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Synthesis and Structure–Affinity Relationships of 4-(5-Aryl-1,2,3,6-tetrahydropyridino)pyrimidine Derivatives as Corticotropin-Releasing Factor$_1$ Receptor Antagonists

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Abstract—Recently, various non-peptide corticotropin-releasing factor$_1$ (CRF_1) receptor antagonists have been reported. 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). We previously presented 4-aryl-1,2,3,6-tetrahydropyridinopyrimidine derivatives including potent CRF receptor ligands **1a** and **1b** and proposed that the 4-aryl-1,2,3,6-tetrahydropyridino moiety might be useful as a substituent in the Up-Area. Our interest shifted to 5-aryl-1,2,3,6-tetrahydropyridinopyrimidine derivatives **2**, among which compound **2m** (CRA0165) had highest affinity for CRF_1 receptors ($IC_{50} = 11$ nM). We report here the design, synthesis and SARs of derivatives **2**.

Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide,[1] is a principal modulator of the responses to stress and plays an essential role in regulating the activity of the hypothalamus-pituitary-adrenal (HPA) axis.[2] CRF mediates 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 posttraumatic stress disorder[15] suggest that CRF receptor antagonists may be useful for the treatment of depression, anxiety, or other diseases related to stress.

Recently, various non-peptide CRF_1 receptor antagonists have been presented.[16–28] The structure-affinity relationships (SARs) of these compounds suggest that non-peptide CRF_1 receptor antagonists are constructed of three parts, a hydrophobic unit (Up-Area), a proton accepting unit (Central-Area) and an aromatic unit (Down-Area) (Fig. 1). *N*,*N*-Dialkylamino groups containing an alkoxyalkyl group may be typical moieties in the Up-Area. The various presented CRF_1 antagonists contain *N*,*N*-dialkylamino groups in the Up-Area. Notably, however, compounds **1a** (CRA1000) and **1b** (CRA1001) containing a 4-aryl-1,2,3,6-tetrahydropyridinopyrimidino group in the Up-Area exhibit high affinities for CRF_1 receptors ($IC_{50} = 10$ and 22 nM, respectively).[28] The evidence suggests that the SARs of substituents in the Up-Area may not have been fully elucidated. Therefore, our interest shifted to changing the substituted position of the aryl group on the 4-aryl-1,2,3,6-tetrahydropyridino group of compounds **1a** and **1b** from C4 to C5 on the 1,2,3,6-tetrahydropyridine ring. Compound **2c**, which was produced by changing the substituted position of the 3-fluorophenyl group of compound **1a** from C4 to C5 on the 1,2,3,6-tetrahydropyridine ring, had 8 times less CRF_1 receptor affinity than compound **1a** (**1a**: 10 nM, **2c**: $IC_{50} = 82$ nM). Compound **2m**, which included 2-methylphenyl group on C5 of the 1,2,3,6-tetrahydropyridine ring, exhibited high affinity for CRF_1 receptors ($IC_{50} = 11$ nM). In this paper, we report the synthesis and SARs of derivative **2** (Fig. 1).

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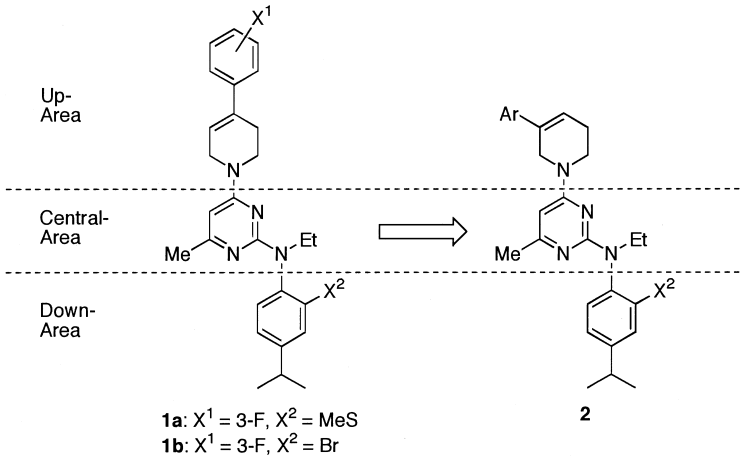


Figure 1.

