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The Development of a Potential Single Photon Emission Computed Tomography (SPECT) Imaging Agent for the Corticotropin-Releasing Hormone Receptor Type 1

Xinrong Tian,[a,†] Ling-Wei Hsin,[a,‡] Elizabeth L. Webster,[b,§] Carlo Contoreggi,[c] George P. Chrousos,[d] Philip W. Gold,[b] Kamal Habib,[b] Alejandro Ayala,[b] William C. Eckelman,[e] Arthur E. Jacobson[a] and Kenner C. Rice[a,*]

[a]*Laboratory of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892, USA*
[b]*Clinical Neuroendocrinology Branch, NIMH, Clinical Center, NIH, Bethesda, MD 20892, USA*
[c]*Brain Imaging Unit, NIDA, Baltimore, MD 21224, USA*
[d]*Pediatric Endocrinology Section, PREB, NICHD, Clinical Center, NIH, Bethesda, MD 20892, USA*
[e]*PET Department, Clinical Center, NIH, Bethesda, MD 20892, USA*

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Abstract—A high-affinity radioligand for $CRHR_1$ has been prepared that can serve as a template for the development of SPECT imaging agents. The 5-chloro-*N*-cyclopropylmethyl-*N*-(2,6-dichloro-4-iodophenyl)-2-methyl-*N*-propylpyrimidine-4,6-diamine (**6b**, $K_i = 14$ nM), and the corresponding 4-bromophenyl analogue (**6a**, $K_i = 21$ nM), were synthesized in four steps from compound **3**. Published by Elsevier Science Ltd.

Corticotropin-releasing hormone (CRH) is a 41 amino-acid peptide secreted in response to stress by several brain nuclei involved in emotions, memory, endocrine and autonomic regulation. Overstimulation of CRH type-1 receptor ($CRHR_1$) may be the underlying factor in the pathogenesis of a variety of mental disorders that include major depression, anxiety and substance withdrawal.[1–4] Nonpeptide $CRHR_1$ antagonists are therefore being developed for their potential of treating these conditions. Moreover, the advent of a specific radioligand to label $CRHR_1$ in vivo would be an invaluable diagnostic and prognostic tool for several illnesses. We have previously shown that antalarmin[5] (*N*-butyl-*N*-ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo-[2,3-*d*]pyrimidin-4-yl]-amine, **1**), initially patented by Pfizer,[6] is a selective high affinity $CRHR_1$ antagonist that exerts anti-anxiety effects in rodents and nonhuman primates. We have also reported the synthesis of potential PET imaging agents for $CRHR_1$.[7] Here we report the synthesis and binding affinity of a potential candidate for single photon emission computed tomography (SPECT). SPECT, like positron emission tomography (PET), is used for in vivo measurement of hormone receptors, and has proven to be a non-invasive technique with wide applicability, particularly in neuroscience (Fig. 1).[8–11]

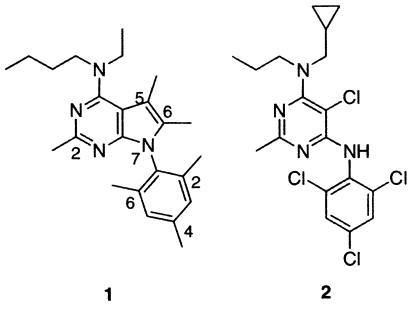

Figure 1.

*Corresponding author. Tel.: +1-301-496-1856; fax: +1-301-402-0589; e-mail: kr21f@nih.gov
†Current Address: Procter & Gamble Pharmaceuticals, HCRC, Mason, OH 45040, USA.
‡Current Address: University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Department of Pharmacology, Piscataway, NJ 08854, USA.
§Current Address: University of North Carolina, Department of Psychiatry, Chapel Hill, NC 27599, USA.

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We based our synthesis of a potential SPECT ligand on a nonpeptide compound (5-chloro-*N*-cyclopropylmethyl-2-methyl-*N*-(2,4,6-trichlorophenyl)-pyrimidine-4,6-diamine, **2**) known to be a high affinity ligand for $CRHR_1$, and known to have CRH antagonist activity as shown by a functional assay (inhibition of stimulated cAMP).[12] Our initial work towards the goal of obtaining a template for a $CRHR_1$ SPECT ligand was based on the synthesis and the determination of the $CRHR_1$ binding affinity of compounds **6a** and **6b** in which a bromine or iodine atom replaced the chlorine atom in the C4 position of the substituted phenyl ring of **2**. If the iodo compound was found to have reasonable affinity for the receptor, the further synthesis of a ^{125}I compound based on **6b** would be attempted.

