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Potential CRF_1R PET imaging agents: 1-Fluoroalkylsubstituted 5-halo-3-(arylamino)pyrazin-2(1*H*)-ones

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ABSTRACT

A series of pyrazinones were prepared and evaluated as potential CRF_1R PET imaging agents. Optimization of their CRF_1R binding potencies and octanol–phosphate buffer phase distribution coefficients are discussed herein.

Corticotropin-releasing factor, a 41-amino acid neuropeptide produced in the hypothalamus, plays a central role in the coordination of neuroendocrine, autonomic, behavioral and immune responses to stress.[1,2] Secreted from the hypothalamus in response to acute physical or psychological stress, CRF activates the transcription of a gene which results in the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. In turn, ACTH stimulates the release of the steroid hormone cortisol from the adrenal gland. To restore the balance of the hypothalamic–pituitary–adrenal (HPA) axis, cortisol exerts negative feedback control on the secretion of CRF in the hypothalamus.[3] One hypothesis is that severe or prolonged stress results in an excessive secretion of CRF and a long-term activation of the HPA axis, which may lead to a variety of stress-related illnesses, such as anxiety, depression, obsessive–compulsive and posttraumatic stress disorders.[4a–c]

CRF mediates its function through the CRF_1 and CRF_2 receptor subtypes,[5] of which the CRF_1 receptor appears to play a significant role in the stress-related responses. It has been hypothesized that selective CRF_1R antagonists may be useful for the treatment of the psychiatric disorders. In the past decade and a half, a number of potent non-peptide CRF_1R antagonists have been reported, reflecting significant efforts of many research groups in this area.[6,7]

In light of this progress, the development of a selective CRF_1R Positron Emission Tomography (PET) radioligand could provide scientists with a powerful tool to assess receptor occupancy in clinical trials of CRF_1R receptor antagonists in normal subjects and patients. Development of PET radioligands, however comes with its own set of unique challenges, requiring compounds with potent and selective target affinity, and low non-specific binding.[8]

In 2000, Rice and co-workers[9] published the synthesis of unlabelled fluorinated pyrrolo[2,3-*d*]pyrimidines as high-affinity potential CRF_1R PET ligands **1** (Fig. 1). A year later, Martarello et al.[10] described the radiosynthesis, in vitro binding studies and in vivo rat tissue distribution of [^{18}F] FBPPA **2**, the first reported CRF_1R ^{18}F ligand. The initial level of accumulation of radioactivity of **2** in the rat pituitary was fairly high (5.59%) but the ligand exhibited fast washout (79.2% after 60 min). Very poor levels of radioactivity and retention were displayed in the hypothalamus, amygdala and cerebellum: the brain areas rich in CRF_1 receptor sites. The authors speculated that the high lipophilicity of **2** ($c\log P$ >6) and/or the insolubility of the radiopharmaceutical in blood could account for its exceedingly low blood–brain barrier penetration. In 2003, Eckelman et al.[11] disclosed the synthesis of [^{76}Br]-MJL-1-109-2 **3**, a high-affinity CRF_1R potential PET radioligand (K_i ~2 nM) with appropriate lipophilicity ($c\log P = 3$). In rat biodistribution studies, this compound penetrated the blood–brain barrier with cerebellum and cortex uptakes of 0.29 ± 0.01% ID/g and 0.32 ± 0.03% ID/g after 30 min. More recently, Kumar and Sullivan

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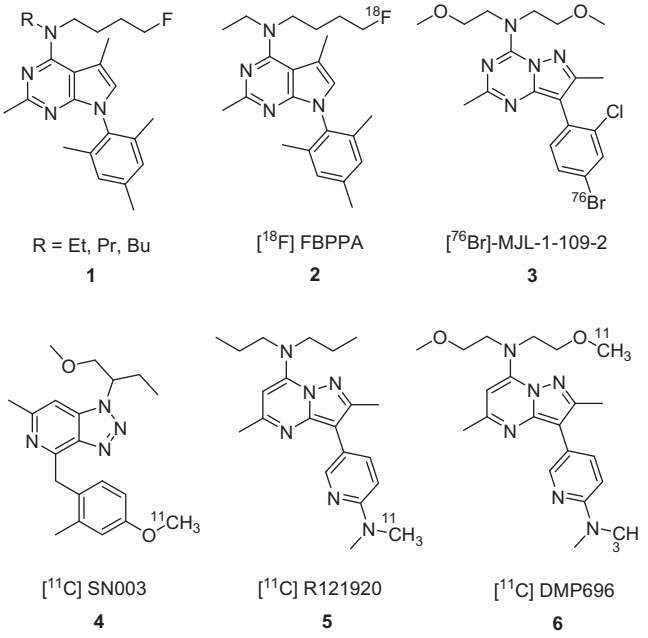


