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Synthesis and Biological Activity of Fluoro-Substituted Pyrrolo[2,3-*d*]pyrimidines: The Development of Potential Positron Emission Tomography Imaging Agents for the Corticotropin-Releasing Hormone Type 1 Receptor

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Abstract—A series of fluoro-substituted 4-(dialkylamino)pyrrolo[2,3-*d*]pyrimidines was synthesized and their binding affinity for corticotropin-releasing hormone type 1 receptor ($CRHR_1$) was investigated. Compounds **11a** and **11b** possessed very high $CRHR_1$ affinity ($K_i = 3.5$, 0.91 nM, respectively). They are promising candidates for the development of ^{18}F-containing nonpeptide PET radioligands for $CRHR_1$. Published by Elsevier Science Ltd.

Corticotropin-releasing hormone (CRH), a 41-amino acid neuropeptide, coordinates the overall response of the body to stress through the release of adrenocorticotropic hormone (ACTH) and regulates stress-induced changes in the autonomic nervous system, neuroendocrine functions, immune system, and behavior.[1–3] Overproduction of CRH in the brain has been associated with mental disorders such as anxiety[4] and depression,[5] and substance abuse.[6] Thus, nonpeptide CRH type 1 receptor ($CRHR_1$) antagonists would be useful as pharmacological tools to further study these disorders. A number of selective and potent $CRHR_1$ antagonists (**1**–**4**) have been reported,[7–10] and **1**–**3** also displayed significant pharmacological effects in behavioral studies (Fig. 1).[11–14]

Positron emission tomography (PET) is a non-invasive imaging technology that has been adapted for localization and quantification of brain and somatic receptors in living animals and humans. Therefore, the development of specific and potent radiolabelled PET tracers for $CRHR_1$ would provide invaluable research tools to investigate the physiologic functions of CRH system in normal subjects and patients with various neuropsychiatric and neurodegenerative diseases. Furthermore, such a compound may be also useful in the discovery and development of potential therapeutics for the treatment of these disorders as it would allow direct measurement of drug effects.

The structure–activity relationships (SAR) of structurally diverse nonpeptide $CRHR_1$ antagonists have been examined.[15] These SAR studies have not focused on the development of PET imaging agents for the $CRHR_1$. We have found that *N*-butyl-*N*-ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine (**2**, antalarmin, first described by Pfizer)[16] has high affinity and selectivity for $CRHR_1$, and it is able to block the in vitro and in vivo biological actions of CRH.[8] Thus, antalarmin was chosen as the template for the design of F-(unlabeled) substituted analogues for evaluation as potential PET tracers for $CRHR_1$. In this paper, we discuss the synthesis and SAR of these fluorine-containing antalarmin derivatives (**11a**–**e**).

The key intermediate, 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidine (**5**), was synthesized in five steps according to a modified literature method[16] in 37% total yield from commercially available

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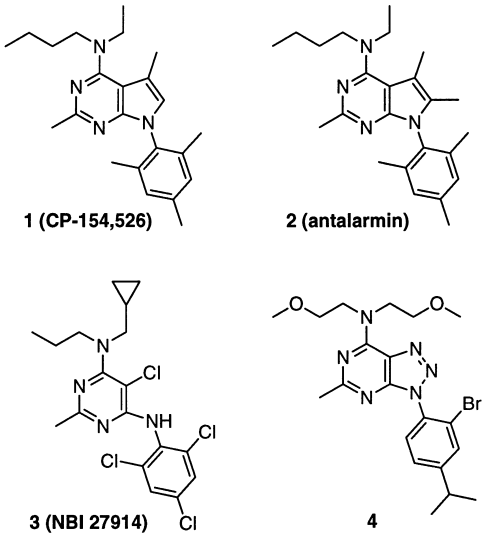
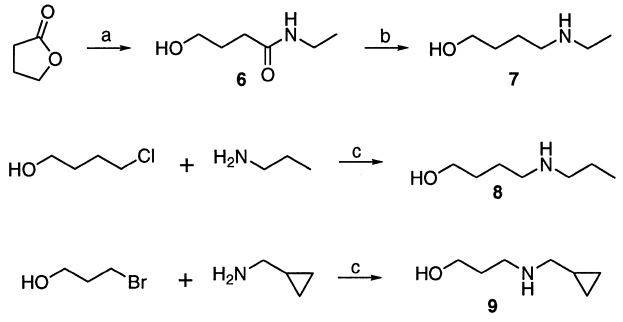


Figure 1.