The synthesis was carried out using a route analogous to one reported by Chen.[12] In addition to **2**, bromo (**6a**) and iodo (**6b**) analogues were prepared in four steps from 4,4-dichloro-2-methyl pyrimidine (**3**), in overall yields of 62 and 47%, respectively (Scheme 1). Coupling of **3** with 4-bromo and 4-iodo-2,6-dichloroaniline gave the pyrimidines **4a** and **4b**. Amination with *N*-cyclopropylmethylpropylamine in DMSO at 140 °C gave **5a,b**, followed by chlorination, which afforded the desired 4-halo derivatives (*N*-(4-bromo-2,6-dichlorophenyl)-5-chloro-*N*-cyclopropylmethyl-2-methyl-*N*-propylpyrimidine-4,6-diamine (**6a**) and 5-chloro-*N*-cyclopropylmethyl-*N*-(2,6-dichloro-4-iodophenyl)-2-methyl-*N*-propylpyrimidine-4,6-diamine (**6b**).

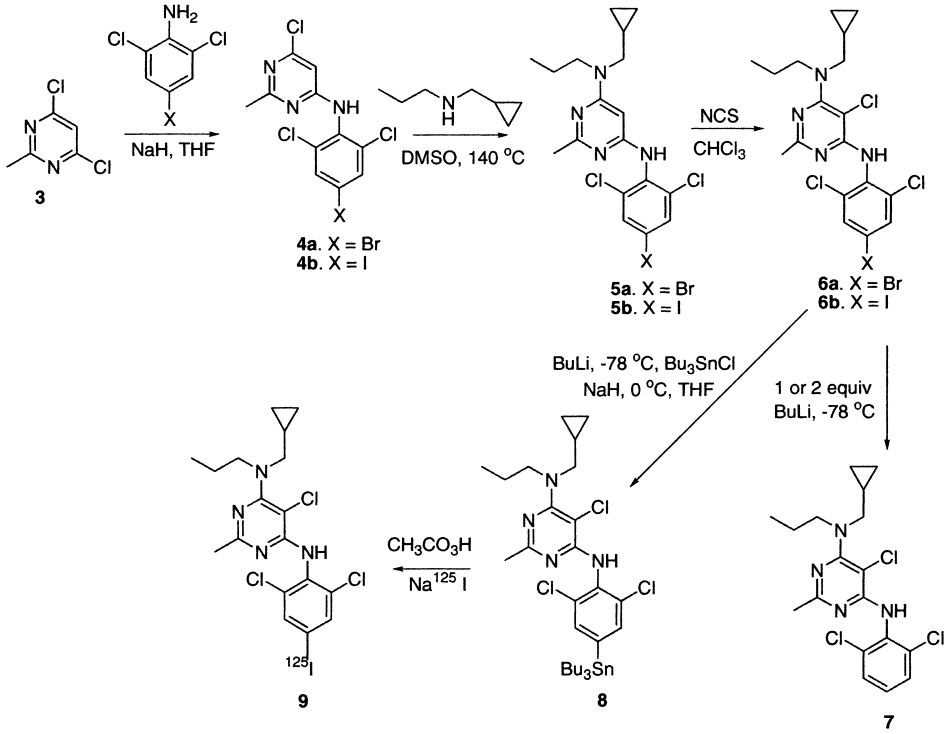
Table 1. CRH_1 binding affinity

Compound	K_i (nM)
2	31±9.7[a]
6a	21±7.9[a]
6b	14±4.6[a]
8	3.0[b]

[a]Three binding curves conducted in duplicate were generated for each compound and the K_i values represent the mean of the three experiments±SEM.
[b]The result of a single experiment.

The CRH_1 affinity of **2**, **6a**, **6b**, and the tributyltin analogue **8** was determined using a previously reported procedure.[7] The binding affinity of these compounds in rat cerebellum against radioligand [^{125}I]Tyr^0-sauvagine is shown in Table 1. Iodo analogue **6b** exhibited higher affinity than the formerly known[12] chloro analogue **2**. The affinity decreased in the order of I > Br > Cl. This corresponds with the decrease in lipophilicity (I > Br > Cl). The effect of lipophilicity on $CRHR_1$ affinity was further demonstrated with compound **8**. This tributyltin analogue, the most lipophilic among the four synthesized compounds, showed the highest binding affinity.

