: 2969–2973.
6. Lovenberg TW, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. *Proc Natl Acad Sci USA* 1995; **92**: 836–840.
7. Perrin MH, Donaldson CJ, Chen R, Lewis KA, Vale WW. *Endocrinology* 1993; **133**: 3058–3061.
8. Liaw CW, Lovenberg TW, Barry G, Oltersdorf T, Grigoriadis DE, De Souza EB. *Endocrinology* 1996; **137**: 72–77.
9. Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB. *Trends Pharmacol Sci* 1996; **17**: 166–172.
10. Hsin LW, Webster EL, Chrousos GP, Gold PW, Eckelman WC, Contoreggi C, Rice KC. *J Label Compd Radiopharm* 2000; **43**: 899–908, and unpublished observations.
11. Bremner JD, Licinio J, Darnell A, Krystal JH, Owens MJ, Southwick SM, Nemeroff CB, Charney DS. *Am J Psychiatry* 1997; **154**: 624–629.
12. Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, Kilts CD, Loosen PT, Vale W. *Science* 1984; **226**: 1342–1344.
13. Zhou Y, Spangler R, LaForge KS, Maggos CE, Ho A, Kreek MJ. *J Pharmacol Exp Ther* 1996; **279**: 351–358.
14. Richter RM, Weiss F. *Synapse* 1999; **32**: 254–261.
15. Hernandez-Escalante V, Trava-Garcia M, Bastarrachea-Sosa R, Laviada-Molina H. *Salud Mental* 2003; **26**: 9–15.
16. Habib KE, Weld KP, Rice KC, Pushkas J, Champoux M, Listwak S, Webster EL, Atkinson AJ, Schulkin J, Contoreggi C, Chrousos GP, McCann SM, Suomi SJ, Higley JD, Gold PW. *Proc Natl Acad Sci USA* 2000; **97**: 6079–6084.
17. Chen YL. International Patent WO 94/13676, 1994.
18. Chen YL, Mansbach RS, Winter SM, Brooks E, Collins J, Corman ML, Dunaiskis AR, Faraci WS, Gallaschun RJ, Schmidt A, Schulz DW. *J Med Chem* 1997; **40**: 1749–1754.

DOI: 10.1002/jlcr

19. Lundkvist J, Chai Z, Teheranian R, Hasanvan H, Bartfai T, Jenck F, Widmer U, Moreau J-L. *Eur J Pharmacol* 1996; **309**: 195–200.
20. Shaham Y, Erb S, Leung S, Buczek Y, Stewart J. *Psychopharmacol* 1998; **137**: 184–190.
21. Deak T, Nguyen KT, Ehrlich AL, Watkins LR, Spencer RL, Maier SF, Licinio J, Wong M-L, Chrousos GP, Webster E, Gold PW. *Endocrinology* 1999; **140**: 79–86.
22. Griebel G, Perrault G, Sanger DJ. *Psychopharmacol* 1998; **138**: 55–66.
23. Webster EL, Lewis DB, Torpy DJ, Zachman EK, Rice KC, Chrousos GP. *Endocrinology* 1996; **137**: 5747–5750.
24. Grigoriadis DE, Liu XJ, Vaughn J, Palmer SF, True CD, Vale WW, Ling N, De Souza EB. *Mol Pharmacol* 1996; **50**: 679–686.
25. Zhang G, Huang N, Li Y-W, Qi X, Marshall AP, Yan X-X, Hill G, Rominger C, Prakash SR, Bakthavatchalam R, Rominger DH, Gilligan PJ, Zaczek R. *J Pharmacol Exp Ther* 2003; **305**: 57–69.
26. Hsin LW, Tian XR, Webster EL, Coop A, Caldwell TM, Jacobson AE, Chrousos GP, Gold PW, Habib KE, Ayala A, Eckelman WC, Contoreggi C, Rice KC. *Bioorg Med Chem* 2002; **10**: 175–183.

DOI: 10.1002/jlcr