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Design, Synthesis and Structure–Affinity Relationships of 4-Methylidenepiperidine and 4-Aryl-1,2,3,6-tetrahydropyridine 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). Our interest focused on the Up-Area in deriving the novel methylidenepiperidine derivatives **8**–**10** and 4-aryl-1,2,3,6-tetrahydropyridine derivatives **11**–**13** as non-peptide CRF_1 receptor antagonists. Compounds **8a** and **11a** had moderate affinity for CRF_1 receptor, but compounds **9**, **10**, **12** and **13** did not exhibit CRF_1 receptor affinity. Modification of derivatives **11** afforded compounds **11i** (CRA1001) and **11x** (CRA1000), which had high affinity and selectivity for CRF_1 receptors with potent anxiolytic-like and antidepressant-like properties in some experimental animal models. These findings suggest that the hydrophonic unit (Up-Area) may be useful for design of CRF_1 antagonists. We report here the design, synthesis and SARs of the derivatives **8** and **11** and isosteres **9**, **10**, **12** and **13**.

Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide originally isolated and characterized from ovine hypothalamic origin,[1] plays an essential role in regulating the activity of the hypothalamic–pituitary–adrenal (HPA) axis[2] and coordinates various physiological responses to sustained stress.[3–7] CRF exerts physiological effect 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 data, including elevated concentrations of CRF in patients with depression[12] or chronic post-traumatic stress disorder[13] and 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. This suggestion may be supported by the efficacy of selective non-peptide CRF_1 receptor antagonist, butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-yl]ethylamine (CP154,526),[16] in modifying behavioral responses to stress.[17–20] Various non-peptide CRF_1 receptor antagonists have recently been presented.[16,21–30] Among them, typical selective non-peptide CRF_1 receptor antagonists are shown in Figure 1. 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). In the Down-Area, aryl moieties attached with 2- and/or 6- and 4-positional substituents might yield high affinity for CRF_1 receptors, since 2- and/or 6-positional substituents might yield an orthogonal conformation of moieties in the Central-Area and Down-Area, and the 4-positional substituent might interact with a hydrophobic pocket of CRF_1 receptor. An aromatic and basic nitrogen in the Central-Area might be essential for hydrogen bonding between receptor and ligand, and the aromatized ring plays a role in maintaining the orthogonal conformation

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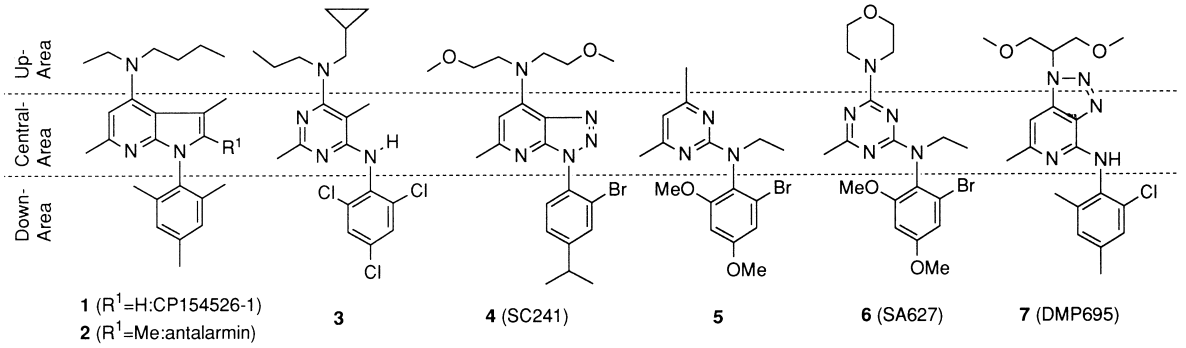


Figure 1.

with the moiety in the Down-Area. *N*,*N*-Dialkylamino groups containing an alkoxyalkyl group may be typical moieties in the Up-Area. Notably, however, compound **5** containing a methyl group in the Up-Area exhibits a high affinity for CRF_1 receptors ($K_i = 12$ nM).[28] The SARs of substituents in the Up-Area might not been fully elucidated. Our interest focused on the chemical modification of substituent in the Up-Area of compounds **3**, **5** and **6** to derive novel methylidenepiperidine derivatives **8** and 4-aryl-1,2,3,6-tetrahydropyridine derivatives **11** as non-peptide CRF_1 receptor antagonists. Compound **8a** and **11a** had moderate affinity for CRF_1 receptors, but compounds **9**, **10**, **12** and **13** did not exhibit CRF_1 receptor affinity. The design, synthesis and SARs of the derivatives **8** and **11** and isosteres **9**, **10**, **12** and **13** are presented (Fig. 2).

Chemistry

The sequence of synthesis of methylidenepiperidinopyrimidine derivatives **8**, **9** and **10** is shown in Scheme 1. Dichloropyrimidine or dichlorotriazine derivative **14**, **15** or **16** was treated with methylidenepiperidine (4-(R^4,R^5C=) piperidine) in the presence of diisopropylethylamine to afford derivative **17**, **18** or **19**, respectively. In the case of 2,4-dichloropyrimidine **14**, the treatment produced a mixture of polar isomer **17** and less polar isomer **17′** (>5:1). The substituted position of the methylidenepiperidino group on the pyrimidine ring was determined by NOEs analysis. Thus, as depicted in Figure 3, NOEs were observed between the 5-positional proton on the pyrimidine ring and 2- and 6-positional protons on the piperidine ring of isomer **17a**, while no NOEs were observed between the 5-positional proton on the pyrimidine ring and 2-and 6-positional protons on the piperidine ring of isomer **17′a**. Derivative **17** separated by chromatography, **18** or **19** was reacted with *N*-non-substituted aniline compound (X^1, X^2, X^3-Ph-NH_2), and the resulting secondary amino group of **20**, **21** or **22** was alkylated to the methylidenepiperidinopyrimidine derivative **8**, **9** or **10**, respectively (Method A). The methylidenepiperidinopyrimidine derivative **8** was prepared by condensation of 4-oxypiperidinopirimidine derivative **26** with Wittig reagent (Method C). Derivative **26** was provided by deprotecting the ethylene ketal of derivative **25**, which was prepared from 1,4-dioxa-8-azaspiro[4,5]decane as in Method A (Method B). Compound **8h** was prepared by treating derivative **26** with freshly distilled cyclopentadiene under basic conditions (Method D).

The procedure of synthesis of 4-aryl-1,2,3,6-tetrahydropyridinopyrimidine derivatives **11**, **12** and **13** is shown in Scheme 2. Treatment of dichloropyrimidine or dichlorotriazine derivative **14**, **15** or **16** with 4-aryl-1,2,3,6-tetrahydropyridine in the presence of diisopropylethylamine afforded derivative **27**, **28** or **29**, respectively. Treatment of 2,4-dicloropyrimidine yielded a mixture of polar isomer **27** and less polar isomer **27′** (5:1) separated easily

