Bioorganic & Medicinal Chemistry 19 (2011) 5955–5966

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Pyrazolo[1,5-*a*]pyrimidines, triazolo[1,5-*a*]pyrimidines and their tricyclic derivatives as corticotropin-releasing factor 1 (CRF_1) receptor antagonists

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ARTICLE INFO

Article history:
Received 26 July 2011
Revised 24 August 2011
Accepted 25 August 2011
Available online 27 August 2011

Keywords:
Corticotrophin-releasing factor 1 receptor
Antagonist

ABSTRACT

To identify structurally novel CRF1 receptor antagonists, a series of bicyclic core antagonists, pyrazolo [1,5-a]pyrimidines, triazolo[1,5-*a*]pyrimidines, imidazo[1,2-*a*]pyrimidines and pyrazolo[1,5-*a*][1,3,5]triazines were designed, synthesized and evaluated as CRF1 receptor antagonists. Compounds **2**–**27** showed binding affinity (IC_{50} = 4.2–418 nM) and antagonist activity (EC_{50} = 4.0–889 nM). Compound **5** was found to show oral efficacy in an Elevated Plus Maze test in rats. Further chemical modification of them led us to discovery of the tricyclic core antagonists pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidines. The discovery process of these compounds is presented, as is the study of the structure–activity relationship.

1. Introduction

Major depression is a complex and recurrent lifelong illness, characterized by repeated periods of exacerbation and remission. Despite the number of treatments available (which focus mainly on addressing modulation of monoamine neurotransmitters), significant unmet needs remain in terms of efficacy, onset of effect, and tolerability. Currently-available antidepressant drugs take 6–8 weeks to exert their effect, and ~30% of patients are non-responders; moreover drug-induced side effects reduce patient compliance. Extensive attempts have been made to identify the relevant biological targets contributing to the main pathophysiological mechanisms of anxiety and depressive disorders with the aim of getting more efficacious and safer treatments with rapid relief of symptoms.

It is well-known that corticotropin-releasing factor (CRF) exerts its biological functions through binding to type-1 (CRF_1) and/or type-2 (CRF_2) receptors.[1] Several clinical evidences suggest the association of a high level of CRF and the onset of anxiety and depressive disorders.[2–7]

Significant contributions have been made in the past two decades by several pharmaceutical research groups to discover non-peptide CRF_1 receptor antagonists as a potential treatment for stress-related illnesses (including depression). 7*H*-Pyrrolo[2,3-*d*]pyrimidine (CP-154,526)[8] as well as pyrazolo[1,5-*a*]pyrimidine (R121919)[8] and pyrazolo[1,5-*a*][1,3,5]triazine (DMP-696)[8] are among the first and most representative compounds identified.

In an effort to discover structurally novel CRF_1 receptor antagonists exhibiting improved in vivo profiles, many scaffolds have been designed and synthesized. In this report, we describe novel bicyclic core CRF_1 antagonists **2–3** (Fig. 1). These are designed based on the tricyclic core CRF_1 antagonist **1** reported in our previous study.[9] Cleavage of the C-ring of the tricyclic core structure consisting of A-, B- and C-rings resulted in a novel bicyclic core structure consisting of A- and B-rings (Fig. 1). Bicyclic core structures consisting of B- and C-rings were previously reported with dihydroimidazoles,[10] dihydropyrrolopyrimidines,[11,12] tetrahydroimidazopyrimidines[13] and imidazoimidazoles[14] while the bicyclic core structures in this report consist of A- and B-rings.

2. Chemistry

Test compounds listed in Tables 1–3 were synthesized as shown in Schemes 1–3. The synthesis of analogs **2–19** is described in Scheme 1a. The cyclization reaction of 5-aminopyrazole **34** with optional alkyl diethyl malonates **37a–i** followed by chlorination with phosphorous oxychloride afforded dichlorides **38a–i**, respectively. Aminolysis of **38a–b** and **38i** with 1-ethylpropylamine resulted in aminochlorides **39a–b** and **39i**, respectively. Aminolysis of **39a** and **39b** with 2-chloro-4-methoxyaniline afforded **2** and **3**, respectively. Chemoselective aminolysis of **38a–i** with dipropylamine afforded **40a–h**, respectively. Aminolysis of **40a–h** with 2-chloro-4-methoxyaniline afforded **4–11**, 5-*N*-alkylation of which with optional alkyl halides in the presence of sodium hydride produced **12–19**, respectively.

The synthesis of **20–23** is outlined in Scheme 1b. The cyclization reaction of 3-amino-1,2,4-triazole **35** with optional diethyl alkyl malonates **37a–b** resulted in dichlorides **41a–b**. Monoamin-

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doi:10.1016/j.bmc.2011.08.055

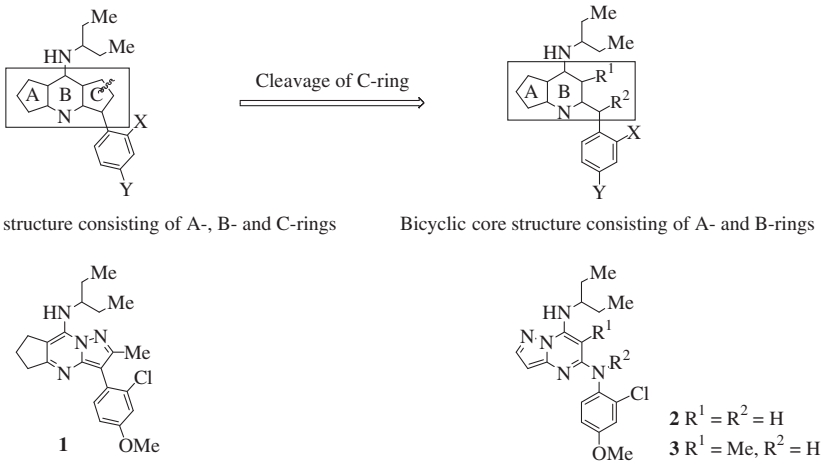
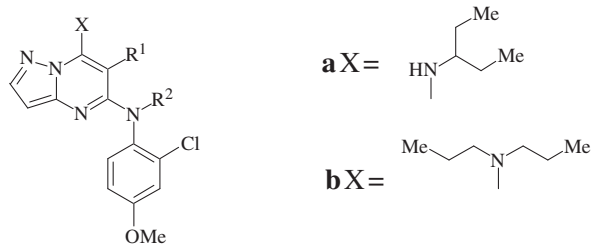


Figure 1. Molecular design of a novel bicyclic core CRF1 antagonist.

Table 1
Activity profiles of novel bicyclic core CRF_1 antagonists



Compd	R^1	R^2	X	Binding affinity IC_{50} (nM) Human	Antagonist activity EC_{50} (nM) Human
2	H	H	a	14	113
4	H	H	b	61	53
3	Me	H	a	7.4	7.4
5	Me	H	b	8	4 (81)[a]
6	Et	H	b	4.2	8.6
7	n-Pr	H	b	32	95
8	i-Pr	H	b	113	234
9	n-Bu	H	b	340	NT[b]
10	c-Pn	H	b	308	NT[b]
11	OMe	H	b	13	883
12	H	Me	b	20	97
13	H	Et	b	31.6	18.6
14	H	n-Pr	b	418	75.7
15	H	Methallyl	b	76.7	856.1
16	H	Allyl	b	4.4	28.9
17	H	CH2c-Pr	b	213	NT[b]
18	H	CH_2CH_2OMe	b	680	NT[b]
19	H	CH_2OMe	b	15	4.4

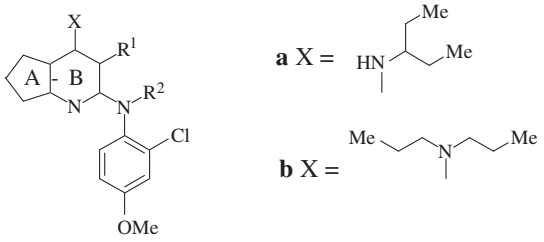
[a] cAMP assay for rat.
[b] Not tested.

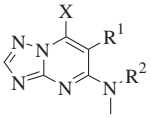
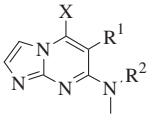
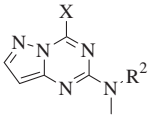
ation of **41a** and **41b** with 1-ethylpropylamine afforded **42a** and **42b**, respectively. Aminolysis of **42a–b** with 2-chloro-4-methoxyaniline and *N*-methyl-2-chloro-4-methoxyaniline afforded **20** and **21**, respectively. Chemoselective monoaminolysis of **41a** with dipropylamine afforded **43a**, further aminolysis of which with 2-chloro-4-methoxyaniline afforded **22**. *N*-Alkylation of **22** with methyl iodide in the presence of sodium hydride resulted in **23**.

The synthesis of analogs **24–26** is described in Scheme 1c. The cyclization reaction of 2-aminoimidazole **36** with **37a–b** followed by chlorination with phosphorous oxychloride afforded **44a–b**. Chemoselective monoamination of **44a–b** with dipropylamine afforded **45a–b**, respectively. Aminolysis of **45b** with 2-chloro-4-methoxyaniline afforded **24**. Aminolysis of **45a** with 2-chloro-4-methoxyaniline followed by *N*-alkylation with methyl iodide and ethyl iodide afforded *N*-methyl analog **25** and *N*-ethyl analog **26**, respectively.

Pyrazolotriazine analog **27** was prepared from 3-aminopyrazole **34** as shown in Scheme 2. Reaction of **34** with ethoxycarbonyl isothiocyanate afforded a thiourea **46**, treatment of which under alkaline condition provided a cyclized compound **47**. *S*-Methylation of **47** with methyl iodide afforded **48**, chlorination of which with phosphorous oxychloride in *N,N*-dimethylformamide provided **48**. Aminolysis of the chloride **49** with dipropylamine afforded **50**, oxidation of which with *m*-CPBA provided a methylsulfonyl compound **51**. Aminolysis of the methylsulfone **51** with 2-chloro-4-methoxyaniline resulted in a pyrazolotriazine analog **27**.

