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Pyrrolo[1,2-*b*]pyridazines, pyrrolo[2,1-*f*]triazin-4(3*H*)-ones, and related compounds as novel corticotropin-releasing factor 1 (CRF_1) receptor antagonists

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ABSTRACT

To identify structurally novel corticotropin-releasing factor 1 (CRF_1) receptor antagonists, a series of bicyclic core analogs pyrrolo[1,2-*b*]pyridazines and pyrrolo[2,1-*f*]triazin-4(3*H*)-ones, which were designed based on a monocyclic core antagonist, was synthesized and evaluated. Among the compounds tested, 2-difluoromethoxy-4-methylpyridin-5-yl analog **27** was found to show efficacy in a dose-dependent manner in an elevated plus maze test in rats. The discovery process and structure–activity relationship is presented.

1. Introduction

Recent studies have shown that corticotropin-releasing factor (CRF) is a key regulator of the hypothalamic–pituitary–adrenal (HPA) axis, coordinating the endocrine, behavioral and autonomic responses to stress. It has been postulated that hypersecretion of CRF may be involved in stress-related disorders, including anxiety and depression.[1]

Extensive preclinical studies on CRF agonists and antagonists (as well as limited clinical studies on CRF) have provided the theoretical foundation for the hypothesis that abnormal secretion or the synthesis of CRF may underlie a diverse range of stress-related disorders such as anxiety, depression, obsessive–compulsive disorder, and post-traumatic stress disorder.

CRF is a 41 amino-acid peptide first isolated in 1981.[2] It is a major modulator of the body's responses to stress. Modulation of the function of CRF receptors has become an important target for drug discovery.

Peptide ligands are valuable tools to investigate CRF-mediated physiology. Studies with peptide ligands have provided overwhelming support for a role for CRF in coordination of the body's responses to stress. Corticotropin-releasing factor 1 (CRF_1) receptor has been the most extensively studied CRF receptor as a potential therapeutic target. Preclinical and clinical studies have suggested that antagonists of CRF_1 may offer promise in the treatment of stress-related disorders.[3–6]

Considerable progress in the identification of non-peptide modulators of CRF function has been made in the last decade, providing opportunities for definitive clinical studies on treatments for some of the disorders described above. The small-molecule CRF_1 receptor antagonist R121919 had some efficacy in a small open-label clinical study focusing on anxiety and depression.[7] However, another CRF_1 receptor antagonist, CP316311, failed in a trial for depression.[8]

Structurally diverse CRF_1 antagonists may have different profiles; additional studies may be needed to define the clinical utility of CRF_1 antagonists. Thus, the design and synthesis of non-peptide small-molecule CRF_1 antagonists continues to be an attractive area of research. Investigations of structure–activity relationships (SAR) from various chemotypes led to the development of a pharmacophore model for small-molecule CRF_1 antagonists.[9,10]

In an effort to design structurally novel small-molecule CRF_1 antagonists, we focused on the monocyclic antagonist **I** as illustrated by structure **1** (Fig. 1) reported by Neurogen group in 2001.[11] The structure is distinct from our previously designed tricyclic and bicyclic core antagonists (Fig. 2),[12,13] which include two atoms between the sp^2 nitrogen acting as a hydrogen-bond acceptor and the aryl ring in an orthogonal orientation to the core ring, whereas the newly designed bicyclic core antagonist **II** (as illustrated by **2**, **3** and **4**) includes one carbon atom between the sp^2 nitrogen and the aryl ring (Fig. 1).

In these bicyclic core structures **2**–**4**, conformational freedom of the 3-pentyl and methyl on the newly fused pyrrole moiety was predicted to be more restricted relative to that of the monocyclic structure **1**. Restriction of such conformational freedom of the

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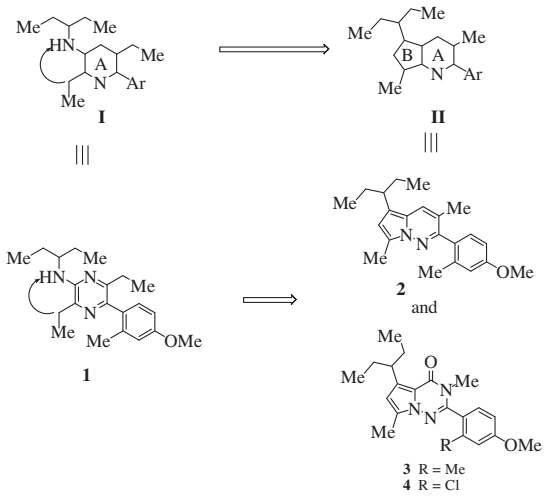


Figure 1. Molecular design of bicyclic core CRF_1 antagonists based on the monocyclic core structure.

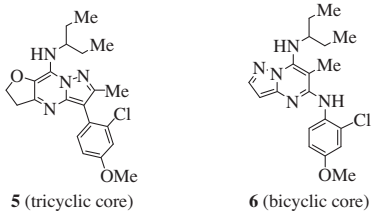


Figure 2.

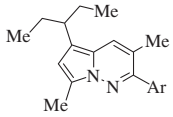
critical substituents on the bicyclic core system was of great interest in the optimization process.

We report here the full details of another series of structurally novel CRF_1 antagonists **2–4** which show strong activities in in vitro and in vivo evaluations.

2. Chemistry

The synthesis of test compounds listed in Tables 1–4 is outlined in Schemes 1–5. The synthesis of **2** and **7–12** is described in Scheme 1. Pyrrolo[1,2-*b*]pyridazine **43** was prepared by the coupling reaction of ethyl 1-*N*-amino-2-methylpyrrole carboxylate **41** and optionally substituted aryl β-keto ester **42** under reflux conditions in the presence of *p*-toluenesulfonic acid. Chlorination of **43** with triphenylphosphine and carbon tetrachloride under reflux conditions resulted in a chloride **44**. Removal of the chloro group was carried out by catalytic hydrogenation, resulting in **45**. Reaction of **45** with an ethyl magnesium bromide followed by acidic dehydration afforded an *E*/*Z* mixture **46**. Catalytic hydrogenation of **46** afforded analogs **2** and **7–12**. The synthesis of the imidazo[1,5-*a*]pyrimidine analog **13** is outlined in Scheme 2. A chemoselective cross-coupling reaction of 2,4-dichloro-5-methylpyrimidine **47** and 2-methyl-4-methoxyphenylboronic acid in the presence of a palladium catalyst afforded **48**. Replacement of another chloro residue with a nitrile was carried out with potassium cyanide in DMSO to afford **49**. Reaction of **49** with methyl magnesium bromide followed by reduction with titanium tetraisopropoxide in ammonia afforded **50**. N-acylation of **50** with 2-ethylbutanoyl chloride and triethylamine afforded **51**, a cyclization reaction of which was carried out with phosphorous oxychloride in toluene at 90 °C, resulting in **13**. The synthesis of the imidazo[1,5-*b*]pyridazine analog **14** is described in Scheme 3. 2-Ethylbutanal **52** was converted to a N-protected amino acid **53** by the following sequential reactions: aminocyanation of 2-ethyl butanal; acidic hydrolysis; then N-protection with benzyloxycarbonyl chloride. Activation of the carboxylic acid of **53** with carbonyldiimidazole followed by the reaction with the lithium anion of *t*-butyl acetate afforded β-keto ester **54**, C-alkylation of which with ethyl 2-(trifluoromethanesulfonyloxy)propionate in the presence of sodium hydride provided **55**. Acidic deprotection of **55** followed by decarboxylation afforded **56**. Cyclization of the γ-keto ester **56** with hydrazine hydrate afforded a six-membered ring product **57**. Bromination of **57** followed by dehydrobromination provided oxidative product **58**, chlorination of which with phosphorous oxychloride afforded **59**. A cross-coupling reaction of **59** with 2-methyl-4-methoxyphenylboronic acid in the presence of a palladium catalyst afforded **14**. The synthesis of **3**, **4**, **15–29**, **31**, **33**, **34**, **36** and **37** is described in Scheme 4. Reaction of ethyl 2-methylpyrrole-4-carboxylate **60** with an alkyl Grignard reagent afforded **61**, reduction of which with lithium aluminum hydride provided **62**. C-acylation of the pyrroles **62** with triphosgene followed by the addition of optional alkylamine resulted in amides **63**. 1-N-amination of the pyrrole-2-carboxyamides **63** was carried out by the reaction with chloroamine in the presence of sodium hydride. Cyclization of **64** with optionally substituted aryl aldehydes in the presence of oxygen and Darco-KB resulted in bicyclic products **3**,**4**, **15–29**, **31**, **33**,**34**, **36** and **37**, respectively. Pyrrolo[2,1-*f*]triazinones **30**, **32**, **35** and **38** were prepared as described in Scheme 5. The Wittig reaction of 2-methylbutanal **65** with the phosphorane afforded α,β-unsaturated methyl ketone **66**. Michael addition of ethyl nitroacetate to **66** in the

Table 1
Activity profiles of novel bicyclic core CRF_1 antagonists



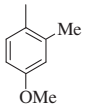
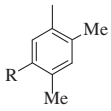
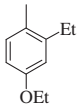
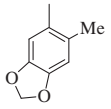
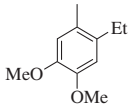
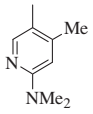
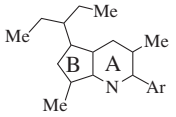
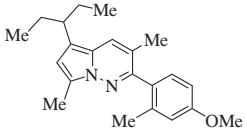
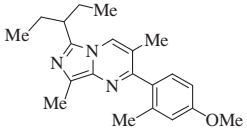
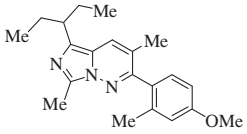
Compd	Ar	Binding affinity IC_{50} (nM) human	Antagonist activity EC_{50} (nM) human
2		16.4	4.9
7	 **a** R = H	11.6	1.8
8	**b** R = Me	16.6	3.3
9		13.4	6.9
10		18.3	2.5
11		8.9	1.9
12		7.4	11.5

Table 2
Activity profiles of novel bicyclic core CRF_1 antagonists



Compd	Structure	Binding affinity IC_{50} (nM) human	Antagonist activity EC_{50} (nM) human
2		16.4	4.9
13		311	NT[a]
14		122	566

[a] Not tested.

presence of triethylamine provided **67**. Reduction of the nitro group of **67** followed by intra-molecular cyclization afforded a pyrrole ester **68**. Alkaline hydrolysis of **68** followed by acidification provided decarboxylated product **69**. Reaction of **69** with triphosgene followed by the addition of methylamine afforded *N*-methyl amide **70**, N-amination of which with chloroamine in the presence of sodium hydride resulted in **71**. A coupling reaction of **71** with optionally substituted aryl aldehydes afforded **30**, **32**, **35** and **38**, respectively.

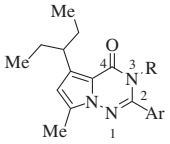
3. Results and discussion

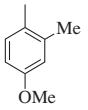
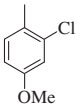
Synthesized bicyclic core CRF_1 antagonists (Tables 1–4) were studied in a series of in vitro and in vivo tests to identify potential preclinical candidates. Test compounds were first evaluated for their binding affinity to human CRF_1 and antagonist activity in a CRF-stimulated adenylate cyclase assay.[14] Representative compounds with higher receptor binding and antagonist activity were then evaluated in rat pharmacokinetic studies. They were also assessed for their anxiolytic efficacy in elevated plus maze models in rats.[15–17]

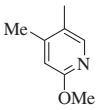
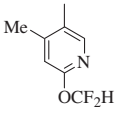
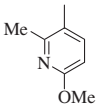
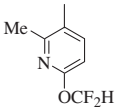
Data for human CRF_1 binding and antagonist activity for pyrrolo[1,2-*b*]pyridazines are summarized in Table 1. As depicted in Figure 1, ring closure between the two atoms of the monocyclic 1,4-pyrazine antagonist **1** results in analogs **2**–**4** with moderate-to-potent binding affinity and antagonist activity (**2**: Table 1; **3** and **4**: Table 3).

Our optimization process of the chemical lead **2** was initiated with chemical modification of the aryl (Ar) moiety (Table 1). Replacement of the 2-methyl-4-methoxyphenyl group of **2** with 2,4-dimethylphenyl, 2,4,5-trimethylphenyl, 2-ethyl-4-ethoxyphenyl, 2-methyl-4,5-methylenedioxyphenyl and 2-ethyl-4,5-dimethoxyphenyl resulted in analogs **7**–**11**, respectively. They retained equipotent binding affinity for human CRF_1 receptor, whereas

Table 3
Activity profiles of novel bicyclic core CRF_1 antagonists



Ar  **a**  **b**

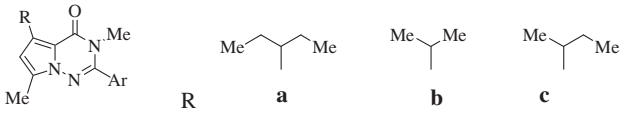
Compd	R	Ar	Binding affinity IC_{50} (nM) human	Antagonist activity EC_{50} (nM) human
15	H	a	149	688
16	H	b	14.3	4.0
3	Me	a	2.6	1.7
4	Me	b	3.8	1.2
17	Et	a	9.7	1.2
18	Et	b	4.6	0.7
19	CH_2CH_2OH	a	7.8	4.2
20	CH_2CH_2OH	b	4.4	3.5
21	*i*Pr	a	19.2	5.1
22	*c*Pr	a	5.7	3.7
23	*c*Pr	b	5.9	1.0
24	CH_2*i*Pr	a	111	47.2
25	CH_2*c*Pr	a	3.3	5.0
26	Me		8.6	17.2
27	Me		5.3 (8.4)[a]	3.2 (2.6)[b]
28	Me		3.8	1.1
29	Me		4.5	0.3

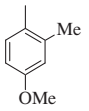
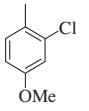
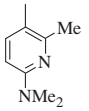
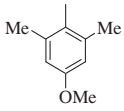
[a] Binding assay for rat CRF.
[b] cAMP assay for rat.

some of them exhibited more potent antagonist activity than expected from their binding affinity. Replacement of the Ar moiety of **2** with 2-*N*,*N*-dimethylamino-4-methylpyridin-5-yl afforded **12** with equipotent binding affinity, whereas it tended to show slightly less potent antagonist activity relative to **2** and **7**–**11**. The pyrrolo[1,2-*b*]pyridazine analog **2** was found to be effective in the elevated plus maze test in rats (3 mg/kg, po) as well as swim-stress induced anxiety-like behavior. Further optimization of this series of analogs was discontinued because of their chemical instability (Fig. 3). Compound **2** was found to cause a decomposition reaction on a TLC plate at room temperature under atmospheric conditions, resulting in **39** (13%) and **40** (trace) as a hydrolysis product and an oxidative product, respectively. They were isolated in addition to the recovered **2** (80%). The stereochemistry of the enone system of **39** was determined to be '*Z*' because of an observed NOE between the methyl protons and the vinyl proton circled (Fig. 3).