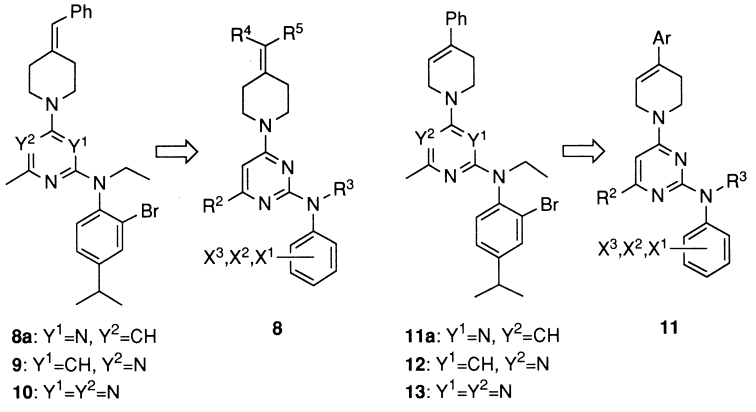
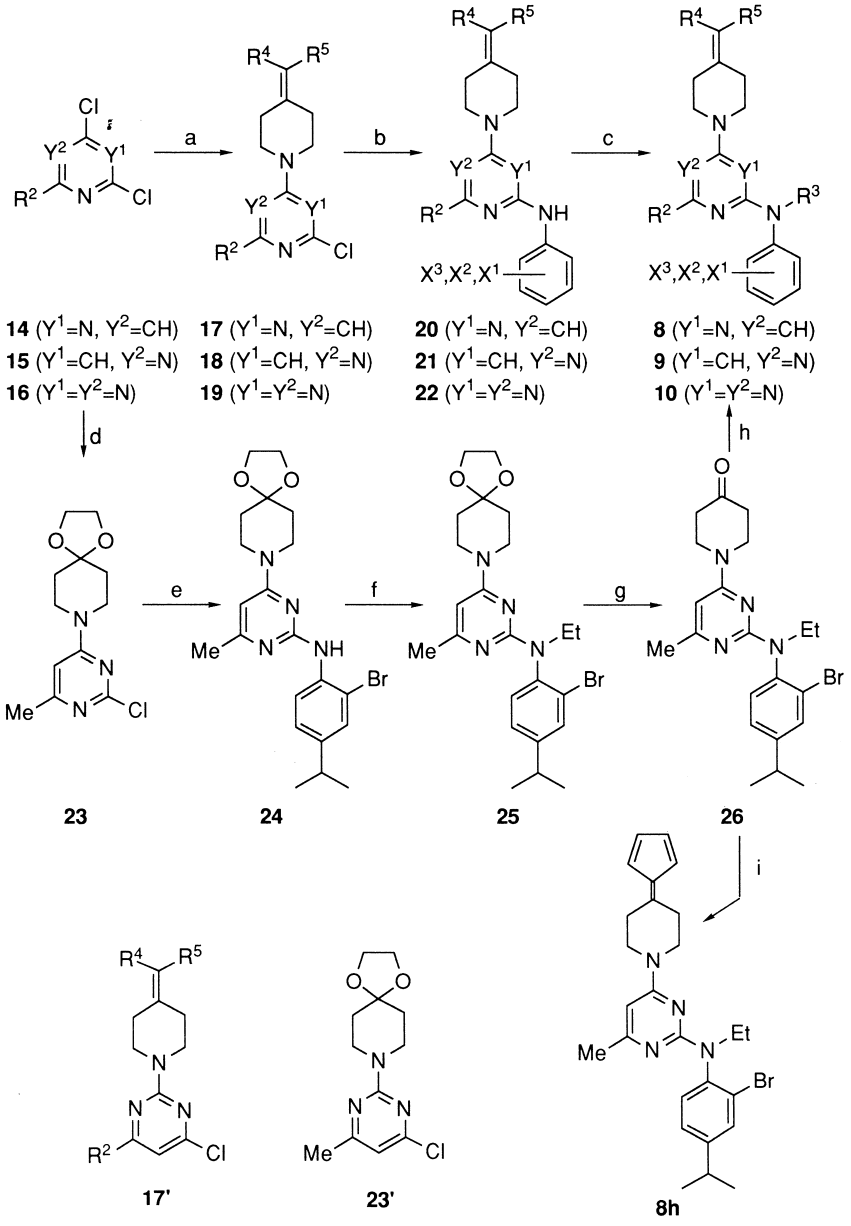


Figure 2.



Scheme 1. Reagents and reaction conditions: (a) 4-(R^4,R^5C=)piperidine, isoPr$_2$NEt, EtOH; (b) X^3,X^2,X^1-Ar-NH$_2$, *iso*Pr$_2$NEt, $(CH_2OH)_2$, reflux; (c) R^3-l or -Br, NaH, DMF; (d) 4-(1,3-dioxolan2-yl)piperadine, *iso*Pr$_2$NEt, EtOH; (e) 2-Br-4 *iso*Pr-Ar-NH$_2$, *iso*Pr$_2$NEt, $(CH_2OH)_2$, reflux; (f) Et-l, NaH, DMF; (g) 1 N Cl, THF; (h) R^4,R^5 = PPh_3, THF; (i) cyclopentadiene, Et_3N. Method A: a, b, c; Method B: d, e, f, g; Method C: h; Method D: i.

by chromatography as well as a mixture of **17** and **17′** (Method E). The substituted position of the tetrahydropyridino group on the pyrimidine ring, and that of the methylidenepiperidino group, were determined by NOEs analysis. Derivative **27**, **28** or **29** was reacted with *N*-non-substituted aniline (X^1, X^2, X^3-Ph-NH_2) and then with halide (R^3-Br or-I) (Method F) or with *N*-substituted aniline (X^1, X^2, X^3-Ph-NHR^3) (Method G) to afford the derivatives **11**, **12** or **13**, respectively. On the other hand, the 4-oxypiperidinopirimidine derivative **26** was transformed to the derivatives **11**, except for furan (Ar = furanyl) and thiophene (Ar = thienyl) derivatives, by treatment of Grignard reagent (ArMg-Br or-I) and then dehydration of the resulting hydroxyl group (Method H). The furan derivative **11d** or thiophene derivative **11e** was prepared by treatment of derivative **26** with the corresponding litio-salts followed by methanesulfonyl chloride under basic conditions (Method I) or formic acid (Method J), respectively.

Results and Discussion

Derivatives **8**–**13** (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] the obtained IC_{50} values are shown in Tables 1 and 2. Compounds **8** and **11** did not exhibit corticotropin-releasing factor$_2$ (CRF_2) receptor binding affinity in rat heart against radioligand [^{125}I]-sauvagine.[32]

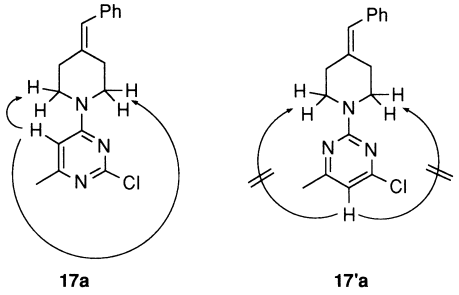
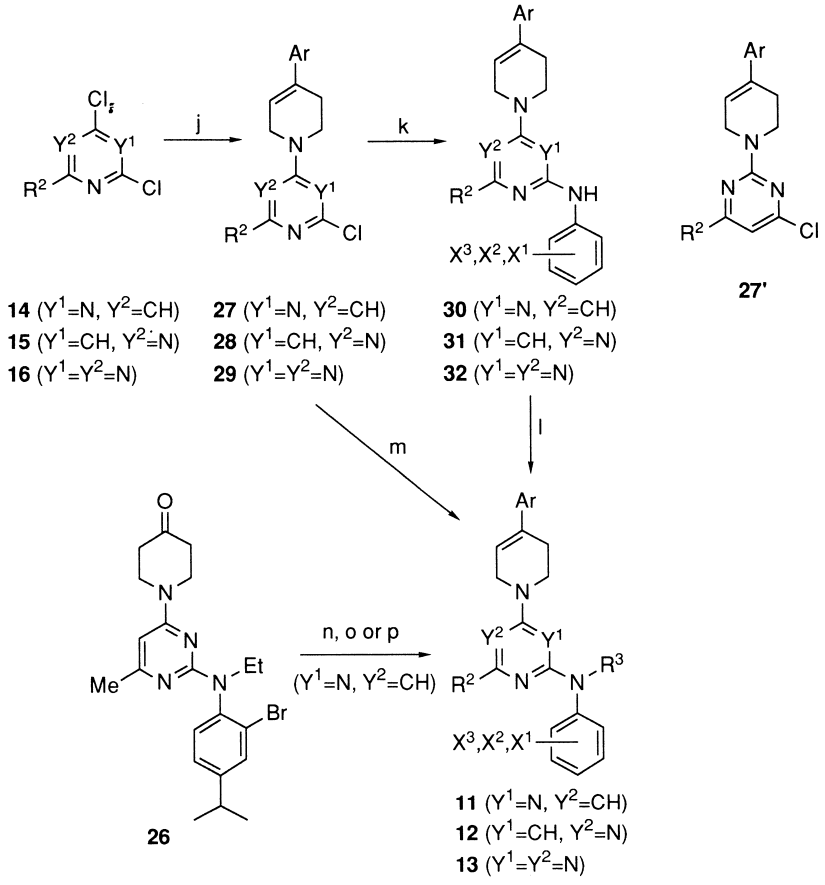


Figure 3.