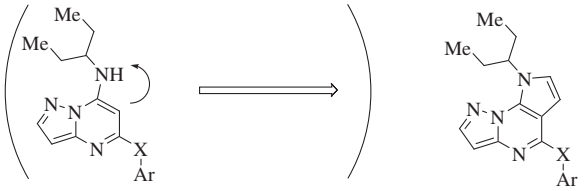
Table 2
Activity profiles of miscellaneous bicyclic core CRF_1 antagonists

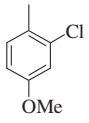
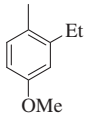
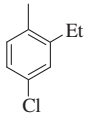
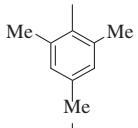
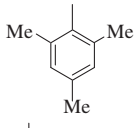
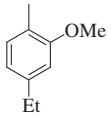


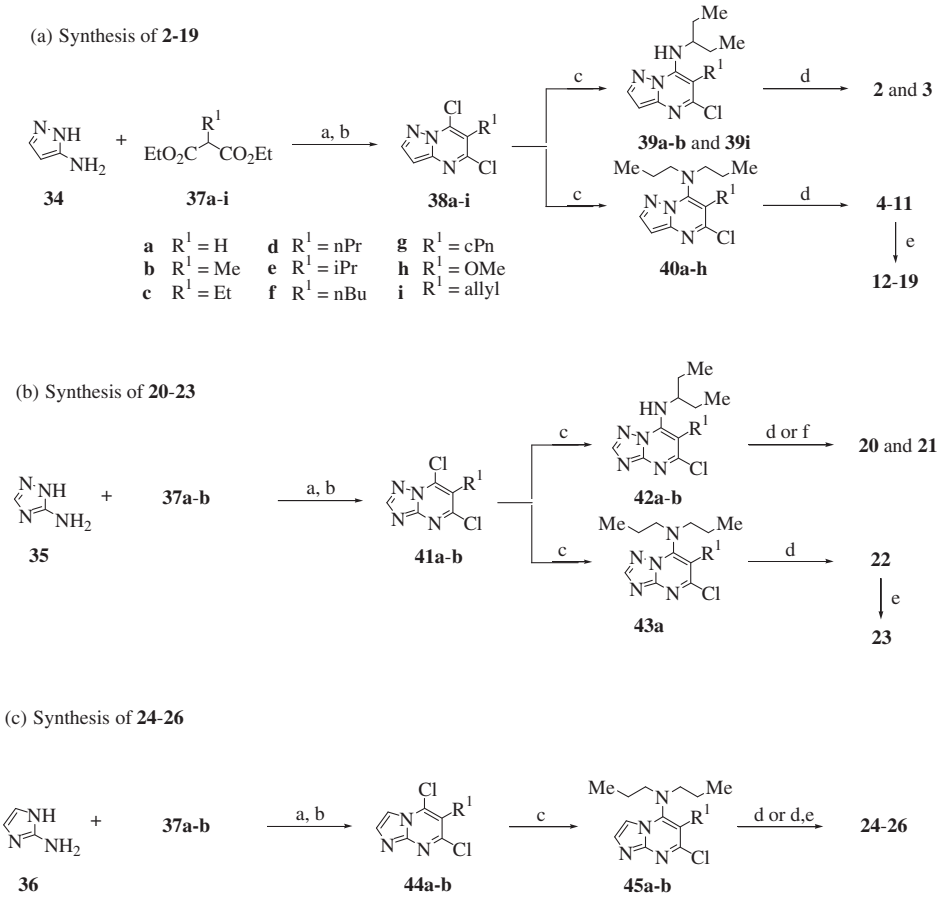
Compd	A–B	X	R^1	R^2	Binding affinity IC_{50} (nM) Human	Antagonist activity EC_{50} (nM) Human
20		**a**	Me	H	16	58
21		**a**	H	Me	42	367
22		**b**	Me	H	8	9
23		**b**	H	Me	33	889
24		**b**	Me	H	12	46
25		**b**	H	Me	67	NT[a]
26		**b**	H	Et	42	76
27		**b**	—	Me	20	26

[a] Not tested.

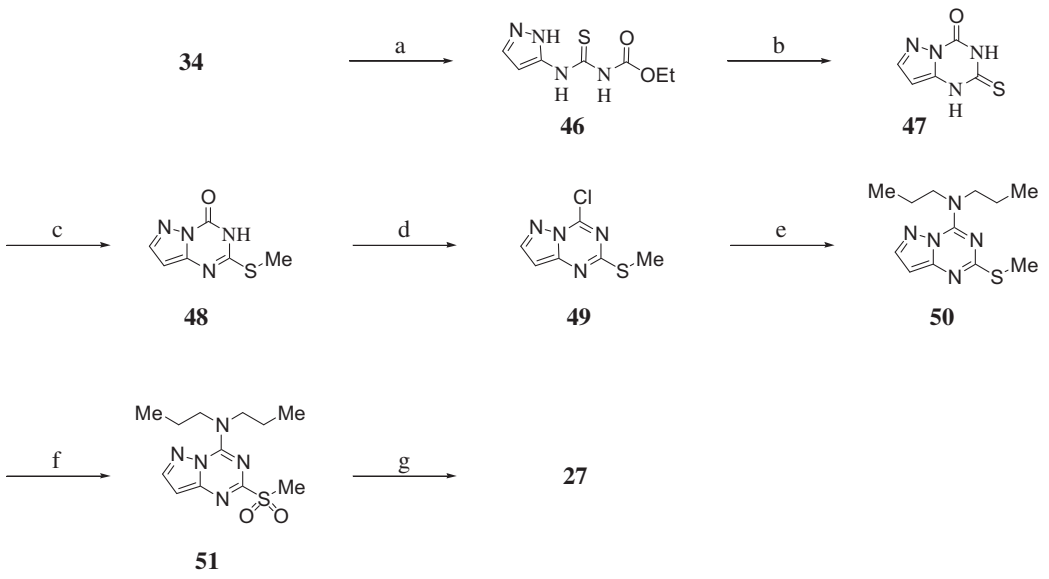
Table 3
Activity profiles of newly designed tricyclic core CRF_1 antagonists



Compd	Ar	X	Binding affinity IC_{50} (nM) Human	Antagonist activity EC_{50} (nM) Human
28		NH	3.2	0.7
29		NH	2.4	3.5
30		NH	4.7	3.9
31		CH_2	7.1	32.2
32		NH	6.1	4.1
33		O	7.0	5.6

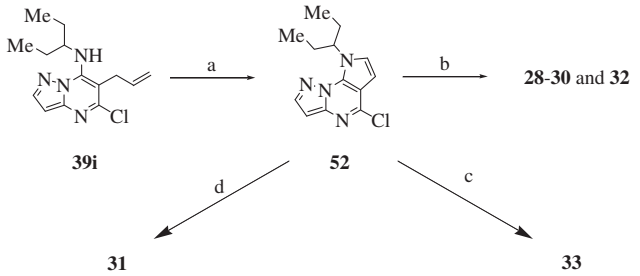


Scheme 1. Synthesis of **2–26**. Reagents and conditions: (a) NaOEt, EtOH, reflux; (b) $POCl_3$, reflux; (c) *N*,*N*-dipropylamine or 1-ethylpropylamine, THF, 0 °C to rt; (d) 2-chloro-4-methoxyaniline, 150∼180 °C; (e) NaH, alkyl halide, THF, 0 °C to rt; (f) *N*-methyl-2-chloro-4-methoxyaniline, 150–200 °C.



Scheme 2. Synthesis of **27**. Reagents and conditions: (a) ethoxycarbonyl isothiocyanate, EtOAc/benzene, rt; (b) NaOH aq, rt; (c) MeI, NaOH aq, EtOH, rt; (d) $POCl_3$, *N*,*N*-dimethylaniline, reflux; (e) *N*,*N*-dipropylamine, triethylamine, THF, rt; (f) *m*-CPBA, CH_2Cl_2, 0 °C to rt; (g) *N*-methyl-2-chloro-4-methoxyaniline, 130–160 °C.

The synthesis of tricyclic analogs **28**–**33** is described in Scheme 3. Oxidative C–C bond cleavage of the terminal olefin of **39i** afforded a tricyclic product **52** without isolation of an aldehyde expected as an intermediate. Aminolysis of the chloride **52** with optionally substituted anilines afforded **28**–**30** and **32**. Replacement of the chlorine of **52** with 4-ethyl-2-methoxyphenol in the presence of a base resulted in the corresponding ether analog **33** (Table 3: X = O). The substitution reaction of **52** with 2,4,6-trimethylbenzyl zinc bromide afforded the corresponding methylene analog **31** (Table 3: X = CH_2).



Scheme 3. Synthesis of **28–33**. Reagents and conditions: (a) cat OsO_4-$NaIO_4$, THF/H_2O, rt; (b)optionally substituted anilines, 100 °C; (c) 2-methoxy-4-ethylphenol, K_2CO_3, DMF, 100 °C; (d) 2,4,6-trimethylbenzyl bromide, Zn, 1,2-dibromoethane, TMSCl, THF then $Pd(Ph_3P)_4$, 60 °C.

3. Results and discussion

The compounds listed in Tables 1–3 were tested for their binding affinity to the human CRF_1 receptor and antagonist activity in a CRF-stimulated adenylate cyclase assay.[15] One of the leading compounds with higher receptor binding and antagonist activity was then evaluated in a cAMP assay and pharmacokinetic study in rats. Anxiolytic efficacy was also assessed in the Elevated Plus Maze model for rats.[16–18]

The human CRF_1 receptor binding and antagonist activity data for pyrazolo[1,5-*a*]-1,5-pyrimidines **2–19** are summarized in Table 1. Pyrazolo[1,5-*a*]pyrimidin-5-amine core analog **2** and its corresponding tertiary amine analog **4** showed moderate antagonist activity and binding affinity. Introduction of a methyl group into position 6 of the pyrazolo[1,5-*a*]pyrimidin-5-amine core of **2** and **4** afforded **3** and **5**, respectively. Analogs **3** and **5** resulted in an improvement in their binding affinity and antagonist activity relative to **2** and **4**, respectively. Introduction of bulkier groups such as ethyl, *n*-propyl, *i*-propyl, *n*-butyl, cyclopentyl and methoxy groups instead of the methyl group of **5** afforded **6–11**, respectively. The most potent analog **6** had an ethyl group at position 6. The *n*-propyl analog **7** exhibited moderate binding affinity and antagonist activity. The methoxy analog **11** showed quite good improvement in its binding affinity but showed unexpectedly weak antagonist activity for its strong binding affinity. The corresponding *i*-propyl, *n*-butyl and *c*-pentyl analogs **8–10** resulted in a significant reduction of binding affinity. Replacement of the NH moiety at position 5 of the pyrazolopyrimidine core of **4** with *N*-methyl, *N*-ethyl, *N*-propyl, *N*-methallyl, *N*-allyl, *N*-cyclopropylmethyl, *N*-methoxyethyl and *N*-methoxymethyl groups afforded analogs **12–19**, respectively.

Among them, the most potent binding affinity was obtained in the *N*-allyl analog **16** whereas the most potent antagonist activity was obtained in the *N*-methoxymethyl analog **19**. Analogs **12–15** showed moderate binding affinity and antagonist activity, respectively. Analogs **17** and **18** exhibited moderate binding affinity. Remarkably weaker potency of **18** relative to **19** was ascribed to the relatively more Lewis basic and more hydrophilic methoxy moiety of **18**, which is more exposed outside of the molecule relative to that of **19**. As a result, small substituents such as methyl and ethyl groups were found as a favored R^1, where R^2 is hydrogen. *N*-Allyl and *N*-methoxymethyl groups were found as a favored R^2, where R^1 is hydrogen.

Miscellaneous bicyclic core structures were also synthesized and evaluated for their CRF_1 binding affinity and antagonist activity. The human CRF_1 receptor binding and antagonist activity data for triazolopyrimidines **20–23**, imidazolopyrimidines **24–26** and pyrazolotriazine **27** are summarized in Table 2. Triazolopyrimidine analogs **20–23** exhibited moderate-to-strong binding affinity. Among them, the NH analogs **20** and **22** exhibited relatively more potency than the corresponding *N*-methyl analogs **21** and **23**, respectively. In a series of imidazolopyrimidine analogs **24–26**, the tertiary amine analog **27** showed quite potent activity in both evaluations. The *N*-Methyl analog **25** and the corresponding *N*-ethyl analog **26** showed moderate potency in their binding affinity and/or antagonist activity. Thus, *N*-methyl analogs **23** and **25** tended to show less potent in vitro activities relative to the corresponding NH analogs **22** and **24**, respectively. The same structure–activity relationship was observed between *N*-methyl analog **12** and NH analog **5** (Table 1). The pyrazolotriazine analog **27** showed quite potent activities in receptor affinity and antagonist activity. Hence, introduction of an additional nitrogen atom into the bicyclic core moiety of **3** afforded **20** with a reduction of in vitro activity. Introduction of another nitrogen atom into the core structures of **5** and **12** afforded **22** and **23**, respectively also with a tendency of reduction of in vitro activities. The pyrazolotriazine analog **27** also with an additional nitrogen atom showed close receptor affinity to that of **12**. Imidazopyrimidine analogs **24–25** showed relatively weaker in vitro activities than the corresponding triazolopyrimidine analogs **20**, **22** and **23**, respectively.