Chemistry

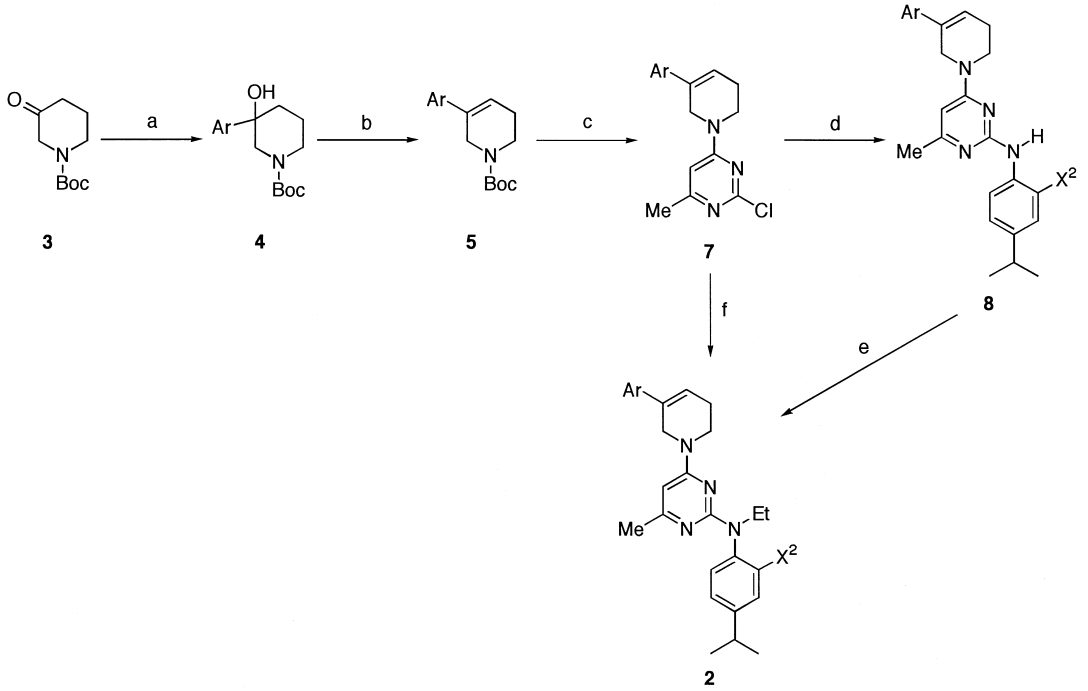
The synthesis of compounds **2a**–**2t** is shown in Scheme 1.

A crude 5-aryl-1,2,3,6-tetrahydropyridine **5**, which was prepared by treatment of 3-piperidone **3** with Grignard reagent (ArMg-Br or-Cl) followed by acid, was coupled with 2,4-dichloro-6-methylpyrimidine **6** in the presence of diisopropylethylamine to afford 4-(5-aryl-1,2,3,6-tetrahydropyridino)-2-chloro-6-methylpyrimidine **7**. The substituted position of the tetrahydropyridino group on the pyrimidine ring of derivative **7** was determined by NOEs analysis, with NOEs observed between the proton on C5 of the pyrimidine ring and protons on C2 and C6 of the piperidine ring of compound **7a**. Compound **7** was reacted with anilines having the formula 2-X^2-4-isoPr-Ph-NH_2 and the resulting amines **8a**–**8i** were ethylated to give compounds **2a**–**2i** (Method A).

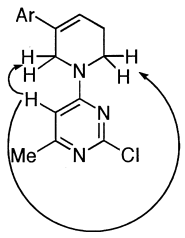
Compounds **2j**–**2r** were prepared by coupling *N*-ethyl-4-isopropyl-2-methylthioaniline (2-MeS-4-isoPr-Ph-NHEt) with 4-(5-aryl-1,2,3,6-tetrahydropyridino)-2-chloro-6-methylpyrimidine **7** prepared by the same procedure as the 1st–3rd steps of Method A from 3-piperidone **3** (Method B).

Results and Discussion

Compounds **2a**–**2r** (Fig. 2) were evaluated for corticotropin-releasing factor$_1$ (CRF_1) receptor binding affinity in rat frontal cortex against radioligand [^{125}I]-ovine CRF,[31] and the obtained IC_{50} values are shown in Table



Scheme 1. Reagents and reaction conditions: (a) ArMg-Br, THF; (b) TFA-CH_2Cl_2 or concd HCl; (c) 2,4-dichloro-6-methylpyrimidine (**6**), iso-Pr_2NEt, EtOH.; (d) 4-isoPr-2-X^2-Ar-NH_2, $(CH_2OH)_2$, reflux.; (e) Et-I, NaH, DMF.; (f) 4-isoPr-2-MeS-Ph-NHEt, $(CH_2OH)_2$, reflux. Method A: a, b, c, d, e; Method B: a, b, c, f.



7a

Figure 2.