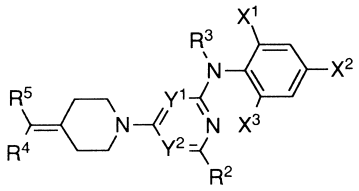
Benzylidene derivative **8a** had moderate affinity at CRF_1 receptors but the isosteres **9** and **10** did not exhibit CRF_1 receptor affinity. This finding suggests that the steric repulsion between the 5-positional proton (Y^2=CH) in the Central-Area and the 2-and/or 6-positional protons of piperidino group may be important in yielding the molecular conformation producing CRF_1 receptor affinity. Mono-alkyl-substituted methylidene derivatives **8b**, **8c** and **8d** and the smallest cyclic-alkyl-substituted methylidene derivatives **8g** have moderate affinity for CRF_1 receptors as does compound **8a**. Di-alkyl-subsutituted methylidene derivatives **8e** and **8f** exhibited reduced CRF_1 receptor affinity, as did compound **8h** containing a larger cyclic moiety, compared with **8g**. This suggests that there may be steric restriction for the second substituent on the methylidene moiety.

In light of these findings, the 4-aryl-1,2,3,6-tetrahydropyridine derivative **11** and the isosteres **12** and **13** were selected as the next targets, since 4-phenyl-1,2,3,6-tetrahydropyridino moiety was slightly smaller than benzylidenepiperidino moiety and phenyl group of 4-phenyl-1,2,3,6-tetrahydropyridino moiety might show a subtly different conformation compared with phenyl group of benzylidenepiperidino moiety. Compound **11a** exhibited higher CRF_1 receptor affinity than **8a**. The isosteres **12** and **13** did not have CRF_1 receptor affinity, nor did **9** and **10**, isosteres of **8a**. The 5-positional proton (Y^2=CH) is essential for CRF_1 receptor affinity. A larger aryl group reduced CRF_1 receptor affinity (**11b**, **11c**), but substituents of the same size retained CRF_1 receptor affinity (**11d**, **11e**), compared with the phenyl group of **11a**. This dramatic result suggests that there may be steric restriction, with a very narrow space, around the phenyl group of **11a**. Of the phenyl groups with substituent(s) (**11a**–**11s**), the 3-chloro and 3-fluorophenyl group yielded the most binding affinity for CRF_1 receptors (**11g**, **11i**). The 2-positional substituents, 2-methyl and 2-methoxy groups, had lower CRF_1 receptor affinity than 3- or 4-methyl and methoxy groups (**11l**



Scheme 2. Reagents and reaction conditions: (j) 4-Ar-1,2,3,6-tetrahydropyridine, *iso*Pr_2NEt, EtOH; (k) X^3, X^2, X^1-Ar-NH_2, *iso*Pr_2NEt, $(CH_2OH)_2$, reflux; (l) R^3-I or -Br, NaH, DMF; (m) X^3, X^2, X^1-Ar-NHR^3, *iso*Pr_2NEt, $(CH_2OH)_2$, reflux; (n) ArMg-Br or -I, THF, and then TFA-CH_2Cl_2; (o) furanel, LDA, THF and then MsCl, Et_3N, DMAP, CH_2Cl_2; (p) thiophene, LDA, THF and then HCO_2H. Method E: j; Method F: k, l; Method G: m; Method H: n; Method I: o; Method J: p.

Table 1. 4-Methylidenepiperidine derivatives: physical and binding data



No.	Y^1	Y^2	R^2	R^3	R^4	R^5	X^1	X^2	X^3	Salt	Method[a]	Mp (°C)	Analysis[b]	CRF_1 receptor IC_{50} (nM)[c]
8a	N	CH	Me	Et	Ph		Br	*i*-Pr	H	HCl	A	149–152[d]	$C_{28}H_{33}BrN_4$·HCl	280
9	CH	N	Me	Et	Ph		Br	*i*-Pr	H	HCl	A	Amorphous	$C_{28}H_{33}BrN_4$·HCl	> 1000
10	N	N	Me	Et	Ph		Br	*i*-Pr	H	—	A	Amorphous	$C_{27}H_{32}BrN_5$	> 1000
8b	N	CH	Me	Et	Me		Br	*i*-Pr	H	HCl	B,C	181–184[e]	$C_{23}H_{31}BrN_4$·HCl	270
8c	N	CH	Me	Et	*n*-Pr		Br	*i*-Pr	H	HCl	B,C	161–163[f]	$C_{25}H_{35}BrN_4$·HCl	250
8d	N	CH	Me	Et	*n*-Pen		Br	*i*-Pr	H	HCl	B,C	167–170[d]	$C_{27}H_{39}BrN_4$·HCl	280
8e	N	CH	Me	Et	Ph		Br	*i*-Pr	H	HCl	A	162–165[g]	$C_{34}H_{37}BrN_4$·HCl	> 1000
8f	N	CH	Me	Et	Me		Br	*i*-Pr	H	HCl	B,C	183–186[g]	$C_{24}H_{33}BrN_4$·HCl	420
8g	N	CH	Me	Et	-CH_2-CH_2		Br	*i*-Pr	H	HCl	B,C	175–178[g]	$C_{24}H_{31}BrN_4$·HCl	160
8h	N	CH	Me	Et	-CH=CH-CH=CH-		Br	*i*-Pr	H	HCl	B,D	Amorphous	$C_{26}H_{31}BrN_4$·HCl	> 1000

[a]Methods A–D are described in the text.
[b]Elemental analyses for all compounds are within ±0.4% of the theoretical values for the indicated formula. Analyses were performed for all elements except O.
[c]IC_{50} values from duplicate determination.
[d–g]Recrystallization or crystallization solvents are depicted: *iso*PrOH-*iso*Pr_2O; [e]Recrystallization or crystallization solvents are depicted: AcOEt-hexanes; [f]Recrystallization or crystallization solvents are depicted: *iso*Pr_2O; [g]Recrystallization or crystallization solvents are depicted: AcOEt.

versus **11k**, **11j** and **11o** versus **11n**, **11m**). This result might be due to the conformation of the phenyl group, since the 2-positional substituents have more steric hindrance against 3-and/or 5-positinal protons of the 1,2,3,6-terahydropyridino group than the 3-or 4-substituents on the phenyl group. Changing the 2-bromo-4-isopropylphenyl group of the aniline moiety to 2,4,6-trimethyl, 2,4-dimethoxy, or 2,4-dichlorophenyl decreased CRF_1 receptor affinity (**11f**–**11i** versus **11ac**–**11af**, **11ag**–**11aj**, **11al**). However, displacement of the bromo group of the aniline moiety of **11f** with an iodo group retained CRF_1 receptor affinity (**11t**), while displacement with a methylthio group increased CRF_1 receptor affinity (**11f**–**11i** versus **11u**–**11x**). Compound **11x** (CRA1000) had the highest CRF_1 receptor affinity among 4-aryl-1,2,3,6-tetrahydropyridine derivatives **11**. The methylthio group might be a better substituent than a bromo or iodo group for obtaining orthogonal conformation between the pyrimidine ring and benzene ring of the anilino group and/or for elevating an electron density on the molecular for yielding high affinity for CRF_1 receptors. For the 4-positional substituent (X^2) on aniline moiety, there may be steric restriction, since a dimethylamino and a bromo group each yielded the same CRF_1 receptor affinity as an isopropyl group (**11f**–**11i** versus **11y**–**11ab**, **11ak**), but a smaller, longer or bigger group than an isopropyl group decreased CRF_1 receptor affinity (**11u**, **11g** versus **11am**–**11ao**, **11ap**–**11ar**, respectively). On the other hand, the modification of ethyl group (R^3) yielded lower CRF_1 receptor affinity (**11at**–**11ay**), except for a propagyl group (**11as**). R^3 might be important in yielding an orthogonal conformation between moieties in the Central-Area and Down-Area. Notably, however, there is steric restriction, with very narrow space, for the *N*-substituent of the anilino moiety (R^3). Furthermore, the modification of methyl group (R^2) on the pyrimidine ring decreased CRF_1 receptor affinity (**11f** versus **11az**, **11ba**). There might thus be narrow steric restriction for R^2. The methyl group may be essential for R^2 to yield CRF_1 receptor affinity.

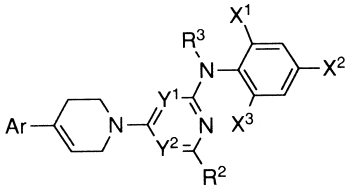
In vitro and in vivo pharmacological profiles of compounds **11i** (CRA1001) and **11x** (CRA1000), typical compounds among derivatives **8**–**13**, have already been presented.[33,34] These findings suggest that compounds **11i** and **11x** are potent and selective antagonists of CRF_1 receptors with potent anxiolytic-like and antidepressant-like properties in various experimental animal models.