The synthesis of bicyclic core analogs **13**–**14** containing three nitrogen atoms and their biological evaluations were carried out (Table 2). Replacement of the pyrrolo[1,2-*b*]pyridazine core of **2** with imidazo[1,5-*a*]pyrimidine afforded **13** with significant reduction of binding affinity. Replacement of the pyrrolo[1,2-*b*]pyridazine core of **2** with imidazo[1,5-*b*]pyridazine resulted in **14**, also with reduction of binding affinity and antagonist activity, whereas

Table 4
Activity profiles of novel bicyclic core CRF_1 antagonists

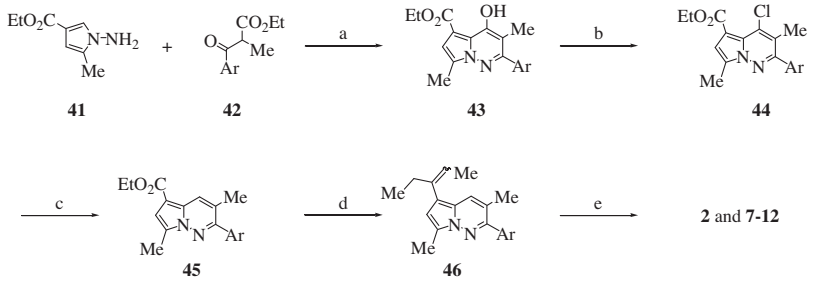


Compd	Ar	R	Binding affinity IC_{50} (nM) human	Antagonist activity EC_{50} (nM) human
3		a	2.6	1.7
30		c	5.0	2.4
4		a	3.8	1.2
31		b	8.7	7.4
32		c	4.9	1.1
33		a	7.8	5.5
34		b	131	NT[a]
35		c	81.2	36.5
36		a	2.0	0.6
37		b	13.1	4.5
38		c	4.2	3.5

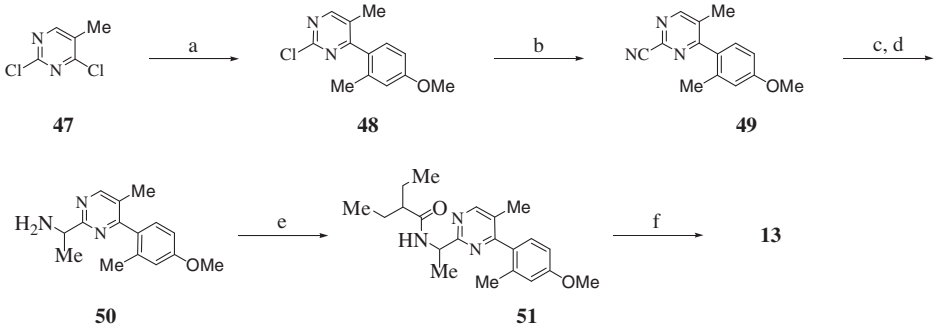
[a] Not tested.

14 showed more potency in its binding affinity relative to **13**. As a result, pyrrolo[1,2-*b*]pyridazine was considered to be the most favored template among the tested three bicyclic cores to make better interaction with CRF_1, whereas analogs **13** and **14** were guessed to make undesired ionic interaction with untargeted proteins because of the additional basic nitrogen contained in their imidazole rings. Our objective was identification of a chemically stable structure with oral efficacy as a CRF_1 antagonist, so focus was placed on further chemical modification of the unstable bicyclic core of **2** to make it more stable. The pyrrolo[1,2-*b*]pyridazine structure of **2** was found to decompose through the pathway described in Figure 3 because of its electron-rich property. We considered that the decomposition pathway could be avoided by replacing the unstable electron-rich template with a relatively more electron-deficient one such as pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one, resulting in **3** and **4** (Fig. 1). A series of pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one analogs did not cause a decomposition reaction under the same condition as described above because of the electron-withdrawing carbonyl moiety attached to the electron-rich pyrrole moiety.

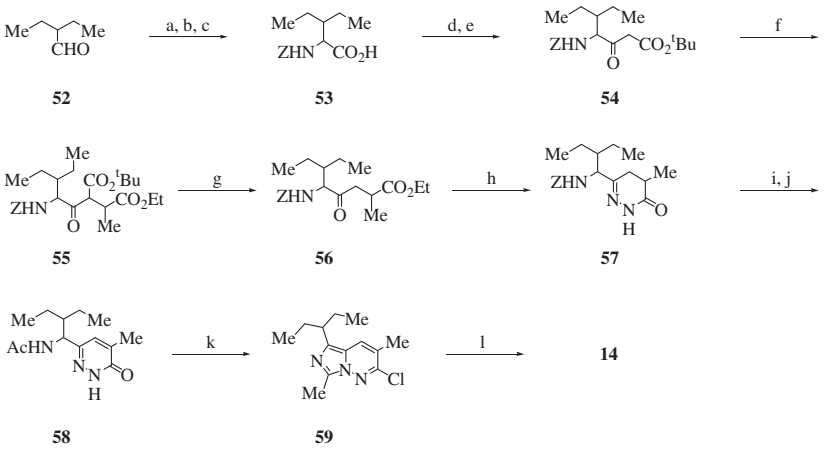
As such, optimization was carried out with respect to the pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one series (which were designed and synthesized as additional chemically stable bicyclic core antagonists) with the aim of finding a chemically stable antagonist. N-unsubstituted pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one analogs **15** and **16** were synthesized and evaluated for their in vitro activities. As shown in Table 3, the 2-methyl-4-methoxyphenyl analog **15** exhibited weaker activities in both of the evaluations relative to the corresponding 2-chloro-4-methoxyphenyl analog **16**, whereas **16** exhibited tenfold more potency and nearly 170-fold more potency in binding affinity and antagonist activity, respectively. Introduction of lower *N*-alkyl groups such as *N*-methyl and *N*-ethyl into the 3-nitrogen of the triazinone moiety of **15** and **16** resulted in **3**,**4** and **17**,**18**, respectively, with increased in vitro activities. Replacement of the 2-methyl-4-methoxyphenyl moiety of **3** and **17** with 2-chloro-4-methoxyphenyl (which was expected to increase the binding affinity) afforded **4** and **18**, respectively, with no substantial increase of in vitro activities. Accordingly, introduction of a lower alkyl group into the 3-nitrogen of the triazinone



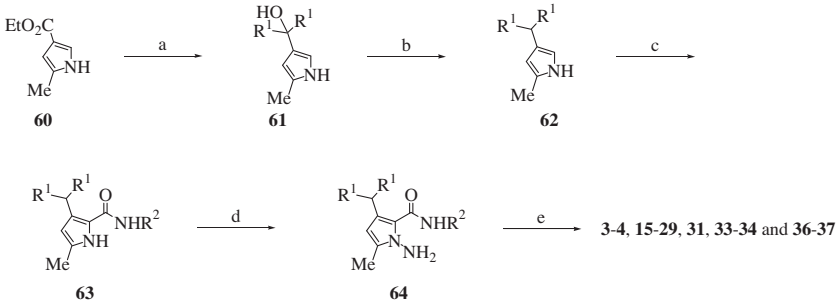
Scheme 1. Synthesis of **2** and **7**–**12**. Reagents and conditions: (a) *p*-TsOH, toluene, reflux; (b) PPh_3, Celite, CCl_4, reflux; (c) 10% Pd-C, HCO_2NH_4, EtOH, rt; (d) EtMgBr, THF, 0 °C to rt; (e) H_2, 10% Pd-C, EtOH, rt.



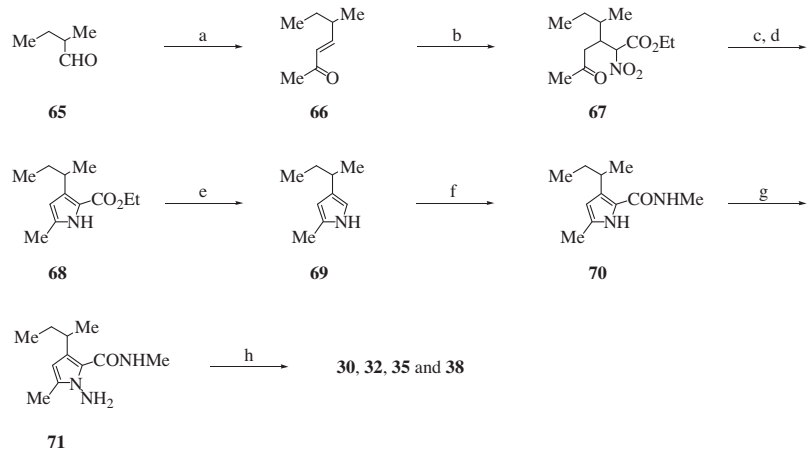
Scheme 2. Synthesis of **13**. Reagents and conditions: (a) 2-Methyl-4-methoxyphenylboronic acid, $PdCl_2(dppf)_2$–CH_2Cl_2, K_2CO_3, DME–H_2O, 80 °C; (b) KCN, DMSO, 100 °C; (c) MeMgBr, Et_2O, 0 °C; (d) NH_3 in 1,4-dioxane, Ti(O*i*Pr)4, EtOH then $NaBH_4$, rt; (e) 2-ethylbutanoyl chloride, Et_3N, CH_2Cl_2, 0 °C; (f) $POCl_3$, toluene, 90 °C.



Scheme 3. Synthesis of **14**. Reagents and conditions: (a) NaCN, $(NH_4)_2CO_3$, $NaHSO_3$, MeOH–H_2O, 60 °C; (b) 47% HBr, reflux; (c) ZCl, NaOH, THF–H_2O, rt; (d) CDI, THF, rt; (e) LiHMDS, $CH_3CO_2{}^tBu$, THF, −78 °C to rt; (f) ethyl 2-(trifluoromethylsulfonyloxy)propionate, NaH, THF–CH_2Cl_2, 0 °C to rt; (g) TFA, CH_2Cl_2, rt; (h) $NH_2NH_2{\cdot}H_2O$, AcOH/EtOH, reflux; (i) Br_2, AcOH, 50 °C; (j) Ac_2O, pyridine, rt; (k) $POCl_3$, 80 °C; (l) 2-methyl-4-methoxyphenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O, 80 °C.



Scheme 4. Synthesis of **3**–**4**, **15**–**29**, **31**, **33**–**34** and **36**–**37**. Reagents and conditions: (a) R^1MgBr, THF, 50 °C; (b) $LiAlH_4$, THF, reflux; (c) $ClCOCCl_3$, THF, 0 °C then optional alkylamine, 0 °C; (d) NaH, DMF, 0 °C then NH_2Cl in Et_2O, 0 °C; (e) ArCHO, O_2, Darco-KB, xylene, 130 °C.



Scheme 5. Synthesis of **30**, **32**, **35** and **38**. Reagents and conditions: (a) $Ph_3P{=}CHCOMe$, CH_2Cl_2, rt; (b) $O_2NCH_2CO_2Et$, Et_3N, CH_3CN, 65 °C; (c) formamidinesulfinic acid, Et_3N, DMF, 80 °C; (d) $AcONH_4$, AcOH, 100 °C; (e) NaOH, H_2O–EtOH, rt to 60 °C; (f) $ClCOCCl_3$, THF, 0 °C then $MeNH_2$, rt; (g) NaH, DMF, 0 °C then NH_2Cl in Et_2O, rt; (h) ArCHO, O_2, Darco-KB, xylene, 130 °C.

ring (which keeps the Ar ring in an orthogonal orientation to the bicyclic core) was considered to be very effective for the increase of in vitro activities. Introduction of hydrophilic *N*-(2-hydroxy)ethyl instead of the lower *N*-alkyl into the corresponding position-3 resulted in **19** and **20** with retained binding affinity and slightly decreased antagonist activity relative to **3**,**4** and **17**,**18**. Introduction of a bulkier *N*-alkyl group into the corresponding 3-position of the triazinone ring afforded **21**–**25** with more complicated results. The *N*-isopropyl analog **21** showed potent antagonist activity for its relatively weaker binding affinity. *N*-Cyclopropyl analogs **22** and **23** exhibited strong binding affinity and antagonist activity. The *N*-isobutyl analog **24** showed a remarkable decrease in both of the evaluations whereas the less bulky *N*-cyclopropylmethyl analog **25** showed much stronger activities relative to **24**. As such, the N-branched alkyl group tended to show a deleterious effect on the binding affinity and/or antagonist activity, as illustrated by **21** and **24**, because of their bulkiness. Replacement of the 2,4-substituted phenyl (Ar) moiety of the above-described analogs

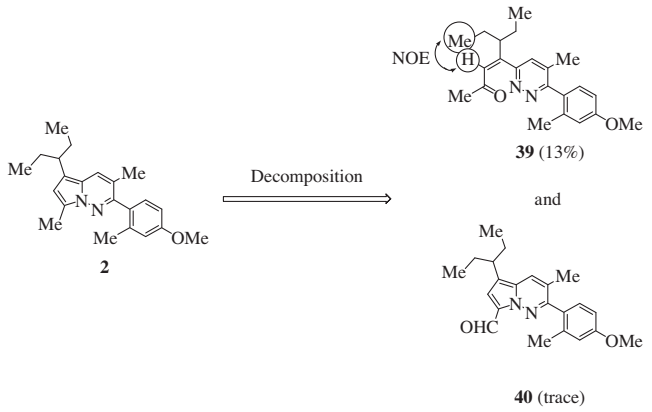


Figure 3. Decomposition of **2**.

with an appropriately substituted pyridine moiety resulted in **26**–**29** with moderate-to-potent activities. The 2-methoxy-4-methylpyridin-5-yl analog **26** exhibited reduced activity relative to the corresponding phenyl analog **3**. The 2-methyl-6-methoxypyridin-3-yl analog **28** (in which the hydrophilic nitrogen atom of the pyridine ring was covered by the two *ortho*-substituents relative to **26**) showed the more potent in vitro activity. To block the predicted metabolic demethylation of the pyridine moiety, replacement of the methoxy of **26** and **28** with difluoromethoxy afforded **27** and **29**, respectively, with a tendency of increase in their antagonist activity. Appropriately substituted pyridine moieties in which the hydrophilic nitrogen was covered by the two *ortho*-substituents (e.g., methoxy and difluoromethoxy) were found to be beneficial as Ar moieties.

The effect of the lipophilic alkyl chain 'R' on activity profiles was investigated (Table 4). The 1-methylpropyl analog **30** showed nearly equipotent in vitro activity with the 1-ethylpropyl analog **3**. The same SAR was observed between the corresponding 2-chloro-4-methoxyphenyl analogs **4** and **32**, whereas the isopropyl analog **31** showed a tendency of reduction in its potency relative to **4** and **32**. Among the 6-(*N*,*N*-dimethyl)amino-2-methylpyridin-3-yl analogs **33**–**35**, the 1-ethylpropyl analog **33** ($c\log P = 4.1$) exhibited the most potent activity relative to others, whereas the 1-methylpropyl analog **35** ($c\log P = 3.6$) showed less potency than **33** and more potency than **34** ($c\log P = 3.0$) in binding affinity and/or antagonist activity. This SAR (R = 1-ethylpropyl **a** > 1-methylpropyl **c** > isopropyl **b**) seemed to be mainly dependent upon the lipophilicity of the 'R' moiety. The same SAR as described above was observed in the trisubstituted phenyl analogs **36**–**38**. 1-Ethylpropyl analogs **36** tended to show the strongest potency among the tested three analogs, whereas the isopropyl analog **37** and 1-methylpropyl analog **38** tended to show the least potency and medium potency, respectively. Thus, the effect of the lipophilicity of the 'R' on activity profiles was found to be particularly remarkable in the 2-aminopyridine analogs **33**–**35** relative to the phenyl analogs **4**, **31**, **32** and **36**–**38**.