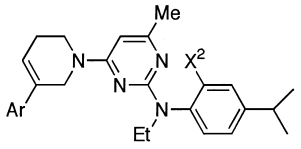
1. Compounds **2a**–**2k** and **2m**–**2r** did not exhibit corticotropin-releasing factor$_2$ (CRF_2) receptor binding affinity in rat heart against radioligand [^{125}I]-sauvagine.[29]

We recently presented 4-aryl-1,2,3,6-tetrahydropyridine derivative **1**.[22] In that study, the introduction of fluorine or chlorine atom to moderately good CRF_1 receptor antagonist **1c** (X^1 = H, X^2 = Br, IC_{50} = 66 nM) resulted in increased affinity for CRF_1 receptors (**1a**: X^1 = 3-F, X^2 = MeS, IC_{50} = 10 nM. **1b**: X^1 = 3-F, X^2 = Br, IC_{50} = 22 nM). Similarly, 5-phenyl-1,2,3,6-tetrahydropyridine compound **2a** exhibited moderately good CRF_1 receptor affinity (IC_{50} = 89 nM). However, the introduction of a fluorine or chlorine atom onto the 3 or 4-position of the phenyl group of **2a** did not increase affinity for CRF_1 receptors (compounds **2b**–**2l**), at variance with the case of 4-aryl-1,2,3,6-tetrahydropyridine derivative **1**. Enhancement of CRF_1 affinity was found only in the case of introduction of a methyl group onto the 2-position of the phenyl group of **2a** (**2m**: IC_{50} = 11 nM). Change in the position of the methyl group from the 2- to the 3- or the 4-position decreased CRF_1 receptor binding affinity (**2p**: IC_{50} = 70 nM, **2q**: IC_{50} = 70 nM). This finding is very interesting, since 2-methyl analogue of 4-aryl-1,2,3,6-tetrahydropyridine derivative **1f** (X^1 = 2-Me, X^2 = MeS) exhibited lower affinity for CRF_1 receptors (IC_{50} = 130 nM) than 3-methyl isostere **1d** (X^1 = 3-Me, X^2 = MeS, IC_{50} = 66 nM) and 4-methyl isostere **1e** (X^1 = 4-Me, X^2 = MeS, IC_{50} = 66 nM). Prolongation of the methyl group slightly decreased CRF_1 receptor affinity (**2n**: IC_{50} = 28 nM, **2o**: IC_{50} = 44 nM), but these affinities were higher than those of the 3- and the 4-methyl isosteres **1d** and **1e**. These results suggest that the 2-substituent might control the relative stereochemistry of the phenyl group, and that the stereochemistry of the phenyl group might be important for interaction with CRF_1 receptors. Furthermore, the difference in SARs between 4-aryl-1,2,3,6-tetrahydropyridine derivatives **1** and 5-aryl-1,2,3,6-tetrahydropyridine derivative **2** suggests that steric restriction might exist, with a very narrow space for the phenyl group of derivatives **1** and **2** in the molecular structure of CRF_1 receptors.

Conclusions

The successful discovery of 5-aryl-1,2,3,6-terahyropyridinopirimidine derivative **2** as a selective CRF_1 receptor antagonist suggests that chemical modification of the Up-Area may be useful for design of CRF_1 antagonists, and that steric restriction might exist with a very narrow space for the phenyl group of derivative **2** in the molecular

Table 1. 5-Aryl-1,2,3,6-tetrahydropyridine derivatives: physical and binding data



Compd	Ar	X^2	Method[a]	Salt	mp (°C)	CRF_1 Receptor IC_{50} (nM)[g]
2a	Ph	2-Br	A	HCl	138–143[b]	89
2b	3-F-Ph	2-Br	A	HCl	167–171[b]	96
2c	3-F-Ph	2-MeS	A	HCl	140–142[c]	82
2d	4-F-Ph	2-Br	A	HCl	122–123[b]	82
2e	4-F-Ph	2-MeS	A	HCl	144–147[c]	58
2f	3-Cl-Ph	2-Br	A	HCl	165–170[b]	180
2g	3-Cl-Ph	2-MeS	A	HCl	141–147[c]	98
2h	4-Cl-Ph	2-Br	A	—	Amorphase	360
2i	4-Cl-Ph	2-MeS	A	HCl	136–140[c]	160
2j	3,4-F_2-Ph	2-MeS	B	HCl	128–130[b]	340
2k	3,5-F_2-Ph	2-MeS	B	HCl	132–135[c]	82
2l	3,4-Cl_2-Ph	2-MeS	B	HCl	106–109[d]	> 1000
2m	2-Me-Ph	2-MeS	B	HCl	128–132[c]	11
2n	2-Et-Ph	2-MeS	B	HCl	142–146[e]	28
2o	2-isoPr-Ph	2-MeS	B	HCl	136–140[e]	44
2p	3-Me-Ph	2-MeS	B	HCl	117–119[c]	70
2q	4-Me-Ph	2-MeS	B	HCl	148–152[f]	70
2r	4-MeO-Ph	2-MeS	B	HCl	111–115[b]	110