On the basis of the findings described above, a series of heterotricyclic analogs were designed and synthesized. The human CRF_1 receptor binding and antagonist activity data for newly designed heterotricyclic core antagonists **28–33** are described in Table 3. Most of the tricyclic core analogs listed in Table 3 exhibited very potent binding affinity, including analog **33** (X = O) and **31** (X = CH_2). Only **31** (X = CH_2) exhibited relatively weaker antagonist activity relative to others for its strong binding affinity. The significant reduction of the antagonist activity of **31** relative to the potency of the corresponding NH analog **32** was thought to be caused by the relatively higher lipophilicity of the methylene linker (X = CH_2). The most potent antagonist **28** among this series had a 2-chloro-4-methoxyphenyl group as the aryl moiety connected by the linker X = NH.

The anxiolytic efficacy of **5**, which showed potent antagonist activity in a rat cyclic AMP assay (half maximal effective concentration (EC_{50}) = 81 nM, Table 1), was assessed by the Elevated Plus Maze test (Figure 2). Vehicle-treated rats significantly decreased the time spent in open arms (p <0.05) relative to control animals. Pretreatment with compound **5** (10 mg/kg, po) significantly increased the spent time in open arms (p = 0.050) relative to vehicle-treated animals.

Pharmacokinetic data for compound **5** were investigated after its administration in single doses to rats (Table 4). Intravenous administration of **5** to rats (1 mg/kg, n = 4) resulted in detectable plasma levels (half-life ($T_{1/2}$) = 1.7 h), whereas oral administration of **5** to rats (10 mg/kg, n = 4) resulted in a $T_{1/2}$ of 3.4 h. The area under the curve (AUC) value of **5** was 96 ng· h/mL after intravenous administration *versus* 153 ng· h/mL after oral administration. The

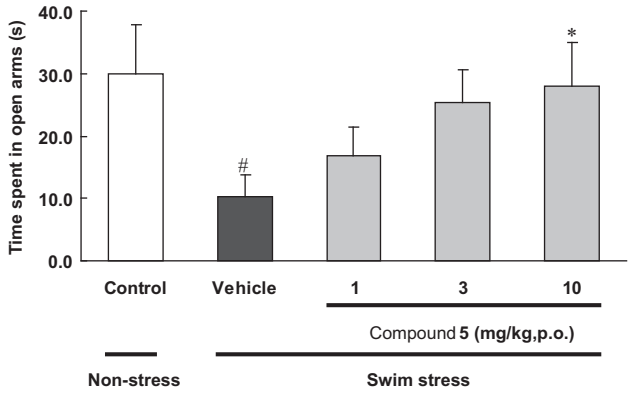


Figure 2. Effect of compound **5** in the rat elevated plus-maze test in swim stress-loaded rats Each column represents mean ± standard errors of 16 rats $^{\#}p$ <0.05 vs. Control group (t-test) $^{*}P$ <0.05 vs.Vehicle group (Dunnett test)

Table 4
Pharmacokinetic parameters of compound **5**

Parameter	iv	po
Dose (mg/kg)	1	10
AUCinfinity (ng·h/ml)	96	153
Tmax (h)		4.0
C_{max} (ng/ml)		27.4
CLtotal (ml/min/kg)	186	
$T_{1/2}$ (h)	1.7	3.4
Vss (ml/kg)	14423	
F (%)		16

steady state volume of distribution (V_{ss}) was calculated to be 14423 mL/kg, indicating that this compound showed good distribution in tissues. Systemic clearance (CL) was 186 ml/min/kg, indicating that this compound was susceptible to being metabolized. The C_{max} value after oral dosing was 27.4 ng/mL, whereas the T_{max} value was 4.0 h. The bioavailability of **5** was 16%. K_p value (brain content of **5**/plasma concentration of **5**) was 6.2 (1.0 h after oral dosing).

4. Conclusion

A series of bicyclic core antagonists, pyrazolo[1,5-*a*]pyrimidines, triazolo[1,5-*a*]pyrimidine-5,7-diamines, imidazo[1,2-*a*]-pyrimidines and pyrazolo[1,5-*a*][1,3,5]triazines were designed, synthesized and evaluated as CRF_1 antagonists. Some of the pyrazolo[1,5-*a*]pyrimidines **3**, **5**, **6** and **19** showed strong in vitro activities. Among them, **5** showed oral efficacy at 10 mg/kg in the Elevated Plus Maze test in rats. Further design and synthesis for structural diversity led us to discover a series of tricyclic core antagonists, pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidines. Despite their very strong in vitro activities, they did not show oral efficacy because of the presumed pharmacokinetic problems.

5. Experimental

5.1. Chemistry

5.1.1. General procedures

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. Fast atom bombardment (FAB-MS, HRMS) and electron ionization (EI) mass spectra were obtained on a JEOL JMS-DX303HF spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined on a HITACHI MI200H spectrometer. Infrared spectra (IR) were measured in a Perkin-Elmer FT-IR 1760X spectrometer. Melting points and results of elemental analyses were uncorrected. Column chromatography was carried out on silica gel [Merck Silica gel 60 (0.063–0.200 mm), Wako gel C-200, or Fuji Silysia FL60D]. Thin layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, Silica gel 60 F254). The following abbreviations for solvents and reagents are used *N*,*N*-dimethylformamide (DMF), ethyl acetate (EtOAc), acetic acid (AcOH), methanol (MeOH), ethanol (EtOH), dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), trimethylsilyl chloride (TMSCl), sodium hydride (NaH).

5.1.2. 5,7-Dichloropyrazolo[1,5-*a*]pyrimidine (38a)

To a stirred solution of sodium ethoxide in EtOH, which was prepared from sodium (2.77 g, 120 mmol) and EtOH (90 mL) by the conventional method, were added diethyl malonate (9.64 g, 60 mmol) at ambient temperature and then 3-aminopyrazole (5.0 g, 60 mmol). The reaction mixture was refluxed for 24 h. After cooling to room temperature, the precipitates were collected by filtration and dissolved in water. The aqueous solution was acidified with 2 N HCl (pH ~2). The resulting precipitates were collected by filtration and dried under reduced pressure to afford 4*H*-pyrazolo[1,5-*a*]pyrimidine-5,7-dione (6.56 g) as a white powder, which was used for the next reaction without further purification. MS (FAB, Pos) *m*/*z* 152 $(M+H)^+$

A stirred suspension of 4*H*-pyrazolo[1,5-*a*]pyrimidine-5,7-dione (6.56 g) and *N*,*N*-diethylaniline (8.3 mL, 52 mmol) in phosphorus oxychloride (30 mL) was refluxed for 4 h. After cooling, the reaction mixture was poured into ice-water, stirred for 30 min, neturalized with saturated aqueous sodium carbonate and extracted with EtOAc. The combined organic layers were washed with water, brine and dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel using EtOAc/hexane (1/10) to give **38a** (6.60 g, 81% yield in 2steps) as a yellow solid. TLC R_f = 0.51 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (d, *J* = 2.4 Hz, 1H), 7.00 (s, 1H), 6.75 (d, *J* = 2.4 Hz, 1H).

5.1.3. 5,7-Dichloro-6-methylpyrazolo[1,5-*a*]pyrimidine (38b)

41% yield in 2steps; a pale yellow powder. TLC R_f = 0.46 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 202 and 204 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, *J* = 2.38 Hz, 1H), 6.70 (d, *J* = 2.38 Hz, 1H), 2.55 (s, 3H).

5.1.4. 5,7-Dichloro-6-ethylpyrazolo[1,5-*a*]pyrimidine (38c)

54% yield in 2steps; a yellow solid. TLC R_f = 0.44 (EtOAc/hexane, 1/4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 2.20 Hz, 1H), 6.70 (d, *J* = 2.20 Hz, 1H), 3.00 (q, *J* = 7.51 Hz, 2H), 1.29 (t, *J* = 7.51 Hz, 3H).

5.1.5. 5,7-Dichloro-6-propylpyrazolo[1,5-*a*]pyrimidine (38d)

47% yield in 2steps; a beige powder. TLC R_f = 0.39 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 2.20 Hz, 1H), 6.70 (d, *J* = 2.20 Hz, 1H), 2.87–2.97 (m, 2H), 1.64–1.80 (m, 2H), 1.07 (t, *J* = 7.32 Hz, 3H).

5.1.6. 5,7-Dichloro-6-isopropylpyrazolo[1,5-*a*]pyrimidine (38e)

46% yield in 2steps; a yellow oil. TLC R_f = 0.50 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 2.4 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, H1), 3.38 (m, 1H), 1.51 (d, *J* = 5.5 Hz, 6H).

5.1.7. 5,7-Dichloro-6-*n*-butylpyrazolo[1,5-*a*]pyrimidine (38f)

TLC R_f = 0.36 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (d, *J* = 2.38 Hz, 1H), 6.70 (d, *J* = 2.38 Hz, 1H), 2.88–3.00 (m, 2H), 1.58–1.73 (m, 2H), 1.41–1.56 (m, 2H), 1.00 (t, *J* = 7.23 Hz, 3H).

5.1.8. 5,7-Dichloro-6-cyclopentylpyrazolo[1,5-*a*]pyrimidine (38g)

62% yield in 2 steps; a yellow oil. TLC R_f = 0.32 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (d, *J* = 2.4 Hz, 1H), 6.69 (d, *J* = 2.4 Hz, 1H), 3.84 (m, 1H), 2.21–2.12 (m, 2H), 2.12–1.91 (m, 4H), 1.77 (m, 2H).

5.1.9. 5,7-Dichloro-6-methoxypyrazolo[1,5-*a*]pyrimidine (38h)

22% yield in 2 steps; an ivory solid. TLC R_f = 0.54 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 2.4 Hz, 1H), 6.73 (d, *J* = 2.4 Hz, 1H), 4.00 (s, 3H).

5.1.10. 6-Allyl-5,7-dichloropyrazolo[1,5-*a*]pyrimidine (38i)

48% yield in s steps; a colorless oil. TLC R_f = 0.33 (EtOAc/hexane, 1/9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (d, *J* = 2.1 Hz, 1H), 6.72 (d, *J* = 2.1 Hz, 1H), 5.92 (m, 1H), 5.11–5.21 (m, 2H), 3.72 (m, 2H).

5.1.11. 5,7-Dichloro-6-methylimidazo[1,2-*a*]pyrimidine (44b)

73% yield; a white powder. TLC R_f = 0.76 (EtOAc); MS (APCI, Pos 20 V) *m*/*z* 202 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 1.5 Hz, 1H), 7.65 (d, *J* = 1.5 Hz, 1H), 2.54 (s, 3H).