We evaluated oral efficacy of many compounds, which showed potent in vitro activity profiles. As a result, we selected compound **27** as the representative compound. The anxiolytic efficacy of compound **27**, which showed binding affinity to rat brain membranes (half-maximal inhibitory concentration (IC_{50}) = 8.4 nM) and antagonist activity in a cyclic AMP assay (half-maximal effective concentration (EC_{50}) = 2.6 nM), was assessed by the rat elevated plus maze test (Fig. 4). Vehicle-treated rats significantly decreased the time spent in open arms ($p < 0.05$) relative to control animals. Pretreatment with compound **27** at 10 mg/kg significantly increased the time spent in open arms ($p = 0.047$) relative to vehicle-treated animals.

Pharmacokinetic data for compound **27** were investigated after single-dose administration to rats (Table 5). Intravenous administration of compound **27** to rats (1 mg/kg, $n = 3$) resulted in its detection in plasma (half-life ($T_{1/2}$) = 4.3 h), whereas oral administration of **27** to rats (1 mg/kg, $n = 3$) resulted in a $T_{1/2}$ of 2.4 h. The AUC value of **27** was 518 ng h/mL after intravenous administration *versus* 70.2 ng h/mL after oral administration. The steady state volume of distribution (V_{ss}) was 2947 mL/kg, indicating that this compound showed good distribution to the tissues. Systemic clearance (CL) was 34.7 mL/min/kg. The maximum concentration that the drug achieved after dosing (C_{max}) after oral dosing was 21.5 ng/mL, whereas the time to maximum concentration (T_{max}) was 1.0 h. The bioavailability of **27** was 13.5%.

Table 5
Pharmacokinetic parameters of compound **27**

Parameter	iv	po
Dose (mg/kg)	1	1
$AUQ_{infinity}$ (ng·h/mL)	518	70.2
T_{max} (h)		1.0
C_{max} (ng/mL)		21.5
CL_{total} (mL/min/kg)	34.7	
$T_{1/2}$ (h)	4.3	2.4
V_{ss} (mL/kg)	2947	
F (%)		13.5

$n = 3$.

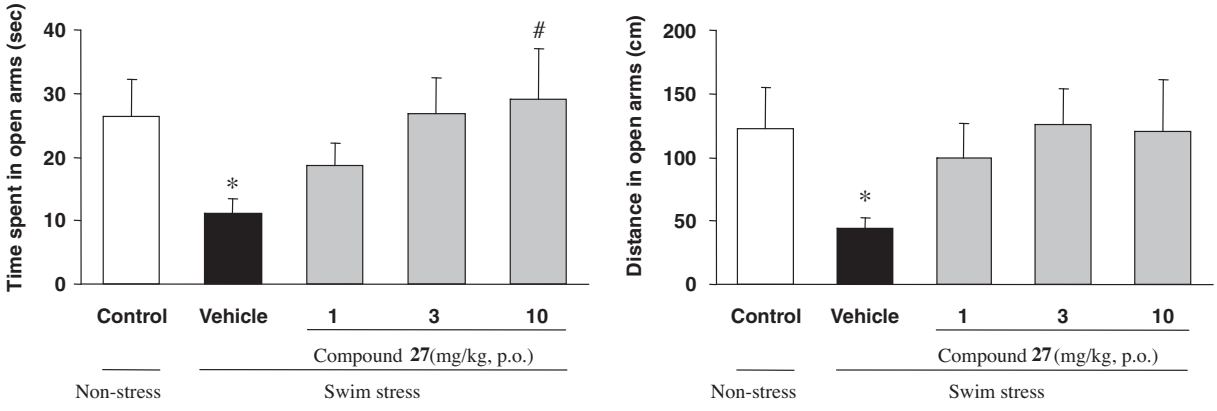


Figure 4. Effect of compound **27** in the rat elevated plus-maze test in swim stress-loaded rats. Each column represents mean ± standard errors of 16 rats. $^{\#}p < 0.05$ versus control group (*t*-test). $^{*}P < 0.05$ versus vehicle group (Dunnett test).

4. Conclusion

Based on the monocyclic core antagonist **1**, bicyclic core antagonist **2** was designed and synthesized. The pyrrolo[1,2-*b*]pyridazine analog **2** showed potent in vitro activity and was found to be effective in the elevated plus maze test in rats as well as swim-stress induced anxiety-like behavior, whereas **2** was too unstable to be developed further as a clinical candidate. After substantial chemical modification, pyrrolo[2,1-*f*]triazin-4(3*H*)-ones **3, 4** were designed and synthesized as additional, chemically stable bicyclic core antagonists. Continued synthetic work focused mainly on optimization of the Ar moiety to identify orally effective compounds in this series of analogs. Among the compounds tested in vivo, the 2-difluoromethoxy-4-methylpyridin-5-yl analog **27** was found to show efficacy in a dose–response manner in the elevated plus maze test in rats.

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 ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. Fast atom bombardment (FABMS, 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 F_{254}). The following abbreviations for solvents and reagents are used; diethyl ether (Et_2O), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol (EtOH), ethyl acetate (EtOAc), methanol (MeOH), tetrahydrofuran (THF), dichloromethane (CH_2Cl_2).

5.1.2. Decomposition of 2

A solution of compound **2** in CH_2Cl_2 was charged on two plates of TLC (Merck 5744, 0.5 mm, 20 × 20 cm) and kept at room temperature under atmospheric condition for 41 h. After developing the TLC using hexane/EtOAc (1/1), UV (254 nm)-positive fractions were collected and eluted by EtOAc to afford reasonable amount of decomposition products, purification of which gave **39** (2.8 mg: 13%) and **40** (trace) as the isolated products with the recovered starting material **2** (16 mg: 80%).

5.1.2.1. Compound 39. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22–7.14 (m, 2H), 6.88–6.80 (m, 2H), 6.31 (s, 1H), 3.86 (s, 3H), 2.49 (m, 1H), 2.15 (d, *J* = 0.6 Hz, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 1.70–1.40 (m, 4H), 0.98 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 198.53, 161.87, 160.01, 159.64, 155.43, 138.09, 136.12, 130.43, 129.49, 129.05, 128.00, 115.97, 111.41, 55.49, 51.00, 31.37, 25.55, 20.10, 19.20, 11.90; MS (APCI, Pos) *m/z* 353 $(M+H)^+$.

5.1.2.2. Compound 40. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.62 (d, *J* = 0.9 Hz, 1H), 7.37 (s, 1H), 7.17 (d, *J* = 7.8 Hz, 1H), 6.90–6.82 (m, 2H), 3.87 (s, 3H), 2.65 (m, 1H), 2.21 (s, 3H), 2.10 (d, *J* = 0.9 Hz, 3H), 1.88–1.45 (m, 4H), 0.83 (t, *J* = 7.2 Hz, 6H); MS (APCI, Pos) *m/z* 351 $(M+H)^+$.

5.1.3. Ethyl 4-hydroxy-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate (43)

To a stirred solution of **41** (4.11 g, 24.8 mmol) and ethyl 3-(4-methoxy-2-methyl-phenyl)-2-methyl-3-oxopropionate **42** (6.2 g, 25 mmol) in toluene (85 mL) was added *p*-toluenesulfonic acid monohydrate (470 mg, 2.5 mmol) at room temperature and the reaction mixture was heated at reflux temperature removing the resulting water with Dean-Stark apparatus. After being stirred for 36 h, the reaction mixture was cooled, diluted with $NaHCO_3$ aq and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/100–4/21) to give **43** (5.4 g, 61% yield) as a yellow powder. TLC R_f = 0.64 (EtOAc/hexane, 1/3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.03 (s, 1H), 7.16 (m, 1H), 6.91 (d, *J* = 0.9 Hz, 1H), 6.87–6.79 (m, 2H), 4.40 (q, *J* = 7.2 Hz, 2H), 3.85 (s, 3H), 2.46 (d, *J* = 0.9 Hz, 3H), 2.16 (s, 3H), 1.96 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H).

5.1.4. Ethyl 4-hydroxy-2-(2,4-dimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

83% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.01 (s, 1H), 7.15–7.05 (m, 2H), 6.89 (d, *J* = 0.9 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 2.45 (d, *J* = 0.9 Hz, 3H), 2.38 (s, 3H), 2.13 (s, 3H), 1.95 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H).

5.1.5. Ethyl 4-hydroxy-2-(2,4,5-trimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

82% yield; a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.99 (s, 1H), 7.05 (s, 1H), 6.98 (s, 1H), 6.89 (d, *J* = 0.8 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 2.45 (d, *J* = 0.8 Hz, 3H), 2.28 (s, 3H), 2.26 (s, 3H), 2.09 (s, 3H), 1.96 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H).

5.1.6. Ethyl 4-hydroxy-2-(4-ethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

48% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.99 (s, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 6.90 (d, *J* = 1.2 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 6.80 (dd, *J* = 2.4, 8.4 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 2H), 4.08 (q, *J* = 7.2 Hz, 2H), 2.45 (m, 2H), 2.45 (d, *J* = 1.2 Hz, 3H), 1.95 (s, 3H), 1.45 (t, *J* = 7.2 Hz, 3H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.11 (t, *J* = 7.5 Hz, 3H).

5.1.7. Ethyl 4-hydroxy-3,7-dimethyl-2-(6-methyl-1,3-benzodioxol-5-yl)pyrrolo[1,2-*b*]pyridazine-5-carboxylate

51% yield; a yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.04 (s, 1H), 6.90 (m, 1H), 6.76 (m 1H), 6.70 (s, 1H), 5.97 (s, 2H), 4.39 (q, *J* = 7.2 Hz, 2H), 2.46 (s, 3H), 2.06 (s, 3H), 1.97 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H).

5.1.8. Ethyl 4-hydroxy-2-(4,5-diethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

50% yield; a pale yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.02 (s, 1H), 6.91 (d, *J* = 0.6 Hz, 1H), 6.83 (s, 1H), 6.71 (s, 1H), 4.40 (q, *J* = 7.2 Hz, 2H), 3.94 (s, 3H), 3.85 (s, 3H), 2.47 (s, 3H), 2.42 (m, 2H), 1.97 (s, 3H), 1.42 (t, *J* = 7.2 Hz, 3H), 1.11 (t, *J* = 7.5 Hz, 3H).

5.1.9. Ethyl 2-(6-dimethylamino-4-methylpyridin-3-yl)-4-hydroxy-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

27% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.04 (s, 1H), 8.04 (s, 1H), 6.91 (d, *J* = 0.8 Hz, 1H), 6.44 (s, 1H), 4.40 (q, *J* = 7.0 Hz, 2H), 3.14 (s, 6H), 2.46 (d, *J* = 0.8 Hz, 3H), 2.14 (s, 3H), 2.03 (s, 3H), 1.42 (t, *J* = 7.0 Hz, 3H).

5.1.10. Ethyl 4-chloro-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate (44)

To a stirred solution of **43** (4.9 g, 14 mmol) were added triphenylphosphine (11 g, 42 mmol), tetrachloromethane (12.4 mL, 124 mmol) and Celite (2.2 g) in THF (70 mL) at ambient temperature and the reaction mixture was heated at reflux temperature. After being stirred for 2.5 h, the reaction mixture was cooled to room temperature, diluted with EtOAc/hexane (2/1), and then filtered through a pad of Celite. After the filtrate was evaporated, the resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/100–1/4) to give **44** (3.9 g, 76% yield) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (m, 1H), 7.11 (d, J = 0.9 Hz, 1H), 6.88–6.81 (m, 2H), 4.37 (q, J = 6.9 Hz, 2H), 3.86 (s, 3H), 2.49 (d, J = 0.9 Hz, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 1.41 (t, J = 6.9 Hz, 3H).

5.1.11. Ethyl 4-chloro-2-(2,4-dimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

93% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17–7.08 (m, 4H), 4.37 (q, J = 6.9 Hz, 2H), 2.48 (s, 3H), 2.40 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 1.41 (t, J = 6.9 Hz, 3H).

5.1.12. Ethyl 4-chloro-2-(2,4,5-trimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

58% yield; a yellow sold; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (d, J = 0.7 Hz, 1H), 7.09 (s, 1H), 7.00 (s, 1H), 4.37 (q, J = 7.1 Hz, 2H), 2.48 (d, J = 0.7 Hz, 3H), 2.30 (s, 3H), 2.28 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H).

5.1.13. Ethyl 4-chloro-2-(4-ethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

99% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 0.9 Hz, 1H), 6.89 (d, J = 2.4 Hz, 1H), 6.82 (dd, J = 2.4, 8.4 Hz, 1H), 4.37 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 6.9 Hz, 2H), 2.48 (s, 3H), 2.40 (m, 2H), 2.15 (s, 3H), 1.46 (t, J = 6.9 Hz, 3H), 1.41 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.5 Hz, 3H).

5.1.14. Ethyl 4-chloro-3,7-dimethyl-2-(6-methyl-1,3-benzodioxol-5-yl)pyrrolo[1,2-*b*]pyridazine-5-carboxylate

93% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (s, 1H), 6.78 (s, 1H), 6.71 (s, 1H), 6.00 (s, 2H), 4.37 (q, J = 7.2 Hz, 2H), 2.49 (s, 3H), 2.17 (s, 3H), 2.06 (s, 3H), 1.41 (t, J = 7.2 Hz, 3H).

5.1.15. Ethyl 4-chloro-2-(4,5-dimethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

42% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (s, 1H), 6.84 (s, 1H), 6.70 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 3.95 (s, 3H), 3.86 (s, 3H), 2.50 (s, 3H), 2.55–2.20 (m, 2H), 2.17 (s, 3H), 1.41 (t, J = 7.2 Hz, 3H), 1.11 (t, J = 7.5 Hz, 3H).

5.1.16. Ethyl 2-(6-Dimethylamino-4-methylpyridin-3-yl)-4-chloro-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

86% yield; a yellow oil; MS (ESI, Pos) m/z 387 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.11 (s, 1H), 6.44 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 3.15 (s, 6H), 2.48 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H), 1.41 (t, J = 7.2 Hz, 3H).

5.1.17. Ethyl 2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate (45)

To a stirred solution of **44** (8.2 g, 22.0 mmol) in EtOH (160 mL) and dioxane (160 mL) were added ammonium formate (8.2 g, 130 mmol) and 10% Pd-C (800 mg) at room temperature under argon atmosphere. After being stirred for 2 h, the reaction mixture was filtered through a pad of Celite. The filtrate was evaporated and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/100–1/4) to afford **45** (7.4 g, 99% yield) as a yellow viscous oil. TLC R_f = 0.36 (EtOAc/hexane, 1/5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 1.2 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 0.9 Hz, 1H), 6.88–6.78 (m, 2H), 4.36 (q, J = 6.9 Hz, 2H), 3.84 (s, 3H), 2.50 (d, J = 0.9 Hz, 3H), 2.14 (s, 3H), 2.09 (d, J = 1.2 Hz, 3H), 1.41 (t, J = 6.9 Hz, 3H).

5.1.18. Ethyl 2-(2,4-dimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

77% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, J = 0.9 Hz, 1H), 7.17–7.10 (m, 3H), 7.06 (d, J = 0.9 Hz, 1H), 4.38 (q, J = 6.9 Hz, 2H), 2.50 (d, J = 0.9 Hz, 3H), 2.40 (s, 3H), 2.13 (s, 3H), 2.09 (d, J = 0.9 Hz, 3H), 1.42 (t, J = 6.9 Hz, 3H).