[a]Preparation methods are described in the text.
[b]Recrystallization solvents are depicted: isoPrOH–isoPr$_2$O.
[c]AcOEt–Et$_2$O.
[d]AcOEt.
[e]AcOEt–EtOH.
[f]isoPrOH–AcOEt.
[g]IC_{50} values from duplicate determination.

structure of CRF_1 receptors. Furthermore, the difference in SARs in substituents on the phenyl group between 4-aryl-1,2,3,6-tetrahydropyridine derivative **1** and 5-aryl-1,2,3,6-tetrahydropyridine derivative **2** suggests that its substituents might be needed to control the conformation of the phenyl group to yield high CRF_1 receptor affinity.

Compound **2m** (CRA0165), 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. Proton nuclear magnetic resonance (NMR) spectra were obtained using a Varian Gemini 2000 (200 MHz) spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu Profile (EI), HITACHI M-2500 (SIMS) or Micromass Platform LC (Ion Spray and ESI). High-resolution mass spectra (HRMS) were obtained on a JEOL JMS-SX102 (FAB). Elemental analyses were performed by a Perkin-Elmer 2400 (carbon, hydrogen and nitrogen) or Yokogawa IC7000P (halogen and sulfur). Analytical thin-layer chromatography was conducted on precoated silica gel 60 F_{254} plates (Merck). Silica gel (C-200, 100–200 mesh (Wako Pure Chemical)) was used for column chromatography, using the solvent systems (volume ratios) indicated below.

Method A: 4-(5-Phenyl-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2a. To a solution of phenyl magnesium bromide in tetrahydrofurane (THF) prepared from bromobenzene (4.73 g, 30.1 mmol) and magnesium (0.76 g, 31.3 mmol) in THF (50 mL) was added *N*-*tert*-butoxycarbonyl-3-piperidone (5.00 g, 25.1 mmol) with ice cooling. After stirring for 1.5 h with ice cooling, saturated aqueous NH_4Cl was added to the reaction mixture and extraction was performed with AcOEt three times. The combined extract was washed with satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt: hexanes (1:3) afforded 4.21 g (61%) of *N*-*tert*-butoxycarbonyl-3-hydroxy-3-phenylpiperidine **4a** as a light yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.50–1.65 (1H, m), 1.79–2.08 (4H, m), 2.75–2.95 (1H, m), 3.19 (1H, d, J = 13.6 Hz), 3.90–4.18 (2 H, m), 7.24–7.60 (5H, m, ArH); MS (ESI) m/z 300 ($M^+ + Na$).

A solution of **4a** (3.63 g, 16.0 mmol) in trifluoroacetic acid (49 mL) was stirred at room temperature for 16 h and then heated at reflux for 5 h. The mixture was concentrated and the residue was partitioned between satd. aq $NaHCO_3$ and $CHCl_3$. The separated water phase was extracted with $CHCl_3$ three times. The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure.

A mixture of the above residue, 2,4-dichloro-6-methylpyrimidine **6** (2.60 g, 16 mmol) and diisopropylethylamine (5.16 g, 39.9 mmol) in EtOH (30 mL) was stirred at −10 °C for 2 h and then stirred overnight at room temperature. The reaction mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the separated water phase was extracted with AcOEt twice. The combined organic phase was washed satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt:hexanes (1:3) afforded 2.17 g (48%) of 2-chloro-4-(5-phenyl-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine **7a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.25–2.54 (2H, m), 2.35 (3H, s), 3.66–3.97 (2H, m), 4.24–4.51 (2H, m), 6.17–6.39 (1H, m), 6.30 (1H, s), 7.17–7.55 (5H, m); MS (ESI Pos) m/z 310 ($M^+ + 2 + Na$, 39%), 308 ($M^+ + Na$, 100%).

A mixture of **7a** (1.10 g, 3.85 mmol), 2-bromo-4-isopropylaniline HCl (0.97 g, 3.87 mmol) and diisopropylethylamine (0.50 g, 3.87 mmol) in ethylene glycol (11 mL) was heated at reflux for 1.5 h. The reaction mixture was poured into saturated aqueous NaHCO3 and extracted with AcOEt. The 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 (1:4) afforded 1.32 g (74%) of 2-(2-bromo-4-isopropylanilino)-4-(5-phenyl-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine **8a** as a yellow amorphase: ^{1}H NMR ($CDCl_3$) δ 1.24 (6 H, d, J = 7.0 Hz), 2.31 (3 H, s), 2.33–2.52(2 H, m), 2.85 (1 H, sept, J = 7.0 Hz), 3.73–3.87 (2 H, m), 4.41–4.53 (2H, m), 6.02 (1 H, s), 6.21–6.33 (1 H, m), 7.10 (1 H, d d, J = 8.6, 2.2 Hz), 7.14–7.50 (7 H, m), 8.41 (1 H, d, J = 8.6 Hz); MS (ESI Pos) m/z 487 ($M^+ + 2 + Na$, 98%), 485 ($M^+ + Na$, 100%).