Conclusions

The characteristics of 4-aryl-1,2,3,6-tetrahydropyridinopirimidine derivatives **11**, which are selective CRF_1 receptor antagonists, suggest that the chemical modification on the Up-Area may be useful for design of CRF_1 antagonists. Notably, however, there is narrow steric restriction on the Up-Area.

Compounds **11i** (CRA1001) and **11x** (CRA1000), typical compounds among derivatives **8**–**13**, may prove to be effective for treatment of anxiety-and/or depression-related disorders without the side effects observed for currently prescribed related medications.[34]

Table 2. 4-Aryl-1,2,3,6-tetrahydropyridine derivatives: physical and binding data



No	Y^1	Y^2	R^2	R^3	Ar	X^1	X^2	X^3	Salt	Method[a]	Mp (°C)	Analysis[b]	CRF_1 receptor IC_{50} (nM)[c]
11a	N	CH	Me	Et	Ph	Br	*i*-Pr	H	HCl	E,F	124–127[d]	$C_{27}H_{31}BrN_4{\cdot}HCl{\cdot}H_2O$	66
12	CH	N	Me	Et	Ph	Br	*i*-Pr	H	HCl	E,F	Amorphous	$C_{27}H_{31}BrN_4{\cdot}HCl{\cdot}1.1H_2O$	>1000
13	N	N	Me	Et	Ph	Br	*i*-Pr	H	—	E,F	Amorphous	$C_{26}H_{30}BrN_5$	890
11b	N	CH	Me	Et	2-Naph	Br	*i*-Pr	H	HCl	B,H	177–178[e]	$C_{31}H_{33}BrN_4{\cdot}HCl{\cdot}H_2O$	>1000
11c	N	CH	Me	Et	3-Naph	Br	*i*-Pr	H	HCl	B,H	129–130[e]	$C_{31}H_{33}BrN_4{\cdot}HCl{\cdot}H_2O$	>1000
11d	N	CH	Me	Et	2-Fur	Br	*i*-Pr	H	—	B,I	Amorphous	$C_{25}H_{29}BrN_4O$	44
11e	N	CH	Me	Et	2-Thio	Br	*i*-Pr	H	—	B,J	Amorphous	$C_{25}H_{29}BrN_4S$	52
11f	N	CH	Me	Et	4-Cl-Ph	Br	*i*-Pr	H	—	E,F	91–92[f]	$C_{27}H_{30}BrClN_4$	39
11g	N	CH	Me	Et	3-Cl-Ph	Br	*i*-Pr	H	HCl	B,H	174–175[e]	$C_{27}H_{30}BrClN_4{\cdot}HCl{\cdot}H_2O$	25
11h	N	CH	Me	Et	4-F-Ph	Br	*i*-Pr	H	HCl	E,F	183–185[e]	$C_{27}H_{30}BrFN_4{\cdot}HCl{\cdot}H_2O$	79
11i	N	CH	Me	Et	3-F-Ph	Br	*i*-Pr	H	—	E,F	119–120[g]	$C_{27}H_{30}BrFN_4$	22
11j	N	CH	Me	Et	4-Me-Ph	Br	*i*-Pr	H	HCl	B,H	161–164[e]	$C_{28}H_{33}BrN_4{\cdot}HCl{\cdot}H_2O$	63
11k	N	CH	Me	Et	3-Me-Ph	Br	*i*-Pr	H	HCl	B,H	162–165[e]	$C_{28}H_{33}BrN_4{\cdot}HCl{\cdot}H_2O$	66
11l	N	CH	Me	Et	2-Me-Ph	Br	*i*-Pr	H	HCl	B,H	177–179[e]	$C_{28}H_{33}BrN_4{\cdot}HCl{\cdot}1.25H_2O$	130
11m	N	CH	Me	Et	4-MeO-Ph	Br	*i*-Pr	H	HCl	B,H	134–135[e]	$C_{28}H_{33}BrN_4O{\cdot}HCl{\cdot}1.1H_2O$	82
11n	N	CH	Me	Et	3-MeO-Ph	Br	*i*-Pr	H	HCl	B,H	149–152[e]	$C_{28}H_{33}BrN_4O{\cdot}HCl{\cdot}H_2O$	140
11o	N	CH	Me	Et	2-MeO-Ph	Br	*i*-Pr	H	HCl	B,H	160–161[e]	$C_{28}H_{33}BrN_4O{\cdot}HCl{\cdot}H_2O$	240
11p	N	CH	Me	Et	3-CF_3-Ph	Br	*i*-Pr	H	HCl	E,F	127–130[e]	$C_{28}H_{30}BrF_3N_4{\cdot}HCl{\cdot}H_2O$	39
11q	N	CH	Me	Et	3,4-Cl_2-Ph	Br	*i*-Pr	H	HCl	E,F	126–129[e]	$C_{27}H_{29}BrCl_2N_4{\cdot}HCl{\cdot}0.9H_2O$	67
11r	N	CH	Me	Et	3,4-F_2-Ph	Br	*i*-Pr	H	HCl	B,H	173–174[e]	$C_{27}H_{29}BrF_2N_4{\cdot}HCl{\cdot}H_2O$	50
11s	N	CH	Me	Et	3,5-F_2-Ph	Br	*i*-Pr	H	HCl	E,F	126–131[h]	$C_{27}H_{29}BrF_2N_4{\cdot}HCl{\cdot}H_2O$	46
11t	N	CH	Me	Et	3-F-Ph	I	*i*-Pr	H	H_2SO_4	E,F	248–249[g]	$C_{27}H_{30}FIN_4{\cdot}H_2SO_4$	28
11u	N	CH	Me	Et	4-Cl-Ph	MeS	*i*-Pr	H	HCl	E,F	134–135[i]	$C_{28}H_{33}ClN_4S{\cdot}HCl{\cdot}H_2O$	22
11v	N	CH	Me	Et	3-Cl-Ph	Mes	*i*-Pr	H	HCl	E,F	125–128[e]	$C_{28}H_{33}ClN_4S{\cdot}HCl{\cdot}H_2O$	20
11w	N	CH	Me	Et	4-F-Ph	Mes	*i*-Pr	H	HCl	E,F	136–139[e]	$C_{28}H_{33}FN_4S{\cdot}HCl{\cdot}H_2O$	81
11x	N	CH	Me	Et	3-F-Ph	Mes	*i*-Pr	H	—	E,F	107–108[g]	$C_{28}H_{33}FN_4S$	10
11y	N	CH	Me	Et	4-Cl-Ph	Br	Me_2N	H	HCl	E,F	116–119[e]	$C_{26}H_{29}BrClN_5{\cdot}HCl{\cdot}1.3H_2O$	45
11z	N	CH	Me	Et	3-Cl-Ph	Br	Me_2N	H	HCl	E,F	Amorphous	$C_{26}H_{29}BrClN_5{\cdot}2HCl{\cdot}1.5H_2O$	56
11aa	N	CH	Me	Et	4-F-Ph	Br	Me_2N	H	HCl	E,F	122–124[e]	$C_{26}H_{29}BrFN_5{\cdot}HCl{\cdot}1.2H_2O$	74
11ab	N	CH	Me	Et	3-F-Ph	Br	Me_2N	H	HCl	E,F	116–119[i]	$C_{26}H_{29}BrFN_5{\cdot}HCl{\cdot}H_2O$	39
11ac	N	CH	Me	Et	4-Cl-Ph	Me	Me	Me	HCl	E,G	125–127[e]	$C_{27}H_{31}ClN_4{\cdot}HCl{\cdot}H_2O$	430
11ad	N	CH	Me	Et	3-Cl-Ph	Me	Me	Me	HCl	E,G	120–123[e]	$C_{27}H_{31}ClN_4{\cdot}HCl{\cdot}H_2O$	130
11ae	N	CH	Me	Et	4-F-Ph	Me	Me	Me	HCl	E,G	131–134[e]	$C_{27}H_{31}FN_4{\cdot}HCl{\cdot}H_2O$	300
11af	N	CH	Me	Et	3-F-Ph	Me	Me	Me	HCl	E,G	127–129[j]	$C_{27}H_{31}FN_4{\cdot}HCl{\cdot}H_2O$	74
11ag	N	CH	Me	Et	4-Cl-Ph	MeO	MeO	H	—	E,G	159–160[k]	$C_{26}H_{29}ClN_4O_2$	>1000
11ah	N	CH	Me	Et	3-Cl-Ph	MeO	MeO	H	—	E,G	133–145[k]	$C_{26}H_{29}ClN_4O_2$	360
11ai	N	CH	Me	Et	4-F-Ph	MeO	MeO	H	—	E,G	182–183[i]	$C_{26}H_{29}FN_4O_2$	>1000
11aj	N	CH	Me	Et	3-F-Ph	MeO	MeO	H	—	E,G	127–129[k]	$C_{26}H_{29}FN_4O_2$	190
11ak	N	CH	Me	Et	4-Cl-Ph	Br	Br	H	HCl	E,F	111–114[e]	$C_{24}H_{23}Br_2ClN_4{\cdot}HCl{\cdot}H_2O$	66
11al	N	CH	Me	Et	4-Cl-Ph	Cl	Cl	H	HCl	E,F	112–114[e]	$C_{24}H_{23}Cl_3N_4{\cdot}HCl{\cdot}H_2O$	150
11am	N	CH	Me	Et	4-Cl-Ph	MeS	*n*-Pr	H	HCl	E,F	125–128[i]	$C_{28}H_{33}ClN_4S{\cdot}HCl{\cdot}H_2O$	480
11an	N	CH	Me	Et	4-Cl-Ph	MeS	*n*-Bu	H	HCl	E,F	120–123[i]	$C_{29}H_{35}ClN_4S{\cdot}HCl{\cdot}H_2O$	430
11ao	N	CH	Me	Et	4-Cl-Ph	MeS	*c*-Pen	H	HCl	E,G	132–137[h]	$C_{30}H_{35}ClN_4S{\cdot}HCl{\cdot}H_2O$	130
11ap	N	CH	Me	Et	3-Cl-Ph	Br	Me	H	HCl	E,F	140–141[e]	$C_{25}H_{26}BrClN_4S{\cdot}HCl{\cdot}1.5H_2O$	140
11aq	N	CH	Me	Et	3-Cl-Ph	Br	n-Pr	H	HCl	E,F	156–157[e]	$C_{27}H_{30}BrClN_4{\cdot}HCl{\cdot}H_2O$	360
11ar	N	CH	Me	Et	3-Cl-Ph	Br	t-Bu	H	HCl	E,F	145–150[e]	$C_{28}H_{32}BrClN_4{\cdot}HCl{\cdot}H_2O$	≥1000
11as	N	CH	Me	HC≡C-CH_2	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	121–123[i]	$C_{28}H_{28}BrClN_4{\cdot}HCl{\cdot}H_2O$	31
11at	N	CH	Me	H_2C=CH-CH_2	4-Cl-Ph	Br	*i*-PrE,F	H	HCl	E,F	109–112[i]	$C_{28}H_{30}BrClN_4{\cdot}HCl{\cdot}H_2O$	240
11au	N	CH	Me	Me	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	106–109[i]	$C_{26}H_{28}BrClN_4{\cdot}HCl{\cdot}H_2O$	290
11av	N	CH	Me	N-Pr	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	137–140[i]	$C_{28}H_{32}BrClN_4{\cdot}HCl{\cdot}H_2O$	140
11aw	N	CH	Me	N-Pen	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	118–121[i]	$C_{30}H_{36}BrClN_4{\cdot}HCl{\cdot}H_2O$	>1000
11ax	N	CH	Me	i-Bu	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	124–127[i]	$C_{29}H_{34}BrClN_4{\cdot}HCl{\cdot}H_2O$	810
11ay	N	CH	Me	Ph-CH_2	4-Cl-Ph	Br	*i*-Pr	H	HCl	E,F	137–139[i]	$C_{32}H_{32}BrClN_4{\cdot}HCl{\cdot}H_2O$	>1000
11az	N	CH	H	Et	4-Cl-Ph	Br	*i*-Pr	H	—	E,F	Amorphous	$C_{26}H_{28}BrClN_4$	>1000
11ab	N	CH	*i*-Pr	Et	4-Cl-Ph	Br	*i*-Pr	H	—	E,F	Amorphous	$C_{29}H_{34}BrClN_4$	>1000