Figure 1.

evaluated [^{11}C]SN003 **4**,[12] [^{11}C]R121920 **5** and [^{11}C]DMP696 **6**[13] in baboons. Metabolism of all three radioligands was rapid in baboon, with compounds **5** and **6** showing just 50% of the parent molecule remaining after 9 and 13 min, respectively. There was a lack of detectable specific binding with all three ligands, and this was attributed to the lower density of CRF_1R receptors in primate brain as compared to rat or human brain.

The goal of the present work was to identify an effective CRF_1R antagonist PET ligand with appropriate physico-chemical properties that would be useful in assessing the receptor occupancy of clinical candidates in vivo. In particular, we turned to our pyrazinone series[7c] of potent CRF_1R antagonists to find a receptor ligand having an $IC_{50} \leqslant 5$ nM with lipophilicity as measured by $\log D \leqslant 4$.

Our initial efforts focused on the incorporation of a fluorine label in the side chain of 1-substituted 5-halo-3-(arylamino)pyrazin-2(1*H*)-ones[7d] with compounds **7** and **8** as the PET ligand targets (Fig. 2). One hypothesis was that the monocyclic 5-halo-3-(arylamino)pyrazin-2(1*H*)-one core would be advantageous in producing target compounds with reduced lipophilicity compared to the literature bicyclic core CRF_1R antagonist chemotypes. In addition, an optimal balance between potency and polarity might be achieved by a systematic modulation of the steric and electronic properties of aryl rings, as well as by a variation in substituent X.

The synthetic pathway to derivatives **7** is illustrated in Scheme 1. Previously described (*R*)-2-cyclopropyl-2-((*R*)-1-phenylethylamino)ethanol[7d] **9** was treated with NaH and bromofluoroethane to give fluoroethyl ether **10**. Mild hydrogenolysis followed by

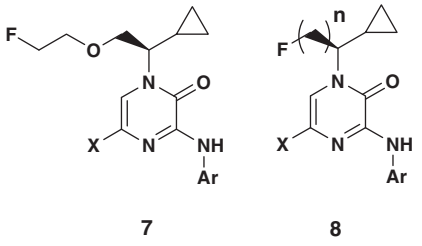
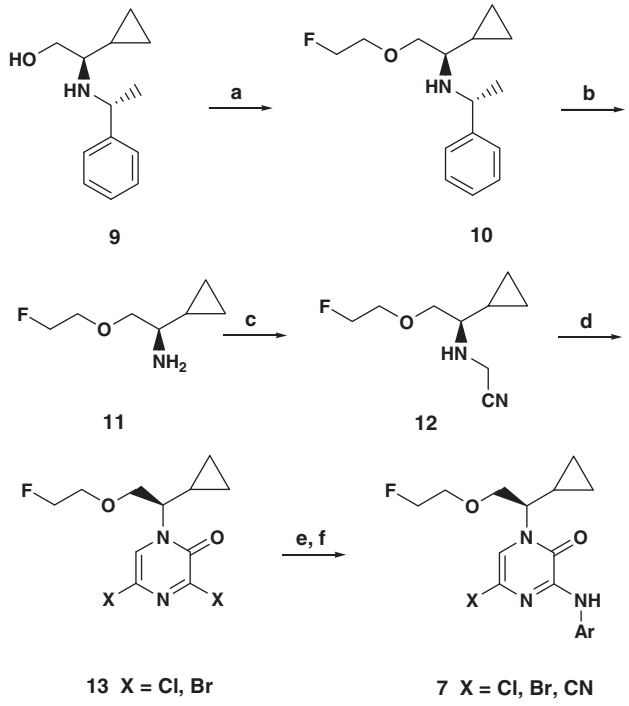
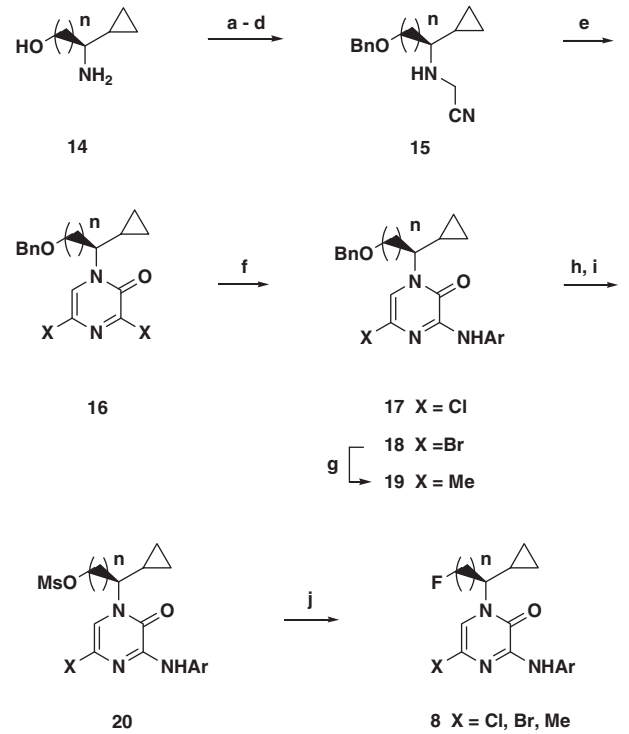


