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Synthesis, binding affinity, radiolabeling, and microPET evaluation of 4-(2-substituted-4-substituted)-8-(dialkylamino)-6-methyl-1-substituted-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-ones as ligands for brain corticotropin-releasing factor type-1 (CRF_1) receptors

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ABSTRACT

Compounds **1**–**14** were synthesized in a search for high-affinity CRF_1 receptor ligands that could be radiolabeled with ^{11}C or ^{18}F for use as positron emission tomography (PET) radiotracers. Derivatives of **2** were developed which contained amide *N*-fluoroalkyl substituents. Compounds [^{18}F]**24** and [^{18}F]**25** were found to have appropriate lipophilicities of $\log P_{7.4} = 2.2$ but microPET imaging with [^{18}F]**25** demonstrated limited brain uptake.

The neuropeptide corticotropin-releasing factor (CRF)[1,3] coordinates the neuroendocrine response to stress through the hypothalamic–pituitary–adrenal axis by acting on the CRF Type-1 (CRF_1) receptor.[4] Dysregulation of brain CRF signaling has been proposed to be involved in stress, anxiety, depression, and addiction; and brain regional alterations in CRF_1 density have been detected in depressed patients and victims of suicide.[5–8] This evidence has resulted in a more than 20-year search for small molecule, brain bioavailable CRF_1 antagonists that could be used as therapeutics for treating CRF_1-related disorders.[9–11] Therapeutic medication development can be uniquely aided by the availability of validated positron emission tomography (PET) and single-photon emission computed tomography (SPECT) radiotracers that can be used to both support target occupancy measurements of candidate therapeutics and to explore mechanisms of pathophysiology and treatment response by measurement of receptor density and expression levels.[12–15] Numerous attempts to develop a viable brain CRF_1 receptor PET radiotracer have been reported over the years but no candidates to date have been successful due to problems such as high lipophilicity and inability to cross the blood–brain barrier (BBB), rapid metabolism, or insufficient specific receptor binding.[16–19] As part of an effort to develop a viable brain CRF_1 receptor PET tracer we have been exploring various structural motifs[20] and report here our results with compounds **1**–**15** (Scheme 1), and derivatives thereof, which are based on a previous report that compounds **2**, **6**, and **9** displayed high binding affinity (IC_{50} = 0.70, 0.49, and 0.92 nM, respectively) at the rat brain CRF_1 receptor.[21] We anticipated that the reported high binding affinity of **2**, **6**, and **9** would enable radiotracer derivatives of this structural class to achieve specific binding at CRF_1 and that the presence of the carbonyl group would enhance water solubility, thereby reducing log*P* and increasing BBB passage.[22–25] Furthermore, the synthetic route for this class of compounds[21] (Scheme S1, Supplementary material) allows the aromatic core and the *N*,*N*-dialkyl group to be held constant while the

Abbreviations: CRF, corticotropin-releasing factor; CRF_1, CRF type-1 receptor; PET, positron emission tomography; SPECT, single-photon emission computed tomography; BBB, blood–brain barrier; DMA, dimethylacetamide; SUV, standard uptake value; TACs, time–activity curves; CPCU, chemical processing control unit; rcy, radiochemical yield; EOB, end-of-bombardment.

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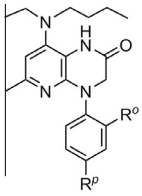


1 R^o = Br, R^p = Br
2 R^o = Br, R^p = *i*-Pr
3 R^o = Et, R^p = Br
4 R^o = Br, R^p = Cl
5 R^o = Cl, R^p = Br
6 R^o = Cl, R^p = Cl
7 R^o = Me, R^p = I
8 R^o = Me, R^p = Me
9 R^o = Me, R^p = OMe
10 R^o = Br, R^p = *t*-Bu
11 R^o = Me, R^p = Br
12 R^o = Br, R^p = Me
13 R^o = Cl, R^p = Me
14 R^o = Me, R^p = Cl
15 R^o = Me, R^p = OH

Scheme 1.

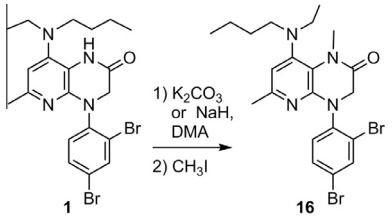
2,4-substituted pendant aryl ring is varied to produce a library of high-affinity candidates that are amenable to radiolabeling with ^{11}C or ^{18}F.

The binding affinities of compounds **1**–**14** at the CRF_1 and CRF_2 receptors were determined by in vitro competition binding assays at 23 °C using HEK293T cells transfected with the human CRF_1 or CRF_2 receptor (Table 1). Compounds **1**–**14** all bound to the CRF_1 receptor with high affinity (K_i = ~1.3–5.4 nM). Thus, although these compounds have various combinations of substituents on the pendant aryl ring, they all have binding affinities within a narrow range that differs by ~4 nM. The difference in affinities of **2**, **6**, and **9** between our results and the previously reported results[21] is presumably due to the difference between human and rat CRF_1 receptors as well as the fact that we are reporting K_i values and the previous work reported IC_{50} values. Sauvagine and R121919[26] were used as positive controls in our assays: R121919 was found to have an affinity of K_i = 2.6 ± 0.4 nM for the CRF_1 receptor which is comparable to the previously reported values of K_i = 3.5 nM[26] and K_i = 3.0 ± 0.16 nM;[27] and sauvagine was found to have an affinity of K_i = 0.8 ± 0.1 nM which is in agreement with the previously reported value of K_i = 0.8–1 nM.[28] The competing radioligand for these studies, [^{125}I]-Tyr^0-sauvagine, has a reported affinity of 0.2–0.4 nM for the CRF_1 receptor.[28] Compounds **1**–**3**, **6**, and **8**–**12**