A mixture of **8a** (1.21 g, 2.61 mmol) and 60% NaH in oil (136 mg, 3.40 mmol) in *N*,*N*-dimethylformamide (DMF) (12 mL) was stirred at room temperature for 30 min followed by the addiction of ethyl iodide (057 g, 3.65 mmol). After stirring overnight for 16 h, the reaction mixture was partitioned between AcOEt and water. The separated organic phase was washed with satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt:hexanes (1:4) afforded the free base of **2a** (1.03 g). The free base was treated with 4 M HCl/AcOEt in isopropanol and recrystallized from a mixture of isopropanol and diisopropyl ether to afford 1.03 g (74%) of **2a** as a light yellow crystal: ^{1}H NMR ($CDCl_3$) δ 1.13–1.43 (9H, m), 2.00–2.57 (2H, m), 2.81 (3 H, br s), 2.93 (1H, sept, J = 6.8 Hz), 3.25–3.77 (2H, m), 3.98–4.42 (3H, m), 4.50–4.82 (1H, m), 5.87–6.06 (1H, m), 6.08–6.29 (1H, m), 6.95–7.43 (7H, m, ArH), 7.46–7.60 (1H, m), 13.74 (1H, br s); MS (ESI, Pos) m/z 515 ($M^+ + 2 + Na$, 71%), 513 ($M^+ + Na$, 69%), 493 ($M^+ + 2$, 100%), 493 (M^+, 98%); IR (KBr) 2962, 2868,

2652, 1646, 1608, 1586, 1552, 1496, 1446, 1356, 1258 cm^{-1}. Anal. ($C_{27}H_{31}BrN_4 \cdot HCl \cdot 0.7H_2O$) C, H, N, Br, Cl.

Using a corresponding procedure, the following compounds **2b**–**2i** were prepared.

4-(5-(3-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2b. ^1H NMR ($CDCl_3$) δ 1.10–1.45 (9H, m), 1.97–2.56 (2H, m), 2.64–3.04 (4H, m), 3.21–3.78 (2H, m), 3.90–4.38 (3H, m), 4.50–4.88 (1H, m), 5.89–6.06 (1H, m), 6.10–6.32 (1H, m), 6.67–7.46 (6H, m), 7.56 (1H, br s), 13.79 (1H, br s); MS (ESI Pos) *m*/*z* 533 ($M^+ + 2 + Na$, 100%), 531 ($M^+ + Na$, 95%); IR (KBr) 2964, 1655, 1613, 1591, 1549, 1492, 1443, 1257 cm^{-1}. Anal. ($C_{27}H_{30}BrFN_4 \cdot HCl$) C, H, N, Br, Cl, F.

4-(5-(3-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2c. ^1H NMR ($CDCl_3$) δ 1.10–1.42 (9H, m), 2.00–2.55 (5H, m), 2.80 (3H, br s), 2.96 (1H, sept, $J = 6.9$ Hz), 3.29–3.76 (2H, m), 3.83–4.38 (3H, m), 4.43–4.80 (1H, m), 5.83–6.04 (1H, m), 6.09–6.32 (1H, m), 6.64–7.42 (7H, m), 13.63 (1H, br s); MS (ESI Pos) *m*/*z* 499 ($M^+ + Na$, 100%); IR (KBr) 2963, 1655, 1610, 1548, 1486, 1443, 1257 cm^{-1}. Anal. ($C_{28}H_{33}FN_4S \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl, F, S.

4-(5-(4-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2d. ^1H NMR ($CDCl_3$) δ 1.16–1.42 (9H, m), 1.95–2.56 (2H, m), 2.80 (3H, br s), 2.74–3.06 (1H, m), 3.50–3.75 (2H, m), 3.94–4.38 (3H, m), 4.47–4.76 (1H, m), 5.98–6.21 (2H, m), 6.90–7.40 (6H, m), 7.43–7.59 (1H, m), 13.77 (1H, br s); MS (ESI Pos) *m*/*z* 533 ($M^+ + 2 + Na$, 100%), 531 ($M^+ + Na$, 98%); IR (KBr) 2963, 1655, 1610, 1590, 1542, 1493, 1396, 1231 cm^{-1}. Anal. ($C_{27}H_{30}BrFN_4 \cdot HCl \cdot 1.5H_2O$) C, H, N, Br, Cl, F.