Figure 2.

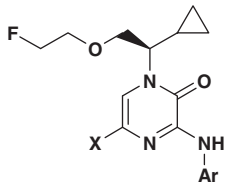


Scheme 1. Reagents and conditions: (a) 1-Bromo-2-fluoroethane, NaH, DMF, rt, 6 h, 20–84%; (b) H_2, 10% Pd–C, EtOH, 5 psi, rt, 30 min, 100%; (c) $ClCH_2CN$, KI, K_2CO_3, CH_3CN, 550 °C, 18 h, 52–82%; (d) $(COX)_2$, toluene, −78 °C, 15 min, then 55 °C, 15 h, 12–55%; (e) NaHMDS, $ArNH_2$, THF, rt, 1.5 h, 8–97%; (f) for **13** (X = Br) to **7** (X = CN) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 175 °C, 18 h, 49%.



Scheme 2. Reagents and conditions: (a) Boc_2O, K_2CO_3, CH_2Cl_2, H_2O, rt, 91–96%; (b) NaH, BnBr, DMF, rt, 63–75%; (c) TFA, rt, 90–95%; (d) $ClCH_2CN$, KI, K_2CO_3, CH_3CN, 55 °C, 18 h, 88–95%; (e) $(COX)_2$, toluene, −78 °C, 15 min, then 55 °C, 15 h, 53–81%; (f) NaHMDS, $ArNH_2$, THF, rt, 1.5 h, 40–91%; (g) $SnMe_4$, $Pd(PPh_3)_4$, DMF, 175 °C, 18 h, 25%; (h) BBr_3, CH_2Cl_2, −78 °C, 81–85%; (i) MsCl, Et_3N, CH_2Cl_2, rt, 77–88%; (j) KF, DMSO, 90 °C, 3–10%.

Table 1
CRF_1R binding affinities and phase distribution coefficients of amines **7a–k**