5.1.12. 7-*N*,*N*-Dipropylamino-5-chloro-pyrazolo[1,5-*a*]pyrimidine (40a)

To a stirred solution of **38a** (6.60 g, 35 mmol) in THF (60 mL) was added dipropylamine (14.5 mL, 106 mmol) at 0 °C. Stirring was continued at ambient temperature for 3.5 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated to give **40a** (8.94 g, 100% yield) as a yellow solid, which was used for the next step without further purification. TLC R_f = 0.65 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, *J* = 2.1 Hz, 1H), 6.38 (d, *J* = 2.1 Hz, 1H), 5.87 (s, 1H), 3.75 (m, 4H), 1.74 (m, 4H), 0.95 (t, *J* = 7.4 Hz, 6H).

5.1.13. (5-Chloro-6-methylpyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40b)

94% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 3.0 Hz, 1H), 6.51 (d, *J* = 3.0 Hz, 1H), 3.45 (m, 4H), 1.50 (m, 4H), 0.84 (t, *J* = 6.0 Hz, 6H).

5.1.14. (5-Chloro-6-ethylpyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40c)

92% yield; a yellow oil; TLC R_f = 0.60 (EtOAc/hexane, 1/10); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 2.20 Hz, 1H), 6.52 (d, *J* = 2.20 Hz, 1H), 3.35–3.44 (m, 4H), 2.86 (q, *J* = 7.41 Hz, 2H), 1.42–1.58 (m, 4H), 1.29 (t, *J* = 7.41 Hz, 3H), 0.85 (t, *J* = 7.32 Hz, 6H).

5.1.15. (5-Chloro-6-propylpyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40d)

91% yield; yellow oil; TLC R_f = 0.47 (EtOAc/hexane, 1/10); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 2.38 Hz, 1H), 6.52 (d, *J* = 2.38 Hz, 1H), 3.33–3.44 (m, 4H), 2.69–2.79 (m, 2H), 1.60–1.76 (m, 2H), 1.41–1.56 (m, 4H), 1.07 (t, *J* = 7.32 Hz, 3H), 0.86 (t, *J* = 7.32 Hz, 6H).

5.1.16. (5-Chloro-6-isopropylpyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40e)

74% yield; a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, *J* = 2.3 Hz, 1H), 6.25 (d, *J* = 2.3 Hz, 1H), 3.79 (m, 1H), 3.36 (m, 4H), 1.46 (d, *J* = 9.0 Hz, 6H), 1.45 (m, 4H), 0.85 (t, *J* = 7.5 Hz, 6H).

5.1.17. (6-Butyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40f)

20% yield in 3 steps; a yellow oil; TLC R_f = 0.48 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, CDCl3) δ 8.01 (d, *J* = 2.20 Hz, 1H), 6.52 (d, *J* = 2.20 Hz, 1H), 3.33–3.43 (m, 4H), 2.74–2.82 (m, 2H), 1.58–1.70 (m, 2H), 1.41–1.55 (m, 6H), 1.00 (t, *J* = 7.32 Hz, 3H), 0.86 (t, *J* = 7.41 Hz, 6H).

5.1.18. (5-Chloro-6-cyclopentylpyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40g)

75% yield; a yellow oil; ^{1}H NMR (300 MHz, CDCl3) δ 8.02 (d, *J* = 2.4 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 3.79 (m, 1H), 3.38 (m, 4H), 2.26–2.08 (m, 2H), 2.02–1.80 (m, 4H), 1.80–1.64 (m, 2H), 1.47 (m, 4H), 0.85 (t, *J* = 7.5 Hz, 6H).

5.1.19. (5-Chloro-6-methoxypyrazolo[1,5-*a*]pyrimidin-7-yl)-dipropylamine (40h)

100% yield; a colorless oil.; TLC R_f = 0.72 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, CDCl3) δ 8.01 (d, *J* = 2.4 Hz, 1H), 6.47 (d, *J* = 2.4 Hz, 1H), 3.81 (s, 3H), 3.68 (m, 4H), 1.59 (m, 4H), 0.87 (t, *J* = 7.2 Hz, 6H).

5.1.20. (5-Chloropyrazolo[1,5-*a*]pyrimidin-7-yl)-(1-ethylpropyl)-amine (39a)

100% yield; a yellow oil. TLC R_f = 0.33 (EtOAc/hexane, 1/8); MS (APCI, Pos) *m*/*z* 239 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 2.38 Hz, 1H), 6.43 (d, *J* = 2.20 Hz, 1H), 6.26 (d, *J* = 9.52 Hz, 1H), 5.94 (s, 1H), 3.36–3.52 (m, 1H), 1.59–1.88 (m, 4H), 1.00 (t, *J* = 7.51 Hz, 6H).

5.1.21. (5-Chloro-6-methylpyrazolo[1,5-*a*]pyrimidin-7-yl)-(1-ethylpropyl)-amine (39b)

100% yield; a pale yellow oil. TLC R_f = 0.46 (EtOAc/hexane, 1/8); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 2.20 Hz, 1H), 6.39 (d, *J* = 2.20 Hz, 1H), 6.25 (d, *J* = 10.98 Hz, 1H), 3.90–4.07 (m, 1H), 2.45 (s, 3H), 1.53–1.81 (m, 4H), 0.98 (t, *J* = 7.41 Hz, 6H).

5.1.22. (6-Allyl-5-chloropyrazolo[1,5-*a*]pyrimidin-7-yl)-(1-ethylpropyl)-amine (39i)

94% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, *J* = 2.2 Hz, 1H), 6.41 (d, *J* = 2.2 Hz, 1H), 6.29 (d, *J* = 10.3 Hz, 1H), 6.13–5.89 (m, 1H), 5.28–5.15 (m, 1H), 5.08–4.94 (m, 1H), 4.06–3.87 (m, 1H), 3.66–3.53 (m, 2H), 1.80–1.49 (m, 4H), 0.96 (t, *J* = 7.4 Hz, 6H).

5.1.23. (5-Chloro-6-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)-(1-ethylpropyl)-amine (42b)

98% yield; a white powder, TLC R_f = 0.25 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 254 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 5.71 (d, *J* = 9.9 Hz, 1H), 4.31 (m, 1H), 2.45 (s, 3H), 1.63–1.57 (m, 4H), 0.95 (t, *J* = 7.3 Hz, 6H).

5.1.24. (7-Chloro-imidazo[1,2-*a*]pyrimidin-5-yl)-dipropylamine (45a)

63% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.7.65 (d, *J* = 1.7 Hz, 1H), 7.37 (d, *J* = 1.7 Hz, 1H), 6.20 (s, 1H), 3.29 (m, 4H), 1.66 (m, 4H), 0.92 (t, *J* = 7.3 Hz, 6H).

5.1.25. (7-Chloro-6-methylimidazo[1,2-*a*]pyrimidin-5-yl)-dipropylamine (45b)

A yellow oil. TLC R_f = 0.50 (EtOAc/hexane, 1/1); MS (APCI, Pos) *m*/*z* 267 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 1.7 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 3.24 (m, 4H), 2.35 (s, 3H), 1.55 (m, 4H), 0.86 (t, *J* = 7.4 Hz, 6H).

5.1.26. N^5-(2-Chloro-4-methoxyphenyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (4)

A mixture of **40a** (200 mg, 0.79 mmol) and 2-chloro-4-methoxyaniline (374 mg, 2.38 mmol) was heated at 200 °C for 3 h. The reaction mixture was cooled and diluted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, and then evaporated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/10–1/4) to afford **4** (199 mg, 67% yield). TLC R_f = 0.32 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 374 $(M+H)^+$; FABHRMS calcd for $C_{19}H_{25}ClN_5O$: 374.1748. Found: 374.1746; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94 (d, *J* = 9.0 Hz, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 6.99 (d, *J* = 3.0 Hz, 1H), 6.86 (dd, *J* = 3.0, 9.0 Hz, 1H), 6.50 (br s, 1H), 6.12 (d, *J* = 2.1 Hz, 1H), 5.36 (s, 1H), 3.81 (s, 3H), 3.62 (m, 4H), 1.73–1.58 (m, 4H), 0.90 (t, *J* = 7.5 Hz, 6H); mp 169–170 °C.

5.1.27. N^5-(2-chloro-4-methoxyphenyl)-N^7-(1-ethylpropyl)-pyrazolo[1,5-*a*]pyrimidine-5,7-diamine (2)

76% yield; brown powder. TLC R_f = 0.24 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 360 $(M+H)^+$; FABHRMS calcd for $C_{18}H_{23}ClN_5O$: 360.1591. Found: 360.1591; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, *J* = 8.97 Hz, 1H), 7.83 (d, *J* = 2.20 Hz, 1H), 6.99 (d, *J* = 2.93 Hz, 1H), 6.87 (dd, *J* = 8.97, 2.93 Hz, 1H), 6.51 (s, 1H), 6.13 (d, *J* = 2.20 Hz,

1H), 5.98 (d, J = 8.97 Hz, 1H), 5.29 (s, 1H), 3.82 (s, 3H), 3.29 (m, 1H), 1.66 (m, 4H), 0.97 (t, J = 7.51 Hz, 6H); mp 135–137 °C.

5.1.28. N^5-(2-chloro-4-methoxyphenyl)-N^7-(1-ethylpropyl)-6-methylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (3)

18% yield; a white powder. TLC R_f = 0.38 (EtOAc/hexane, 1/8); MS (APCI, Pos) 374 $(M+H)^+$; FABHRMS calcd for $C_{19}H_{25}ClN_5O$: 374.1748. Found: 374.174; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, J = 9.15 Hz, 1H), 7.81 (d, J = 2.20 Hz, 1H), 6.97 (d, J = 2.93 Hz, 1H), 6.90 (dd, J = 9.15, 2.93 Hz, 1H), 6.74 (s, 1H), 6.16 (d, J = 2.20 Hz, 1H), 5.78 (d, J = J = 10.25 Hz, 1H), 3.80 (s, 3H), 3.72 (m, 1H), 2.30 (s, 3H), 1.66 (m, 4H), 0.98 (t, J = 7.41 Hz, 6H); mp 118–119 °C.

5.1.29. N^5-(2-chloro-4-methoxyphenyl)-6-methyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (5)

56% yield; a white powder. TLC R_f = 0.52 (EtOAc/hexane, 1/8); MS (APCI, Pos) m/z 388 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{27}ClN_5O$: 388.1904. Found: 388.1902; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (d, J = 8.97 Hz, 1H), 7.86 (d, J =2 .20 Hz, 1H), 6.98 (d, J = 2.93 Hz, 1H), 6.85–6.96 (m, 2H), 6.23 (d, J = 2.20 Hz, 1H), 3.81 (s, 3H), 3.21–3.47 (m, 4H), 2.35 (s, 3H), 1.38–1.57 (m, 4H), 0.87 (t, J = 7.32 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.96, 12.09, 22.35, 54.57, 56.05, 93.28, 102.57, 113.43, 114.69, 122.75, 124.05, 130.01, 142.74, 148.01, 148.61, 153.28, 155.20; IR (KBr) 495.62, 635.43, 657.61, 694.25, 741.50, 756.92, 838.88, 860.10, 903.49, 1006.66, 1034.62, 1050.05, 1101.15, 1180.22, 1225.54, 1273.75, 1289.18, 1311.36, 1341.25, 1381.75, 1440.56, 1459.85, 1503.24, 1532.17, 1570.74, 1628.59, 2871.49, 2930.31, 2961.16, 3099.05 cm^{-1}; Anal. Calcd for $C_{20}H_{26}ClN_5O$; C, 61.93; H, 6.76; N, 18.05. Found: C, 61.93; H, 6.85; N, 18.32; mp 124–125 °C.

