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Potential CRF_1R PET imaging agents: *N*-Fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethyl-*N*-alkylpyrazolo[1,5-*a*][1,3,5]triazin-4-amines

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ABSTRACT

A series of *N*-fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-dimethyl-*N*-alkylpyrazolo[1,5-*a*][1,3,5]triazin-4-amines 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 resulted in discovery of analog **7e** (IC_{50} = 6.5 nM, log *D* = 3.5).

Corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide produced by hypothalamic nuclei in brain, plays a central role in the coordination of neuroendocrine, autonomic, behavioral and immune responses to stress.[1,2] Secreted from hypothalamus in response to acute physical or psychological stress, CRF activates the transcription of the pro-opiomelanocortin gene, resulting in the secretion of adrenocorticotropin hormone (ACTH) from the pituitary gland. In turn, ACTH enters the circulation and stimulates the release of cortisol, the principal primate adrenal steroid hormone, from the adrenal gland. To restore the homeostasis 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 uncontrolled 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 nonpeptide 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 both to assess receptor occupancy in clinical trials of CRF_1R receptor antagonists, and to monitor changes in CRF concentration in normal and diseased patients. However, the discovery of such a ligand has proved to be challenging.

In 2000, Rice[8] 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.[9] described the radiosynthesis, in vitro binding studies and in vivo rat tissue distribution of [^{18}F] FBPPA **2**, the first reported CRF_1R PET ligand. Although the initial level of accumulation of radioactivity of **2** in the rat pituitary was fairly high (5.59%), the ligand exhibited fast washout (79.2% after 60 min). Very poor levels and retention of radioactive ligand were displayed by the hypothalamus, amygdala and cerebellum, which are 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[10] disclosed the synthesis of [^{76}Br]-MJL-1-109-2 **3**, a high affinity CRF_1R PET radioligand

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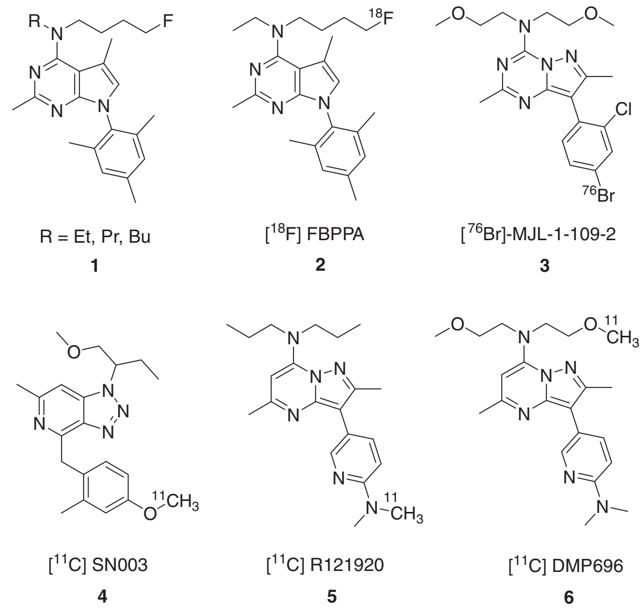


R = Et, Pr, Bu
1

$[^{18}F]$ FBPPA
2

$[^{76}Br]$-MJL-1-109-2
3

$[^{11}C]$ SN003
4

$[^{11}C]$ R121920
5

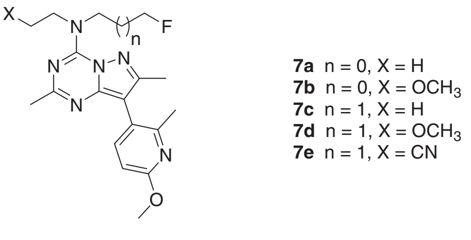
$[^{11}C]$ DMP696
6

Figure 1.

($K_i \sim 2$ nM) with appropriate lipophilicity ($c\log P = 3$). In rat biodistribution studies, this compound proved to be able to penetrate 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, Dileep Kumar and Sullivan evaluated $[^{11}C]$SN003 **4**,[11] $[^{11}C]$R121920 **5** and $[^{11}C]$DMP696 **6**[12] in baboons. The lack of detectable specific binding in each study was attributed to the lower density of CRF_1R receptors in primate brain as compared to rat or human brain. This group also determined that all three radioligands underwent rapid metabolism in baboon, with compounds **5** and **6** showing just 50% of the parent molecule remaining after 9 and 13 min, respectively.