5.1.19. Ethyl 2-(2,4,5-trimethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

99% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 1.2 Hz, 1H), 7.08 (s, 1H), 7.05 (d, J = 0.7 Hz, 1H), 6.99 (s, 1H), 4.37 (q, J = 7.0 Hz, 2H), 2.50 (d, J = 0.9 Hz, 3H), 2.29 (s, 3H), 2.27 (s, 3H), 2.10 (d, J = 1.2 Hz, 3H), 2.08 (s, 3H), 1.41 (t, J = 7.0 Hz, 3H).

5.1.20. Ethyl 2-(4-ethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

91% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 1.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 0.6 Hz, 1H), 6.90 (d, J = 2.7 Hz, 1H), 6.83 (dd, J = 8.4, 2.7 Hz, 1H), 4.38 (q, J = 6.9 Hz, 2H), 4.09 (q, J = 6.9 Hz, 2H), 2.50 (d, J = 0.6 Hz, 3H), 2.09 (d, J = 0.9 Hz, 3H), 1.46 (t, J = 6.9 Hz, 3H), 1.42 (t, J = 6.9 Hz, 3H), 1.11 (t, J = 6.9 Hz, 3H).

5.1.21. Ethyl 4-chloro-3,7-dimethyl-2-(6-methylbenzo [1,3] dioxol-5-yl)pyrrolo[1,2-*b*]pyridazine-5-carboxylate

99% yield; a green oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (d, J = 1.2 Hz, 1H), 7.07 (m, 1H), 6.79 (d, J = 0.6 Hz, 1H), 6.71 (s, 1H), 6.00 (s, 2H), 4.38 (q, J = 6.9 Hz, 2H), 2.51 (d, J = 0.6 Hz, 3H), 2.11 (d, J = 1.2 Hz, 3H), 2.06 (s, 3H), 1.42 (t, J = 6.9 Hz, 3H).

5.1.22. Ethyl 2-(4,5-dimethoxy-2-ethylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

76% yield; a yellow powder; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, J = 1.2 Hz, 1H), 7.07 (d, J = 0.9 Hz, 1H), 6.85 (s, 1H), 6.72 (s, 1H), 4.38 (q, J = 6.9 Hz, 2H), 3.95 (s, 3H), 3.86 (s, 3H), 2,51 (d, J = 0.9 Hz, 3H), 2.40 (m, 2H), 2.11 (d, J = 1.2 Hz, 3H), 1.42 (t, J = 6.9 Hz, 3H), 1.11 (t, J = 7.5 Hz, 3H).

5.1.23. Ethyl 2-(6-dimethylamino-4-methylpyridin-3-yl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine-5-carboxylate

81% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.29 (d, J = 1.1 Hz, 1H), 8.03 (s, 1H), 7.06 (d, J = 0.9 Hz, 1H), 6.44 (s, 1H), 4.37 (q, J = 7.1 Hz, 2H), 3.14 (s, 6H), 2.50 (d, J = 0.9 Hz, 3H), 2.16 (d, J = 1.1 Hz, 3H), 2.13 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H).

5.1.24. 5-(1-Ethylpropenyl)-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine (46)

To a stirred solution of **45** (7.3 g, 22 mmol) in THF (72 mL) was added ethyl magnesium bromide (22 mL, 65 mmol, 3.0 M/diethyl ether) at 0 °C. After being stirred for 3.5 h at room temperature, ethyl magnesium bromide (7.0 mL, 3.0 M/Et_2O) was added further to the reaction mixture. After being stirred for 1 h, the reaction mixture was cooled to 0 °C, quenched with 2 M HCl (50 mL) and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/100–3/17) to give **46** (6.87 g, 95% yield) as a yellow oil. TLC

R_f = 0.53 (EtOAc/hexane, 1/5); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 1.2 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.86–6.76 (m, 2H), 6.55 (d, J = 0.9 Hz, 1H), 5.66 (q, J = 6.9 Hz, 1H), 3.83 (s, 3H), 2.52 (q, J = 7.2 Hz, 2H), 2.49 (s, 3H), 2.18 (s, 3H), 1.98 (d, J = 0.9 Hz, 3H), 1.84 (d, J = 6.9 Hz, 3H), 1.05 (t, J = 7.2 Hz, 3H).

5.1.25. 2-(2,4-Dimethylphenyl)-5-(1-ethylpropenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine

81% yield; a brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 and 7.31 (m, total 1H), 7.17–7.05 (m, 3H), 6.58 and 6.49 (m, total 1H), 5.68 and 5.60 (q, J = 6.0 Hz, total 1H), 2.54 (m, 2H), 2.52 and 2.49 (s, 3H), 2.38 (s, 3H), 2.18 and 2.16 (s, 3H), 1.98 (s, 3H), 1.85 and 1.62 (m, total 3H), 1.05 and 1.00 (t, J = 6.0 Hz, total 3H).

5.1.26. 2-(2,4,5-Trimethylphenyl)-5-(1-ethylpropenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine

91% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 and 7.67 (m, total 1H), 7.06 (s, 1H), 7.00 and 7.02 (s, total 1H), 6.48 and 6.57 (s, total 1H), 5.60 and 5.68 (m, total 1H), 2.55 (m, 1H), 2.49 and 2.53 (s, 3H), 2.40 (m, 1H), 2.28 (s, 3H), 2.26 (s, 3H), 2.12 and 2.14 (s, total 3H), 1.99 (d, J = 0.7 Hz, 3H), 1.62 and 1.85 (m, total 3H), 0.99 and 1.05 (t, J = 7.5 Hz, total 3H).

5.1.27. 2-(4-Ethoxy-2-ethylphenyl)-5-(1-ethylpropenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine

63% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 0.6 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.4, 2.4 Hz, 1H), 6.58 (d, J = 0.6 Hz, 1H), 5.68 (q, J = 6.9 Hz, 1H), 4.08 (q, J = 6.9 Hz, 2H), 2.50 (m, 4H), 2.50 (s, 3H), 1.97 (s, 3H), 1.84 (d, J = 6.9 Hz, 3H), 1.45 (t, J = 6.9 Hz, 3H), 1.13 (t, J = 7.5 Hz, 3H), 1.05 (t, J = 7.5 Hz, 3H).

5.1.28. 5-(1-Ethylpropenyl)-3,7-dimethyl-2-(6-methylbenzo[1,3]dioxol-5-yl)-pyrrolo[1,2-*b*]pyridazine

92% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 and 7.31 (m, total 1H), 6.77 and 6.76 (m, total 1H), 6.59 and 6.50 (s, total 1H), 5.98 (m, 2H), 5.68 and 5.61 (m, total 1H), 2.54 (s, 3H), 2.60–2.48 (m, 5H), 2.10 (m, 3H), 2.00 (s, 3H), 1.85 and 1.63 (m, total 3H), 1.02 (m, 3H).

5.1.29. 2-(4,5-Dimethoxy-2-ethylphenyl)-5-(1-ethylpropenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine

72% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 and 7.32 (s, total 1H), 6.84 and 6.83 (s, total 1H), 6.59 and 6.50 (s, total 1H), 5.74–5.53 (m, 1H), 3.94 (s, 3H), 3.86 (s, 3H), 2.53 (m, 3H), 2.50–2.34 (m, 4H), 2.00 (s, 3H), 1.85 and 1.63 (m, total 3H), 1.19–0.95 (m, 6H).

5.1.30. {5-[5-(1-Ethylpropenyl)-3,7-dimethylpyrrolo[1,2-*b*] pyridazin-2-yl]-4-methylpyridin-2-yl}-dimethylamine

93% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.68 (d, J = 1.1 Hz, 1H), 6.58 (s, 1H), 6.44 (s, 1H), 5.67 (q, J = 6.8 Hz, 1H), 3.13 (s, 6H), 2.51–2.59 (m, 2H), 2.50 (s, 3H), 2.16 (s, 3H), 2.05 (s, 3H), 1.84 (d, J = 6.8 Hz, 3H), 1.04 (t, J = 7.5 Hz, 3H).

5.1.31. 5-(1-Ethylpropyl)-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine (2)

To a stirred solution of **46** (6.87 g, 20.5 mmol) in EtOH (70 mL) was added 10% Pd-C (345 mg) under argon atmosphere. The reaction mixture was stirred at room temperature under hydrogen gas. After being stirred for 3 h, the reaction mixture was filtered through a pad of Celite and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 0/1–22/78) to afford **2** (7.3 g, 77% yield) as a yellow powder. TLC R_f = 0.65 (EtOAc/hexane, 1/3); MS (APCI, Pos) m/z 337 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{29}N_2O$: 337.2280. Found: 337.2291; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.14 (d, J = 8.1 Hz, 1H), 6.75–6.84 (m, 2H), 6.41 (s, 1H), 3.83 (s, 3H), 2.55–2.68 (m, 1H), 2.50 (s, 3H), 2.19 (s, 3H), 1.96 (s, 3H), 1.50–1.83 (m, 4H), 0.83 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 11.5, 12.6, 19.2, 20.0, 29.7, 40.1, 55.3, 109.6, 111.0, 114.8, 115.5, 115.6, 123.3, 123.3, 124.1, 130.1, 130.2, 137.8, 152.4, 159.3; IR (KBr) 2959, 2922, 2870, 2853, 1611, 1573, 1502, 1463, 1456, 1442, 1291, 1281, 1265, 1239, 1160, 1122, 1094, 1054, 999, 865, 810, cm^{-1}; mp 83–85 °C.

5.1.32. 2-(2,4-Dimethylphenyl)-5-(1-ethylpropyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine (7)

A yellow oil. TLC R_f = 0.64 (EtOAc/hexane, 1/9); MS (APCI, Pos) m/z 321 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{29}N_2$: 321.2331. Found: 321.2325; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 1.1 Hz, 1H), 7.46 (d, J = 1.1 Hz, 1H), 7.04–7. 17 (m, 3H), 6.43 (s, 1H), 2.55–2.69 (m, 1H), 2.50 (s, 3H), 2.38 (s, 3H), 2.18 (s, 3H), 1.97 (d, J = 1.1 Hz, 3H), 1.50–1.84 (m, 4H), 0.83 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.55, 12.58, 19.15, 19.67, 21.34, 29.72, 40.13, 109.51, 114.77, 115.40, 123.29, 123.34, 124.10, 126.31, 128.91, 130.85, 134.67, 136.05, 137.84, 152.68; IR (neat) 2961, 2921, 2871, 1615, 1550, 1505, 1454, 1408, 1377, 1302, 1263, 1217, 1145, 1094, 1035, 998, 885, 824, 758, 620 cm^{-1}; mp 213–214 °C.

5.1.33. 5-(1-Ethylpropyl)-3,7-dimethyl-2-(2,4,5-trimethylphenyl)pyrrolo[1,2-*b*]pyridazine (8)

A yellow powder. TLC R_f = 0.48 (EtOAc/hexane, 1/20); MS (APCI, Pos) m/z 335 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{31}N_2$: 335.2487. Found: 335.2467; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.06 (s, 1H), 7.01 (s, 1H), 6.42 (s, 1H), 2.56–2.67 (m, 1H), 2.50 (s, 3H), 2.28 (s, 3H), 2.26 (s, 3H), 2.13 (s, 3H), 1.97 (s, 3H), 1.68–1.82 (m, 2H), 1.54–1.67 (m, 2H), 0.83 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.86, 12.86, 19.40, 19.45, 19.67, 19.90, 30.02, 40.42, 109.72, 114.99, 115.74, 123.55, 123.64, 124.29, 130.37, 131.66, 133.62, 133.89, 135.23, 136.74, 153.09; IR (KBr) 2958, 2915, 2868, 1616, 1550, 1504, 1478, 1457, 1450, 1407, 1385, 1368, 1353, 1304, 1283, 1262, 1213, 1144, 1088, 1076, 1032, 1021, 1008, 982, 967, 892, 877, 811, 691, 636, 552, 459 cm^{-1}; Anal Calcd for $C_{23}H_{30}N_2$; C, 82.59; H, 9.04; N, 8.37; Found: C, 82.41; H, 9.30; N, 8.38; mp 77 °C.

5.1.34. 2-(4-Ethoxy-2-ethylphenyl)-5-(1-ethylpropyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine(9)

A yellow solid. TLC R_f = 0.54 (EtOAc/hexane, 1/10); MS (APCI, Pos) m/z 365 $(M+H)^+$; FABHRMS calcd for $C_{24}H_{33}N_2O$: 365.2593. Found: 365.2579; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J = 1.1 Hz, 1H), 7.13 (d, J = 8.2 Hz, 1H), 6.88 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.3, 2.6 Hz, 1H), 6.43 (s, 1H), 4.08 (q, J = 6.9 Hz, 2H), 2.57–2.69 (m, 1H), 2.44–2.55 (m, 5H), 1.96 (d, J = 0.9 Hz, 3H), 1.51–1.84 (m, 4H), 1.44 (t, J = 7.0 Hz, 3H), 1.15 (t, J = 7.5 Hz, 3H), 0.84 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.58, 12.59, 15.03, 15.43, 19.43, 26.50, 29.70, 40.10, 63.36, 109.45, 111.21, 114.72, 114.74, 115.78, 123.20, 123.32, 124.06, 129.40, 130.27, 143.99, 152.34, 158.85; IR (KBr) 814, 871, 998, 1037, 1064, 1093, 1111, 1144, 1168, 1223, 1258, 1278, 1294, 1355, 1375, 1390, 1407, 1436, 1453, 1462, 1477, 1496, 1568, 1608, 2853, 2871, 2913, 2962, 3426, 3485, 3500 cm^{-1}; Anal. Calcd for $C_{24}H_{32}N_2O$; C, 79.08; H, 8.85; N, 7.67; Found: C, 79.54; H, 9.08; N, 7.75; mp 55–59 °C.

5.1.35. 5-(1-Ethylpropyl)-3,7-dimethyl-2-(6-methyl-1,3-benzodioxol-5-yl)pyrrolo[1,2-*b*]pyridazine (10)

A yellow solid. TLC R_f = 0.51 (EtOAc/hexane, 1/10); MS (APCI, Pos) m/z 351 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{27}N_2O_2$: 351.2073.

Found: 351.2070; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 0.9 Hz, 1H), 6.76 (s, 1H), 6.73 (s, 1H), 6.44 (s, 1H), 5.97 (s, 2H), 2.56–2.70 (m, 1H), 2.51 (s, 3H), 2.11 (s, 3H), 1.98 (d, J = 0.7 Hz, 3H), 1.49–1.85 (m, 4H), 0.83 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.59, 12.57, 19.03, 19.55, 29.71, 40.11, 100.94, 109.25, 109.68, 110.19, 114.92, 115.41, 123.33, 123.37, 124.25, 129.99, 130.36, 145.41, 147.38, 152.38; IR (KBr) 820.56, 834.06, 846.60, 875.52, 934.34, 970.02, 1010.52, 1037.52, 1073.19, 1080.91, 1140.69, 1222.65, 1235.18, 1259.29, 1294.97, 1362.46, 1407.78, 1438.64, 1463.71, 1487.81, 1503.24, 1617.98, 2867.63, 2916.81, 2951.52, 3443.28, 3499.20 cm^{-1}; Anal Calcd for $C_{22}H_{26}N_2O_2$; C, 75.40; H, 7.48; N, 7.99; Found: C, 75.58; H, 7.67; N, 7.93; mp 108–110 °C.