5.1.30. N^5-(2-chloro-4-methoxyphenyl)-6-ethyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (6)

20% yield; a yellow powder. TLC R_f = 0.34 (EtOAc/hexane, 1/9); MS (APCI, Pos) m/z 402 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{29}ClN_5O$: 402.2061. Found: 402.2060; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.64 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 2.2 Hz, 1H), 7.04 (s, 1H), 6.98 (d, J = 2.9 Hz, 1H), 6.92 (dd, J = 9.0, 2.9 Hz, 1H), 6.22 (d, J = 2.2 Hz, 1H), 3.81 (s, 3H), 3.24–3.39 (m, 4H), 2.92 (q, J = 7.7 Hz, 2H), 1.41–1.58 (m, 4H), 1.35 (t, J = 7.7 Hz, 3H), 0.88 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.73, 13.24, 18.75, 22.31, 54.64, 55.77, 93.00, 109.71, 113.15, 114.40, 122.47, 123.74, 129.69, 142.57, 147.57, 148.40, 152.46, 154.88; IR (KBr) 3438, 2961, 2930, 2871, 2853, 2835, 1621, 1590, 1571, 1532, 1498, 1466, 1439, 1409, 1375, 1342, 1286, 1277, 1244, 1224, 1214, 1180, 1100, 1051, 893, 882, 863, 858, 837, 808, 750 cm^{-1}; Anal. Calcd for $C_{21}H_{28}ClN_5O$; C, 62.75; H, 7.02; N, 17.42. Found: C, 63.03; H, 6.88; N, 17.22; mp 62–64 °C.

5.1.31. N^5-(2-chloro-4-methoxyphenyl)-N^7,N^7,6-*n*-propylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (7)

25% yield; a vory powder. TLC R_f = 0.32 (EtOAc/hexane, 1/10); MS (APCI, Pos) m/z 416 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{31}ClN_5O$: 416.2217. Found: 416.2229; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, J = 9.15 Hz, 1H), 7.86 (d, J = 2.01 Hz, 1H), 7.05 (s, 1H), 6.98 (d, J = 2.93 Hz, 1H), 6.91 (dd, J = 9.15, 2.93 Hz, 1H), 6.22 (d, J = 2.01 Hz, 1H), 3.81 (s, 3H), 3.29 (m, 4H), 2.82 (m, 2H), 1.72 (m, 2H), 1.50 (m, 4H), 1.13 (t, J = 7.32 Hz, 3H), 0.88 (t, J = 7.32 Hz, 6H); mp 74–75 °C.

5.1.32. N^5-(2-chloro-4-methoxyphenyl)-6-isopropyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (8)

20% yield; a yellow oil. TLC R_f = 0.64 (EtOAc/hexane, 1/10); MS (APCI, Pos) m/z 416 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{31}ClN_5O$: 416.2217. Found: 416.2224; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (d, J = 8.97 Hz, 1H), 7.85 (d, J = 2.01 Hz, 1H), 7.09 (s, 1H), 6.98 (d, J = 2.75 Hz, 1H), 6.91 (dd, J = 8.97, 2.75 Hz, 1H), 6.20 (d, J = 2.01 Hz, 1H), 4.24 (m, 1H), 3.81 (s, 3H), 3.38 (m, 2H), 3.20 (m, 2H), 1.50 (m, 10H), 0.88 (t, J = 7.32 Hz, 6H).

5.1.33. 6-butyl-N^5-(2-chloro-4-methoxyphenyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (9)

23% yield; a yellow oil. TLC R_f = 0.30 (EtOAc/hexane, 1/10); MS (APCI, Pos) m/z 430 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{33}ClN_5O$: 430.2374. Found: 430.2368; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 9.15 Hz, 1H), 7.86 (d, J = 2.20 Hz, 1H), 7.05 (s, 1H), 6.98 (d, J = 2.75 Hz, 1H), 6.92 (dd, J = 9.15, 2.75 Hz, 1H), 6.22 (d, J = 2.20 Hz, 1H), 3.81 (s, 3H), 3.30 (m, 4H), 2.84 (m, 2H), 1.58 (m, 8H), 1.03 (t, J = 7.23 Hz, 3H), 0.88 (t, J = 7.41 Hz, 6H).

5.1.34. N^5-(2-chloro-4-methoxyphenyl)-6-cyclopentyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (10)

29% yield; an orange oil. TLC R_f = 0.28 (EtOAc/hexane, 1/9); MS (APCI, Pos) m/z 442 $(M+H)^+$; FABHRMS calcd for $C_{24}H_{33}ClN_5O$: 442.2374. Found: 442.2378; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (d, J = 9.15 Hz, 1H), 7.85 (d, J = 2.20 Hz, 1H), 6.97 (d, J = 2.75 Hz, 1H), 6.90 (dd, J = 9.15, 2.75 Hz, 1H), 6.69 (s, 1H), 6.18 (d, J = 2.20 Hz, 1H), 4.22 (m, 1H), 3.81 (s, 3H), 3.31 (m, 4H), 1.98 (m, 6H), 1.81 (m, 2H), 1.46 (m, 4H), 0.88 (t, J = 7.41 Hz, 6H).

5.1.35. N^5-(2-chloro-4-methoxyphenyl)-6-methoxy-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (11)

45% yield; a colorless oil. TLC R_f = 0.38 (EtOAc/hexane, 1/8); MS (APCI, Pos) m/z 404 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{27}ClN_5O_2$: 404.1853. Found: 404.1853; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (d, J = 9.1 Hz, 1H), 7.85 (d, J = 2.2 Hz, 1H), 7.66 (s, 1H), 6.98 (d, J = 2.7 Hz, 1H), 6.91 (dd, J = 9.0, 2.7 Hz, 1H), 6.20 (d, J = 2.2 Hz, 1H), 3.84 (s, 3H), 3.81 (s, 3H), 3.52–3.66 (m, 4H), 1.51–1.69 (m, 4H), 0.88 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.43, 21.91, 52.80, 55.77, 61.36, 93.14, 113.15, 114.51, 121.69, 123.48, 124.29, 129.61, 141.76, 142.85, 146.77, 149.16, 154.70; IR (neat) 3397, 2962, 2934, 2873, 1622, 1613, 1583, 1546, 1538, 1531, 1488, 1463, 1441, 1413, 1395, 1381, 1278, 1257, 1221, 1207, 1182, 1095, 1043, 1010, 756 cm^{-1}.

5.1.36. N^5-(2-chloro-4-methoxyphenyl)-N^7-(1-ethylpropyl)-6-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-5,7-diamine (20)

8% yield; an ivory powder. TLC R_f = 0.28 (EtOAc/hexane, 1/1); MS (APCI, Pos) m/z 377 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, J = 9.15 Hz, 1H), 8.08 (s, 1H), 6.97 (m, 2H), 6.89 (dd, J = 9.15, 2.84 Hz, 1H), 5.35 (d, J = 9.89 Hz, 1H), 3.88 (m, 1H), 3.81 (s, 3H), 2.31 (s, 3H), 1.66 (m, 4H), 0.98 (t, J = 7.41 Hz, 6H); Anal. Calcd for $C_{18}H_{23}ClN_6O$; C, 57.67; H, 6.18; N, 22.42. Found: C, 57.83; H, 6.15; N, 22.07; mp 142–148 °C.

5.1.37. N^5-(2-chloro-4-methoxyphenyl)-N^7-(1-ethylpropyl)-N^5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-5,7-diamine (21)

4% yield; a yellow wax. TLC R_f = 0.23 (EtOAc/hexane, 1/8); MS (APCI, Pos) m/z 377 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.24 (d, J = 8.79 Hz, 1H), 7.09 (d, J = 2.93 Hz, 1H), 6.91 (dd, J = 8.79, 2.93 Hz, 1H), 5.50 (d, J = 8.24 Hz, 1H), 4.78 (s, 1H), 3.87 (s, 3H), 3.46 (s, 3H), 3.00 (m, 1H), 1.51 (m, 4H), 0.85 (t, J = 7.32 Hz, 6H).

5.1.38. N^5-(2-chloro-4-methoxyphenyl)-6-methyl-N^7,N^7-dipropyl[1,2,4]triazolo[1,5-*a*]pyrimidine-5,7-diamine (22)

56% yield; off-white needles. TLC R_f = 0.14 (EtOAc/hexane, 1/2); MS (APCI, Pos) m/z 389 $(M+H)^+$; FABHRMS calcd for $C_{19}H_{26}ClN_6O$: 389.1857. Found: 389.1858; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.78 (d, J = 8.97 Hz, 1H), 8.12 (s, 1H), 7.11 (s, 1H), 6.97 (d, J = 2.93 Hz, 1H), 6.89 (dd, J = 8.97, 2.93 Hz, 1H), 3.80 (s, 3H), 3.37 (dd, J = 8.15,

6.68 Hz, 4H), 2.33 (s, 3H), 1.49 (m, 4H), 0.85 (t, *J* = 7.32 Hz, 6H); mp 165–168 °C.

5.1.39. N^7-(2-chloro-4-methoxyphenyl)-6-methyl-N^5,N^5-dipropylimidazo[1,2-*a*]pyrimidine-5,7-diamine (24)

11% yield in 2 steps; a white powder. TLC R_f = 0.42 (MeOH/CH_2Cl_2, 1/9); MS (APCI, Pos) *m*/*z* 388 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{27}ClN_5O$: 388.1904. Found: 388.1905; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.95 (d, *J* = 9.15 Hz, 1H), 7.40 (d, *J* = 1.46 Hz, 1H), 7.27 (d, *J* = 1.46 Hz, 1H), 7.03 (s, 1H), 6.96 (d, *J* = 2.93 Hz, 1H), 6.90 (dd, *J* = 9.15, 2.93 Hz, 1H), 3.81 (s, 3H), 3.18 (m, 4H), 2.29 (s, 3H), 1.52 (m, 4H), 0.87 (t, *J* = 7.32 Hz, 6H); mp 189–191 °C.

5.1.40. N^5-(2-Chloro-4-methoxyphenyl)-N^5-(methoxymethyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (19)

To a stirred solution of **4** (200 mg, 536 μmol) in DMF (3.0 mL) was added NaH (26 mg, 643 μmol, 60% in mineral oil) at ambient temperature under argon atmosphere. After stirring for 30 min, the reaction mixture was treated with methoxymethylchloride (49 μL, 643 μmol), then stirred for 1 h, quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/9–1/3) to give **19** (207 mg, 92% yield) as a yellow oil. TLC R_f = 0.57 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 418 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{29}ClN_5O_2$: 418.2010. Found: 418.2002; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, *J* = 2.4 Hz, 1H), 7.34 (d, *J* = 9.0 Hz, 1H), 7.07 (d, *J* = 3.0 Hz, 1H), 6.90 (dd, *J* = 3.0, 9.0 Hz, 1H), 6.13 (d, *J* = 2.4 Hz, 1H), 4.81 (s, 1H), 3.84 (s, 3H), 3.48 (s, 3H), 3.46 (m, 4H), 1.53 (m, 4H), 0.77 (t, *J* = 7.2 Hz, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 11.26, 20.73, 53.02, 55.81, 56.50, 79.57, 79.96, 92.12, 113.84, 115.54, 132.25, 133.12, 134.51, 143.01, 149.65, 150.88, 156.71, 159.23; IR (neat) 2962, 2934, 1614, 1550, 1469, 1465, 1442, 1383, 1283, 1231, 1119, 1068 cm^{-1}.