4-(5-(4-Fluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2e. ^1H NMR ($CDCl_3$) δ 1.18–1.44 (9H, m), 1.95–2.56 (5H, m), 2.80 (3H, br s), 2.96 (1H, sept, $J = 6.9$ Hz), 3.29–3.78 (2H, m), 3.90–4.38 (3H, m), 4.48–4.82 (1H, m), 5.85–6.26 (2H, m), 6.86–7.40 (7H, m), 13.61 (1H, br s); MS (ESI Pos) *m*/*z* 499 ($M^+ + Na$, 100%); IR (KBr) 2963, 1655, 1608, 1544, 1507, 1394, 1254 cm^{-1}. Anal. ($C_{28}H_{33}FN_4S \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl, F, S.

4-(5-(3-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2f. ^1H NMR ($CDCl_3$) δ 1.00–1.46 (9H, m), 2.00–2.54 (2H, m), 2.81 (3H, br s), 2.91 (1H, sept, $J = 6.8$ Hz), 3.21–3.78 (2H, m), 3.87–4.01 (3H, m), 4.46–4.77 (1H, m), 5.90–6.05 (1H, m), 6.08–6.35 (1H, m), 6.80–7.45 (6H, m), 7.50–7.63 (1H, m), 13.81 (1H, br s); MS (ESI Pos) *m*/*z* 551 ($M^+ + 4 + Na$, 27%), 549 ($M^+ + 2 + Na$, 100%), 547 ($M^+ + Na$, 77%); IR (KBr) 2966, 1650, 1612, 1592, 1547, 1493, 1270 cm^{-1}. Anal. ($C_{27}H_{30}BrClN_4 \cdot HCl$) C, H, N, Br, Cl.

4-(5-(3-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2g. ^1H NMR ($CDCl_3$) δ 1.10–1.44 (9H, m), 1.92–2.58 (5H, m), 2.62–3.08 (4H, m), 3.26–3.76 (2H, m), 3.85–4.39 (3H, m), 4.43–4.82 (1H, m), 5.82–6.03 (1H, m), 6.08–6.35 (1H, m), 6.76–7.41 (7H, m), 13.65 (1H, br s); MS (ESI Pos) *m*/*z* 517 ($M^+ + 2 + Na$, 43%), 515 ($M^+ + Na$, 100%); IR (KBr) 2963, 1653, 1609, 1547, 1484, 1258 cm^{-1}. Anal. ($C_{28}H_{33}ClN_4S \cdot HCl \cdot 0.5H_2O$) C, H, N, Cl, S.

4-(5-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 2h. ^1H NMR ($CDCl_3$) δ 1.21 (3H, t, $J = 7.1$ Hz), 1.26 (6H, d, $J = 6.9$ Hz), 2.12–2.37 (5H, m), 2.91 (1H, sept, $J = 6.9$ Hz), 3.40–4.29 (6H, m), 5.85 (1H, s), 6.08–6.20 (1H, m), 7.10–7.35 (6H, m), 7.48 (1H, d, $J = 0.9$ Hz); IR (Neat) 2962, 2361, 1574, 1557, 1538, 1505, 1455, 1247, 1208 cm^{-1}; FAB-HRMS *m*/*z* calcd for $C_{27}H_{30}BrClN_4$: 527.1401 ($M^+ + 2 + H$) and 525.1421 ($M^+ + H$), found: 527.1396 ($M^+ + 2 + H$) and 525.1418 ($M^+ + H$).

4-(5-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2i. ^1H NMR ($CDCl_3$) δ 1.14–1.42 (9H, m), 1.97–2.56 (5H, m), 2.80 (3H, br s), 2.96 (1H, sept, $J = 6.9$ Hz), 3.22–3.77 (2H, m), 3.84–4.38 (3H, m), 4.49–4.86 (1H, m), 5.85–6.30 (2H, m), 6.83–7.47 (7H, m), 13.63 (1H, br s); MS (SIMS Pos) *m*/*z* 495 ($M^+ + 2 + H$, 41%), 493 ($M^+ + H$, 100%); IR (KBr) 2866, 2673, 1642, 1608, 1584, 1553, 1489, 1355, 1261 cm^{-1}. Anal. ($C_{28}H_{33}ClN_4S \cdot HCl$) C, H, N, Cl, S.