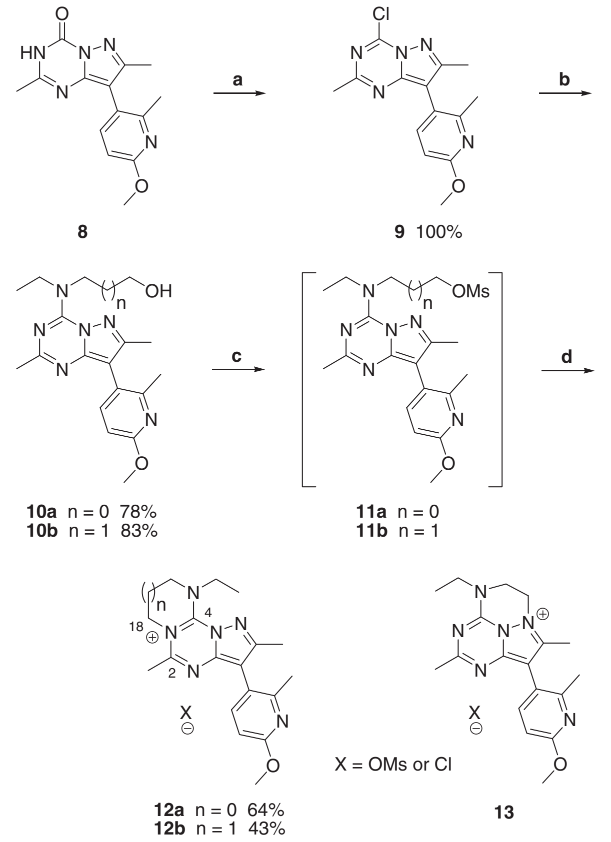
In the course of our search for a suitable CRF_1 PET radioligand, we viewed the amino side chain of pyrazolo[1,5-*a*][1,3,5]triazin-4-amines[13] as suitable site for the introduction of a radiolabel, particularly an ^{18}F isotope. We also believed that the introduction of a polar 8-(6-methoxy-2-methylpyridin-3-yl) substituent and a methoxy- or a cyano-group in the amino side chain could provide compounds with reduced lipophilicity. In the present publication, we describe the synthesis of analogs **7a–e**, and their evaluation as potential radioligands by optimization of CRF_1R binding potencies and phase distribution coefficients (Fig. 2).

Synthesis of fluorinated amines **7a–e** commenced from known pyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-one **8**,[14] which was converted to chloride **9** by reaction with phosphorus oxychloride and DIPEA in toluene (Scheme 1). Subsequent treatment of **9** with 2-(ethylamino)ethanol and 3-(ethylamino)propanol afforded amines **10a** and **10b** in 78% and 83% respective yields. We anticipated that the conversion of **10a** and **10b** to mesylates **11a** and **11b**, followed by a fluoride displacement of the mesyl group would provide desired fluoroethylamines **7a** and **7c**. Instead, **11a** and **11b** underwent spontaneous intramolecular cyclization to form pyrazolo[1,5-*a*][1,3,5]triazinium salts **12a** and **12b**. While the cyclization of **11a** to **12a** was almost instantaneous, the conversion of **11b** to **12b** proceeded slower, as was observed by the LCMS method.[15]



7a n = 0, X = H
7b n = 0, X = OCH_3
7c n = 1, X = H
7d n = 1, X = OCH_3
7e n = 1, X = CN

Figure 2.



8

9 100%

10a n = 0 78%
10b n = 1 83%

11a n = 0
11b n = 1

12a n = 0 64%
12b n = 1 43%

X = OMs or Cl

13

Scheme 1. Reagents and conditions: (a) $POCl_3$, DIPEA, toluene, 115 °C, 4 h, 100%; (b) 2-(ethylamino)ethanol or 3-(ethylamino)propanol, THF, rt, overnight; (c) MsCl, Et_3N, CH_2Cl_2, rt, overnight; (d) spontaneous cyclization, rt.

We initially attempted to determine the structure of **12a** by X-ray crystallographic analysis, however all our efforts to crystallize this salt were unsuccessful. Thus, we took advantage of a proton–carbon correlation HMBC NMR experiment to elucidate the structure of the molecule. Indeed, the observed C–H long range correlations between H18 and C2 as well as H18 and C4 (Scheme 1) were consistent with structure **12a**, not structure **13**, indicating that the cyclization took place via the nitrogen atom N3 of the triazine ring.

Treatment of **12a** and **12b** with potassium fluoride in ethylene glycol or TBAF in THF did not result in conversion to **7a** and **7c**, which necessitated introduction of the fluorine earlier in the synthesis.

2-Fluoroethylamine hydrochlorides **14a–b** were prepared by a literature method,[16] and 3-fluoropropylamines **15a–c** were prepared by reaction of 1-bromo-3-fluoropropane with the corresponding ethylamine hydrochlorides. Fluoroalkylamines **7a–e** were ultimately synthesized by reaction of chloride **9** with **14a–b** and **15a–c** (Scheme 2).

We found that upon concentration of the acetonitrile–water–TFA fractions of purified 2-fluoroethylamines **7a** and **7b**, partial N3-cyclization (9–12%) to the corresponding pyrazolo[1,5-*a*][1,3,5]triazinium salts took place. In contrast, fluoropropyl derivatives **7c–e** were stable to the solvent evaporation conditions and were isolated without any noticeable cyclization. The CRF_1R