5.1.41. N^5-(2-chloro-4-methoxyphenyl)-N^5-methyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (12)

61% yield; a pale yellow powder. TLC R_f = 0.30 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 388 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{27}ClN_5O$: 388.1904. Found: 388.1901; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 2.20 Hz, 1H), 7.23 (d, *J* = 8.60 Hz, 1H), 7.06 (d, *J* = 2.93 Hz, 1H), 6.89 (dd, *J* = 8.60, 2.93 Hz, 1H), 6.15 (d, *J* = 2.20 Hz, 1H), 4.78 (s, 1H), 3.85 (s, 3H), 3.41 (m, 7H), 1.53 (m, 4H), 0.77 (t, *J* = 7.41 Hz, 6H); mp 95–96 °C.

5.1.42. N^5-(2-chloro-4-methoxyphenyl)-N^5-ethyl-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (13)

94% yield; a white powder. TLC R_f = 0.20 (EtOAc/hexane, 1/8); MS (APCI, Pos) *m*/*z* 402 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 2.20 Hz, 1H), 7.20 (d, *J* = 8.79 Hz, 1H), 7.08 (d, *J* = 2.93 Hz, 1H), 6.90 (dd, *J* = 8.79, 2.93 Hz, 1H), 6.12 (d, *J* = 2.20 Hz, 1H), 4.72 (s, 1H), 4.19 (m, 1H), 3.85 (s, 3H), 3.78 (m, 1H), 3.39 (m, 4H), 1.51 (m, 4H), 1.20 (t, *J* = 7.14 Hz, 3H), 0.76 (t, *J* = 7.41 Hz, 6H); Anal. Calcd for $C_{21}H_{28}ClN_5O$; C, 62.75; H, 7.02; N, 17.42. Found: C, 62.75; H, 7.09; N, 17.21; mp 107–108 °C.

5.1.43. N^5-(2-chloro-4-methoxyphenyl)-N^5,N^7,N^7-tripropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (14)

98% yield; an ivory wax. TLC R_f = 0.56 (EtOAc/hexane, 1/3); MS (APCI, Pos) *m*/*z* 416 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{31}ClN_5O$: 416.2217. Found: 416.2222; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 2.2 Hz, 1H), 7.20 (d, *J* = 8.6 Hz, 1H), 7.07 (d, *J* = 2.7 Hz, 1H), 6.89 (dd, *J* = 8.7, 2.7 Hz, 1H), 6.11 (d, *J* = 2.2 Hz, 1H), 4.66–4.75 (m, 1H), 3.95–4.23 (m, 1H), 3.85 (s, 3H), 3.51–3.72 (m, 1H), 3.34–3.45 (m, 4H), 1.42–1.70 (m, 6H), 0.94 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.18, 11.47, 20.45, 21.19, 50.41, 52.80, 55.76, 80.74, 91.66, 113.91, 115.69, 132.21, 134.00, 135.22, 142.94, 149.42, 151.32, 157.03, 159.09; IR (KBr) 580, 627, 752, 842, 880, 903, 938, 1033, 1047, 1103, 1138, 1161, 1181, 1226, 1260, 1285, 1326, 1380, 1390, 1441, 1463, 1497, 1525, 1550, 1615, 2873, 2962, 3094 cm^{-1}; .

5.1.44. N^5-(2-chloro-4-methoxyphenyl)-N^5-(2-methyl-2-propenyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (15)

95% yield; a yellow oil. TLC R_f = 0.25 (EtOAc/hexane, 1/8); MS (APCI, Pos) *m*/*z* 428 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{31}ClN_5O$: 428.2217. Found: 428.2218; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 2.20 Hz, 1H), 7.22 (d, *J* = 8.79 Hz, 1H), 7.06 (d, *J* = 2 .93 Hz, 1H), 6.86 (dd, *J* = 8.79, 2.93 Hz, 1H), 6.10 (d, *J* = 2.20 Hz, 1H), 5.07 (m, 1H), 4.80 (m, 3H), 3.99 (m, 1H), 3.84 (s, 3H), 3.42 (m, 4H), 1.82 (s, 3H), 1.55 (m, 4H), 0.77 (t, *J* = 7.41 Hz, 6H).

5.1.45. N^5-allyl-N5-(2-chloro-4-methoxyphenyl)-N^7,N^7-dipropyl pyrazolo[1,5-*a*]pyrimidine-5,7-diamine (16)

100% yield; a white powder. TLC R_f = 0.21 (EtOAc/hexane, 1/8); MS (APCI, Pos) *m*/*z* 414 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.81 (d, *J* = 2.20 Hz, 1H), 7.34 (d, *J* = 8.79 Hz, 1H), 7.22 (d, *J* = 2.93 Hz, 1H), 7.02 (dd, *J* = 8.79, 2.93 Hz, 1H), 6.00 (d, *J* = 2.20 Hz, 1H), 5.92 (m, 1H), 5.09 (m, 2H), 4.73 (m, 2H), 4.16 (m, 1H), 3.80 (s, 3H), 3.43 (m, 4H), 1.46 (m, 4H)0.69 (t, *J* = 7.41 Hz, 6H); Anal. Calcd for $C_{22}H_{28}ClN_5O$; C, 63.83; H, 6.82; N, 16.92. Found: C, 63.70; H, 6.82; N, 16.61; mp 85–86 °C.

5.1.46. N^5-(2-chloro-4-methoxyphenyl)-N^5-(cyclopropylmethyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (17)

90% yield; a pink powder. TLC R_f = 0.50 (EtOAc/hexane, 1/6); MS (APCI, Pos) *m*/*z* 428 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{31}ClN_5O$: 428.2217. Found: 428.2222; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 2.20 Hz, 1H), 7.29 (d, *J* = 8.60 Hz, 1H), 7.06 (d, *J* = 2.93 Hz, 1H), 6.89 (dd, *J* = 8.79, 2.93 Hz, 1H), 6.11 (d, *J* = 2.20 Hz, 1H), 4.73 (s, 1H), 4.03–4.23 (m, 1H), 3.85 (s, 3H), 3.33–3.56 (dd, *J* = 8.33, 6.87 Hz, 5H), 1.42–1.60 (m, 4H), 1.00–1.18 (m, 1H), 0.76 (t, *J* = 7.41 Hz, 6H), 0.34–0.46 (m, 2H), 0.06–0.20 (m, 2H); IR (KBr) 772, 789, 834, 880, 905, 1020, 1039, 1072, 1105, 1149, 1181, 1202, 1226, 1259, 1289, 1329, 1388, 1439, 1461, 1499, 1518, 1550, 1614, 2873, 2962, 3007, 3433 cm^{-1}; Anal. Calcd for $C_{23}H_{30}ClN_5O$; C, 64.55; H, 7.07; N, 16.36. Found: C, 64.73; H, 7.13; N, 16.26; mp 61–66 °C.

5.1.47. N^5-(2-chloro-4-methoxyphenyl)-N^5-(2-methoxyethyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (18)

91% yield; a yellow oil. TLC R_f = 0.57 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 432 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{31}ClN_5O_2$: 432.2166. Found: 432.2165; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 2.2 Hz, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 7.05 (d, *J* = 2.7 Hz, 1H), 6.89 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.11 (d, *J* = 2.2 Hz, 1H), 4.73 (s, 1H), 4.35–4.55 (m, 1H), 3.84 (s, 3H), 3.55–3.81 (m, 3H), 3.40 (dd, *J* = 8.5, 6.1 Hz, 4H), 3.32 (s, 3H), 1.43–1.59 (m, 4H), 0.76 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ11.17, 20.47, 48.29, 52.83, 55.75, 58.60, 70.45, 80.62, 91.76, 113.90, 115.52, 132.50, 134.14, 135.01, 142.95, 149.49, 151.23, 156.90, 159.17; IR (KBr) 627, 754, 861, 903, 1032, 1046, 1104, 1124, 1192, 1223, 1260, 1286, 1387, 1464, 1497, 1524, 1550, 1614, 2874, 2962 cm^{-1};

5.1.48. N^5-(2-chloro-4-methoxyphenyl)-N^5-(methoxymethyl)-N^7,N^7-dipropylpyrazolo[1,5-*a*]pyrimidine-5,7-diamine (23)

90% yield; a pale yellow oil. TLC R_f = 0.57 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 418 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{29}ClN_5O_2$: 418.2010. Found: 418.2002; ^{1}H NMR (300 MHz, DMOSO-d_6) δ 7.80 (d, *J* = 2.2 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 2.9 Hz, 1H), 7.04 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.00 (d, *J* = 2.2 Hz, 1H), 5.19 (s, 2H), 4.92 (s, 1H), 3.84 (s, 3H), 3.48–3.57 (m, 4H), 3.37 (s, 3H),

1.44–1.60 (m, 4H), 0.76 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, DMOSO-d_6) δ 11.26, 20.73, 53.02, 55.81, 56.50, 79.57, 79.96, 92.12, 113.84, 115.54, 132.25, 133.12, 134.51, 143.01, 149.65, 150.88, 156.71, 159.23; IR (neat) 647, 741, 754, 843, 861, 904, 1028, 1046, 1068, 1118, 1193, 1231, 1262, 1283, 1329, 1382, 1441, 1464, 1496, 1530, 1550, 1555, 1614, 2874, 2962 cm^{-1}.

5.1.49. N^7-(2-chloro-4-methoxyphenyl)-N^7-methyl-N^5,N^5-dipropylimidazo[1,2-*a*]pyrimidine-5,7-diamine (25)

57% yield in 2 steps; a purple oil. TLC R_f = 0.36 (MeOH/EtOAc, 1/10); MS (APCI, Pos) m/z 388 (M+H)$^+$; FABHRMS calcd for $C_{20}H_{27}ClN_5O$: 388.1904. Found: 388.1903; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, J = 1.65 Hz, 1H), 7.21 (d, J = 8.79 Hz, 1H), 7.12 (d, J = 1.65 Hz, 1H), 7.06 (d, J = 2.93 Hz, 1H), 6.89 (dd, J = 8.79, 2.93 Hz, 1H), 5.09 (s, 1H), 3.86 (s, 3H), 3.45 (s, 3H), 2.97 (m, 4H), 1.48 (m, 4H), 0.80 (t, J = 7.41 Hz, 6H).

5.1.50. N^7-(2-chloro-4-methoxyphenyl)-N^7-ethyl-N^5,N^5-dipropylimidazo[1,2-*a*]pyrimidine-5,7-diamine (26)

34% yield in 2 steps; a pale purple powder. TLC R_f = 0.23 (MeOH/EtOAc, 1/9); MS (APCI, Pos) m/z 402 (M+H)$^+$; FABHRMS calcd for $C_{21}H_{28}ClN_5O$: 401.1982. Found: 401.1994; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7 .37 (d, J = 1.65 Hz, 1H), 7.19 (d, J = 8.60 Hz, 1H), 7.12 (d, J = 1.65 Hz, 1H), 7.08 (d, J = 2.93 Hz, 1H), 6.90 (dd, J = 8.60, 2.93 Hz, 1H), 5.02 (s, 1H), 4.14–4.39 (m, 1H), 3.86 (s, 3H), 3.75–3.86 (m, 1H), 2.84–3.06 (m, 4H), 1.36–1.59 (m, 4H), 1.22 (t, J = 7.14 Hz, 3H), 0.80 (t, J = 7.41 Hz, 6H); IR (KBr) 473, 524, 566, 583, 612, 669, 677, 731, 746, 760, 784, 795, 808, 852, 881, 935, 1030, 1049, 1118, 1132, 1151, 1165, 1217, 1231, 1269, 1288, 1381, 1407, 1426, 1442, 1496, 1523, 1530, 1624, 1879, 2053, 2503, 2550, 2872, 2963, 3054, 3085, 3169 cm^{-1}; Anal. Calcd for $C_{21}H_{28}ClN_5O$; C, 62.75; H, 7.02; N, 17.42. Found: C, 62.96; H, 7.21; N, 16.96; mp 111–113 °C.

