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Chemical Modification of Aryl-1,2,3,6-tetrahydropyridinopyrimidine Derivative to Discover Corticotropin-Releasing Factor$_1$ Receptor Antagonists: Aryl-1,2,3,6-tetrahydropyridino-purine, -3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine, -purin-8-one, and -7*H*-pyrrolo[2,3-*d*]pyrimidine Derivatives

Toshihito Kumagai, Taketoshi Okubo, Hiromi Kataoka-Okubo, Shigeyuki Chaki, Shigeru Okuyama and Atsuro Nakazato*

1st Laboratory, Medicinal Research Laboratories, Taisho Pharmaceutical Co., Ltd.,1-403 Yoshino-cho, Ohmiya, Saitama 330-8530, Japan

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Abstract—Structure–affinity relationships (SARs) of non-peptide CRF_1 antagonists suggest that such antagonists can be constructed of three units: a hydrophobic unit (Up-Area), a proton accepting unit (Central-Area), and an aromatic unit (Down-Area). Recently, various non-peptide corticotropin-releasing factor$_1$ (CRF_1) receptor antagonists obtained by modification of the Central-Area have been reported. In contrast, we modified the Up-Area and presented 4- or 5-aryl-1,2,3,6-tetrahydropyridinopyrimidine derivatives including potent CRF receptor ligands **1a**–**c**, and proposed that the 4- or 5-aryl-1,2,3,6-tetrahydropyridino moiety might be useful as a substituent in the Up-Area. Our interest shifted to the chemical modification in which the pyrimidine ring of **1a**–**c** was replaced by other heterocycles, purine ring of **2**, 3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine ring of **3**, purin-8-one ring of **4** and 7*H*-pyrrolo[2,3-*d*]pyrimidine ring of **5**. Among them, 5-aryl-1,2,3,6-tetrahydropyridinopurine compound **6j** (CRA0186) had the highest affinity for CRF_1 receptors ($IC_{50} = 20$ nM). We report here the synthesis and SARs of derivatives **6**–**9**.

Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, is a principal modulator for the responses to stress through the release of adrenocorticotropic (ACTH) from the anterior pituitary.[1,2] CRF moderates various physiological responses to sustained stress[3–7] by binding to two subtypes of seven-transmembrane G-protein-coupled CRF receptors, CRF_1 and CRF_2 receptors,[8–11] and has higher affinity for CRF_1 receptors than CRF_2 receptors.[10] Clinical evidence, the elevation of CRF concentration in patients with depression[12] or chronic post-traumatic stress disorder,[13] and the blunted corticotropin response to CRF in patients with depression,[14] anxiety, anorexia nervosa, or post-traumatic stress disorder[15] suggest that CRF receptor antagonists may be useful for the treatment of depression, anxiety, or other diseases related to stress.

Structure–affinity relationships (SARs) of non-peptide CRF_1 receptor antagonists suggest that such antagonists can be constructed of three parts, a hydrophobic unit (Up-Area), a proton accepting unit (Central-Area) and an aromatic unit (Down-Area), and *N*,*N*-dialkylamino groups containing an alkoxyalkyl group may be typical Up-Area moieties (Fig. 1).[16] Recently, various non-peptide CRF_1 receptor antagonists produced by modification of the Central-Area have been presented.[16–28] Among them, pyrimidine,[19,20,24,26,28,29] purine,[22] 3*H*-triazolo[4,5-*d*]pyrimidine,[22] purin-8-one[23] and 7*H*-pyrrolo[2,3-*d*]pyrimidine[17] may be typical Central-Area residues.

We found that pyrimidine derivatives **1b** (CRA1001),[28] **1c** (CRA1000)[28] and **1d** (CRA0165)[29] containing a 4- or 5-aryl-1,2,3,6-tetrahydropyridino group in the Up-Area

*Corresponding author. Tel.: +81-48-663-1111; fax: +81-48-652-7254; e-mail: s10968@ccm.taisho.co.jp

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exhibited high affinities for CRF_1 receptors ($IC_{50} = 10$, 22 and 11 nM, respectively). As part of our ongoing effort to discover novel CRF_1 receptor antagonists, we have continued to change the pyrimidine ring of derivative **1** with other Central-Area moieties, purine, 3*H*-triazolo [4,5-*d*]pyrimidine, purin-8-one and 7*H*-pyrrolo[2,3-*d*] pyrimidine rings. Among the modified derivatives **6–9**, purine derivatives **6** exhibited moderately good or high affinity for CRF_1 receptors (typical compound **6j**: $IC_{50} =$ 20 nM). Interestingly, 5-aryl-1,2,3,6-tetrahydropyridino derivatives generally exhibited higher affinity for CRF_1 receptors than 4-aryl-1,2,3,6-tetrahydropyridino derivatives. In this paper, we report the synthesis and SARs of derivatives **6–9** (Fig. 1).