5.1.36. 2-(2-Ethyl-4,5-dimethoxyphenyl)-5-(1-ethylpropyl)-3,7-dimethylpyrrolo[1,2-*b*]pyridazine (11)

A yellow powder. TLC R_f = 0.59 (EtOAc/hexane, 1/3); MS (APCI, Pos) m/z 381 (M+H)$^+$; FABHRMS calcd for $C_{24}H_{33}N_2O_2$: 381.2542. Found: 381.2520; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 1.1 Hz, 1H), 6.84 (s, 1H), 6.74 (s, 1H), 6.44 (s, 1H), 3. 94 (s, 3H), 3.85 (s, 3H), 2.57–2.70 (m, 1H), 2.52 (s, 3H), 2.39–2.54 (m, 2H), 1.98 (s, 3H), 1.51–1.85 (m, 4H), 1.15 (t, J = 7.5 Hz, 3H), 0.84 (t, J = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.64, 13.62, 17.03, 20.38, 27.09, 30.75, 41.16, 56.98, 57.04, 110.62, 112.63, 113.30, 115.90, 116.62, 124.32, 124.39, 125.20, 129.84, 136.03, 147.68, 149.85, 153.35; IR (KBr) 2961, 2924, 2853, 1606, 1517, 1465, 1450, 1355, 1299, 1262, 1245, 1209, 1148, 1134, 1098, 861, 824 cm^{-1}; mp 97–99 °C.

5.1.37. 5-[5-(1-Ethylpropyl)-3,7-dimethylpyrrolo[1,2-*b*] pyridazin-2-yl]-*N*,*N*,4-trimethyl-2-pyridinamine (12)

A yellow oil. TLC R_f = 0.60 (EtOAc/hexane, 1/3); MS (APCI, Pos) m/z 351 (M+H)$^+$; FABHRMS calcd for $C_{22}H_{31}N_4$: 351.2549. Found: 351.2547; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (s, 1H), 7.46–7.47 (m, 1H), 6.43–6.44 (m, 2H), 3.13 (s, 6H), 2.58–2.67 (m, 1H), 2.51 (s, 3H), 2.17 (d, J = 0.5 Hz, 3H), 2.03 (d, J = 0.9 Hz, 3H), 1.68–1.79 (m, 2H), 1.57–1.67 (m, 2H), 0.83 (t, J = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.51, 12.58, 19.37, 20.11, 29.71, 38.29, 40.12, 106.37, 109.64, 114.81, 116.04, 122.31, 123.24, 123.30, 124.15, 146.64, 147.73, 150.40, 159.17; IR (neat) 2958, 2921, 2869, 1606, 1517, 1439, 1398, 1375, 1356, 1306, 1264, 1225, 1171, 1150, 1093, 1075, 1064, 1036, 995, 971, 880, 830, 816, 739, 636, 535 cm^{-1}.

5.1.38. 2-Chloro-4-(4-methoxy-2-methylphenyl)-5-methylpyrimidine (48)

To a stirred solution of **47** (1.0 g, 6.1 mmol) and 2-methyl-4-methoxyphenylboronic acid (1.1 g, 6.7 mmol) in DME-water (10 mL-2 mL) were added $PdCl_2(dppf)\cdot CH_2Cl_2$ (250 mg, 0.31 mmol) and potassium carbonate (1.3 g, 9.20 mmol) at ambient temperature under argon atmosphere and the reaction mixture was heated at 80 °C. After being stirred for 22 h, the reaction mixture was cooled to room temperature and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 16/84–36/64) to give **48** (1.2 g, 79% yield) as a white powder. TCL R_f = 0.42 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (m, 1H), 7.07 (dd, J = 7.8, 0.9 Hz, 1H), 6.85–6.75 (m, 2H), 3.83 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H).

5.1.39. 4-(4-Methoxy-2-methylphenyl)-5-methylpyrimidine-2-carbonitrile (49)

To a stirred solution of **48** (3.36 g, 13.5 mmol) in DMSO (27 mL) was added potassium cyanide (1.75 g, 27.0 mmol) at room temperature under argon atmosphere and the reaction mixture was heated at 100 °C. After being stirred for 23 h, the reaction mixture was cooled to room temperature, poured into water and extracted with EtOAc. The organic layer was washed with water, bri6ne, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 25/75–34/55) to afford **49** (1.95 g, 60% yield) as a yellow powder. TLC R_f = 0.49 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, J = 0.9 Hz, 1H), 7.09 (d, J = 8.1 Hz, 1H), 6.89–6.81 (m, 2H), 3.85 (s, 3H), 2.26 (d, J = 0.9 Hz, 3H), 2.15 (s, 3H).

5.1.40. 1-[4-(4-Methoxy-2-methylphenyl)-5-methylpyrimidin-2-yl]ethanone

To a stirred solution of **49** (100 mg, 0.42 mmol) in Et_2O (1.4 mL) was added dropwise methyl magnesium bromide (0.17 mL, 0.51 mmol, 3.0 M/Et_2O) at 0 °C under argon atmosphere. After being stirred for 10 min, the reaction mixture was quenched with 1 M HCl (1.5 mL), treated with $NaHCO_3$ 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, 47/53–67/33) to give the title compound (75 mg, 73% yield) as a colorless oil. TLC R_f = 0.24 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.79 (d, J = 0.6 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 6.89–6.81 (m, 2H), 3.86 (s, 3H), 2.77 (s, 3H), 2.23 (d, J = 0.6 Hz, 3H), 2.16 (s, 3H).

5.1.41. 1-[4-(4-Methoxy-2-methylphenyl)-5-methylpyrimidin-2-yl]ethylamine (50)

To a stirred solution of 1-[4-(4-methoxy-2-methylphenyl)-5-methylpyrimidin-2-yl]ethanone (75 mg, 0.307 mmol) in EtOH(1.0 mL) were added titanium tetraisopropoxide (110 μl, 0.614 mmol) and ammonia (3.1 mL, 1.55 mmol, 0.5 M/dioxane) at room temperature under argon atmosphere. After being stirred for 15 h, the reaction mixture was treated with sodium borohydride (18 mg, 0.46 mmol) and stirred for additional 4 h, and then treated with ammonium hydroxide (28%). The reaction mixture was filtered through a pad of Celite and washed with EtOAc. The filtrate was extracted with 1 M HCl, washed with EtOAc. By adding 2 M sodium hydroxide, pH value of the solution was adjusted to 12. The aqueous solution was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give **50** (57.4 mg, 72% yield) as a brown oil. MS (APCI, Pos) m/z 258 (M+H)$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 0.9 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 6.88–6.70 (m, 2H), 4.25 (q, J = 6.6 Hz, 1H), 3.84 (s, 3H), 2.14 (s, 3H), 2.14 (s, 3H), 1.48 (d, J = 6.6 Hz, 3H).

5.1.42. 2-Ethyl-*N*-{1-[4-(4-methoxy-2-methylphenyl)-5-methylpyrimidin-2-yl]ethyl}butyramide (51)

To a stirred solution of **50** (55 mg, 0.213 mmol) in CH_2Cl_2 (2.0 mL) were added triethylamine (90 μl, 0.641 mmol) and 2-ethylbutyryl chloride (35 mg, 0.256 mmol) at 0 °C under argon atmosphere. After being stirred for 1.5 h, the reaction mixture was quenched with $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 60/40–80/20) to give **51** (34 mg, 45% yield) as a yellow oil. TLC R_f = 0.25 (EtOAc/hexane, 2/1); MS (APCI, Pos) m/z 356 (M+H)$^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 0.9 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H), 6.92 (d, J = 8.1 Hz, 1H), 6.88–6.76 (m, 2H), 5.31 (m, 1H), 3.85 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H), 1.96 (m, 1H), 1.76–1.38 (m, 4H), 1.53 (d, J = 6.6 Hz, 3H), 0.92 (t, J = 7.5 Hz, 3H), 0.85 (t, J = 7.5 Hz, 3H).

5.1.43. 6-(1-Ethylpropyl)-2-(4-methoxy-2-methylphenyl)-3,8-dimethylimidazo[1,5-*a*]pyrimidine (13)

To a stirred solution of **51** (16 mg, 45 μmol) in toluene (0.45 mL) was added phosphorus oxychloride (20 μl, 224 μmol) at room temperature under argon atmosphere and the reaction mixture was

heated at 90 °C. After being stirred for 4 h, the reaction mixture was cooled to room temperature, quenched with ice and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by TLC (EtOAc/hexane, 2/1) to give **13** (7.1 mg, 47% yield) as a yellow oil. TLC R_f = 0.50 (EtOAc/hexane, 2/1); MS (ESI, Pos) *m*/*z* 338 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{28}N_3O$: 338.2232. Found: 338.2233; ^1H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.2 Hz, 1H), 7.13–7.16 (m, 1H), 6.80–6.84 (m, 2H), 3.84 (s, 3H), 2.67 (s, 3H), 2.61–2.71 (m, 1H), 2.20 (s, 3H), 1.95 (d, *J* = 1.2 Hz, 3H), 1.74–1.84 (m, 4H), 0.82 (t, *J* = 7.3 Hz, 6H); IR (neat) 585, 702, 736, 791, 821, 848, 999, 1024, 1053, 1102, 1127, 1162, 1240, 1265, 1281, 1294, 1312, 1351, 1381, 1397, 1465, 1507, 1574, 1609, 2871, 2927, 2959 cm^{-1}.

5.1.44. 5-(1-Ethylpropyl)imidazolidine-2,4-dione

To a stirred solution of 2-ethylbutyraldehyde **52** (20.4 g, 204 mmol) in MeOH–H_2O (75–75 mL) were added sodium hydrogen sulfite (25 g, 240 mmol), ammonium carbonate (50 g, 521 mmol) and sodium cyanide (25 g, 510 mmol) at room temperature and the reaction mixture was heated at 60 °C. After being stirred for 12 h, the reaction mixture was quenched with water. The resulting precipitates were collected by filtration, and then dried under reduced pressure to give the title compound (20.1 g, 58% yield) as a white solid.; ^1H NMR (300 MHz, DMSO-d_6) δ 10.57 (br s, 1H), 7.87 (s, 1H), 4.08 (dd, *J* = 2.7, 1.1 Hz, 1H), 1.10–1.65 (m, 4H), 0.88 (t, *J* = 7.4 Hz, 3H), 0.84 (t, *J* = 7.4 Hz, 3H).

5.1.45. 2-Amino-3-ethylpentanoic acid hydrogen bromide salt

A mixture of 5-(1-ethylpropyl)imidazolidin-2,4-dione (2.79 g) and hydrobromic acid (12 mL, 47%) was stirred for 2 days at 150 °C. The reaction mixture was cooled to room temperature, and then the resulting precipitates were collected by filtration, dried under reduced pressure to afford 2-amino-3-ethylpentanoic acid hydrogen bromide salt (2.35 g, 63% yield) as a white solid.

^1H NMR (300 MHz, DMSO-d_6) δ 13.82 (br s, 1H), 8.14 (br s, 2H), 3.90 (br s, 1H), 1.67 (m, 1H), 1.57–1.11 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.88 (t, *J* = 7.3 Hz, 3H).

5.1.46. 2-Benzyloxycarbonylamino-3-ethylpentanoic acid (53)

To a stirred solution of 2-amino-3-ethylpentanoic acid hydrogen bromide salt (2.35 g, 10.4 mmol) were added 2 M NaOH (15 mL, 30 mmol) and benzyloxycarbonyl chloride (1.5 mL, 10.5 mmol) at ambient temperature. After being stirred for 12 h, benzyloxycarbonyl chloride (1.0 mL) was added and stirring was continued for 5 h. The reaction mixture was quenched with 2 M HCl and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 25/75–75/25) to give **53** (600 mg, 21% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.32 (m, 5H), 5.13 (s, 2H), 4.57 (m, 1H), 1.77 (br s, 1H), 1.51–1.17 (m, 4H), 1.07–0.85 (m, 6H).

5.1.47. *tert*-Butyl 4-benzyloxycarbonylamino-5-ethyl-3-oxo-heptanoate (54)

To a stirred solution of lithium hexamethyldisilazane (3.2 mL, 1.0 M in THF) was added dropwise *tert*-butyl acetate (0.43 mL, 3.2 mmol) at −78 °C. After being stirred for 30 min, the reaction mixture was treated with **53** (280 mg, 1.00 mmol) and CDI (170 mL, 1.05 mmol) in THF (4 mL) at −78 °C and allowed to warm up to room temperature. After being stirred for 1.5 h, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 5/95–25/75) to afford **54** (264 mg, 70% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.31 (m, 5H), 5.11 (s, 2H), 3.45 (m, 2H), 1.74 (br s, 1H), 1.46 (s, 9H), 1.34–1.06 (m, 4H), 1.02 (m, 3H), 0.88 (m, 3H).

5.1.48. 1-*tert*-Butyl 4-ethyl 2-(2-benzyloxycarbonylamino-3-ethylpentanoyl)-3-methylsuccinate (55)

To a stirred suspension of sodium hydride (30 mg, 0.75 mmol, 60% in mineral oil) in THF was added dropwise a solution of **54** (264 mg, 0.70 mmol) in THF (3.5 mL) at 0 °C. After being stirred for 10 min, the reaction mixture was treated with a solution of ethyl 2-(trifluoromethylsulfonyloxy)propionate (350 mg, 1.40 mmol) in CH_2Cl_2 (3.5 mL) at room temperature and stirred for 2 h. The reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated to give **55** (524 mg), which was used for the next reaction without purification.

5.1.49. Ethyl 5-benzyloxycarbonylamino-6-ethyl-2-methyl-4-oxo-octanoate (56)

To a stirred solution of **55** (524 mg, 0.699 mmol) in CH_2Cl_2 (3.5 mL) was added trifluoromethanesulfonic acid (0.5 mL) at room temperature. After being stirred for 2 days, the reaction mixture was quenched with $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/3) to afford **56** (75 mg, 29%). TLC R_f = 0.37 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 400 $(M+Na)^+$, 378 $(M+H)^+$.

5.1.50. Benzyl [2-ethyl-1-(5-methyl-6-oxo-1,4,5,6-tetrahydropyridazin-3-yl)butyl]carbamate (57)

To a stirred solution of **56** (75 mg, 0.20 mmol) in EtOH (2.0 mL) was added hydrazine monohydrate (50 μl, 1.0 mmol) at reflux temperature. After being stirred for 12 h, the reaction mixture was treated with AcOH (0.2 mL) at reflux temperature and stirred for 7 h, and then evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 4/6–7/3) to give **57** (58 mg, 84% yield). TLC R_f = 0.33 (EtOAc/hexane, 1/1); MS (ESI, Pos) *m*/*z* 346 $(M+H)^+$.