7

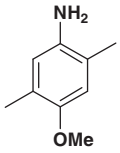
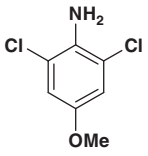
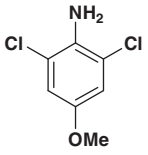
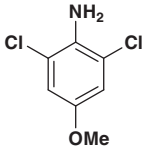
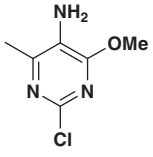
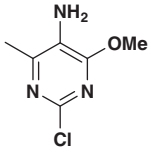
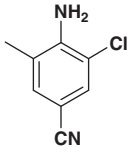
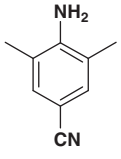
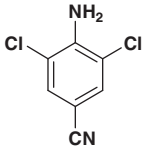
Compd	Ar-NH_2	X	IC_{50} (nM)	Log*D*
7a		Cl	0.46	4.6
7b		Cl	0.60	4.0
7c		Br	0.46	4.1
7d		CN	7.7	3.4
7e		Cl	19	3.3
7f		Br	14	3.4
7g		Cl	4.5	3.7
7h		Cl	7.4	3.4
7i		Cl	5.0	3.6

Table 1 (*continued*)

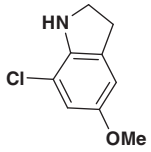
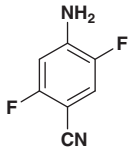
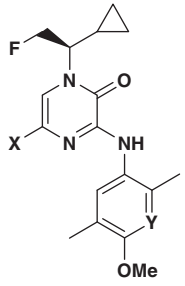
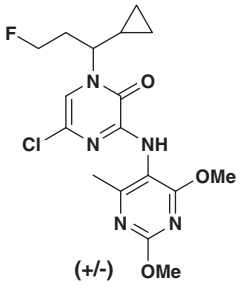
Compd	Ar-NH_2	X	IC_{50} (nM)	Log*D*
7j		Cl	3.2	4.0
7k		Cl	>1000	4.6

Table 2
CRF_1R binding affinities and phase distribution coefficients of amines **8a–d**



8a-c



8d

Compd	X	Y	IC_{50} (nM)	Log*D*
8a	Cl	CH	1.1	4.5
8b	Me	CH	0.61	4.2
8c	Me	N	4.7	4.1
8d	—	—	53	2.9

alkylation of the resulting amine **11** with chloroacetonitrile produced cyanomethylamine **12**. Upon treatment with either oxalyl chloride or oxalyl bromide, **12** underwent cyclization to pyrazinones **13**, which were coupled with a number of anilines to provide target compounds **7** (X = Cl or Br). For target compound **7** with X = CN, the corresponding bromide was substituted with cyanide using zinc cynanide and a palladium catalyst.

Fluoroalkyl pyrazinones **8** were synthesized as shown in Scheme 2. Hydroxyalkylamines **14** were converted to cyanomethylamines **15** by a sequence of standard transformations.[7c] Upon treatment with either oxalyl chloride or oxalyl bromide, derivatives **15** were cyclized to the corresponding pyrazinones **16**. Coupling of the latter compounds with a variety of anilines conveniently provided pyrazinones **17** and **18** in moderate to high yields. 5-Bromo derivatives **18** were converted to 5-methylpyrazinones **19** by a palladium-catalyzed coupling with tetramethylstannane. The benzyl groups in **17**, **18** and **19** were carefully removed by the action of boron tribromide in dichloromethane at low temperature, and the resulting alcohols were converted to the corresponding mesylates **20**. Consecutive displacement with potassium fluoride provided target compounds **8**, albeit in low yields.

The CRF_1R binding affinities of analogs **7a–k** and **8a–d** were determined by measuring the inhibition of specific binding of [^{125}I]-*o*-CRF in a CRF_1 receptor binding assay using rat frontal cortex homogenate.[14] The log*D* values of the compounds were determined by a classical octanol–phosphate buffer (pH ~7.4)

partitioning of the compounds.[15] Both sets of data are presented in Tables 1 and 2.

The most potent compounds in the fluoroethoxy series—**7a**, **7b**, and **7c**—all had subnanomolar CRF_1R binding affinities. Unfortunately, the log*D* values of these compounds were among the highest in the series. Variations in a trisubstitution pattern of **7a** and **7b** as well as changes in the size of a halogen atom X in **7b** and **7c** gave compounds with similar binding affinity. The introduction of a polar cyano-group to the pyrazinone core of **7d** and to the aromatic rings of **7g**, **7h** and **7i** resulted in some loss of potency, however, these compounds were still near the appropriate potency and lipophilicity range. A further increase in polarity led to a significant reduction in potency, as evidenced by the data for pyrimidinyl analogs **7e** and **7f**, the compounds with the lowest log*D* values in this subseries. This inverse relationship between CRF_1R IC_{50} and log*D* values has been also observed in a related series.[16] Conformational constraint of the aniline sector (as in **7j**) did not provide a significant improvement of the potency-lipophilicity balance. The total loss of activity observed for 4-cyano-2,5-difluoro-analog **7k** suggested the importance of a larger substituent (i.e., a chlorine atom or a methyl group) at the 2-position of the phenyl ring.