Chemistry

The synthesis of derivatives **6–9** is shown in Scheme 1. A crude 4- or 5-aryl-1,2,3,6-tetrahydropyridine **12**, which was prepared by treatment of compound **10** with Grignard reagent (ArMg-Br or -Cl) followed by acid in the same fashion as previously presented,[28,29] was coupled with known chloride, 6-chloro-2-methylpurines **13**,[22] 7-chloro-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidines **14**[22] 6-chloro-2,7-dimethylpurin-8-ones **15**[23] or 4-chloro-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines **16**,[17] to afford novel 4- or 5-aryl-1,2,3,6-tetrahydropyridine derivatives **6–9**.

Results and Discussion

Compounds **6–9** (Fig. 1) were evaluated for corticotropin-releasing factor$_1$ (CRF_1) receptor binding affinity in rat frontal cortex against radioligand [^{125}I]-ovine CRF,[30] and the IC_{50} obtained values are shown in Table 1. Compound **6j** did not exhibit corticotropin-releasing factor$_2$ (CRF_2) receptor binding affinity in rat heart against radioligand [^{125}I]-sauvagine.[31]

Purine derivative **6a** substituted with a 4-phenyl-1,2,3,6-tetrahydropyridino group exhibited moderate affinity for CRF_1 receptors ($IC_{50} = 385$ nM), but less than that of pyrimidine derivative **1a** ($IC_{50} = 66$ nM). The introduction of a halogen atom (F or Cl) on the phenyl group in the Up-Area slightly increased or did not affect CRF_1 receptor affinity (**6a** versus **6b**, **6c**), similar to pyrimidine derivative **1** (**1a** versus **1b**). However, replacing the bromine atom of **6b** or **6c** with a MeS group on the phenyl group in Down-Area did not affect CRF_1 receptor affinity (**6b**, **6c** versus **6d–6g**), unlike the case for pyrimidine derivative **1** (**1b** versus **1c**). 4-(2-Methylphenyl)-1,2,3,6-tetrahydropyridine compound **8h** exhibited no affinity for CRF_1 receptors ($IC_{50} > 1000$ nM), and this decrease in affinity was larger than that for pyrimidine derivative **1**. These findings suggested that a purine moiety was less desirable for obtaining CRF_1 receptor ligands using a 4-aryl-1,2,3,6-tetrahydropyridino group for the Up-Area

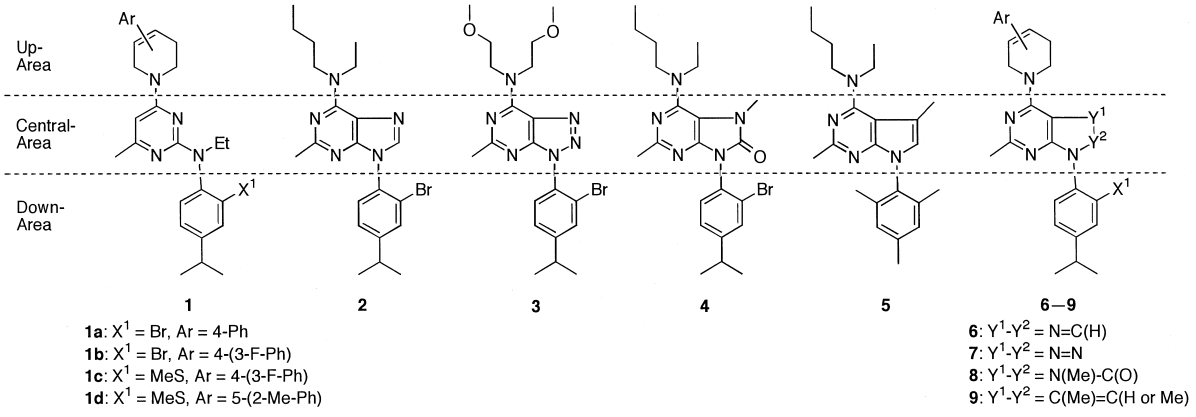
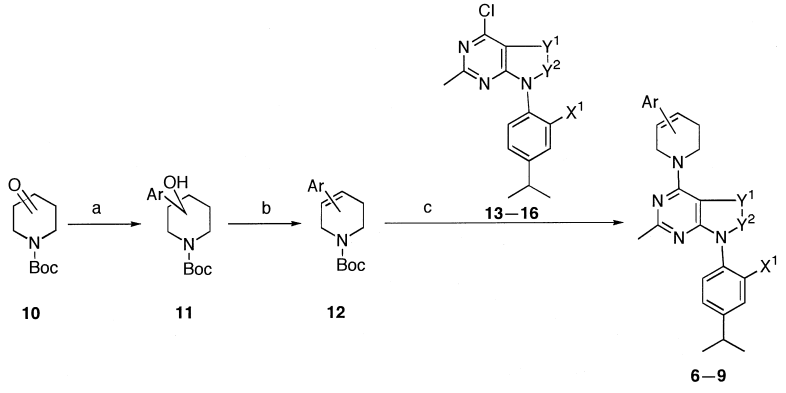
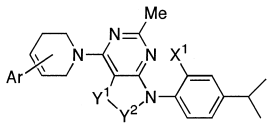


Figure 1.