[a]Methods B, E–J are described in the text.
[b]Elemental analyses for all compounds are within ±0.4% of the theoretical values for the indicated formula. Analyses were performed for all elements except 0.
[c]IC_{50} values from duplicate determination.
[d–k]Recrystallization or crystallization solvents are depicted: Et_2O–AcOEt; [e]Recrystallization or crystallization solvents are depicted: *iso*PrOH–*iso*Pr_2O; [f]Recrystallization or crystallization solvents are depicted: AcOET–hexanes; [g]Recrystallization or crystallization solvents are depicted: EtOH; [h]Recrystallization or crystallization solvents are depicted: EtOH–*iso*Pr_2O; [i]Recrystallization or crystallization solvents are depicted: AcOET; [j]Recrystallization or crystallization solvents are depicted: AcOET–*iso*Pr_2O; [k]Recrystallization or crystallization solvents are depicted: Et_2O.

Experimental

Chemistry

Melting points (mp) were determined on a Yanaco MP-500D mp apparatus and are uncorrected. Infrared (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 relative to tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Simazu/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.

Method A. 4-(4-Benzylidenepiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 8a. To a stirred mixture of diisopropylethylamine (5.30 g, 41.0 mmol) and 4-benzylidenepiperidine HCl (4.20 g, 20.0 mmol) in EtOH (60 mL), cooled in an ice-bath, was added 2,4-dichloro-6-methylpyrimidine **14** (3.33 g, 20.0 mmol). After 2 h, the mixture was stirred for 15 h at room temperature and then concentrated in vacuo. The residue was partitioned between AcOEt and water. The separated organic phase was washed with saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:10–1:3) afforded 5.13 g (85%) of 4-(4-benzylidenepiperidino)-2-chloro-6-methylpyrimidine **17a** as a light yellow oil and 0.84 g (14%) of 2-(4-benzylidenepiperidino)-4-chloro-6-methylpyrimidine **17′a** as light yellow oil.

17a: ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s, CH_3), 2.47 (2H, br t, J = 5.8 Hz, CCH_2C), 2.59 (2H, br t, J = 5.8 Hz, CCH_2C), 3.66 (2H, br t, J = 5.8 Hz, NCH_2), 3.76 (2H, br t, J = 5.8 Hz, NCH_2), 6.28 (1H, s, PhCH=), 6.44 (1H, s, H of pyrimidine), 7.15–7.42 (5H, m, ArH); MS (FAB) m/z 302 (M^{++}3, 33%), 300 (M^{++}1, 100%).

17′a: ^{1}H NMR ($CDCl_3$) δ 2.31 (3H, s, CH_3), 2.42 (2H, br t, J = 5.9 Hz, CCH_2C), 2.55 (2H, br t, J = 5.9 Hz, CCH_2C), 3.83 (2H, br t, J = 5.9 Hz, NCH_2), 3.93 (2H, br t, J = 5.9 Hz, NCH_2), 6.38 (1H, s, PhCH=), 6.41 (1H, s, H of pyrimidine), 7.18–7.40 (5H, m, ArH); MS (FAB) m/z 302 (M^+ + 3, 33%), 300 (M^+ + 1, 100%).

A mixture of **17a** (3.00 g, 10.0 mmol), 2-bromo-4-isopropylaniline HCl (2.51 g, 10.0 mmol) and diisopropylethylamine (1.42 g, 11.0 mmol) in ethylene glycol (20 mL) was heated at reflux for 1.5 h. The mixture was partitioned between AcOEt and 1 N aqueous NaOH. The separated organic phase was washed with saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:7) afforded 3.25 g (68%) of 4-(4-benzylidenepiperidino)-2-(2-bromo-4-isopropylanilino)-6-methylpyrimidine **20a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, d, J = 7.0 Hz, CH_3 of *iso*Pr), 2.30 (3H, s, CH_3 of pyrimidine), 2.45 (2H, br t, J = 5.8 Hz, CCH_2C), 2.58 (2H, br t, J = 5.8 Hz, CCH_2C), 2.76–2.95 (1H, m, CH of *iso*Pr), 3.60–3.80 (4H, m, NCH_2), 5.99 (1H, s, PhCH=), 6.42 (1H, br s, H of pyrimidine), 7.09–7.41 (7H, m, ArH), 8.39 (1H, d, J = 8.6 Hz, ArH); MS (FAB) m/z 479 (M^+ + 3, 100%), 477 (M^+ + 1, 98%).