5.1.51. *N*-[2-Ethyl-1-(5-methyl-6-oxo-1,6-dihydropyridazin-3-yl)butyl]acetamide (58)

To a stirred solution of **57** (58 mg, 0.17 mmol) in AcOH (1.0 mL) was added bromine (30 mg, 0.19 mmol) at 50 °C. After being stirred for 12 h, the reaction mixture was cooled to room temperature, quenched with $NaHCO_3$ aq and washed with EtOAc. To a stirred aqueous layer were added acetic anhydride (1.0 mL) and pyridine (5.0 mL) at room temperature. After being stirred for 12 h, the reaction mixture was quenched with 2 M HCl and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to **58** (12 mg, 28% yield). TLC R_f = 0.39 (MeOH/eEtOAc, 1/9); MS (ESI, Pos) *m*/*z* 252 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 11.69 (br s, 1H) 7.07 (m, 1H), 6.08 (d, *J* = 8.6 Hz, 1H), 5.03 (dd, *J* = 9.2, 5.6 Hz, 1H), 2.33 (d, *J* = 1.3 Hz, 3H), 2.04 (s, 3H), 1.60 (m, 1H), 1.47–1.16 (m, 4H), 0.95 (t, *J* = 7.5 Hz, 3H), 0.88 (t, *J* = 7.5 Hz, 3H).

5.1.52. 2-Chloro-5-(1-ethylpropyl)-3,7-dimethylimidazo[1,5-*b*]pyridazine (59)

A solution of **58** (12 mg, 48 μmol) in phosphorus oxychloride (1.0 mL) was stirred at 80 °C for 6 h. The reaction mixture was cooled to room temperature, quenched with ice-water, neturalized with $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated to give **59** (14 mg, 100% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 7.47 (q, *J* = 1.2 Hz, 1H), 2.68 (s, 3H), 2.59 (m, 1H), 2.30 (d, *J* = 1.1 Hz, 3H), 1.75 (quin, *J* = 7.3 Hz, 4H), 0.76 (t, *J* = 7.4 Hz, 6H).

5.1.53. 5-(1-Ethylpropyl)-2-(4-methoxy-2-methylphenyl)-3,7-dimethylimidazo[1,5-*b*]pyridazine (14)

To a stirred solution of **59** (14 mg, 0.048 mmol) in DME (0.2 mL) were added 2-methyl-4-methoxyphenylboronic acid (8 mg, 0.05 mmol), $Pd(PPh_3)_4$(6 mg, 0.005 mmol) and 1 M Na_2CO_3 (0.1 mL) at 80 °C. After being stirred for 1 h, the reaction mixture was cooled and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by silica gel chromatography (EtOAc/hexane, 15/85–35/65) to give **14** (3.6 mg, 23% yield) as a yellow oil. TLC R_f = 0.50 (EtOAc/hexane, 1/2); MS (ESI, Pos) *m*/*z* 338 (M+H)+; FAB-HRMS calcd for $C_{21}H_{28}N_3O$: 338.2232. Found: 338.2233; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.2 Hz, 1H), 7.15 (m, 1H), 6.80–6.84 (m, 2H), 3.84 (s, 3H), 2.67 (s, 3H), 2.66 (m, 1H), 2.20 (s, 3H), 1.95 (d, *J* = 1.2 Hz, 3H), 1.74–1.84 (m, 4H), 0.82 (t, *J* = 7.3 Hz, 6H).

5.1.54. 3-(5-Methyl-1*H*-pyrrol-3-yl)-pentan-3-ol (61)

To a stirred solution of **60** (4.6 g, 30 mmol) in THF (60 mL) was added dropwise ethyl magnesium bromide (40 mL, 3.0 M/Et_2O) at 0 °C and heated at 50 °C. After being stirred for 1 day, the reaction mixture was treated with ethyl magnesium bromide (60 mL, 3.0 M/Et_2O) and stirred for another 6 h. The reaction mixture was cooled to 0 °C, poured into NH_4Cl aq carefully at 0 °C and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated to give **61** (4.98 g, 99% yield) as a brown solid. TLC R_f = 0.80 (EtOAc/hexane, 1/2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 (m, 1H), 6.53 (m, 1H), 5.79 (m, 1H), 2.25 (d, *J* = 0.7, 2H), 1.75 (q, *J* = 7.5 Hz, 4H), 0.85 (t, *J* = 7.5 Hz, 6H).

5.1.55. 3-(5-Methyl-1*H*-pyrrol-3-yl)-propan-2-ol

98% yield; a brown oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (br s, 1H), 6.60 (m, 1H), 5.93 (m, 1H), 2.25 (d, *J* = 0.9 Hz, 3H), 1.55 (s, 6H).

5.1.56. 4-(1-Ethylpropyl)-2-methyl-1*H*-pyrrole (62)

To a stirred suspension of lithium aluminum hydride (3.0 g, 79.1 mmol) in THF (150 mL) was added dropwise a solution of **61** (3.31 g, 19.8 mmol) in THF (50 mL) in 30 min at reflux temperature. After being stirred for 17 h, the reaction mixture was cooled to 0 °C, quenched with Na_2SO_4 aq. The resulting precipitates were removed by filtration through a Celite and the filtrate was evaporated to give **62** (3.38 g) as a brown oil, which was used for the next reaction without further purification. TLC R_f = 0.46 (EtOAc/hexane, 1/9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (m, 1H), 6.38 (t, *J* = 2.0 Hz, 1H), 5.72 (m, 1H), 2.25 (m, 3H), 2.22 (m, 1H), 1.64–1.38 (m, 4H), 0.84 (t, *J* = 7.4 Hz, 6H).

5.1.57. 4-(1-Methylethyl)-2-methyl-1*H*-pyrrole

90% yield; a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 1H), 6.42 (m, 1H), 5.81 (m, 1H), 2.79 (m, 1H), 2.25 (m, 3H), 1.21 (s, 3H), 1.18 (s, 3H).

5.1.58. 3-(1-Ethylpropyl)-5-methyl-1*H*-pyrrole-2-caboxylic acid methylamide (63)

To a stirred solution of **62** (2.76 g, 18.2 mmol) in THF (36 mL) was added trichloroacethyl chloride (2.0 mL, 18.2 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was treated with methylamine (36 mL, 40%/water), stirred for 1 h at room temperature, and then extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 50/50) to afford **63** (1.55 g, 41% yield in 2 steps) as an off-white powder. TLC R_f = 0.26 (EtOAc/hexane, 1/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (m, 1H), 5.75 (d, *J* = 3.1 Hz, 1H), 5.67 (br s, 1H), 2.97 (d, *J* = 4.9 Hz, 3H), 2.48 (m, 1H), 2.24 (s, 3H), 1.76–1.45 (m, 4H), 0.84 (t, *J* = 7.3 Hz, 6H).

5.1.59. 3-(1-Ethylpropyl)-5-methyl-1*H*-pyrrole-2-caboxylic acid ethylamide

51% yield; a brown solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (br s, 1H), 5.75–5.74 (m, 1H), 5.66 (br s, 1H), 3.51–3.41 (m, 2H), 2.46 (m, 1H), 2.24 (s, 3H), 1.73–1.52 (m, 4H), 1.25 (t, *J* = 7.2 Hz, 3H), 0.85 (t, *J* = 7.2 Hz, 6H).

5.1.60. 3-(1-Ethylpropyl)-5-methyl-1*H*-pyrrole-2-caboxylic acid cyclopropylamide

22% yield; a brown soild; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (br s, 1H), 6.75 (br s, 1H), 5.99 (d, *J* = 2.6 Hz, 1H), 3.36 (m, 1H), 2.35(s, 3H), 2.23 (s, 3H), 1.77–1.45 (m, 4H), 0.96–0.87 (m, 1H), 0.83 (m, 6H), 0.67 (m, 2H), 0.56 (m, 1H).

5.1.61. 3-(1-Methylethyl)-5-methyl-1*H*-pyrrole-2-caboxylic acid methylamide

51% yield; an ivory solid; MS (APCI, Pos) *m*/*z* 181 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.98 (m, 1H), 5.84 (d, *J* = 3.3 Hz, 1H), 5.64 (m, 1H), 3.01 (m, 1H), 2.98 (d, *J* = 4.8 Hz, 3H), 2.24 (s, 3H), 1.26 (d, *J* = 6.9 Hz, 6H).

5.1.62. 1-Amino-3-(1-ethylpropyl)-5-methyl-1*H*-pyrrole-2-carboxylic acid methylamide (64)

To a stirred suspension of sodium hydride (200 mg, 60% in mineral oil) in DMF (15 mL) were added a solution of **63** (1.04 g, 5.0 mmol) in DMF (10 mL) and a solution of NH_2Cl in Et_2O (50 mL) at 0 °C. After being stirred for 15 min, the reaction mixture was quenched with $Na_2S_2O_3$ aq and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 25/75–1/1) to afford **64** as a beige powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.64 (m, 1H), 5.65 (s, 1H), 5.14 (br s, 2H), 2.95 (d, *J* = 4.7 Hz, 3H), 2.85 (m, 1H), 2.23 (d, *J* = 0.7 Hz, 2H), 1.71–1.39 (m, 4H), 0.82 (t, *J* = 7.4 Hz, 6H).

5.1.63. 1-Amino-3-(1-ethylpropyl)-5-methyl-1*H*-pyrrole-2-carboxylic acid ethylamide

31% yield; a pale yellow soild; MS (APCI, Pos) *m*/*z* 238 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (br s, 1H), 5.64 (s, 1H), 5.19 (s, 2H), 3.49–3.40 (m, 2H), 2.78 (m, 1H), 2.23 (s, 3H), 1.66–1.46 (m, 4H), 1.23 (t, *J* = 7.2 Hz, 3H), 0.83 (t, *J* = 7.2 Hz, 6H).

5.1.64. 1-Amino-3-(1-ethylpropyl)-5-methyl-1*H*-pyrrole-2-carboxylic acid cyclopropylamide

63% yield; a brown solid; MS (APCI, Pos) *m*/*z* 250 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.66 (br s, 1H), 5.63 (s, 1H), 5.18 (s, 2H), 2.90–2.70 (m, 1H), 2.74 (m, 1H), 2.23 (s, 3H), 1.70–1.40 (m, 4H), 0.86–0.82 (m, 2H), 0.81 (t, *J* = 7.2 Hz, 6H). 0.56–0.53 (m, 2H)

5.1.65. 5-(1-Ethylpropyl)-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (3)

To a stirred solution of **64** (200 mg, 0.90 mmol) in xylene (2.0 mL) were added 4-methoxy-2-methylbenzaldehyde (144 mg, 0.96 mmol) and Darco-KB (114 mg) at room temperature. The reaction mixture was vigorously stirred under oxygen atmosphere. After being stirred for 10 h at 130 °C, the reaction mixture was cooled to room temperature. The Darck-KB was removed by filtration through a pad of Celite and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 1/4) to give **3** (194 mg, 61% yield) as a white solid. TLC R_f = 0.45 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 354 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{28}N_3O_2$: 354.2182. Found: 354.2185; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, *J* = 8.97 Hz, 1H), 6.80–6.90 (m, 2H), 6.17 (s, 1H), 3.85 (s, 3H), 3.40 (m, 1H), 3.14 (s,

3H), 2.39 (s, 3H), 2.29 (s, 3H), 1.52–1.82 (m, 4H), 0.79–0.95 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.18, 12.14, 19.75, 28.91, 30.69, 39.62, 55.37, 108.16, 111.59, 114.17, 115.88, 125.02, 128.86, 129.99, 130.42, 138.16, 147.33, 155.86, 160.48; mp 117 °C.

5.1.66. 2-(2-Chloro-4-methoxyphenyl)-5-(1-ethylpropyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (4)

A white powder. TLC R_f = 0.46 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 374 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{25}ClN_3O_2$: 374.1635. Found: 374.1634; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 6.94 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.18 (s, 1H), 3.87 (s, 3H), 3.30–3.51 (m, 1H), 3.21 (s, 3H), 2.39 (s, 3H), 1.44–1.88 (m, 4H), 0.58–1.08 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.15, 12.13, 12.20, 28.89, 28.96, 30.33, 39.60, 55.78, 108.30, 113.26, 114.22, 115.17, 124.50, 129.00, 130.58, 131.27, 134.33, 145.63, 155.55, 161.24; IR (KBr) 454, 595, 674, 709, 741, 756, 801, 834, 857, 878, 1003, 1016, 1041, 1069, 1109, 1172, 1187, 1213, 1237, 1275, 1294, 1331, 1348, 1375, 1410, 1427, 1459, 1504, 1565, 1606, 1666, 2872, 2931, 2962 cm^{-1}; mp 90–93 °C.

5.1.67. 5-(1-Ethylpropyl)-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (15)

A white powder. TLC R_f = 0.32 (EtOAc/hexane, 1/3); MS (APCI, Pos) *m*/*z* 340 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{26}N_3O_2$: 340.2025. Found: 340.2023; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.11 (s, 1H), 7.38–7.67 (m, 1H), 6.70–7.00 (m, 2H), 6.16 (s, 1H), 3.85 (s, 3H), 3.15–3.45 (m, 1H), 2.53 (s, 3H), 2.44 (d, *J* = 0.7 Hz, 3H), 1.44–1.86 (m, 4H), 0.83 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.33, 12.17, 21.03, 28.88, 39.99, 55.32, 108.38, 111.23, 113.78, 116.81, 123.61, 130.08, 130.32, 131.10, 138.97, 145.07, 156.50, 160.69; IR (KBr) 523.58, 585.29, 597.82, 665.32, 741.50, 809.96, 862.02, 883.24, 966.16, 1047.16, 1057.76, 1133.94, 1161.90, 1239.04, 1283.39, 1335.46, 1348.00, 1365.35, 1484.92, 1499.38, 1509.03, 1572.66, 1649.80, 2753.85, 2857.02, 2869.56, 2925.48, 2958.27, 3058.55, 3154.01 cm^{-1}; mp 195 °C.

5.1.68. 2-(2-Chloro-4-methoxyphenyl)-5-(1-ethylpropyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (16)

A yellow powder. TLC R_f = 0.44 (EtOAc/hexane, 1/2); MS (APCI, Pos) *m*/*z* 360 $(M+H)^+$; FABHRMS calcd for $C_{19}H_{23}ClN_3O_2$: 360.1479. Found: 360.1484; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.75 (s, 1H), 7.64 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 6.92 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.18 (s, 1H), 3.86 (s, 3H), 3.15–3.32 (m, 1H), 2.45 (s, 3H), 1.45–1.79 (m, 4H), 0.82 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.33, 12.15, 28.87, 39.91, 55.75, 108.52, 113.23, 113.90, 115.71, 122.86, 130.38, 131.45, 132.14, 133.44, 143.30, 155.64, 161.49; IR (KBr) 524, 596, 663, 812, 857, 966, 1046, 1063, 1116, 1183, 1220, 1240, 1270, 1290, 1336, 1347, 1365, 1388, 1409, 1434, 1484, 1501, 1564, 1605, 1649, 2769, 2851, 2923, 2959, 3057, 3184, 3366 cm^{-1}; mp 213 °C.

5.1.69. 3-Ethyl-5-(1-ethylpropyl)-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (17)

A pale yellow viscous oil, TLC R_f = 0.29 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 368 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{30}N_3O_2$: 368.2338. Found: 368.2337; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.38 (m, 1H), 6.80–6.94 (m, 2H), 6.16 (s, 1H), 3.98–4.18 (m, 1H), 3.86 (s, 3H), 3.30–3.51 (m, 2H), 2.38 (d, *J* = 0.7 Hz, 3H), 2.28 (s, 3H), 1.51–1.85 (m, 4H), 1.05 (t, *J* = 7.0 Hz, 3H), 0.80–0.96 (m, 6H); IR (neat) 674, 737, 759, 777, 808, 825, 849, 1012, 1036, 1058, 1113, 1124, 1163, 1182, 1208, 1244, 1267, 1284, 1295, 1314, 1346, 1378, 1413, 1425, 1437, 1455, 1460, 1507, 1573, 1608, 1633, 1675, 2838, 2872, 2932, 2961 cm^{-1}.