Scheme 1. Reagents and conditions: (a) ArMg-Br, THF; (b) TFA–CH_2Cl_2 or concd HCl; (c) isoPr$_2$NEt, EtOH.

Table 1. 4- or 5-Aryl-1,2,3,6-tetrahydropyridine derivatives: binding data



Compound no.	Ar	Y^1–Y^2	X^1	CRF_1 receptor IC_{50} (nM)[a]
6a	4–Ph	N=C(H)	Br	390
6b	4-(4-F–Ph)	N=C(H)	Br	350
6c	4-(4-Cl–Ph)	N=C(H)	Br	170
6d	4-(4-F–Ph)	N=C(H)	MeS	110
6e	4-(4-Cl–Ph)	N=C(H)	MeS	340
6f	4-(3-F–Ph)	N=C(H)	MeS	110
6g	4-(3-Cl–Ph)	N=C(H)	MeS	150
6h	4-(2-Me–Ph)	N=C(H)	MeS	> 1000
6i	5-(4-F–Ph)	N=C(H)	MeS	60
6j	5-(2-Me–Ph)	N=C(H)	MeS	20
7a	4-(4-Cl–Ph)	N=N	Br	> 1000
7b	4-(3-F–Ph)	N=N	MeS	154
7c	5-(2-Me–Ph)	N=N	MeS	96
8a	4-(4-Cl–Ph)	N(Me)–CO	MeS	860
8b	4-(3-F–Ph)	N(Me)–CO	MeS	630
8c	5-(4-F–Ph)	N(Me)–CO	MeS	860
8d	5-(2-Me–Ph)	N(Me)–CO	MeS	290
9a	4-(4-Cl–Ph)	C(Me)=C(Me)	MeS	> 1000
9b	4-(3-F–Ph)	C(Me)=C(Me)	MeS	> 1000
9c	5-(2-Me–Ph)	C(Me)=C(Me)	MeS	> 1000
9d	4-(3-F–Ph)	C(Me)=C(H)	MeS	> 1000
9e	5-(2-Me–Ph)	C(Me)=C(H)	MeS	210
1a[b]				66
1b[b]				22
1c[b]				10
1d[c]				11

[a]IC_{50} values from duplicate determination.
[b]Ref 28.
[c]Ref 29.

than a pyrimidine moiety. Notably, however, introduction of a 5-aryl-1,2,3,6-tetrahydropyridino group onto the purine ring enhanced CRF_1 receptor affinity (**6i**: $IC_{50} = 60$ nM). 5-(2-Methylphenyl)-1,2,3,6-tetrahydropyridinopurine **6j** exhibited high affinity for CRF_1 receptors ($IC_{50} = 20$ nM). This notable enhancement of CRF_1 receptor affinity by replacement of the 4-phenyl group with 5-(2-methylphenyl) group (**6j/6a**: 19-fold, **6j/6f**: 5.6-fold) was larger than in the case of pyrimidine derivative **1** (**1d/1a**: 6-fold, **1d/1c**: 0.9-fold). This difference in SARs between aryl-1,2,3,6-tetrahydropyridinopyrimidine and aryl-1,2,3,6-tetrahydropyridinopurine derivatives suggests that the molecular structure of CRF_1 receptor might have a narrow and non-flexible space for the phenyl group of derivatives **1** and **6** and that this space might require steric control of the phenyl group in the phenyl-1,2,3,6-tetrahydropyridine derivative to yield high affinity for CRF_1 receptors.

3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine derivative **7**, purin-8-one derivative **8** and 7*H*-pyrrolo[2,3-*d*]pyrimidine derivative **9** all exhibited lower affinities for CRF_1 receptors than purine derivative **6** (**6c**, **6f**, **6j** versus **7a**–**7c**; **6e**, **6f**, **6i**, **6j** versus **8a**–**8d**; **6e**, **6f**, **6j** versus **9a**–**9e**). In particular, marked decline in CRF_1 receptor affinity was noted with derivative **9**. Notably, however, 5-(2-methylphenyl) derivatives **6j** ($IC_{50} = 20$ nM), **7c** ($IC_{50} = 96$ nM), **8d** ($IC_{50} = 287$ nM) and **9e** ($IC_{50} = 210$ nM) exhibited the highest affinity for CRF_1 receptors in the derivatives **6**–**9**, respectively. These findings suggest that the substituent on the phenyl group (Ar) might stereochemically control the positioning of the phenyl group (Ar) in aryl-1,2,3,6-tetrahydropyridine derivatives **6**–**9**, and that this stereochemistry might be important for interaction with CRF_1 receptors. Furthermore, the decreased CRF_1 receptor affinity noted for derivatives **7**, **8** and **9** suggests that the CRF_1 receptor affinity of the aryl-1,2,3,6-tetrahydropyridine derivative might be more affected by the Central-Area moiety than that of *N*,*N*-dialkylamine derivatives[16] such as compounds **2**–**5** (Fig. 1).