To a stirred solution of **20a** (2.67 g, 5.59 mmol) in dry *N*,*N*-dimethylformamide (DMF) (26 mL) was added 60% NaH in oil (0.91 g, 7.28 mmol). After 30 min at room temperature, to the stirred mixture was added ethyl iodide (1.22 g, 7.82 mmol). After 15 h at room temperature, the mixture was poured into water and extracted with AcOEt. The extract was washed with water and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:9), treatment with 4 N HCl in AcOEt and recrystallization from AcOEt afforded 2.37 g (78%) of **8a** as a light yellow crystal: mp 149–152 °C; ^{1}H NMR ($CDCl_3$) δ 1.19–1.41 (9H, m, CH_3 of *iso*Pr and CH_3 of Et), 2.77 (3H, br s, CH_3 of pyrimidine), 2.10–3.75 (9H, m, CCH_2CH_2N and CH of *iso*Pr), 4.04–4.74 (2H, m, CH_2 of Et), 5.94 (1H, br s, PhCH=), 6.41 (1H, br s, H of pyrimidine), 7.08–7.40 (7H, m, Ar), 7.54 (1H, br s, ArH); MS (CI) m/z (M^+ + 3, 100%), (M^+ + 1, 98%).

Method B. 4-(4-Oxopiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 26. By the same procedure as in the first step of Method A, treatment of 2,4-dichloro-6-methylpyrimidine **14** (10.55 g, 64.7 mmol) with 1,4-dioxa-8-azaspiro[4,5]decane (9.27 g, 64.7 mmol) and diisopropylethylamine (8.78 g, 68.0 mmol) in EtOH (200 mL) afforded 13.71 g (79%) of 2-chloro-4-(1,4-dioxa-8-azaspiro[4,5]decan-8-yl)-6-methylpyrimidine **23** as colorless crystal and 2.92 g (17%) of 4-chloro-2-(1,4-dioxa-8-azaspiro[4,5]decan-8-yl)-6-methylpyrimidine **23′** as a colorless crystal.

23: mp 127–128°; ^{1}H NMR ($CDCl_3$) δ 1.75 (4H, br t, J = 5.9 Hz, CCH_2C), 2.33 (3H, s, CH_3), 3.75 (4H, br t, J = 5.9 Hz, NCH_2), 4.01 (4H, s, $(CH_2O)_2$), 6.29 (1H, s, H of pyrimidine); MS (CI) m/z 272 (M^+ + 3, 34%), 270 (M^+ + 1, 100%).

23′: mp 102–105 °C; ^{1}H NMR ($CDCl_3$) δ 1.72 (4H, br t, J = 5.8 Hz, CCH_2C), 2.30 (3H, s, CH_3), 3.94 (4H, br t, J = 5.8 Hz, NCH_2), 4.00 (4H, s, $(CH_2O)_2$), 6.38 (1H, s, H of pyrimidine); MS (CI) m/z 272 (M^+ + 3, 31%), 270 (M^+ + 1, 100%).

Compound **23** (15.03 g, 55.7 mmol) was treated with 2-bromo-4-isopropylaniline HCl (13.96 g, 55.7 mmol) and diisopropylethylamine (7.92 g, 61.3 mmol) in ethylene glycol (60 mL) using the same procedure as in the second step of Method A to afford 19.43 g (78%) of 2-(2-bromo-4-isopropylanilino)-4-(1,4-dioxa-8-azaspiro[4,5]-decan-8-yl)-6-methylpyrimidine **24** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, d, J = 6.8 Hz, CH_3 of *iso*Pr), 1.75 (4H, br t, J = 5.8 Hz, CCH_2C), 2.29 (3H, s, CH_3), 2.75–2.93 (1H, m, CH of *iso*Pr), 3.74 (4H, br t,

J = 5.8 Hz, NCH_2), 4.01 (4H, s, $(CH_2O)_2$), 5.99 (1H, s, H of pyrimidine), 7.13 (1H, d d, J = 2.0, 8.6 Hz, ArH), 7.18 (1H, s, NH), 7.38 (1H, d, J = 2.0 Hz, ArH), 8.37 (1H, d, J = 8.6 Hz, ArH); MS (CI) m/z 449 ($M^+ + 3$, 96%), 447 ($M^+ + 1$, 100%).

Compound **24** (18.11 g, 40.5 mmol) was treated with ethyl iodide (10.10 g, 64.8 mmol) and 60% NaH in oil (2.43 g, 60.7 mmol) in dry DMF (100 mL) using the same procedure as in the third step of Method A to afford 14.63 g (76%) of 2-(*N*-ethyl-2-bromo-4-isopropylanilino)-4-(1,4-dioxa-8-azaspiro[4,5]decan-8-yl)-6-methylpyrimidine **25** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, t, J = 7.0 Hz, CH_3 of Et), 1.26 (6H, d, J = 7.0 Hz, CH_3 of *iso*Pr), 1.54–1.69 (4H, m, CCH_2C), 2.20 (3H, s, CH_3), 2.81–2.99 (1H, m, CH of *iso*Pr), 3.39–3.61 (4H, m, NCH_2), 3.68–4.23 (2H, m, CH_2 of Et), 3.96 (4H, s, $(CH_2O)_2$), 5.82 (1H, s, H of pyrimidine), 7.13–7.19 (2H, m, ArH), 7.48 (1H, br s, ArH); MS (CI) m/z 477 ($M^+ + 3$, 91%), 475 ($M^+ + 1$, 100%).

To a solution of **25** (14.25 g) in tetrahydrofurane (THF) (75 mL) was added 4 N aqueous HCl (75 mL) and the solution was stirred for 6 h at room temperature. The reaction mixture was concentrated in vacuo to remove THF and the residue was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The separated organic phase was washed with saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:7–1:6) afforded 11.69 g (90%) of **26** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.1 Hz, CH_3 of Et), 1.26 (6H, d, J = 7.0 Hz, CH_3 of *iso*Pr), 2.26 (3H, s, CH_3), 2.20–2.48 (4H, m, $C(O)CH_2$), 2.83–3.00 (1H, m, CH of *iso*Pr), 3.48–4.28 (6H, m, NCH_2 and CH_2 of Et), 5.91 (1H, s, H of pyrimidine), 7.15 (1H, d, J = 8.1 Hz, ArH), 7.19 (1H, d d, J = 1.5, 8.1 Hz, ArH), 7.49 (1H, d, J = 1.5 Hz, ArH); MS (CI) m/z 433 ($M^+ + 3$, 100%), 431 ($M^+ + 1$, 100%).

Method C. 4-(4-Ethylidenepiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 8b. To a stirred suspension of ethyltriphenylphosphonium bromide (1.90 g, 5.11 mmol) in dry THF (6 mL), cooled to −20 °C, was added dropwise 1.6 M solution of *n*-butyllithium in hexane (3.0 mL, 4.9 mmol). After 30 min, the mixture was warmed to 0 °C and to the stirred mixture was added dropwise a solution of compound **26** (0.40 g, 0.93 mmol) in dry THF (3 mL). After 30 min, the mixture was warmed to room temperature and stirring was continued for 1.5 h. The reaction mixture was partitioned between AcOEt and water. The separated organic phase was washed with saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:8–1:6) and treatment with 4 N HCl in AcOEt in a mixture of AcOEt and hexanes afforded 0.28 g (62%) of **8b** as a yellow crystal: mp 181–184 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (6H, d, J = 7.0 Hz, CH_3 of *iso*Pr), 1.33 (3H, t, J = 7.1 Hz, CH_3 of Et), 1.59 (3H, d, J = 6.4 Hz, CH_3 of ethylidene), 1.86–2.44 (4H, m, CCH_2C), 2.76 (3H, s, CH_3 of pyrimidine), 2.86–3.04 (1H, m, CH of *iso*Pr), 3.04–3.64 (4H, m, NCH_2), 4.00–4.84 (2H, m, CH_2 of Et), 5.24–5.44 (1H, m, C = CH), 5.91 (1H, s, H of pyrimidine), 7.06–7.36 (2H, m, ArH), 7.53 (1H, br s, ArH); MS (EI) m/z 444 ($M^+ + 2$), 442 (M^+), 363 (M^+ - Br, 100%); IR (KBr) 3435, 2960, 2710, 1651, 1609, 1589, 1555 cm^{-1}; anal. ($C_{23}H_{31}BrN_4 \cdot HCl$) C, H, N, Br, Cl.