5.1.51. Mathyl 2*H*-pyrazol-3-ylaminocarbothioylcarbamate (46)

To a stirred suspension of **34** (5.0 g, 60 mmol) in EtOAc (36 mL) and benzene (180 mL) was added dropwise a solution of ethoxycarbonyl isothiocyanate (7.9 g, 60 mmol) in benzene (60 mL) at 5 °C under argon atmosphere. After being stirred for 21 h at ambient temperature, the resulting precipitates were collected by filtration, and purified by recrystallization from EtOAc/hexane to give **46** (7.20 g, 56% yield) as an ivory powder. TLC R_f = 0.34 (MeOH/CH_2Cl_2, 1/9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.92 (br s, 1H), 10.57 (br, 1H), 8.41 (br s, 1H), 7.55 (d, J = 2.3 Hz, 1H), 7.05 (br s, 1H), 4.29 (q, J = 7.1 Hz, 2H), 1.34 (t, J =7.1 Hz, 3H).

5.1.52. 2-Thioxo-2,3-dihydro-1*H*-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (47)

A solution of **46** (7.2 g) in 2 N NaOH (70 mL) was stirred for 1.5 h at ambient temperature. To the stirred reaction mixture was added 2 N H_2SO_4 (100 mL). The resulting precipitates were collected by filtration, washed with water, and then dried under vacuum to give **47** (5.3 g, 100% yield) as a yellow powder. TLC R_f = 0.16 (MeOH/CH_2Cl_2, 1/9); ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.42 (s, 1H), 12.70 (s, 1H), 7.86 (d, J = 1.8 Hz, 1H), 5.88 (d, J = 1.8 Hz, 1H).

5.1.53. 2-Methylsulfanyl-3*H*-pyrazolo[1,5-*a*][1,3,5]triazin-4-one (48)

To a stirred solution of **47** (5.30 g, 34 mmol) in EtOH (136 mL) and 1.73 N NaOH (39.2 mL) was added methyl iodide (2.1 mL, 34 mmol) at ambient temperature, the resulting precipitates were collected by filtration, and then dissolved in water (210 mL). To the resulting solution was added 2 N H_2SO_4 (20 mL). The resulting precipitates were collected by filtration, washed with water, and then dried under vacuum to give **48** (4.63 g, 75% yield) as a white plate. TLC R_f = 0.45 (MeOH/CH_2Cl_2, 1/9); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.86 (s, 1H), 7.96 (d, J = 2.0 Hz, 1H), 6.34 (d, J = 2.0 Hz, 1H), 2.52 (s, 3H).

5.1.54. 4-Chloro-2-methylsulfanyl-pyrazolo[1,5-*a*][1,3,5]-triazine (49)

To a stirred suspension of **48** (2.0 g, 11 mmol) in $POCl_3$ (20 mL) was added *N,N*-diethylaniline (0.7 mL) at ambient temperature. The reaction mixture was allowed to heat at reflux temperature. After 1.5 h, the reaction mixture was cooled to ambient temperature and evaporated. The residue was dissolved in toluene. The organic layer was washed with brine and evaporated to give **49** (2.0 g, 91% yield). TLC R_f = 0.57 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, J = 2.2 Hz, 1H), 6.50 (d, J = 2.2 Hz, 1H), 2.60 (s, 3H).

5.1.55. 4-Dipropylamino-2-methylsulfanyl-pyrazolo[1,5-*a*]-[1,3,5]triazine (50)

To a stirred solution of **49** (1.0 g, 5.0 mmol) in THF (5.0 mL) were added triethylamine (1.0 mL, 7.5 mmol) and dipropylamine (1.0 mL, 7.5 mL) at ambient temperature and stirring was continued for 14 h. After filtration, solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/10) to give **50** (1.1 g, 83% yield) as a white powder. TLC R_f = 0.85 (EtOAc/Hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (d, J = 2.2 Hz, 1H), 6.13 (d, J = 2.2 Hz, 1H), 3.94 (m, 4H), 2.52 (s, 3H), 1.75 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H).

5.1.56. 4-Dipropylamino-2-methylsulfonyl-pyrazolo[1,5-*a*][1,3,5]triazine (51)

To a stirred solution of **50** (600 mg, 2.26 mmol) in CH_2Cl_2 (11 mL) was added *m*-CPBA (1.1 g, 4.52 mmol) at 0 °C, and then stirring was continued for 4 h at ambient temperature. The reaction mixture was quenched with sodium sulfite aqueous solution and extracted with dichloromethane. The combined organic layers were washed with 2 N NaOH, brine and dried over $MgSO_4$. Removal of the solvent in vacuo afforded **55** (590 mg, 88% yield) as a white powder, which was used for the next step without further purification. TLC R_f = 0.62 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, J = 2.2 Hz, 1H), 6.54 (d, J = 2.2 Hz, 1H), 4.30 (m, 2H), 3.74 (m, 2H), 3.27 (s, 3H), 1.80 (m, 4H), 0.98 (t, J = 7.4 Hz, 6H).

5.1.57. N^2-Methyl-N^2-(2-chloro-4-methoxypnenyl)-N^4,N^4-dipropyl-pyrazolo[1,5-*a*][1,3,5]triazine-2,4-diamine (27)

A mixture of **51** (250 mg, 0.84 mmol) and *N*-methyl-2-chloro-4-methoxyaniline (151 mg, 0.88 mmol) was heated up to 130 °C for 21 h, and then 160 °C for 12 h. After cooling to ambient temperature, the reaction mixture was purified by column chromatography on silica gel (EtOAc/hexane, 1/3) to give **27** (69 mg, 22% yield) as a white powder. TLC R_f = 0.67 (EtOAc/hexane, 1/3); MS (APCI, Pos) m/z 389 (M + H) $^+$; FABHRMS calcd for $C_{19}H_{26}ClN_6O$: 389.1857. Found: 389.1859;^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J = 2.2 Hz, 1H), 7.17 (d, J = 8.6 Hz, 1H), 6.98 (d, J = 2.9 Hz, 1H), 6.81 (dd, J = 8.6, 2.9 Hz, 1H), 5.96 (m, 1H), 3.80 (s, 3H), 3.57 (m, 4H), 3.38 (s, 3H), 1.53 (m, 4H), 0.75 (m, 6H); mp 80–81 °C

5.1.58. 8-(1-Ethylpropyl)-5-chloro-8*H*-pyrazolo[1,5-*a*]pyrrolo-[3,2-*e*]pyrimidine (52)

To a stirred solution of **39i** (500 mg, 1.8 mmol) in THF (6.0 mL) and water (6.0 mL) were added osmium tetraoxide (349 mg, 4 wt% water solution) and sodium periodade (770 mg, 3.6 mmol) at ambient temperature and stirring was continued for 4 h. The reaction mixture was diluted with water and extracted with ethyl acetate. The combine organic layers were washed with water, brine, dried over $MgSO_4$, and then

evaporated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/20) to give **52** (228 mg, 48% yield) as a colorless oil. TLC R_f = 0.48 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 263 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 2.4 Hz, 1H), 6.93 (d, *J* = 3.6 Hz, 1H), 6.71 (d, *J* = 3.6 Hz, 1H), 6.62 (d, *J* = 2.4 Hz, 1H), 5.60 (m, 1H), 2.07–1.75 (m, 4H), 0.82 (t, *J* = 7.5 Hz, 6H).

5.1.59. *N*-(2-Chloro-4-methoxyphenyl)-8-(1-ethylpropyl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-amine (28)

A mixture of **52** (228 mg, 0.87 mmol) and 2-chloro-4-methoxyaniline (0.6 mL) was heated at 100 °C for 2 days. The reaction mixture was cooled and diluted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, and then evaporated. The residue was purified by chromatography on silica gel (EtOAc/hexane, 1/7) to give **28** (154 mg, 46% yield) as a brown solid. TLC R_f = 0.38 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 384 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 8.58 (d, *J* = 9.3 Hz, 1H), 7.86 (d, *J* = 2.1 Hz, 1H), 7.07 (br s, 1H), 7.00 (d, *J* = 2.7 Hz, 1H), 6.92 (dd, *J* = 2.7, 9.3 Hz, 1H), 6.84 (d, *J* = 3.6 Hz, 1H), 6.48 (d, *J* = 3.6 Hz, 1H), 6.31 (d, *J* = 2.1 Hz, 1H), 5.91 (m, 1H), 3.82 (s, 3H), 2.05–1.74 (m, 4H), 0.84 (t, *J* = 7.5 Hz, 6H); IR (KBr) 3429, 2966, 1620, 1575, 1496, 1425, 1284, 1049 cm^{-1}; Anal. Calcd for $C_{20}H_{22}ClN_5O$; C, 62.58; H, 5.78; N, 18.24. Found: C, 62.42; H, 5.80; N, 18.00; mp 132–134 °C.

5.1.60. *N*-(2-ethyl-4-methoxyphenyl)-8-(1-ethylpropyl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-amine (29)

9% yield; an ivory powder. TLC R_f = 0.51 (EtOAc/hexane, 1/1); MS (APCI, Pos) *m*/*z* 378 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{28}N_5O$: 378.2294. Found: 378.2300; ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 6.86 (d, *J* = 2.7 Hz, 1H), 6.78 (dd, *J* = 8.7, 3.0 Hz, 1H), 6.62 (d, *J* = 3.1 Hz, 1H), 6.52 (s, 1H), 6.17 (s, 1H), 5.91 (s, 1H), 5.71 (s, 1H), 3.85 (s, 3H), 2.67 (q, *J* = 7.5 Hz, 2H), 1.62–2.03 (m, 4H), 1.20 (t, *J* = 7.5 Hz, 3H), 0.81 (t, *J* = 7.3 Hz, 6H); IR (KBr) 3181, 2996, 2966, 2934, 2874, 2833, 1617, 1573, 1548, 1522, 1497, 1455, 1421, 1381, 1367, 1324, 1291, 1273, 1261, 1234, 1215, 1209, 1190, 1128, 1029, 870, 765, 750, 716 cm^{-1}; mp 165–170 °C.

5.1.61. *N*-(4-chloro-2-ethylphenyl)-8-(1-ethylpropyl)-8*H*-pyrazolo[1,5-*a*]pyrrolo[3,2-*e*]pyrimidin-5-amine (30)

20% yield; a white powder. TLC R_f = 0.53 (EtOAc/hexane, 1/2); MS (MALDI, Pos) *m*/*z* 382 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{25}ClN_5$: 382.1798. Found: 382.1788; ^1H NMR (300 MHz, $CDCl_3$) δ7.78–7.90 (m, 2H), 7.17–7.31 (m, 2H), 6.76 (d, *J* = 3.5 Hz, 1H), 6.60 (s, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 6.11 (d, *J* = 3.5 Hz, 1H), 5.93 (s, 1H), 2.70 (q, *J* = 7.5 Hz, 2H), 1.72–2.04 (m, 4H), 1.27 (t, *J* = 7.5 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 6H); IR (KBr) 2966, 2933, 2875, 1617, 1586, 1573, 1537, 1499, 1486, 1473, 1426, 1414, 1381, 1303, 1277, 1252, 1212, 1126, 910, 874, 834, 808, 743, 710, 692, 629 cm^{-1}; mp 147–149 °C.