Conclusions

The structural similarity of the known CRF_1 receptor antagonists appears to include two alkyl groups of *N*,*N*-dialkylamino or *N*-(secondary-alkyl)amino moiety in the Up-Area.[16] In contrast, 4- or 5-aryl-1,2,3,6-terahyropyridine CRF_1 receptor antagonists have unique structures without two alkyl groups. This present study suggests various possibilities and limitations of the use of 4- or 5-aryl-1,2,3,6-terahyropyridino moiety for discovery of CRF_1 receptor antagonists. However, it appears that Up-Area might be a useful target as the position of chemical modification and that chemical modification of the Up-Area may be useful for discovery of CRF_1 receptor antagonists.

Furthermore, 5-aryl-1,2,3,6-tetrahydropyridinopurine compound **6j** (CRA0186), a selective and potent CRF_1 receptor antagonist, might be useful not only for exploring the functions of CRF_1 receptor but also in the treatment of central nervous system disorders such as depression and/or anxiety-related disorders.

Experimental

Chemistry

Melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. Infra-red (IR) spectra were obtained on a Perkin-Elmer 1760 spectrometer. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian VXR-200 spectrometer. Chemical shifts are reported in parts per million relatives to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimazu/Kratos HV-300. Elemental analyses were performed by a Perkin-Elmer 240C (for carbon, hydrogen and nitrogen) or Yokokawa-Denki IC7000P (for halogen and sulfur). Analytical thin-layer chromatography was conducted on precoated silica gel 60 F254 plates (Merck). Chromatography was performed on silica gel C-200, 100–200 mesh (Wako Pure Chemical), using the solvent systems (volume ratios) indicated below.

***N-tert*-Butoxycarbonyl-3-hydroxy-3-(2-methylphenyl)piperidine 11a.** To a solution of 2-methylphenyl magnesium bromide in tetrahydrofuran (THF) prepared from 2-bromotoluene (18.03 g, 105 mmol) and magnesium (2.35 g, 96.7 mmol) in THF (120 mL) was added a solution of *N-tert*-butoxycarbonyl-3-piperidone (17.50 g, 87.8 mmol) in THF (60 mL) with ice cooling. After stirring for 1.5 h with ice cooling, saturated aqueous NH_4Cl was added to the reaction mixture and extracttion was performed with AcOEt three times. The combined extract was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:5) afforded 9.68 g (38%) of *N-tert*-butoxycarbonyl-3-hydroxy-3-(2-methylphenyl)piperidine **11a** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.49 (9 H, s), 1.54–1.75 (1H, m), 1.88–2.28 (4H, m), 2.66 (3H, s), 2.75–2.98 (1H, m), 3.20 (1H, d, J = 13.7 Hz), 4.00–4.22 (1H, m), 4.27 (1H, d, J = 13.7 Hz), 7.13–7.43 (5H, m, ArH); MS (ESI, Pos.) m/z 314 (M^+ + Na).

2-Methyl-6-(5-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6j. To a solution of **11a** (590 mg, 2.02 mmol) in dioxane (0.84 mL) was added concentrated HCl (8.4 mL), and the resulting mixture was stirred at room temperature for 30 min and then heated at reflux for 3 h. The mixture was concentrated and the resulting solid was dried under reduced pressure.

A mixture of the above solid, 2-methyl-6-chloro-9-(2-methylthio-4-isopropylphenyl)purine **13a** (200 mg, 0.60 mmol) and diisopropylethylamine (5.0 mL, 28.7 mmol) was stirred at reflux for 1 h. After concentration of the reaction mixture under reduced pressure, the resulting residue was partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$, and the separated water phase was extracted with $CHCl_3$ twice. The combined organic phase was washed with saturated brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:8) followed by recrystallization from AcOEt/hexane afforded 243 mg (86%) of **6j** as a yellow crystal: mp 150–151 °C (AcOEt/hexane); ^{1}H NMR ($CDCl_3$) δ 1.31 (6H, d, J = 7.0 Hz), 2.34 (3H, s), 2.38 (3H, s), 2.43–2.56 (2H, m), 2.52 (3H, s), 2.99 (1H, sept, J = 7.0 Hz), 4.50–4.66 (2H, m), 4.75–4.90 (2H, m), 5.74–5.82 (1H, m), 7.16–7.32 (7H, m), 7.79 (1H, s); MS (Ion Spray) m/z 470 (M^+ + H); IR (KBr) 2958, 2923, 2836, 1580, 1513 cm^{-1}. Anal. ($C_{28}H_{31}N_5S$) C, H, N, S.