Method B: 4-(5-(2-Methylphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2m. To a solution of 2-methylphenyl magnesium bromide in tetrahydrofurane (THF) prepared from 2-bromotoluene (40.06 g, 234 mmol) and magnesium (5.43 g, 226 mmol) in THF (400 mL) was added *N*-*tert*-butoxycarbonyl-3-piperidone (42.42 g, 213 mmol) with ice cooling. After stirring for 1.5 h with ice cooling, satd aqueous NH_4Cl was added to the reaction mixture and extraction was performed with AcOEt three times. The combined extract was washed with satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt:hexanes (1:5) afforded 16.37 g (26%) of *N*-*tert*-butoxycarbonyl-3-hydroxy-3-(2-methyl-phenyl)piperidine **4b** as a light yellow oil: ^1H NMR ($CDCl_3$) δ 1.49 (9H, 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 ($M^+ + Na$).

To a solution of **4b** (16.37 g, 56.2 mmol) in dioxane (23 mL) was added concentrated HCl (234 mL), and the resulting mixture was stirred at room temperature for 30 min followed by heating at reflux for 3 h. The mixture was concentrated and the resulting crystal was dried under reduced pressure.

A mixture of the above crystal, 2,4-dichloro-6-methylpyrimidine **6** (9.16 g, 56.2 mmol) and diisopropylethylamine (18.15 g, 140.5 mmol) in EtOH (30 mL) was stirred at

−10 °C for 2 h and then stirred overnight at room temperature. The reaction mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$, and the separated water phase was extracted with AcOEt twice. The combined organic phase was washed with satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt:hexanes (1:6–1:4) afforded 16.84 g (81%) of 2-chloro-4-(5-(2-methylphenyl)-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine **7b** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.30—2.49 (2H, m), 2.32 (3H, s), 2.34 (3H, s), 3.80–3.97 (2H, m), 4.01–4.18 (2H, m), 5.74–5.88 (1H, m), 6.24 (1H, s), 7.07–7.29 (4H, m) ; MS (ESI Pos) *m*/*z* 324 ($M^+ + 2 + Na$, 38%), 322 ($M^+ + Na$, 100%).

A mixture of **7b** (13.72 g, 45.8 mmol) and *N*-ethyl-2-methylthio-4-isopropylaniline (9.58 g, 45.8 mmol) in ethylene glycol (70 mL) was heated at reflux for 1 h. The reaction mixture was poured into satd aqueous $NaHCO_3$ and extracted with AcOEt. The extract was washed with satd brine, dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:9–1:4) afforded free base of **2m** (14.48 g). The free base was treated with 4 M HCl/AcOEt CH_2Cl_2 and recrystallized from a mixture of AcOEt and EtOH to afford 9.75 g (67%) of **2m** as a light yellow crystal: ^{1}H NMR ($CDCl_3$) δ 0.86–1.46 (9H, m), 1.88–3.13 (12H, m), 3.26–4.80 (6H, m), 5.54–6.09 (2H, m), 6.73–7.37 (7H, m), 13.55 (1H, br s); MS (Ion Spray) *m*/*z* 473 ($M^+ + H$); IR (KBr) 2956, 1654, 1606, 1586, 1542, 1484, 1436 cm^{-1}. Anal. ($C_{29}H_{36}N_4S$·HCl) C, H, N, Cl, S.

Using a corresponding procedure, the following compounds **2j**–**2l** and **2n**–**2r** were prepared.

4-(5-(3,4–difluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2j. ^{1}H NMR ($CDCl_3$) δ 1.06–1.42 (9H, m), 2.00–2.59 (5H, m), 2.67–3.03 (4H, m), 3.30–4.38 (5H, m), 4.47–4.78 (1H, m), 5.89–6.47 (2H, m), 6.50–7.26 (6H, m), 6.83–7.47 (7H, m), 13.65 (1H, br s); MS (Ion Spray) *m*/*z* 495 ($M^+ + H$); IR (KBr) 2962, 1656, 1610, 1588, 1546, 1486, 1444, 1260 cm^{-1}. Anal. ($C_{28}H_{32}F_2N_4S$·HCl·0.5H_2O) C, H, N, Cl, F, S.

4-(5-(3,5–difluorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2k. ^{1}H NMR ($CDCl_3$) δ 1.14–1.43 (9H, m), 1.98–2.59 (5H, m), 2.80 (3H, br s), 2.97 (1H, sept, *J* = 6.9 Hz), 3.30–4.40 (5H, m), 4.48–4.80 (1H, m), 5.85–6.30 (2H, m), 6.65–7.32 (6H, m), 13.64 (1H, br s); MS (EI) *m*/*z* 494 (M^+, 7%), 447 (M^+–CH_3S, 100%); IR (KBr) 2962, 1650, 1608, 1586, 1546, 1520, 1490, 1270, 1252 cm^{-1}. Anal. ($C_{28}H_{32}F_2N_4S$·HCl·0.5H_2O) C, H, N, Cl, F, S.