2,4,6-trimethylaniline. The amino alcohols **7**–**9** were synthesized in 80–90% yield as shown in Scheme 1. Treatment of γ-butyrolactone with ethylamine in tetrahydrofuran (THF) at room temperature, followed by reduction with $LiAlH_4$ afforded 4-(ethylamino)butan-1-ol (**7**). *N*-Alkylation of propylamine with 4-chlorobutan-1-ol in THF gave 4-propylamino-1-butanol (**8**). Aminoalcohol **9** was prepared by *N*-alkylation of (cyclopropylmethyl)amine with 3-bromo-1-propanol.



Scheme 1. Synthesis of aminoalcohols. (a) Ethylamine, THF, room temperature; (b) THF, $LiAlH_4$, reflux; (c) THF, reflux.

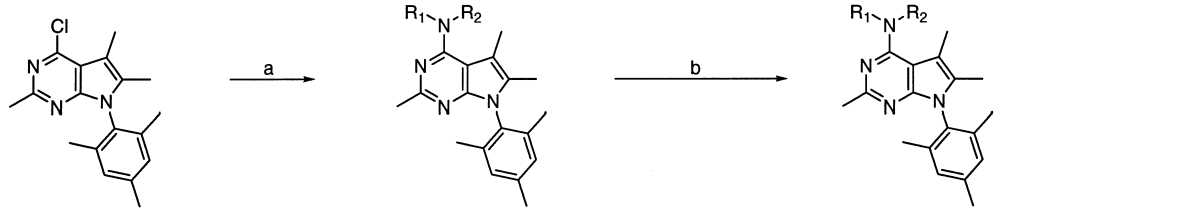
Coupling **5** with an excess amount of the appropriate dialkylamines in dimethyl sulfoxide (DMSO) at 130 °C gave tertiary amines **10a**–**f**. Fluorination of **10a**–**e** was accomplished by refluxing in THF with tetrabutylammonium fluoride (TBAF) and toluenesulfonyl fluoride (TsF) in the presence of 4 Å molecular sieves[17] to afford fluoro-substituted products **11a**–**e** in 65–85% yield (Scheme 2).[18]

Table 1 lists the in vitro $CRHR_1$ binding affinity in rat cerebellum against radioligand [^{125}I]Tyr^0-sauvagine[19] by compounds **10f** and **11a**–**e**. Introducing a fluorine substituent into antalarmin gave compound **11a**, which retained the high affinity of antalarmin for $CRHR_1$. With this promising result, we replaced the ethyl group in **11a** with longer alkyl groups (i.e. propyl, butyl) to improve the $CRHR_1$ potency. Replacing the ethyl group with a propyl substituent afforded fluoride **11b** which showed subnanomolar $CRHR_1$ binding affinity ($K_i = 0.91$ nM). It had a 3-fold higher affinity than antalarmin and it is the most potent compound in this series of antalarmin analogues. Further lengthening the alkyl group to butyl group provided compound **11c** which displayed decreased $CRHR_1$ affinity. In contrast to the *N*-butyl-*N*-ethyl derivatives, the *N*-cyclopropylmethyl-*N*-propyl series displayed a different $CRHR_1$ binding affinity profile. Compound **10f** possessed about the same $CRHR_1$ affinity ($K_i = 2.3$ nM) as antalarmin, whereas its fluoro analogue **11d** showed significantly decreased $CRHR_1$ affinity.

Table 1. Structure–activity relationships for fluoro-substituted antalarmin derivatives

Compound	R_1	R_2	K_i (nM)[a]
2 (antalarmin)	Bu	Et	2.5 ± 0.64
10f	Pr	CH_2-cyclopropane	2.3 ± 0.2
11a	$CH_2CH_2CH_2CH_2F$	Et	3.5 ± 0.89
11b	$CH_2CH_2CH_2CH_2F$	Pr	0.91 ± 0.25
11c	$CH_2CH_2CH_2CH_2F$	Bu	10 ± 3.5
11d	$CH_2CH_2CH_2F$	CH_2-cyclopropane	30[b]
11e	$-CH_2CH_2N(CH_2CH_2F)CH_2CH_2-$		>1000

[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 determination.



10a: $R_1 = (CH_2)_4OH$, $R_2 = Et$
10b: $R_1 = (CH_2)_4OH$, $R_2 = Pr$
10c: $R_1 = (CH_2)_4OH$, $R_2 = Bu$
10d: $R_1 = (CH_2)_3OH$, $R_2 = CH_2$-cyclopropane
10e: $R_1,R_2 = -CH_2CH_2N(CH_2CH_2OH)CH_2CH_2-$
10f : $R_1 = Pr$, $R_2 = CH_2$-cyclopropane

11a: $R_1 = (CH_2)_4F$, $R_2 = Et$
11b: $R_1 = (CH_2)_4F$, $R_2 = Pr$
11c: $R_1 = (CH_2)_4F$, $R_2 = Bu$
11d: $R_1 = (CH_2)_3F$, $R_2 = CH_2$-cyclopropane
11e: $R_1,R_2 = -CH_2CH_2N(CH_2CH_2F)CH_2CH_2-$

Scheme 2. Synthesis of fluoro-substituted antalarmin analogues. (a) HNR_1R_2, DMSO, 130 °C, 5 h; (b) Bu_4NF, TsF, molecular sieves 4 Å, THF, reflux, 20 h.