Using a corresponding procedure, the following compounds **6a–6i**, **7a–7c**, **8a–8d** and **9a–9e** were prepared.

2-Methyl-6-(4-phenyl-1,2,3,6-tetrahydropyridino)-9-(2-bromo-4-isopropylphenyl)purine 6a. Amorphous; ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, d, J = 6.8 Hz), 2.56 (3H, s), 2.70–2.83 (2H, m), 2.98 (1H, sept, J = 6.8 Hz), 4.50–4.68 (2H, m), 4.83–4.98 (2H, m), 6.19–6.28 (1H, m), 7.20–7.49 (7H, m), 7.61 (1H, d, J = 2.0 Hz), 7.83 (1H, s); MS (FAB) m/z 490 (M^+ + 2 + H), 488 (M^+ + H); IR (KBr) 3435, 2961, 1581 cm^{-1}. Anal. ($C_{26}H_{26}BrN_5 \cdot H_2O$) C, H, N, Br.

2-Methyl-6-(4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-bromo-4-isopropylphenyl)purine 6b. Mp 166–167 °C ($CHCl_3$-hexane); ^{1}H NMR ($CDCl_3$) δ 1.31 (6H, d, J = 7.0 Hz), 2.56 (3H, s), 2.66–2.78 (2H, m), 2.98 (1H, sept, J = 7.0 Hz), 4.51–4.68 (2H, m), 4.83–4.98 (2H, m), 6.13–6.23 (1H, m), 7.00–7.09 (2H, m), 7.32–7.44 (4H, m), 7.62 (1H, d, J = 2.0 Hz), 7.83 (1H, s); MS (ion spray) m/z 470 (M^+ + H); IR (KBr) 2964, 2920, 1601, 1574 cm^{-1}. Anal. ($C_{26}H_{25}BrFN_5$) C, H, N, Br, F.

2-Methyl-6-(4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6c. Mp 159–160 °C ($CHCl_3$/hexane); ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, d, J = 6.8 Hz), 2.56 (3H, s), 2.62–2.78 (2H, m), 2.98 (1H, sept, J = 6.8 Hz), 4.50–4.63 (2H, m), 4.82–4.96 (2H, m), 6.17–6.27 (1H, m), 7.29–7.43 (6H, m), 7.61 (1H, d, J = 1.8 Hz), 7.83 (1H, s); MS (APCI) m/z 526 (M^+ + 4 + H), 524 (M^+ + 2 + H), 522 (M^+ + H); IR (KBr) 2962, 2924, 1572, 1516 cm^{-1}. Anal. ($C_{26}H_{25}BrClN_5$) C, H, N, Br, Cl.

2-Methyl-6-(4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6d. Mp 162–163 °C (AcOEt); ^{1}H NMR ($CDCl_3$) δ 1.31 (6H, d, J = 7.0 Hz), 2.35 (3H, s), 2.55 (3H, s), 2.65–2.77 (2H, m), 2.99 (1H, sept, J = 7.0 Hz), 4.50–4.67 (2H, m), 4.83–4.98 (2H, m), 6.12–6.23 (1H, m), 6.98–7.10 (2H, m), 7.16–7.45 (5H, m), 7.81 (1H, s); MS (ion spray) m/z 474 (M^+ + H); IR (KBr) 2963, 2918, 2866, 1578, 1509 cm^{-1}. Anal. ($C_{27}H_{28}FN_5S$) C, H, N, S, F.

2-Methyl-6-(4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6e. Mp 165–166 °C (AcOEt); ^{1}H NMR ($CDCl_3$) δ 1.31 (6H, d, J = 6.8 Hz), 2.35 (3H, s), 2.55 (3H, s), 2.64–2.77 (2H, m),

2.99 (1H, sept, $J=6.8$ Hz), 4.50–4.67 (2H, m), 4.84–4.97 (2H, m), 6.18–6.26 (1H, m), 7.19 (1H, dd, $J=1.9$, 8.0 Hz), 7.27–7.39 (6H, m), 7.81 (1H, s); MS (ion spray) m/z 492 ($M^+ + 2 + H$), 490 ($M^+ + H$); IR (KBr) 2953, 2917, 1578 cm^{-1}. Anal. ($C_{27}H_{28}ClN_5S\cdot 0.2H_2O$) C, H, N, S, Cl.