4-(5-(3,4–dichlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2l. ^{1}H NMR ($CDCl_3$) δ 1.06–1.43 (9H, m), 1.95–2.56 (5H, m), 2.70–3.09 (1H, m), 2.80 (3H, br s), 3.20–3.75 (2H, m), 3.80–4.81 (4H, m), 5.80–6.36 (2H, m), 6.65–7.52 (6H, m), 13.67 (1H, br s); MS (EI) *m*/*z* 530 ($M^+ + 4$, 1%), 528 ($M^+ + 2$, 6%), 526 (M^+, 8%), 483 ($M^+ + 4$-CH_3S, 17%), 481 ($M^+ + 2$-CH_3S, 69%), 479 (M^+–CH_3S, 100%); IR (KBr) 2958, 1650, 1606, 1586, 1538, 1480, 1376, 1266 cm^{-1}. Anal. ($C_{28}H_{32}Cl_2N_4S$·HCl) C, H, N, Cl, S.

4-(5-(2-ethylphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2n. ^{1}H NMR ($CDCl_3$) δ 0.80–1.47 (12H, m), 1.86–3.15 (11H, m), 3.24–4.82 (6H, m), 5.55–6.07 (2H, m), 6.75–7.40 (7H, m), 13.55 (1H, br s); MS (ESI Pos) *m*/*z* 509 ($M^+ + Na$, 100%), 487 ($M^+ + H$, 23%); IR (KBr) 2959, 1654, 1607, 1584, 1551, 1538, 1482, 1435, 1254 cm^{-1}. Anal. ($C_{30}H_{38}N_4S$·HCl) C, H, N, Cl, S.

4-(5-(2-isopropylphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2o. ^{1}H NMR ($CDCl_3$) δ 0.82–1.58 (15H, m), 1.97–3.19 (10H, m), 3.33–4.82 (6H, m), 5.57–6.11 (2H, m), 6.76–7.60 (7H, m), 13.61 (1H, br s); MS (ESI Pos) *m*/*z* 523 ($M^+ + Na$, 100%), 501 ($M^+ + H$, 24%); IR (KBr) 2958, 1656, 1605, 1584, 1539, 1484, 1439, 1343, 1271, 1253 cm^{-1}. Anal. ($C_{31}H_{40}N_4S$·HCl) C, H, N, Cl, S.

4-(5-(3-methylphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2p. ^{1}H NMR ($CDCl_3$) δ 1.11–1.47 (9H, m), 1.87–2.60 (8H, m), 2.78 (3H, br s), 2.94 (1H, sept, *J* = 6.9 Hz), 3.20–4.89 (6H, m), 5.85–6.34 (2H, m), 6.60–7.40 (7H, m), 13.57 (1H, br s); MS (Ion Spray) *m*/*z* 473 ($M^+ + H$); IR (KBr) 2962, 1652, 1606, 1586, 1546, 1484, 1440, 1380, 1346, 1252 cm^{-1}. Anal. ($C_{29}H_{36}N_4S$·HCl·H_2O) C, H, N, Cl, S.

4-(5-(4-methylphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2q. ^{1}H NMR ($CDCl_3$) δ 1.12–1.43 (9H, m), 1.98–2.59 (8H, m), 2.79 (3H, br s), 2.97 (1H, sept, *J* = 6.7 Hz), 3.30–4.80 (6H, m), 5.83–6.29 (2H, m), 6.84–7.40 (7H, m), 13.58 (1H, br s); MS (Ion Spray) *m*/*z* 473 ($M^+ + H$); IR (KBr) 2966, 2922, 2868, 1646, 1606, 1586, 1550, 1486, 1356, 1260 cm^{-1}. Anal. ($C_{29}H_{36}N_4S$·HCl) C, H, N, Cl, S.

4-(5-(4-methoxyphenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-methylthio-4-isopropylanilino)-6-methylpyrimidine hydrochloride 2r. ^{1}H NMR ($CDCl_3$) δ 1.16–1.43 (9H, m), 1.67–2.60 (5H, m), 2.83 (3H, br s), 2.99 (1H, sept, *J* = 6.9 Hz), 3.20–4.80 (6H, m), 3.82 (3H, s), 5.92–6.14 (2H, m), 6.70–7.40 (7H, m), 13.58 (1H, br s); MS (Ion Spray) *m*/*z* 489 ($M^+ + H$); IR (KBr) 2958, 1632, 1606, 1586, 1484, 1374, 1272, 1258 cm^{-1}. Anal. ($C_{29}H_{36}N_4OS$·HCl·0.2H_2O) C, H, N, Cl, S.

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[31,32] 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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