We found that the high lipophilicity of antalarmin caused troublesome problems (e.g., solubility, bioavailability). To increase the hydrophilicity of fluoro-antalarmin derivatives, compound **11e** containing an additional basic piperazine nitrogen was designed and synthesized. The fluoride **11e** did possess much better solubility than antalarmin. However, it had little affinity for the $CRHR_1$ (>1 μM). It was also observed in recent SAR studies[10,20,21] that introducing polar functionalities into a variety of potent $CRHR_1$ antagonists was detrimental to their $CRHR_1$ binding affinity.

In summary, we report the synthesis and $CRHR_1$ binding affinity of a novel series of fluoro-substituted antalarmin derivatives. Among them, compounds **11a** and **11b** are high affinity $CRHR_1$ ligands and are potential candidates for the development of ^{18}F-containing PET tracers for the $CRHR_1$. Furthermore, these nonpeptide fluorides may prove to be useful as research tools for studying the physiological and pathological roles of CRH system, and in the development of new medications for CRH-related disorders. In vivo pharmacological studies of these fluoro-antalarmin analogues are in progress and the results will be reported in due course.

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18. Experimental procedures: (A) Coupling reaction: A mixture of 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo[2,3-*d*]pyrimidine (2.00 g, 6.37 mmol) and 6 to 10 equiv of the corresponding amine in DMSO (15 mL) was heated at 130 °C for 5 h and the crude product was purified using silica gel column chromatography to afford **10a–f** in 90–98% yield; (B) Fluorination: To a solution of tetrabutylammonium fluoride (3.0 mmol) in THF (10 mL) in the presence of molecular sieves 4 Å (3 g) was added a mixture of *p*-toluenesulfonyl fluoride (2 mmol) and the corresponding aminoalcohol (1.0 mmol) in THF (5 mL) at room temperature. The reaction was stirred at reflux for 20 h and the final product was chromatographed to afford **11a–e** in 65–85% yield. All of the intermediates and final products were characterized by ^1H NMR, CIMS, and high-resolution EIMS.
19. $CRHR_1$ binding assay: Frozen whole rat brains, dissected from male Sprague–Dawley rats, 200–300 g, were purchased from Taconic Farms (Germantown, NY). The brains were rapidly frozen, shipped to NIH on dry ice and stored at −70 °C until the time of the assay. At the time of assay, brains were thawed on ice, cerebellar hemispheres were dissected, weighed, and homogenized in 20 volumes of ice cold homogenization buffer containing PBS, 10 mM $MgCl_2$, 2 mM EGTA, and 0.1 mM bacitracin, pH 7.0. The homogenate was centrifuged for 10 min at 40,000 *g*. The pellet was resuspended in cold PBS buffer and recentrifuged for 10 min at 40,000 *g*. The final pellet was resuspended in homogenization buffer at 30 mg tissue wet weight/mL buffer and 0.1 mL homogenate was added to 1.5 mL microfuge tubes containing 0.1 mL of [^{125}I]-Tyr^0-sauvagine (New England Nuclear, Boston, MA) in incubation buffer (homogenization buffer plus 0.01% BSA), and 0.1 mL of incubation buffer containing increasing concentrations (10^{-10} to 10^{-6} M) of the CRH antagonist analogues. Nonspecific binding was defined by 1 μM ovine CRH and 1 μM sauvagine (Peninsula, Belmont, CA) which gave identical nonspecific binding values. Binding reactions were incubated for 2 h at room temperature. Radioactivity bound to tissue was separated from free ligand by centrifugation in a microfuge (Beckman, Palo Alta, CA) for 10 min at 12,000 *g* at 4 °C. The supernatant was aspirated and the radioactivity of the pellet was measured in a gamma counter at approximately 80% efficiency. The computer program GraphPad Prism 2.0a for Power Macintosh (GraphPad Software Inc., San Diego CA) was used to calculate K_i values from the IC_{50} of the [^{125}I]-Tyr^0-sauvagine competitive binding curves for each antagonist analogue. Unless noted in Table 1, three binding curves conducted in duplicate were generated for each analogue and the K_i values represent the mean of the three experiments ± SEM.

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