2-Methyl-6-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6f. Mp 168–169 °C (AcOEt); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J=6.8$ Hz), 2.35 (3H, s), 2.55 (3H, s), 2.65–2.78 (2H, m), 2.99 (1H, sept, $J=6.8$ Hz), 4.50–4.67 (2H, m), 4.85–4.99 (2H, m), 6.24–6.33 (1H, m), 6.90–7.02 (1H, m), 7.08–7.37 (6H, m), 7.82 (1H, s); MS (ion spray) m/z 474 ($M^+ + H$); IR (KBr) 2960, 2918, 2865, 1578 cm^{-1}. Anal. ($C_{27}H_{28}FN_5S$) C, H, N, S, F.

2-Methyl-6-(4-(3-chlorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6g. Mp 155–156 °C (AcOEt); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J=6.8$ Hz), 2.35 (3H, s), 2.55 (3H, s), 2.65–2.78 (2H, m), 2.99 (1H, sept, $J=6.8$ Hz), 4.50–4.68 (2H, m), 4.73–4.98 (2H, m), 6.21–6.30 (1H, m), 7.16–7.32 (6H, m), 7.42 (1H, dd, $J=1.4$, 2.7 Hz), 7.82 (1H, s); MS (ion spray) m/z 492 ($M^+ + 2 + H$), 490 ($M^+ + H$); IR (KBr) 2959, 1578 cm^{-1}. Anal. ($C_{27}H_{28}ClN_5S\cdot 0.2H_2O$) C, H, N, S, Cl.

2-Methyl-6-(4-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6h. Mp 182–183 °C (AcOEt); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J=6.8$ Hz), 2.33 (3H, s), 2.36 (3H, s), 2.50–2.64 (2H, m), 2.56 (3H, s), 2.99 (1H, sept, $J=6.8$ Hz), 4.50–4.66 (2H, m), 4.80–4.93 (2H, m), 5.72–5.77 (1H, m), 7.10–7.24 (5H, m), 7.26–7.33 (2H, m), 7.81 (1H, s); MS (ion spray) m/z 470 ($M^+ + H$); IR (KBr) 2959, 1576, 1515 cm^{-1}. Anal. ($C_{28}H_{31}N_5S$) C, H, N, S.

2-Methyl-6-(5-(4-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purine 6i. Mp 135–136 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J=7.0$ Hz), 2.35 (3H, s), 2.45–2.57 (2H, m), 2.55 (3H, s), 2.99 (1H, sept, $J=7.0$ Hz), 4.43–4.58 (2H, m), 5.04–5.19 (2H, m), 6.16–6.27 (1H, m), 7.01–7.13 (2H, m), 7.19 (1H, dd, $J=1.8$, 8.1 Hz), 7.28 (1H, d, $J=1.8$ Hz), 7.30 (1H, d, $J=8.1$ Hz), 7.43–7.55 (2H, m), 7.81 (1H, s); MS (ion spray) m/z 474 ($M^+ + H$); IR (KBr) 2960, 2922, 2837, 1579 cm^{-1}. Anal. ($C_{27}H_{28}FN_5S\cdot 0.5H_2O$) C, H, N, S, F.

3-(2-Bromo-4-isopropylphenyl)-7-(4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridino)-5-methyl-3*H*-1,2,3-triazolo-[4,5-*d*]pyrimidine 7a. Mp 162–163 °C ($CHCl_3$/hexane); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J=6.8$ Hz), 2.58 (3H, s), 2.63–2.88 (2H, m), 3.00 (1H, sept, $J=6.8$ Hz), 4.30–4.79 (2H, m), 4.87–5.36 (2H, m), 6.18–6.28 (1H, m), 7.31–7.45 (6H, m), 7.65 (1H, d, $J=1.8$ Hz); MS (FAB) m/z 527 ($M^+ + 4 + H$), 525 ($M^+ + 2 + H$), 523 ($M^+ + H$); IR (KBr) 3436, 2963, 2916, 1610, 1593, 1570 cm^{-1}. Anal. ($C_{25}H_{24}BrClN_6$) C, H, N, Br, Cl.

7-(4-(3-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-5-methyl-3(-2-methylthio-4-isopropylphenyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine 7b. Mp 115–116 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.32 (6H, d, $J=7.0$ Hz), 2.40 (3H, s), 2.59 (3H, s), 2.66—2.89 (2H, m), 3.01 (1H, sept, $J=7.0$ Hz), 4.29–4.81 (2H, m), 4.82–5.42 (2H, m), 6.21–6.34 (1H, m), 6.93–7.40 (7H, m); MS (FAB) m/z 475 ($M^+ + H$); IR (KBr) 2962, 2928, 1594, 1564 cm^{-1}. Anal. ($C_{26}H_{27}FN_6S\cdot 0.2H_2O$) C, H, N, S, F.