5.1.70. 2-(2-Chloro-4-methoxyphenyl)-3-ethyl-5-(1-ethylpropyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (18)

A pale yellow viscous oil. TLC R_f = 0.47 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 388 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{27}ClN_3O_2$: 388.1792. Found: 388.1779; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 2.6 Hz, 1H), 6.94 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.17 (s, 1H), 4.03–4.29 (m, 1H), 3.88 (s, 3H), 3.24–3.52 (m, 2H), 2.39 (s, 3H), 1.41–1.86 (m, 4H), 1.07 (t, *J* = 7.0 Hz, 3H), 0.76–0.97 (m, 6H); IR (neat) 2962, 2932, 2872, 1677, 1607, 1566, 1505, 1460, 1439, 1423, 1379, 1347, 1293, 1267, 1233, 1209, 1182, 1089, 1073, 1041, 1026, 822, 810, 738 cm^{-1}.

5.1.71. 5-(1-Ethylpropyl)-3-(2-hydroxyethyl)-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (19)

A pale yellow oil. TLC R_f = 0.60 (EtOAc/hexane, 1/1); MS (APCI, Pos) *m*/*z* 384 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{30}N_3O_3$: 384.2287. Found: 384.2287; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–7.35 (m, 1H), 6.78–6.90 (m, 2H), 6.20 (s, 1H), 4.07–4.29 (m, 1H), 3.85 (s, 3H), 3.57–3.70 (m, 3H), 3.28–3.45 (m, 1H), 2.90 (t, *J* = 5.2 Hz, 1H), 2.39 (d, *J* = 0.7 Hz, 3H), 2.28 (s, 3H), 1.35–1.87 (m, 4H), 0.80–0.93 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.17, 12.03, 12.14, 19.87, 28.73, 28.78, 39.61, 46.46, 55.36, 62.31, 108.66, 111.51, 113.97, 115.91, 124.42, 129.51, 130.41, 131.29, 138.35, 146.95, 157.16, 160.54;IR (neat) 3443, 2961, 2930, 2872, 1675, 1661, 1608, 1507, 1463, 1454, 1435, 1414, 1378, 1347, 1297, 1244, 1055, 1031 cm^{-1}.

5.1.72. 2-(2-Chloro-4-methoxyphenyl)-5-(1-ethylpropyl)-3-(2-hydroxyethyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (20)

A pale yellow powder. TLC R_f = 0.48 (EtOAc/hexane, 1/1); MS (ESI, Pos) *m*/*z* 404 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{27}ClN_3O_3$: 404.1741. Found: 404.1732; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 2.5 Hz, 1H), 6.94 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.20 (s, 1H), 4.28 (m, 1H), 3.87 (s, 3H), 3.75 (m, 1H), 3.45–3.66 (m, 2H), 3.39 (m, 1H), 2.81 (t, *J* = 5.4 Hz, 1H), 2.39 (s, 3H), 1.44–1.91 (m, 4H), 0.76–0.95 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.15, 12.05, 12.12, 28.70, 28.81, 39.60, 46.62, 55.78, 62.21, 108.78, 113.17, 114.03, 115.10, 123.92, 129.64, 131.43, 131.89, 134.36, 145.2, 156.79, 161.30; IR (KBr) 671, 680, 758, 774, 804, 829, 864, 1006, 1019, 1037, 1068, 1133, 1174, 1206, 1235, 1272, 1293, 1348, 1377, 1420, 1433, 1464, 1505, 1561, 1606, 1671, 2852, 2872, 2923, 2959, 3087, 3255, 3358 cm^{-1}; mp 100–120 °C.

5.1.73. 5-(1-Ethylpropyl)-3-isopropyl-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (21)

A pale yellow viscous oil. TLC R_f = 0.34 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 382 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{32}N_3O_2$: 382.2495. Found: 382.2489; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 1H), 6.76–6.92 (m, 2H), 6.13 (s, 1H), 3.76–3.98 (m, 4H), 3.43 (m, 1H), 2.36 (s, 3H), 2.33 (s, 3H), 1.49–1.83 (m, 7H), 1.44 (d, *J* = 6.8 Hz, 3H), 0.79–0.97 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.11, 11.87, 11.96, 20.07, 20.19, 20.83, 28.49, 28.56, 39.15, 52.20, 55.35, 107.93, 111.49, 115.16, 115.82, 125.70, 127.95, 129.56, 130.03, 138.21, 147.53, 156.09, 160.23; IR (neat) 680, 761, 805, 848, 1009, 1037, 1094, 1114, 1124, 1168, 1180, 1203, 1232, 1244, 1262, 1283, 1296, 1316, 1349, 1377, 1415, 1424, 1455, 1462, 1505, 1561, 1574, 1608, 1638, 1673, 1680, 2873, 2931, 2962 cm^{-1}.

5.1.74. 3-Cyclopropyl-5-(1-ethylpropyl)-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (22)

A yellow viscous oil, TLC R_f = 0.38 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 380 $(M+H)^+$; FABHRMS calcd for $C_{23}H_{30}N_3O_2$:

380.2338. Found: 380.2336; ^{1}H NMR (300 MHz, $CDCl_3$) δ7.24–7.37 (m, 1H), 6.73–6.92 (m, 2H), 6.13 (s, 1H), 3.85 (s, 3H), 3.40 (m, 1H), 2.68 (m, 1H), 2.38 (s, 6H), 1.52–1.88 (m, 4H), 0.66–0.97 (m, 8H), 0.43–0.62 (m, 2H); IR (neat) 805, 1041, 1147, 1165, 1217, 1244, 1265, 1284, 1295, 1322, 1351, 1377, 1413, 1455, 1508, 1573, 1608, 1683, 2929, 2960 cm^{-1}.

5.1.75. 2-(2-Chloro-4-methoxyphenyl)-3-cyclopropyl-5-(1-ethylpropyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (23)

A white powder. TLC R_f = 0.38 (EtOAc/hexane, 1/4); MS (APCI, Pos) *m*/*z* 400 (M+H)$^+$; FABHRMS calcd for $C_{22}H_{27}ClN_3O_2$: 400.1792. Found: 400.1790; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 2.6 Hz, 1H), 6.93 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.15 (s, 1H), 3.87 (s, 3H), 3.31–3.50 (m, 1H), 2.76–3.00 (m, 1H), 2.39 (s, 3H), 1.47–1.82 (m, 4H), 0.57–0.98 (m, 9H), 0.43 (m, 1H); IR (KBr) 2957, 2925, 2851, 1686, 1604, 1501, 1462, 1454, 1440, 1415, 1376, 1353, 1330, 1292, 1264, 1213, 1190, 1097, 1052, 1032, 859, 824, 801, 676 cm^{-1}; Anal. Calcd for $C_{22}H_{26}ClN_3O_2$; C, 66.07; H, 6.55; N, 10.51; Found: C, 65.92; H, 6.47; N, 10.56; mp 117 °C.

5.1.76. 5-(1-Ethylpropyl)-3-isobutyl-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (24)

A colorless viscous oil. TLC R_f = 0.29 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 396 (M+H)$^+$; FABHRMS calcd for $C_{24}H_{34}N_3O_2$: 396.2651. Found: 396.2655; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 1H), 6.59–7.18 (m, 2H), 6.16 (s, 1H), 3.69–4.21 (m, 4H), 3.45 (m, 1H), 3.10–3.30 (m, 1H), 2.39 (s, 3H), 2.26 (s, 3H), 1.47–1.99 (m, 5H), 0.80–0.97 (m, 6H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.69 (d, *J* = 6.8 Hz, 3H); IR (neat) 675, 737, 758, 806, 848, 1012, 1043, 1066, 1116, 1125, 1163, 1206, 1232, 1243, 1265, 1286, 1296, 1312, 1346, 1377, 1413, 1421, 1436, 1450, 1463, 1507, 1573, 1608, 1633, 1644, 1676, 1684, 2838, 2871, 2960 cm^{-1}.

5.1.77. 3-(Cyclopropylmethyl)-5-(1-ethylpropyl)-2-(4-methoxy-2-methylphenyl)-7-methylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (25)

A colorless viscous oil. TLC R_f = 0.32 (EtOAc/hexane, 1/9); MS (APCI, Pos) *m*/*z* 394 (M+H)$^+$; FABHRMS calcd for $C_{24}H_{32}N_3O_2$: 394.2495. Found: 394.2479; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 1H), 6.73–6.95 (m, 2H), 6.17 (s, 1H), 3.76–4.04 (m, 4H), 3.18–3.56 (m, 2H), 2.40 (s, 3H), 2.28 (s, 3H), 1.47–1.88 (m, 4H), 0.74–1.08 (m, 7H), 0.24–0.46 (m, 2H), 0.08–0.22 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 4.14, 4.24, 10.94, 11.16, 12.02, 12.14, 19.96, 28.75, 28.81, 39.49, 47.19, 55.34, 108.08, 111.21, 114.36, 115.71, 125.02, 128.69, 130.54, 130.71, 138.52, 147.14, 155.79, 160.41; IR (neat) 674, 737, 757, 794, 809, 847, 937, 986, 1021, 1046, 1128, 1164, 1207, 1232, 1244, 1285, 1296, 1313, 1347, 1357, 1377, 1413, 1428, 1461, 1507, 1573, 1607, 1676, 2871, 2960, 3082 cm^{-1}.

5.1.78. 5-(1-Ethylpropyl)-2-(6-methoxy-4-methyl-3-pyridinyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (26)

A white powder. TLC R_f = 0.60 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 355(M+H)$^+$; FABHRMS calcd for $C_{20}H_{27}N_4O_2$: 355.2134. Found: 355.2133; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.16 (s, 1H), 6.68–6.74 (m, 1H), 6.19 (s, 1H), 3.98 (s, 3H), 3.30–3.48 (m, 1H), 3.18 (s, 3H), 2.39 (d, *J* = 0.73 Hz, 3H), 2.28 (d, *J* = 0.55 Hz, 3H), 1.50–1.84 (m, 4H), 0.87 (t, *J* = 7.41 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.11, 12.16, 19.30, 28.90, 30.82, 39.65, 53.73, 108.42, 111.72, 114.09, 123.04, 129.10, 130.84, 145.06, 146.83, 148.43, 155.60, 164.97; IR (KBr) 453, 677, 701, 761, 799, 861, 945, 1004, 1023, 1051, 1151, 1174, 1196, 1245, 1282, 1312, 1328, 1348, 1357, 1370, 1408, 1431, 1450, 1504, 1557, 1614, 1632, 1667, 2853, 2873, 2924, 2961, 3193, 3363 cm^{-1}; mp 146 °C.

5.1.79. 2-[6-(Difluoromethoxy)-4-methyl-3-pyridinyl]-3,7-dimethyl-5-(3-pentanyl)pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (27)

A white powder. TLC R_f = 0.67 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 391 (M+H)$^+$; FABHRMS calcd for $C_{20}H_{25}F_2N_4O_2$: 391.1946. Found: 391.1946; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.51 (t, *J* = 72.73 Hz, 1H), 6.89 (d, *J* = 0.73 Hz, 1H), 6.20 (s, 1H), 3.40 (m, 1H), 3.18 (s, 3H), 2.37 (dd, *J* = 8.60, 0.73 Hz, 6H), 1.51–1.85 (m, 4H), 0.86 (t, *J* = 7.32 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ11.21, 12.28, 19.59, 29.00, 30.87, 39.71, 108.55, 112.25, 113.71, 113.95, 126.09, 129.21, 131.08, 143.92, 146.71, 150.56, 155.19, 159.60; IR (KBr) 451, 458, 574, 628, 674, 685, 700, 755, 805, 813, 880, 967, 1006, 1032, 1072, 1114, 1135, 1172, 1192, 1213, 1245, 1264, 1310, 1340, 1375, 1395, 1405, 1427, 1455, 1468, 1499, 1563, 1615, 1632, 1662, 1733, 2852, 2922, 2961, 3196, 3363 cm^{-1}; Anal. Calcd for $C_{20}H_{24}F_2N_4O_2$; C, 61.53; H, 6.20; N, 14.35; Found: C, 61.61; H, 6.08; N, 14.33; mp 137 °C.

5.1.80. 5-(1-Ethylpropyl)-2-(6-methoxy-2-methyl-3-pyridinyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (28)

A white powder. TLC R_f = 0.53 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 355 (M+H)$^+$; FABHRMS calcd for $C_{20}H_{27}N_4O_2$: 355.2134. Found: 355.2136; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 1H), 6.70 (dd, *J* = 8.42, 0.55 Hz, 1H), 6.18 (s, 1H), 3.98 (s, 3H), 3.31–3.48 (m, 1H), 3.18 (s, 3H), 2.45 (s, 3H), 2.39 (s, 3H), 1.49–1.84 (m, 4H), 0.87 (t, *J* = 7.23 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.11, 12.16, 22.54, 28.92, 30.74, 39.64, 53.70, 108.10, 108.37, 114.09, 120.74, 129.03, 130.76, 139.10, 146.29, 154.98, 155.68, 163.98; IR (KBr) 451, 634, 675, 695, 755, 771, 798, 828, 922, 996, 1004, 1041, 1119, 1166, 1195, 1265, 1276, 1309, 1325, 1347, 1358, 1376, 1408, 1419, 1443, 1457, 1491, 1515, 1573, 1600, 1609, 1666, 2872, 2929, 2962, 3194, 3362 cm^{-1}.

5.1.81. 2-[6-(Difluoromethoxy)-2-methyl-3-pyridinyl]-3,7-dimethyl-5-(3-pentanyl)pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (29)

A white solid. TLC R_f = 0.50 (EtOAc/hexane, 1/5); MS (ESI, Pos) *m*/*z* 391 (M+H)$^+$; FABHRMS calcd for $C_{20}H_{25}F_2N_4O_2$: 391.1946. Found: 391.1946; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.23 Hz, 1H), 7.62 (t, *J* = 72.64 Hz, 1H), 6.87 (d, *J* = 8.23 Hz, 1H), 6.19 (s, 1H), 6.19 (s, 1H), 3.31–3.48 (m, 1H), 3.18 (s, 3H), 2.47 (s, 3H), 2.38 (s, 3H), 1.50–1.84 (m, 4H), 0.86 (t, *J* = 7.32 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.06, 12.16, 22.42, 28.91, 30.74, 39.66, 108.58, 108.73, 110.33, 113.71, 114.03, 117.10, 124.33, 129.27, 131.15, 140.69, 145.23, 155.40, 155.63, 158.81; IR (KBr) 444, 460, 480, 557, 632, 666, 678, 696, 755, 770, 804, 813, 838, 855, 920, 1003, 1024, 1036, 1070, 1097, 1121, 1145, 1171, 1194, 1261, 1276, 1289, 1328, 1356, 1375, 1406, 1427, 1445, 1482, 1513, 1562, 1585, 1594, 1610, 1661, 2874, 2930, 2963, 3048, 3163, 3411 cm^{-1}; mp 175 °C.