Method D. 4-(4-Cyclopentadienylidenepiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 8h. To a stirred solution of **26** (208 mg, 0.48 mmol) and freshly distilled cyclopentadiene (80 mg, 1.21 mmol) in dry MeOH (0.9 mL) was added pyrrolidine (51 mg, 0.72 mmol). After 3 h at room temperature, the mixture was concentrated in vacuo, chromatographed on silica gel using a mixture of AcOEt and hexanes (1:8), and then treated with 4 N HCl in AcOEt in AcOEt to afford 140 mg (56%) of **8h** as a yellow amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.16 (3H, t, J = 6.0 Hz, CH_3 of Et), 1.26 (6H, d, J = 7.5 Hz, CH_3 of *iso*Pr), 2.27 (3H, br s, CH_3 of pyrimidine), 2.64–3.16 (5H, m, = CCH_2C and CH of *iso*Pr), 3.40–4.35 (6H, m, CH_2NCH_2 and CH_2 of Et), 6.45–6.81 (4H, m, H of cyclopentadiene), 6.51 (1H, s, H of pyrimidine), 7.31–7.60 (2H, m, ArH), 7.72 (1H, br s, ArH); MS (EI) m/z 480 ($M^+ + 2$), 478 (M^+), 399 (M^+ - Br, 100%); IR (KBr) 1651, 1610, 1590, 1546, 1494, 1365, 1257 cm^{-1}. Anal. ($C_{26}H_{31}BrN_4 \cdot HCl$) C, H, N, Br, Cl.

Method E. 2-Chloro-4-(4-phenyl-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine 27a. By the same procedure as in the first step of Method A, treatment of 2,4-dichloro-6-methylpyrimidine **14** (419 mg, 2.57 mmol) with 4-phenyl-1,2,3,6-tetrahydropyridine HCl (503 mg, 2.57 mmol) and diisopropylethylamine (664 mg, 5.14 mmol) in EtOH (4 mL) afforded 491 mg (67%) of **27a** as light yellow crystal and 95 mg (13%) of 4-chloro-2-(4-phenyl-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine **27′a** as a light yellow crystal.

27a: mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (3H, s, CH_3 of pyrimidine), 2.58–2.73 (2H, m, = CCH_2C), 3.94 (2H, t, J = 5.6 Hz, NCH_2), 4.11–4.28 (2H, m, NCH_2), 6.05–6.17 (1H, m, C = CH), 6.28 (1H, s, H of pyrimidine), 7.22–7.47 (5H, m, ArH); MS (CI) m/z 288 ($M^+ + 3$, 32%), 286 ($M^+ + 1$, 100%).

27′a: mp 89–90 °C; ^{1}H NMR ($CDCl_3$) δ 2.34 (3H, s, CH_3 of pyrimidine), 2.55–2.68 (2H, m, = CCH_2C), 4.08 (2H, t, J = 5.7 Hz, NCH_2), 4.41 (2H, d d, J = 2.8, 6.0 Hz, NCH_2), 6.10–6.20 (1H, m, C = CH), 6.42 (1H, s, H of pyrimidine), 7.20–7.50 (5H, m, ArH); MS (CI) m/z 288 ($M^+ + 3$, 33%), 286 ($M^+ + 1$, 100%).

Method F. 4-(4-Phenyl-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 11a. Compound **27a** (466 mg, 1.63 mmol) was treated with 2-bromo-4-isopropylaniline HCl (408 mg, 1.63 mmol) and diisopropylethylamine (232 mg, 1.79 mmol) in ethylene glycol (5 mL) using the same procedure as in the second step of Method A to afford 453 mg (61%) of 2-(2-bromo-4-isopropylanilino)-4-(4-phenyl-1,2,3,6-tetrahydropyridino)-6-methylpyrimidine **30a** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.24 (6H, d, J = 6.8 Hz, CH_3 of

*iso*Pr), 2.32 (3H, s, CH_3 of pyrimidine), 2.58–2.73 (2H, m, $=CCH_2C$), 2.78–2.94 (1H, m, CH of *iso*Pr), 3.91 (2H, t, $J=5.7$ Hz, NCH_2), 4.21 (2H, d d, $J=2.5$, 5.6 Hz, NCH_2), 5.98 (1H, s, H of pyrimidine), 6.11–6.20 (1H, m, $C=CH$), 7.11–7.49 (8H, m, ArH and NH), 8.45 (1H, d, $J=8.6$ Hz, ArH); MS (CI) m/z 465 ($M^+ + 3$, 99%), 463 ($M^+ + 1$, 100%).

Compound **30a** (453 mg, 0.98 mmol) was treated with ethyl iodide (214 mg, 1.37 mmol) and 60% NaH in oil (51 mg, 1.27 mmol) in dry DMF (5 mL) using the same procedure as in the third step of Method A to afford 325 mg (61%) of **11a** as a light yellow crystal: mp 124–127 °C; ^{1}H NMR (DMSO-d_6) δ 1.19 (3H, d, $J=7.0$ Hz, CH_3 of Et), 1.26 (6H, t, $J=7.0$ Hz, CH_3 of *iso*Pr), 2.28 (3H, br s, CH_3 of pyrimidine), 2.55–2.80 (2H, m, $=CCH_2C$), 2.93–3.09 (1H, m, CH of *iso*Pr), 3.30–4.68 (6H, m, NCH_2 and CH_2 of Et), 6.26 (1H, br s, H of pyrimidine), 6.51–6.86 (1H, m, $C=CH$), 7.23–7.63 (7H, m, ArH), 7.73 (1H, br s, ArH); MS (CI) m/z 493 ($M^+ + 3$, 99%), 491 ($M^+ + 1$, 100%); IR (KBr) 3505, 2960, 1652, 1609, 1588, 1546, 1493, 1379, 1255 cm^{-1}. Anal. ($C_{27}H_{31}BrN_4 \cdot HCl \cdot H_2O$) C, H, N, Br, Cl.

Method G. 4-(4-(4-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2,4-dimethoxyanilino)-6-methylpyrimidine 11ag. A mixture of 4-(4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-chloro-6-methylpyrimidine (500 mg, 1.55 mmol), which was prepared by treatment of 2,4-dichloro-6-methylpyrimidine **14** with 4-(4-chlorophenyl)-1,2,3,6-tetrahydropyridine HCl using the same procedure as in Method E, and *N*-ethyl-2,4-dimethoxyaniline (281 mg, 1.55 mmol) in ethylene glycol (2 mL) was heated at reflux for 1.5 h. The mixture was partitioned between AcOEt and saturated aqueous $NaHCO_3$. The separated organic phase was washed with saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:4) and recrystallization from Et_2O afforded 360 mg (50%) of **11ag** as a light yellow crystal: mp 159–160 °C; ^{1}H NMR ($CDCl_3$) δ 1.19 (6H, d, $J=7.0$ Hz, CH_3 of *iso*Pr), 1.15 (3H, t, $J=7.0$ Hz, CH_3 of Et), 2.20 (3H, br s, CH_3 of pyrimidine), 2.41–2.57 (2H, m, $=CCH_2C$), 3.61–4.12 (6H, m, NCH_2 and CH_2 of Et), 3.73 (3H, s, CH_3O), 3.84 (3H, s, CH_3O), 5.77 (1H, s, H of pyrimidine), 6.02–6.12 (1H, m, $C=CH$), 6.45–6.58 (2H, m, ArH), 7.10 (1H, d, $J=8.4$ Hz, ArH), 7.26–7.35 (4H, m, ArH), 7.73 (1H, br s, ArH); MS (EI) m/z 466 ($M^+ + 2$, 3%), 464 (M^+, 8%), 433 (M^+-CH_3O, 100%); IR (KBr) 2954, 2836, 1586, 1438, 1412, 1160 cm^{-1}. Anal. ($C_{26}H_{29}ClN_4O_2$) C, H, N, Cl.

Method H. 4-(4-(3-Chlorophenyl)-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine hydrochloride 11h. A stirred suspension of 3-bromochlorobenzene (427 mg, 2.23 mmol), magnesium (27 mg, 1.12 atoms) and trace iodine in dry THF (5 mL) was heated at reflux under a nitrogen atmosphere, and then cooled in an ice-bath. To the stirred solution was added dropwise a solution of compound **26a** (321 mg, 0.74 mmol). After 1 h, the mixture was warmed to room temperature and stirred for 1 h. The mixture was re-cooled in an ice-bath, and to the mixture was added dropwise a saturated aqueous NH_4Cl. After 10 min, the mixture was extracted with AcOEt. The extract was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:4) afforded 238 mg (59%) of 4-(4-(3-chlorophenyl)-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, $J=7.1$ Hz, CH_3 of Et), 1.24 (6H, d, $J=7.0$ Hz, CH_3 of *iso*Pr), 1.53–1.75 (2H, m, H of CCH_2C), 1.82–2.10 (2H, m, H of CCH_2C), 2.23 (3H, s, CH_3 of pyrimidine), 2.80–3.01 (1H, m, CH of *iso*Pr), 3.08–3.35 (2H, m, H of NCH_2), 3.58–4.35 (4H, m, H of NCH_2 and CH_2 of NEt), 5.86 (1H, s, H of pyrimidine), 7.13–7.21 (2H, m, ArH), 7.21–7.35 (3H, m, ArH), 7.43–7.53 (2H, m, ArH); MS (CI) m/z 545 ($M^+ + 3$, 100%), 543 ($M^+ + 1$, 82%).