5.1.62. 8-(1-ethylpropyl)-*N*-mesityl-8*H*-pyrazolo[1,5-*a*]pyrrolo-[3,2-*e*]pyrimidin-5-amine (32)

18% yield; an ivory powder. TLC R_f = 0.32 (EtOAc/toluene, 1/9); MS (MALDI, Pos) *m*/*z* 362 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{28}N_5$: 362.2345. Found: 362.2337; ^1H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, *J* = 2.2 Hz, 1H), 6.98 (s, 2H), 6.57 (d, *J* = 3.5 Hz, 1H), 6.40 (s, 1H), 6.14 (d, *J* = 2.2 Hz, 1H), 5.92 (s, 1H), 5.45 (s, 1H), 2.35 (s, 3H), 2.22 (s, 6H), 1.67–2.00 (m, 4H), 0.80 (t, *J* = 7.4 Hz, 6H); IR (KBr) 2965, 2922, 2878, 2857, 1622, 1572, 1545, 1505, 1485, 1454, 1429, 1378, 1322, 1287, 747 cm^{-1}; mp 202–203 °C.

5.1.63. 5-(4-Ethyl-2-methoxyphenoxy)-8-(1-ethylpropyl)-8*H*-pyrazolo[1,5-a]pyrrolo[3,2-e]pyrimidine (33)

To a stirred solution of **56** (100 mg, 0.38 mmol) and 4-ethyl-2-methoxyphenol (64 mg, 0.42 mmol) in DMF (0.8 mL) was added potassium carbonate (79 mg, 0.57 mmol). The reaction mixture was allowed to heat up to 100 °C, stirred for 22 h and then cooled to ambient temperature. The reaction mixture was diluted with ethyl acetate. The organic layer was washed with water, brine, dried over $MgSO_4$, and then evaporated. The residue was purified by chromatography on silica gel (EtOAc/hexane, 0/100∼1/4) to give **33** (64 mg, 45% yield) as a white powder. TLC R_f = 0.43 (EtOAc/hexane, 1/4);MS (APCI, Pos) *m*/*z* 379 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{27}N_4O_2$: 379.2134. Found: 379.2155; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, *J* = 2.2 Hz, 1H), 7.15 (d, *J* = 8.1 Hz, 1H), 6.81–6.92 (m, 3H), 6.76 (d, *J* = 3.5 Hz, 1H), 6.30 (d, *J* = 2.2 Hz, 1H), 5.90 (s, 1H), 3.78 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.76–2.04 (m, 4H), 1.30 (t, *J* = 7.6 Hz, 3H), 0.85 (t, *J* = 7.4 Hz, 6H),; IR (KBr) 22961, 2930, 2872, 1619, 1606, 1581, 1507, 1495, 1468, 1461, 1421, 1414, 1402, 1365, 1329, 1280, 1271, 1245, 1225, 1187, 1152, 1124, 1116, 1034, 986, 906, 851, 808, 765, 754, 714 cm^{-1}; mp 166–167 °C.

5.1.64. 8-(1-ethylpropyl)-5-(mesitylmethyl)-8*H*-pyrazolo[1,5-*a*]-pyrrolo[3,2-*e*]pyrimidine (31)

To a stirred solution of **56** (100 mg, 0.38 mmol) in THF (2.0 mL) were added $Pd(PPh_3)_4$ (22 mg, 0.019 mmol) and 2,4,6-trimethylbenzyl zinc bromide (0.95 mL, 0.76 mmol), which was prepared from 2-bromomethyl-1,3,5-trimethyl-benzene (1.0 g, 4.71 mmol) and zinc (350 mg, 5.32 mmol), 1,2-dibromoethane (16 μL, 0.19 mmol) and TMSCl (18μL, 0.14 mmol) in THF (1.1 mL) by the conventional method, at room temperature under argon atmosphere. The reaction mixture was allowed to heat up to 60 °C. After being stirred for 1 h, the reaction mixture was cooled, quenched with NH_4Cl aq, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/100–1/9) to afford **31** (94 mg, 69% yield) as an ivory powder. TLC R_f = 0.50 (EtOAc/toluene, 1/9); MS (MALDI, Pos) *m*/*z* 361 $(M+H)^+$; FABHRMS calcd for C23H29N4: 361.2392. Found: 361.2402; ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, *J* = 2.4 Hz, 1H), 6.91 (s, 2H), 6.73 (d, *J* = 3.7 Hz, 1H), 6.56 (d, *J* = 2.4 Hz, 1H), 6.06 (d, *J* = 3.7 Hz, 1H), 5.93 (s,, 1H), 4.41 (s, 2H), 2.31 (s, 3H), 2.27 (s, 6H), 2.02–1.69 (m, 4H), 0.79 (t, *J* = 7.3 Hz, 6H); IR (KBr) 2965, 2919, 1614, 1566, 1491, 1385, 1364, 1201, 1175, 739, 680 cm^{-1}.

5.2. Biology

5.2.1. Membrane preparations

CHO-K1 cells expressing CRF receptor type 1 were washed with phosphate buffered saline (PBS), scraped and pelleted by centrifugation. Cell pellets were homogenized with binding assay buffer (50 mmol/l Tris–HCl buffer containing 10 mmol/l MgCl, 2 mmol/l EDTA, and 10 TIU/l aprotinin) and centrifuged at 10,000 g for 15 min at 4 °C. The pellet was suspended in the assay buffer, and used as crude membrane preparations for binding studies. Protein concentration was determined according to Bradford 1976

5.2.2. Binding assay

Binding assays for ^{125}I human/rat CRF were done according to reported method (De Souza, 1987;Grigoriadis et al., 1996) but with slight modification. The reaction was initiated by incubating 49 μl of membrane preparations with 50 μl of 0.5 nmol/l ^{125}I human/rat CRF and 1 μl of test compound (1∼10000 nmol/L). The reaction mixture was incubated for 2 h at room temperature, and terminated by centrifugation at 15,000 g for 10 min, and pellet was washed with PBS containing with 0.01% Triton X-100. The radioactivity was measured in a γ-counter. Nonspecific bindings were determined in the presence of unlabeled 1 μmol/l human/rat CRF. Specific binding was determined by subtracting nonspecific binding from total binding. Concentration of the test compound

that caused 50% inhibition of specific radiolabeled ligand binding (IC_{50} values) was determined from each concentration–response curve.

5.2.3. cAMP Assay

CHO-K1 cells expressing CRF receptor type 1 dispersed at 1×10^4 cells/well in 96-well plates were incubated overnight. The culture medium was removed, the cells were washed twice with F-12 nutrient mixture, and then 178 μl of assay medium (F12 nutrient mixture containing 1 mmol/l 3-Isobutyl-1-methylxanthine, a phosphodiesterase inhibitor) was added. After incubation for 10 min at 37 °C, cells were treated with 2 μl of test compound (1–10000 nmol/l) and 20 μl of assay medium containing 10 nmol/l CRF. After the treated cells were incubated for 15 min at 37 °C, supernatants were aspirated, and cells were immediately chilled to terminate further reactions. cAMP formed in the cells was determined using a cAMP enzyme immunoassay system.

The cAMP level under respective treatments was determined by mean of corresponding 2 wells of the Blank group from that of treatment group. Concentration of the test compound that caused 50% inhibition of cAMP production (IC_{50} values) was determined from each concentration–response curve.

5.2.4. Elevated plus-maze test in swim stress-loaded rats

5.2.4.1. 1 Animals. Male Sprague-Dawley rats weighing 230–280 g (Charles River Japan) were used. Rats were accommodated for more than a week in a room at 24 ± 2 °C, 55 ± 15% relative humidity with controlled 12 h dark-light cycles (alternating 12 h cycles with illumination from fluorescent light: 08:00–20:00 h) and were allowed free access to food and water. They were housed in groups of 5 or 6 rats per cage until experiments. All experimental procedures were approved by the Animal Care and Use Committee of Ono Pharmaceutical Co. Ltd. and conducted in accordance with the 'Guidelines on the Use of Experimental Animals'.

5.2.4.2. Elevated plus-maze test in rats, swim-stress induced anxiety-like behavior. The elevated plus-maze apparatus was made of Plexiglas and consisted of four arms (50 cm long × 10 cm wide): two had 40 cm high walls (closed arms), and two had no walls (open arms). The maze was elevated to a height of 50 cm. In the experiments of swim-stress paradigm, rats were forced to swim for 90 sec in a pool (40 × 30 × 38 cm) filled with water (depth of 25 cm) maintained at 22 ± 2 °C prior to the elevated plus-maze test. In non-stress conditions, the rats in the control group were not subjected to forced swim stress. After forced swim stress for 90 s, rats were removed from the pool and dried with a paper towel. For testing, rats were placed individually onto the center of the maze facing a closed arm and behavior was recorded for 5 min with a video camera. The images were analyzed using a computerized behavior tracking and analysis system (EthoVision Version 3.0, Noldus Information Technology). The primary measures were the time spent in open arms and the number of entries in open arms.

Experiments were performed 1 h after oral administration of vehicle (0.5% MC (w/v)), test compounds (1, 3, 10 m/kg).

5.2.4.3. Data and Statistical analysis. The results of the elevated plus-maze test were expressed as mean ± S.E. values. Comparisons between the vehicle and control groups were performed using the *t*-test, while differences between the vehicle and test compound groups were compared with the Dunnett test. Probabilities of < 5% ($p < 0.05$) were considered statistically significant.

5.3. Single dose rat pharmacokinetic study of 5

Single dose pharmacokinetics of **5** was studied in rats. Formulation for intravenous injection was prepared using saline containing 30% HP-β-CD (w/v). Formulation for oral dosing was prepared using saline containing 0.5% MC(w/v). Test compounds (1 mg/kg) were dosed intravenously to the fasted male rats ($n = 4$). Test compounds (10 mg/kg) were dosed orally to the fasted male rats ($n = 4$). After dosing, blood samples (250 μl) were collected from the jugular vein using a heparinized syringe at the selected time points (iv: pre-dosing, 2, 5, 15, 30 min and 1, 2, 3, 4, 8, 24, 48, 72 h; po: 1, 2, 4, 6, 8, 24, 48, 72 h, respectively). The blood samples were ice-chilled and then centrifuged at 12,000 rpm for 2 min at room temperature to obtain plasma, which was preserved at −70 °C in a freezer. The AUC, C_{max}, T_{max}, $T_{1/2}$, V_{ss} and CL were obtained by measuring the time course of the plasma concentration of the test compounds. Bioavailability(F) was calculated according to the following equation:

$$F(\%) = (AUC_{po}/D_{po})/AUC_{iv}/D_{iv}) \times 100$$

AUC_{po}: AUC after oral dosing;
AUC_{iv}: AUC after intravenous dosing;
D_{po}: Dosage of oral administration;
D_{iv}: Dosage of intravenous administration.

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