With the establishment of the high affinity of **6b** for $CRHR_1$, we decided to proceed with the synthesis of the radiolabeled ^{125}I analogue. We envisioned that it could be derived from the corresponding tributyltin compound **8**, which, in turn, should be accessible from the bromo precursor **6a**. As outlined in Scheme 1, conversion of the bromide **6a** to the tributyltin compound **8** was not without problems. Initial treatment of **6a** with 2 equiv of *n*-BuLi followed by tributyltin chloride only gave the debromination product **7**. This was probably due to the bromo-lithium exchange occurring prior to the deprotonation of the secondary amine; the generated aromatic anion was immediately quenched by



Scheme 1.

an acidic anilino proton. The preferred formation of an aromatic anion was confirmed by the use of 1 equiv of *n*-BuLi which also gave the reduction product **7**. We circumvented this problem by a stepwise deprotonation and lithiation sequence. Compound **6a** was first treated with 1 equiv of sodium hydride to deprotonate the aniline followed by 1 equiv of *n*-BuLi for bromo-lithium exchange. The resulting dianionic species was quenched with tributyltin chloride to afford the desired tin compound **8**[13] in 51% yield.

Compound **8** was converted to the targeted ^{125}I compound **9** using the following procedure. To a solution of carrier-free [^{125}I]NaI (5.34 mCi, Amersham-Pharmacia Biotech, Chicago, IL 60611) were added tributyltin precursor (150 μL, 0.33 mg/mL) in ethanol, H_3PO_4 (40 μL, 0.5 M), and peracetic acid (50 μL, 0.2 M). After standing at room temperature for 30 min, sodium bisulfite was added to quench the reaction, and 1 mL of saturated sodium bicarbonate solution was added to the mixture. The [^{125}I] product was extracted with EtOAc (3 × 1 mL) and then passed through a short anhydrous Na_2SO_4 column. The filtrate was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μL of MeOH and 100 μL of mobile phase (MeOH/H_2O/triethylamine, 90:10:0.3), and purified by HPLC on a reverse phase column (C-18 Applied Biosystems Spheri-5 ODS 5 μ, 4.6×250 mm column). The corresponding fraction was collected and evaporated to dryness under a stream of nitrogen to afford pure [^{125}I] product **9** (4.96 mCi) with a radiochemical purity >99%. Since **8** is eluted after **9** using reversed phase HPLC, and proton-destannylation has not been observed under these conditions, we expect the effective specific activity of **9** to be 2200 Ci/mmol.

In summary, we have developed the synthesis of the first nonpeptide potential SPECT ligand for $CRHR_1$. The significant binding affinity exhibited by iodo analogue **6b** makes its ^{125}I analogue **9** an intriguing template for further development of the SPECT imaging agent for the $CRHR_1$. It also holds great potential as a selective nonpeptide radioligand for the $CRHR_1$ binding assay, replacing the currently used peptide radioligands. A study along these lines is in progress and the results will be reported in due course.

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13. The procedure used for the preparation of the tributyltin analogue **8** from bromide **6a** is as follows: To a solution of the bromide **6a** (311 mg, 0.65 mmol) in THF (2 mL) was added sodium hydride (16 mg, 60% in mineral oil) at 0 °C. After being stirred at 0 °C for 15 min, the reaction mixture was cooled to −78 °C and *n*-BuLi (1.6 M in hexane, 0.25 mL) was added. The mixture was stirred for 20 min and treated with tributyltin chloride (288 mg, 0.88 mmol). It was then slowly warmed to room temperature with stirring over 2 h, quenched with aqueous NH_4Cl solution and extracted with EtOAc. The extract was dried over Na_2SO_4 and concentrated. The residue was chromatographed (silica gel, hexanes/EtOAc, 20:1 to 7:1) to give the tributyltin derivative **8** (230 mg, 51% yield) as a clear oil; ^1H NMR (300 MHz, $CDCl_3$) 7.39 (s, 2H), 6.71 (s, 1H), 3.58 (t, 2H, $J=7.8$ Hz), 3.42 (d, 2H, $J=6.9$ Hz), 2.26 (s, 3H), 1.53 (m, 6H), 1.06–1.20 (m, 6H), 0.90 (m, 12H), 0.51 (m, 2H), 0.24 (q, $J=4.8$ Hz); MS (CI+) m/z 689; HRMS calcd for $C_{30}H_{47}N_4Cl_3Sn$ 688.1888, found 688.1892.