5.1.82. 2-(2-Chloro-4-methoxyphenyl)-5-isopropyl-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (31)

A white powder. TLC R_f = 0.57 (EtOAc/hexane, 1/2); MS (ESI, Pos) *m*/*z* 346 (M+H)$^+$; FABHRMS calcd for $C_{18}H_{21}ClN_3O_2$: 346.1322. Found: 346.1317; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 2.6 Hz, 1H), 6.94 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.25 (s, 1H), 3.87 (s, 3H), 3.76 (m, 1H), 3.21 (s, 3H), 2.37–2.41 (m, 3H), 1.26–1.40 (m, 6H); IR (KBr) 811, 1036, 1239, 1271, 1292, 1351, 1411, 1428, 1466, 1503, 1605, 1633, 1680, 2852, 2922, 2960, 3362 cm^{-1}; mp 157 °C.

5.1.83. 2-[6-(Dimethylamino)-2-methyl-3-pyridinyl]-3,7-dimethyl-5-(3-pentanyl)pyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (33)

A white powder. TLC R_f = 0.44 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 368 $(M+H)^+$; FABHRMS calcd for $C_{21}H_{30}N_5O$: 368.2450. Found: 368.2422; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, *J* = 8.6 Hz, 1H), 6.43 (d, *J* = 8.6 Hz, 1H), 6.16 (s, 1H), 3.30–3.49 (m, 1H), 3.21 (s, 3H), 3.14 (s, 6H), 2.39 (s, 3H), 2.38 (s, 3H), 1.47–1.85 (m, 4H), 0.86 (t, *J* = 7.3 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.14, 12.17, 22.94, 28.94, 30.82, 37.87, 39.61, 102.54, 108.13, 114.12, 115.25, 128.79, 130.35, 137.64, 147.27, 154.99, 155.99, 158.97; IR (KBr) 524, 565, 680, 755, 766, 809, 885, 916, 998, 1063, 1137, 1180, 1199, 1244, 1346, 1356, 1377, 1407, 1460, 1511, 1552, 1565, 1598, 1686, 2871, 2959, 3090 cm^{-1}; mp 111 °C.

5.1.84. 2-[6-(Dimethylamino)-2-methyl-3-pyridinyl]-5-isopropyl-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (34)

A white amorphous powder. TLC R_f = 0.50 (EtOAc/hexane, 1/3); MS (ESI, Pos) *m*/*z* 340 $(M+H)^+$; FABHRMS calcd for $C_{19}H_{26}N_5O$: 340.2137. Found: 340.2131; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, *J* = 8.8 Hz, 1H), 6.43 (d, *J* = 8.8 Hz, 1H), 6.24 (s, 1H), 3.75 (m, 1H), 3.21 (s, 3H), 3.13 (s, 6H), 2.39 (s, 3H), 2.36 (s, 3H), 1.30 (d, *J* = 7.0 Hz, 6H); IR (KBr) 458, 485, 495, 508, 524, 541, 551, 562, 678, 694, 720, 757, 766, 804, 850, 884, 912, 1000, 1024, 1059, 1094, 1132, 1195, 1260, 1348, 1376, 1410, 1421, 1467, 1507, 1556, 1599, 1633, 1661, 2077, 2354, 2404, 2478, 2561, 2679, 2852, 2922, 3093, 3193, 3362, 3573, 3585, 3593, 3627, 3646, 3655, 3667, 3673, 3687, 3699, 3709, 3732, 3743, 3748, 3757, 3777, 3794, 3806, 3813, 3851, 3861, 3869, 3879, 3889, 3899, 3914, 3930 cm^{-1}.

5.1.85. 5-(1-Ethylpropyl)-2-(4-methoxy-2,6-dimethylphenyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (36)

A white powder. TLC R_f = 0.48 (EtOAc/hexane, 1/4); MS (ESI, Pos) *m*/*z* 368 $(M+H)^+$; FABHRMS calcd for $C_{22}H_{30}N_3O_2$: 368.2338. Found: 368.2321; ^1H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 2H), 6.17 (s, 1H), 3.83 (s, 3H), 3.40 (m, 1H), 3.10 (s, 3H), 2.39 (s, 3H), 2.21 (s, 6H), 1.53–1.90 (m, 4H), 0.88 (t, *J* = 7.4 Hz, 6H); IR (KBr) 803, 855, 1002, 1023, 1069, 1152, 1193, 1282, 1324, 1347, 1359, 1376, 1406, 1453, 1464, 1493, 1609, 1676, 2869, 2959 cm^{-1}; mp 101 °C.

5.1.86. 5-(1-Methylethyl)-2-(4-methoxy-2,6-dimethylphenyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (37)

A pale yellow viscous oil. TLC R_f = 0.24 (EtOAc/hexane, 1/5); MS (ESI, Pos) *m*/*z* 340 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{26}N_3O_2$: 340.2025. Found: 340.2025; ^1H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 2H), 6.25 (s, 1H), 3.83 (s, 3H), 3.75 (m, 1H), 3.10 (s, 3H), 2.39 (s, 3H), 2.20 (s, 6H), 1.31 (d, *J* = 7.0 Hz, 6H); IR (KBr) 608, 675, 715, 758, 807, 837, 854, 912, 937, 1001, 1023, 1069, 1153, 1179, 1194, 1256, 1287, 1325, 1348, 1377, 1412, 1440, 1464, 1607, 1677, 2347, 2475, 2736, 2866, 2958, 3623, 3633, 3652, 3671, 3706, 3728, 3740, 3747, 3754, 3803, 3811, 3848, 3857, 3866 cm^{-1}.

5.1.87. 5-Methylhepta-3-en-2-one (66)

To a stirred solution of 2-methylbutyraldehyde (68.6 g, 797 mmol) in CH_2Cl_2 (650 mL) was added $Ph_3P{=}CHCOMe$ (254 g, 797 mmol) at room temperature. After being stirred for 3 days, the reaction mixture was evaporated and diluted with hexane. The resulting precipitates were removed by filtration and the filtrate was evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 10/90–33/67) to afford **66** (149 g, 74% yield) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 6.66 (dd, *J* = 16.0, 7.8 Hz, 1H), 6.01 (dd, *J* = 16.0, 1.1 Hz, 1H), 2.22 (s, 3H), 2.21 (m, 1H), 1.47–1.33 (m, 2H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.5 Hz, 3H).

5.1.88. Ethyl 4-methyl-2-nitro-3-(2-oxopropyl)hexanoate (67)

To a stirred solution of **66** (1.36 g, 10.8 mmol) in acetonitrile (10 mL) was added ethyl nitroacetate (1.1 mL, 9.9 mmol) and diisopropylethylamine (1.7 mL, 9.8 mmol) at room temperature. After being stirred for 12 h, the reaction mixture was heated at 60 °C. After being stirred for another 12 h, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was washed with 0.1 M NaOH, water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 5/95–25/75) to give **67** (1.08 g, 42% yield) as an oil.

5.1.89. Ethyl 3-*sec*-butyl-5-methyl-1*H*-pyrrole-2-carboxylate (68)

To a stirred solution of **67** (104 g, 401 mmol) in DMF (800 mL) was added formamidinesulfinic acid (86.5 g, 800 mmol) and triethylamine (56 mL, 400 mmol) at 80 °C. After being stirred for 1.5 h, the reaction mixture was cooled to room temperature, quenched with water and extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$ and evaporated to give the corresponding oxime derivative (64.7 g). To a stirred solution of the oxime derivative (64.7 g) in acetic acid (400 mL) was added ammonium acetate (92.5 g, 1.20 mol) at 100 °C. After being stirred for 1 h, the reaction mixture was cooled to 0 °C and quenched with 5 M NaOH (1.4 L). Insoluble substance was removed by filtration and the filtrate was extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated. The resulting residue was purified by column chromatography on silica gel (EtOAc/hexane, 25/75) to afford **68** (33 g, 39% yield in 2 steps) as an oil. TLC R_f = 0.62 (EtOAc/hexane, 1/3); ^1H NMR (300 MHz, $CDCl_3$) δ 8.61 (br s, 1H), 5.86 (m, 1H), 4.29 (qd, *J* = 7.2, 1.6 Hz, 2H), 3.30 (m, 1H), 2.26 (s, 3H), 1.53 (m, 2H), 1.34 (t, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H).

5.1.90. 3-(1-Metylpropyl)-5-methyl-1*H*-pyrrole (69)

To a stirred soution of **68** (322 mg, 1.54 mmol) in EtOH (3 mL) was added 1 M NaOH (3 mL) at 90 °C. After being stirred for 2 days, the reaction mixture was cooled to room temperature, quenched with 2 M HCl (2 mL) and extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and evaporated to give crude **69** (228 mg) as oil. TLC R_f = 0.47 (EtOAc/hexane, 1/6); ^1H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 1H), 6.40 (m, 1H), 5.77 (s, 1H), 2.52 (m, 1H), 2.24 (s, 3H), 1.64–1.40 (m, 2H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.88 (t, *J* = 7.3 Hz, 3H).

5.1.91. 3-(1-Methylpropyl)-5-methyl-1*H*-pyrrole-2-caboxylic acid methylamide (70)

TLC R_f = 0.66 (EtOAc); ^1H NMR (300 MHz, $CDCl_3$) δ 8.85 (m 1H), 5.81 (d, *J* = 3.1 Hz, 1H), 5.62 (m, 1H), 2.98 (d, *J* = 5.7 Hz, 3H), 2.71 (m, 1H), 2.24 (s, 3H), 1.24 (d, *J* = 6.7 Hz, 3H), 0.90 (t, *J* = 7.3 Hz, 3H).

5.1.92. 1-Amino-3-(1-methylpropyl)-5-methyl-1*H*-pyrrole-2-carboxylic acid *N*-methylamide (71)

TLC R_f = 0.43 (ethyl acetate/hexane, 1/2); ^1H NMR (300 MHz, $CDCl_3$) δ 6.74 (m, 1H), 5.72 (s, 1H), 5.13 (s, 2H), 3.08 (m, 1H), 2.95 (d, *J* = 4.9 Hz, 3H), 2.24 (d, *J* = 0.7 Hz, 3H), 1.55 (m, 2H), 1.18 (d, *J* = 7.0 Hz, 3H), 0.87 (t, *J* = 7.4 Hz, 3H).

5.1.93. 5-(1-Metylpropyl)-2-(4-methoxy-2-methylphenyl)-3,7-dimethylpyrrolo[2,1-f][1,2,4]triazin-4(3*H*)-one (30)

A white powder; TLC R_f = 0.33 (EtOAc/hexane, 1/9); MS (ESI, Pos) *m*/*z* 340 $(M+H)^+$; FABHRMS calcd for $C_{20}H_{26}N_3O_2$: 340.2025.

Found: 340.2025; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17–7.25 (m, 1H), 6.77–6.88 (m, 2H), 6.19 (s, 1H), 3.84 (s, 3H), 3.40–3.73 (m, 1H), 3.14 (s, 3H), 2.39 (s, 3H), 2.27 (s, 3H), 1.57–1.79 (m, 2H), 1.27 (d, J = 7.0 Hz, 3H), 0.77–1.01 (m, 3H); IR (KBr) 454, 676, 755, 792, 826, 846, 935, 960, 1005, 1025, 1049, 1113, 1172, 1194, 1247, 1284, 1309, 1326, 1356, 1375, 1402, 1432, 1455, 1499, 1512, 1562, 1581, 1613, 1667, 2870, 2921, 2959, 2993 cm^{-1}; mp 104 °C.

5.1.94. 5-(1-Metylpropyl)-2-(2-chloro-4-methoxyphenyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (32)

A white powder. TLC R_f = 0.50 (EtOAc/hexane, 1/3); MS (ESI, Pos) m/z 360 (M+H)$^+$; FABHRMS calcd for $C_{19}H_{23}ClN_3O_2$: 360.1479. Found: 360.1473; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, J = 8.5 Hz, 0.5H), 7.36 (d, J = 8.5 Hz, 0.5H), 7.04 (d, J = 2.5 Hz, 1H), 6.94 (dd, J = 8.5, 2.5 Hz, 1H), 6.21 (s, 1H), 3.87 (s, 3H), 3.49–3.63 (m, 1H), 3.21 (s, 3H), 2.39 (s, 3H), 1.57–1.76 (m, 2H), 1.28 (d, J = 7.0 Hz, 1.5H), 1.27 (d, J = 7.0 Hz, 1.5H), 0.93 (t, J = 7.3 Hz, 1.5H), 0.90 (t, J = 7.3 Hz, 1.5H); IR (KBr) 2960, 2930, 2873, 2839, 1680, 1604, 1566, 1502, 1463, 1442, 1428, 1412, 1378, 1354, 1291, 1271, 1238, 1216, 1197, 1175, 1106, 1068, 1037, 1004, 876, 861, 825, 814, 796, 757, 743, 681, 594 cm^{-1}; mp 116–118 °C.

5.1.95. 5-(1-Metylpropyl)-2-[6-(dimethylamino)-2-methyl-3-pyridinyl]-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (35)

A yellow amorphous powder. TLC R_f = 0.48 (EtOAc/hexane, 1/3); MS (ESI, Pos) m/z 354 (M+H)$^+$; FABHRMS calcd for $C_{20}H_{28}N_5O$: 354.2294. Found: 354.229; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (d, J = 8.60 Hz, 1H), 6.41 (d, J = 8.50 Hz, 1H), 6.19 (s, 1H), 3.55 (m, 1H), 3.20 (s, 3H), 3.13 (s, 6H), 2.38 (s, 3H), 2.36 (s, 3H), 1.57–1.76 (m, 2H), 1.27 (d, J = 6.95 Hz, 3H), 0.91 (t, J = 7.41 Hz, 3H); IR (KBr) 501, 525, 565, 583, 684, 692, 757, 766, 808, 887, 982, 1000, 1069, 1140, 1178, 1244, 1266, 1325, 1341, 1352, 1377, 1409, 1422, 1444, 1513, 1553, 1566, 1599, 1686, 2350, 2864, 2922, 2955, 3193, 3369, 3625, 3672, 3747, 3798, 3849, 3897 cm^{-1}.

5.1.96. 5-(1-Metylpropyl)-2-(4-methoxy-2,6-dimethylphenyl)-3,7-dimethylpyrrolo[2,1-*f*][1,2,4]triazin-4(3*H*)-one (38)

A pale yellow viscous oil. TLC R_f = 0.33 (EtOAc/hexane, 1/9); MS (ESI, Pos) m/z 354 (M+H)$^+$; FABHRMS calcd for $C_{21}H_{28}N_3O_2$: 354.2182. Found: 354.2184; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.67 (s, 2H), 6. 20 (s, 1H), 3.82 (s, 3H), 3.53 (m, 1H), 3.10 (s, 3H), 2.39 (s, 3H), 2.09–2.28 (m, 6H), 1.57–1.81 (m, 2H), 1.28 (d, J = 7.0 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H); IR (neat) 3447, 2960, 2871, 1680, 1607, 1495, 1455, 1442, 1413, 1377, 1352, 1326, 1286, 1266, 1250, 1195, 1174, 1153, 1070, 1025, 1002, 858, 840, 807, 758, 737, 676 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 $MgCl_2$, 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 or 2 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-*iso*-butyl-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. 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. Lighting on both ends of the open arm, maintained with constant white illumination and the illumination of the central arena was adjusted to 60 lux. In the experiments of swim-stress paradigm, rats were forced to swim for 120 s 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 120 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 and 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.

5.2.5. Single dose rat pharmacokinetic study of 27

Single dose pharmacokinetics of **27** 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 (1 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}) × 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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