5-Methyl-7-(5-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-3(-2-methylthio-4-isopropylphenyl)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine 7c. Mp 81–83 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.32 (6H, d, $J=6.8$ Hz), 2.38 (3H, s), 2.40 (3H, s), 2.44–2.68 (5H, m), 3.01 (1H, sept, $J=6.8$ Hz), 4.20–4.73 (2H, m), 4.80–5.33 (2H, m), 5.78–5.89 (1H, m), 7.16–7.28 (5H, m), 7.32–7.40 (2H, m); MS (FAB) m/z 471 ($M^+ + H$); IR (KBr) 2956, 2925, 1594, 1563 cm^{-1}. Anal. ($C_{27}H_{30}N_6S\cdot 0.2H_2O$) C, H, N, S.

2,7-Dimethyl-6-(4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purin-8-one 8a. Mp 161–163 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=6.8$ Hz), 2.42 (3H, s), 2.51 (3H, s), 2.67–2.80 (2H, m), 2.97 (1H, sept, $J=6.8$ Hz), 3.50–3.74 (2H, m), 3.67 (3H, s), 4.04–4.13 (2H, m), 6.20–6.28 (1H, m), 7.15–7.41 (7H, m); MS (ion spray) m/z 522 ($M^+ + 2 + H$), 520 ($M^+ + H$); IR (KBr) 2961, 2923, 1731, 1599 cm^{-1}. Anal. ($C_{28}H_{30}ClN_5OS$) C, H, N, S, Cl.

2,7-Dimethyl-6-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purin-8-one 8b. Mp 156–157 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=6.8$ Hz), 2.42 (3H, s), 2.51 (3H, s), 2.70–2.81 (2H, m), 2.98 (1H, sept, $J=6.8$ Hz), 3.50–3.77 (2H, m), 3.66 (3H, s), 4.06–4.14 (2H, m), 6.23–6.33 (1H, m), 6.92–7.04 (1H, m), 7.12–7.38 (6H, m); MS (ion spray) m/z 504 ($M^+ + H$); IR (KBr) 2957, 2925, 1727, 1603 cm^{-1}. Anal. ($C_{28}H_{30}FN_5OS$) C, H, N, S, F.

2,7-Dimethyl-6-(5-(3-fluorophenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purin-8-one 8c. Mp 131–132 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=6.8$ Hz), 2.42 (3H, s), 2.47–2.61 (2H, m), 2.51 (3H, s), 2.98 (1H, sept, $J=6.8$ Hz), 3.43–3.62 (2H, m), 3.64 (3H, s), 4.16–4.23 (2H, m), 6.13–6.24 (1H, m), 6.99–7.12 (2H, m), 7.15–7.31 (3H, m), 7.35–7.43 (2H, m); MS (ion spray) m/z 504 ($M^+ + H$); IR (KBr) 2961, 2923, 1727, 1600, 1510, 1500 cm^{-1}. Anal. ($C_{28}H_{30}FN_5OS$) C, H, N, S, F.

2,7-Dimethyl-6-(5-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-9-(2-methylthio-4-isopropylphenyl)purin-8-one 8d. Mp 164–165 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.30 (6H, d, $J=6.8$ Hz), 2.35 (3H, s), 2.41 (3H, s), 2.44–2.56 (2H, m), 2.48 (3H, s), 2.97 (1H, sept, $J=6.8$ Hz), 3.50–3.63 (2H, m), 3.65 (3H, s), 4.00–4.06 (2H, m), 5.70–5.79 (1H, m), 7.14–7.33 (7H, m); MS (ion spray) m/z 500 ($M^+ + H$); IR (KBr) 2962, 2925, 2886, 1727, 1601 cm^{-1}. Anal. ($C_{29}H_{33}N_5OS$) C, H, N, S.

4-(4-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-7-(2-methylthio-4-isopropylphenyl)-2,5,6-trimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 9a. Mp 173–174 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.33 (6H, d, $J=7.0$ Hz), 2.05 (3H, s), 2.32 (3H, s), 2.42 (3H, s), 2.52 (3H, s), 2.70–2.83 (2H, m), 3.00 (1H, sept, $J=7.0$ Hz), 3.64–3.93 (2H, m), 4.17–4.26 (2H, m), 6.19–6.28 (1H, m), 7.08–7.22 (3H, m),

7.29–7.43 (4H, m); MS (FAB) m/z 519 ($M^+ + 2 + H$), 517 ($M^+ + H$); IR (KBr) 2945, 2920, 1582, 1557, 1546 cm^{-1}. Anal. ($C_{30}H_{33}ClN_4S$) C, H, N, S, Cl.

4-(4-(3-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-7-(2-methylthio-4-isopropylphenyl)-2,5,6-trimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 9b. Amorphous; ^1H NMR ($CDCl_3$) δ 1.32 (6H, d, $J = 6.8$ Hz), 2.05 (3H, s), 2.32 (3H, s), 2.42 (3H, s), 2.53 (3H, s), 2.70–2.85 (2H, m), 2.99 (1H, sept, $J = 6.8$ Hz), 3.63–3.93 (2H, m), 4.16–4.28 (2H, m), 6.24–6.34 (1H, m), 6.89–7.40 (7H, m); MS (FAB) m/z 501 ($M^+ + H$); IR (KBr) 2960, 2921, 1610, 1583, 1547 cm^{-1}. Anal. ($C_{30}H_{33}FN_4S \cdot 0.2H_2O \cdot 0.2CHCl_3$) C, H, N, S, F.