Table 1
Results of in vitro competition binding assays at 23 °C using transfected human CRF_1 and CRF_2 receptors in HEK293T cells

Compd	R^o	R^p	CRF_1		CRF_2	
			K_i (nM) ± SEM[a]	*n*=	% Displacement[b]	*n*=
1	Br	Br	1.3 ± 0.3	3	9.4 ± 2.3	2
2	Br	*i*-Pr	2.4 ± 0.2	3	0.7 ± 3.3	2
3	Et	Br	2.5 ± 0.7	6	2.1 ± 2.5	2
4	Br	Cl	2.5 ± 0.2	3	N/D	N/A
5	Cl	Br	2.7 ± 0.4	3	N/D	N/A
6	Cl	Cl	3.4 ± 0.4	3	0.9 ± 3.4	2
7	Me	I	3.4 ± 0.6	3	N/D	N/A
8	Me	Me	3.9 ± 1.2	3	6.8 ± 1.4	2
9	Me	OMe	4.0 ± 0.6	3	−2.3 ± 3.2	2
10	Br	*t*-Bu	4.2 ± 2.2	3	−3.8 ± 0.5	2
11	Me	Br	4.4 ± 0.9	3	5.9 ± 0.7	2
12	Br	Me	4.4 ± 1.1	3	5.5 ± 4.9	2
13	Cl	Me	4.4 ± 0.4	3	N/D	N/A
14	Me	Cl	5.4 ± 0.9	3	N/D	N/A
R121919	N/A	N/A	2.6 ± 0.4	9	23.8 ± 0.3	2
Sauvagine	N/A	N/A	0.8 ± 0.1	9	100.0 ± 0.0	2
Antisauvagine-30	N/A	N/A	N/D	N/A	99.0 ± 0.6	2

N/D = not determined. N/A = not applicable.
[a] Versus [^{125}I]-Tyr^0-sauvagine.[28]
[b] Mean% displacement of radiotracer ([^{125}I]antisauvagine-30)[29] specific binding at CRF_2 receptors by 1 μM of competing ligand.



Scheme 2.

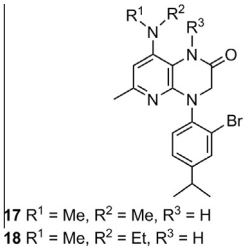
Table 2
Results of in vitro competition binding assays at 23 °C using transfected human CRF_1 receptor in HEK293T cells

Compd	R^o	R^p	R^1	R^2	R^3	K_i (nM) ± SEM[a]	*n*=
16	Br	Br	Bu	Et	Me	2.4 ± 0.6	2
17	Br	*i*-Pr	Me	Me	H	180 ± 5	2
18	Br	*i*-Pr	Me	Et	H	16.4 ± 1.6	3
20	Br	*i*-Pr	Pr	Pr	H	3.1 ± 0.3	3
21	Br	*i*-Pr	Me	Me	CH_2CH_2F	59 ± 9	3
22	Br	*i*-Pr	Et	Et	CH_2CH_2F	7.3 ± 0.7	3
23	Br	*i*-Pr	Me	Me	(*E*)-$CH_2CH=CHCH_2F$	14.2 ± 2.9	3
24	Br	*i*-Pr	Me	Me	$CH_2CH_2CH_2CH_2F$	21.6 ± 2.8	3
25	Br	*i*-Pr	Me	Et	$CH_2CH_2CH_2CH_2F$	13.9 ± 1.9	3

[a] Versus [^{125}I]-Tyr^0-sauvagine.[28]

did not display binding affinity at the CRF_2 receptor (they were unable to significantly displace [^{125}I]antisauvagine-30),[29] thus demonstrating selectivity for the CRF_1 receptor and so further screening of compounds **4**, **5**, **7**, **13**, and **14** at the CRF_2 receptor was not performed.

Compound **1** was found to have the highest binding affinity for the CRF_1 receptor followed by compounds **2**–**5**, but these compounds do not have a position available for radiolabeling with ^{11}C or ^{18}F. Thus, we shifted our focus to derivatives that could potentially be radiolabeled on the amide nitrogen atom. Compound **16** (Scheme 2) was prepared by amide N-methylation of **1** with CH_3I. This resulted in only a small decrease in CRF_1 binding affinity (Table 2) relative to **1** (Table 1). Compounds **17**–**20** (Scheme 3) were synthesized (Scheme S2, Supplementary material) to be used as intermediates in the synthesis of amide *N*-fluoroalkyl target compounds. The shorter *N*,*N*-dialkyl chains of **17**–**19** were expected to compensate for the increased lipophilicity that would result from adding alkyl groups to the amide nitrogen atom, while **20** was included to evaluate the effect on binding affinity of rearranging



17 R^1 = Me, R^2 = Me, R^3 = H
18 R^1 = Me, R^2 = Et, R^3 = H
19 R^1 = Et, R^2 = Et, R^3 = H
20 R^1 = Pr, R^2 = Pr, R^3 = H
21 R^1 = Me, R^2 = Me, R^3 = CH_2CH_2F
22 R^1 = Et, R^2 = Et, R^3 = CH_2CH_2F
23 R^1 = Me, R^2 = Me, R^3 = (*E*)-$CH_2CH=CHCH_2F$
24 R^1 = Me, R^2 = Me, R^3 = $CH_2CH_2CH_2CH_2F$
25 R^1 = Me, R^2 = Et, R^3 = $CH_2CH_2CH_2CH_2F$
26 R^1 = Me, R^2 = Me, R^3 = $CH_2CH_2CH_2CH_2OTs$
27 R^1 = Me, R^2 = Et, R^3 = $CH_2CH_2CH_2CH_2OTs$

Scheme 3.