A solution of 4-(4-(3-chlorophenyl)-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine (170 mg, 0.31 mmol) in trifluoroacetic acid (1.25 mL) was stirred at room temperature for 2 days. The solution was concentrated in vacuo and then partitioned between AcOEt and saturated aqueous $NaHCO_3$. The separated organic phase was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:7), treatment with 4 N HCl in AcOEt in MeOH and recrystallization from a mixture of isopropanol and diisopropyl ether afforded 131 mg (75%) of **11h** as a light yellow crystal: mp 183–185 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, d, $J=7.0$ Hz, CH_3 of *iso*Pr), 1.34 (3H, t, $J=6.8$ Hz, CH_3 of Et), 2.20–2.88 (2H, m, $C=CCH_2C$), 2.80 (3H, br s, CH_3 of pyrimidine), 2.88–3.05 (1H, m, CH of *iso*Pr), 3.30–4.75 (6H, m, NCH_2 and CH_2 of Et), 5.88 (1H, br s, H of pyrimidine), 5.94–6.08 (1H, m, $C=CH$), 7.00–7.61 (7H, m, ArH); MS (CI) m/z 527 ($M^+ + 3$, 100%), 525 ($M^+ + 1$, 84%); IR (KBr) 3504, 2960, 1654, 1610, 1588, 1546, 1492 cm^{-1}. Anal. ($C_{27}H_{30}BrClN_4 \cdot HCl$) C, H, N, Br, Cl.

Method I. 4-(4-Furan-2-yl-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 11d. To a stirred solution of furan (136 mg, 2.00 mmol) in dry THF (1 mL), cooled to −15 °C, was added dropwise 1.63M solution of *n*-butyllithium in hexane (0.9 mL, 1.47 mmol) over 10 min. After 20 min at 5 °C, to the stirred mixture, cooled to −15 °C, was added dropwise a solution of **26** (432 mg, 1.00 mmol) in dry THF over 10 min. After 30 min at −15 °C to 0 °C and then 1 h at room temperature, to the stirred mixture, cooled in an ice-bath, was added dropwise a saturated aqueous NH_4Cl. After 10 min, the mixture was extracted with AcOEt. The extract was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:3) afforded 279 mg (56%) of 4-(4-furan-2-yl-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, t, $J=7.0$ Hz, CH_3 of Et),

1.26 (6H, d, $J=6.8$ Hz, CH_3 of *iso*Pr), 1.77–2.05 (4H, m, H of CCH_2C), 2.21 (3H, s, CH_3 of pyrimidine), 2.81–3.01 (1H, m, CH of *iso*Pr), 3.30–3.58 (2H, m, H of NCH_2), 3.60–4.21 (4H, m, H of NCH_2 and CH_2 of NEt), 5.83 (1H, s, H of pyrimidine), 6.20 (1H, d, $J=3.2$ Hz, ArH), 6.33 (1H, d d, $J=1.8$, 3.2 Hz, ArH), 7.15–7.18 (2H, m, ArH), 7.37 (1H, d d, $J=0.8$, 1.8 Hz, ArH), 7.49 (1H, br s, ArH); MS (CI) m/z 501($M^+ + 3$, 98%), 499 ($M^+ + 1$, 100%).

To a stirred solution of 4-(4-furan-2-yl-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine (104 mg, 0.21 mmol), triethylamine (85 mg, 0.84 mmol) and 4-dimethylaminopyridine (13 mg, 0.11 mmol) in dichloromethane (1.0 mL), cooled in an ice-bath, was added dropwise a solution of methanesulfonyl chloride (48 mg, 0.42 mmol) in dichloromethane (0.5 mL). After 15 min, the mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with AcOEt. The extract was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:9) afforded 70 mg (70%) of **11d** as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, $J=7.0$ Hz, CH_3 of Et), 1.28 (6H, d, $J=6.8$ Hz, CH_3 of *iso*Pr), 2.21 (3H, s, CH_3 of pyrimidine), 2.31–2.47 (2H, m, CCH_2C), 2.84–3.00 (1H, m, CH of *iso*Pr), 3.55–4.30 (6H, m, H of NCH_2 and CH_2 of Et), 5.79 (1H, s, H of pyrimidine), 6.13–6.25 (2H, m, ArH and C=CH), 6.37 (1H, d d, $J=1.8$, 3.3 Hz, ArH), 7.13–7.20 (2H, m, ArH), 7.37 (1H, d, $J=1.8$ Hz, ArH), 7.51 (1H, br s, ArH); MS (FAB) m/z 483($M^+ + 3$, 99%), 481 ($M^+ + 1$, 100%); IR ($CHCl_3$) 2963, 2928, 1656, 1577, 1493, 1451, 1413, 1376 cm^{-1}. Anal. ($C_{27}H_{30}BrN_4O$) C, H, N, Br.

Method J:. 4-(4-Thiophen-2-yl-1,2,3,6-tetrahydropyridino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine 11e. Treatment of **26** (432 mg, 1.00 mmol) with thiophene (163 mg, 2.00 mmol) and 1.63 M solution of *n*-butyllithium in hexane (0.9 mL, 1.47 mmol) in dry THF using the same procedure as in the first step of Method I afforded 228 mg (44%) of 4-(4-thiophen-2-yl-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, $J=7.0$ Hz, CH_3 of Et), 1.26 (6H, d, $J=6.8$ Hz, CH_3 of *iso*Pr), 1.81–2.15 (4H, m, H of CCH_2C), 2.22 (3H, s, CH_3 of pyrimidine), 2.82–2.98 (1H, m, CH of *iso*Pr), 3.20–3.38 (2H, m, H of NCH_2), 3.62–4.18 (4H, m, H of NCH_2 and CH_2 of NEt), 5.84 (1H, s, H of pyrimidine), 6.96–7.00 (2H, m, ArH), 7.16–7.20 (2H, m, ArH), 7.21–7.26 (1H, m, ArH), 7.49 (1H, br s, ArH); MS (CI) m/z 517 ($M^+ + 3$, 100%), 515 ($M^+ + 1$, 99%).

A solution of 4-(4-thiophen-2-yl-4-hydroxypiperidino)-2-(*N*-ethyl-2-bromo-4-isopropylanilino)-6-methylpyrimidine (166 mg, mmol) in 99% formic acid (0.5 mL) was stirred for 2 h at room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with AcOEt. The extract was washed with saturated aqueous $NaHCO_3$ and saturated brine, dried (Na_2SO_4), filtered, and then concentrated in vacuo. Chromatography of the residue on silica gel using a mixture of AcOEt and hexanes (1:9) afforded 132 mg (90%) of **11e** as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, $J=7.1$ Hz, CH_3 of Et), 1.28 (6H, d, $J=6.8$ Hz, CH_3 of *iso*Pr), 2.21 (3H, s, CH_3 of pyrimidine), 2.43–2.60 (2H, m, CCH_2C), 2.85–3.02 (1H, m, CH of *iso*Pr), 3.60–4.30 (6H, m, NCH_2 and CH_2 of Et), 5.80 (1H, s, H of pyrimidine), 6.03–6.15 (1H, m, C=CH), 6.92–7.00 (2H, m, ArH), 7.10–7.20 (3H, m, ArH), 7.51 (1H, br s, ArH); MS (CI) m/z 499($M^+ + 3$, 99%), 497 ($M^+ + 1$, 100%) ; IR ($CHCl_3$) 2962, 2928, 1661, 1577, 1493, 1450, 1414, 1375 cm^{-1}. Anal. ($C_{27}H_{30}BrN_4S$) C, H, N, Br, S.

Binding study

Rats were decapitated and the frontal cortex and heart were rapidly dissected. Tissues were 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 crude membrane preparation for binding studies. Protein concentration was determined using a described method.[35]

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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