4-(5-(2-Methylphenyl)-1,2,3,6-tetrahydropyridino)-7-(2-methylthio-4-isopropylphenyl)-2,5,6-trimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine 9c. Mp 133–134 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.32 (6H, d, $J = 6.8$ Hz), 2.04 (3H, s), 2.31 (3H, s), 2.35 (3H, s), 2.40 (3H, s), 2.45–2.59 (2H, m), 2.49 (3H, s), 2.99 (1H, sept, $J = 6.8$ Hz), 3.62–3.83 (2H, m), 4.11–4.20 (2H, m), 5.70–5.79 (1H, m), 7.08–7.25 (7H, m); MS (FAB) m/z 497 ($M^+ + H$); IR (KBr) 2961, 2922, 2867, 2831, 1593, 1585, 1556 cm^{-1}. Anal. ($C_{31}H_{36}N_4S$) C, H, N, S.

2,5-Dimethyl-4-(4-(3-fluorophenyl)-1,2,3,6-tetrahydropyridino)-7-(2-methylthio-4-isopropylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 9d. Mp 162–163 °C (AcOEt); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J = 6.8$ Hz), 2.32 (3H, s), 2.49 (3H, s), 2.56 (3H, s), 2.74–2.87 (2H, m), 2.98 (1H, sept, $J = 6.8$ Hz), 3.87 (2H, t, $J = 6.8$ Hz), 4.23–4.33 (2H, m), 6.23–6.34 (1H, m), 6.85 (1H, d, $J = 1.2$ Hz), 6.90–7.03 (1H, m), 7.11–7.38 (6H, m); MS (FAB) m/z 487 ($M^+ + H$); IR (KBr) 2959, 2925, 1607, 1575, 1552, 1535, 1500 cm^{-1}. Anal. ($C_{29}H_{31}FN_4S$) C, H, N, S, F.

2,5-Dimethyl-4-(5-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-7-(2-methylthio-4-isopropylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 9e. Mp 120–121 °C (AcOEt/hexane); ^1H NMR ($CDCl_3$) δ 1.31 (6H, d, $J = 7.0$ Hz), 2.31 (3H, s), 2.35 (3H, s), 2.46 (3H, s), 2.48–2.50 (2H, m), 2.51(3H, s), 2.97 (1H, sept, $J = 7.0$ Hz), 3.81 (2H, t, $J = 5.7$ Hz), 4.16–4.25 (2H, m), 5.73–5.80 (1H, m), 6.83 (1H, d, $J = 1.0$ Hz), 7.09–7.28 (7H, m); MS (EI) m/z 482 (M^+); IR (KBr) 2961, 2924, 1552, 1535 cm^{-1}. Anal. ($C_{30}H_{34}N_4S$) C, H, N, S, F.

Binding study

Rats were decapitated and the frontal cortex and heart were rapidly dissected. The frontal cortex or the heart was homogenized with 50 mM Tris–HCl buffer (pH 7.0) containing 10 mM $MgCl_2$ and 2 mM ethylenediaminetetraacetic acid (EDTA), and centrifuged at 48,000 g for 20 min at 4 °C. The pellet was washed twice with the buffer, and the final pellet was suspended in the assay buffer (50 mM Tris–HCl buffer, pH 7.0, containing 10 mM $MgCl_2$, 2 mM EDTA, 0.1% bovine serum albumin (BSA) and 100 KU/mL aprotinin), and used as a crude membrane preparation for binding studies. Protein concentration was determined using a described method.[30]

Binding assays for [^{125}I]-ovine CRF and [^{125}I]-sauvagine were performed according to reported procedures[31] but with slight modifications. The reaction was initiated by incubating 0.5 mL of membrane preparation with 0.2 nM [^{125}I]-ovine CRF or 0.2 nM [^{125}I]-sauvagine. The reaction mixture was incubated for 2 h at 25 °C (for [^{125}I]-ovine CRF binding) or at 23 °C (for [^{125}I]-sauvagine binding), and reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 mL of phosphate-buffered saline (PBS) containing 0.01% Triton X-100. Radioactivity was quantified in a gamma-counter. Nonspecific binding was determined in the presence of unlabeled 1 μM ovine CRF (for [^{125}I]-ovine CRF binding) or 1 μM sauvagine (for [^{125}I]-sauvagine binding). Specific binding was determined by subtracting nonspecific binding from total binding. In the competition-binding assay, the concentration of the test compound that caused 50% inhibition of specific radiolabeled ligand binding (IC_{50} values) was determined from each concentration–response curve.

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