The data for the fluoroalkyl pyrazinones are shown in Table 2. The highest log*D* compound, **8a**, has a potent CRF_1R binding affinity with an IC_{50} of 1.1 nM. Replacement of Cl by Me gives **8b** which has similar potency and a trend to a lower log*D*. Replacement of the lower aryl group with a pyridine residue (**8c**) did not provide a significant improvement in log*D*, but resulted in some loss of activity. Compounds with much lower log*D* such as **8d** showed a large loss of potency, a trend which had also been seen in the fluoroethoxy-series (vide supra).

In summary, the present research produced a number of potent fluorinated pyrazinones as CRF_1R antagonists. While the most potent compounds in both series consistently had higher log*D* values then desired for potential PET ligands, a reasonable compromise between potency and polarity was achieved with cyano-derivatives **7g**, **7h** and **7i**. The data displayed by these three compounds met our established selection criteria, thus making them potential candidates for radiosynthesis and biodistribution studies. The continuation of these efforts will be presented in due course.[17]

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14. *CRF_1R binding assay*. Frozen rat frontal cortex (source of CRF_1 receptor) was thawed rapidly in assay buffer containing 50 mM HEPES (pH 7.0 at 23 °C) 10 mM $MgCl_2$, 2 mM EGTA, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 0.005% Triton X-100, 10 U/mL bacitracin and 0.1% ovalbumin and homogenized. The suspension was centrifuged at 32,000×*g* for 30 min. The resulting supernatant was discarded and the pellet resuspended by homogenization in assay buffer and centrifuged again. The supernatant was discarded and the pellet resuspended by homogenization in assay buffer and frozen at −70 °C. On the day of the experiment aliquots of the homogenate were thawed quickly and homogenate (25 μg/well rat frontal cortex) added to ligand (150 pM ^{125}I-*o*-CRF) and drugs in a total volume of 100 μL of assay buffer. The assay mixture was incubated for 2 h at 21 °C. Bound and free radioligand were then separated by rapid filtration, using glass fiber filters (Whatman GF/B, pretreated with 0.3% PEI) on a Brandel Cell Harvester. Filters were then washed multiple times with ice cold wash buffer (PBS w/o Ca^{2+} and Mg^{2+}, 0.01% Triton X-100 (pH 7.0 at 23 °C)). Nonspecific binding was defined using 1 μM DMP696. Filters were then counted in a Wallac Wizard gamma counter.
15. *Shake-flask log*D *determination assay for lipophilicity*. In a 250 mL separatory funnel were added 25 mM phosphate buffer (200 mL) and octanol (10 mL). The two-phase system was mixed well and let stand overnight to allow complete saturation and separation of both layers. A sample (1.0 mg) was dissolved in octanol (1 mL) and transferred to a volumetric flask, containing 50 mL of the phosphate buffer, saturated with octanol, as described above. The resulting mixture was shaken intensely for 30–40 min and was allowed to stand until two layers separated completely. A sample of each layer was analyzed by an HPLC method twice. The sample area counts were used to calculate the shake-flask log*D*.
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17. For a recently published related series see: Deskus, J. A.; Dischino, D. D.; Mattson, R. J.; Ditta, J. L.; Parker, M. F.; Denhart, D. J.; Zuev, D.; Huang, H.; Hartz, R. A.; Ahuja, V. T.; Wong, H.; Mattson, G. K.; Molski, T. F.; Grace, J. E.; Zueva, L.; Nielsen, J. M.; Dulac, H.; Li, Y.-W.; Guaraldi, M.; Azure, M.; Onthank, D.; Hayes, M.; Wexler, E.; McDonald, J.; Lodge, N. J.; Bronson, J. J.; Macor, J. E. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6651.