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Design, Synthesis, and Structure−Activity Relationships of Novel Pyrazolo[5,1-*b*]thiazole Derivatives as Potent and Orally Active Corticotropin-Releasing Factor 1 Receptor Antagonists

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ABSTRACT: This paper describes the design, synthesis, and structure−activity relationships of a novel series of 7-dialkylamino-3-phenyl-6-methoxy pyrazolo[5,1-*b*]thiazole derivatives for use as selective antagonists of the corticotropin-releasing factor 1 (CRF_1) receptor. The most promising compound, *N*-butyl-3-[4-(ethoxymethyl)-2,6-dimethoxyphenyl]-6-methoxy-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo-[5,1-*b*][1,3]thiazole-7-amine (**6t**), showed high affinity (IC_{50} = 70 nM) and functional antagonism (IC_{50} = 7.1 nM) for the human CRF_1 receptor as well as dose-dependent inhibition of the CRF-induced increase in the plasma adrenocorticotropic hormone (ACTH) concentration at a dose of 30 mg/kg (po). Further, in the light/dark test in mice, the compound **6t** showed anxiolytic activity at a dose of 30 mg/kg (po).

MeO, OMe, MeO, OEt, **6t**

INTRODUCTION

Corticotropin-releasing factor (CRF), a 41-amino-acid neuropeptide, is a key regulator of the hypothalamus−pituitary−adrenal axis, which coordinates the endocrine response to stress by regulating the release of adrenocorticotropic hormone (ACTH) from the pituitary.[1,2] Two receptor subtypes (CRF_1 and CRF_2) belonging to the class B subfamily of G protein-coupled receptors (GPCRs) have been identified.[3−6] They are widely distributed throughout the central and peripheral nervous systems. Several preclinical and clinical studies suggest the therapeutic potential of CRF_1 receptor antagonists in stress-related diseases such as depression, anxiety, and possibly, irritable bowel syndrome;[7−11] the role of the CRF_2 receptor, however, as a target for stress-related disorders has not been fully elucidated. Subsequent to the discovery of the nonpeptide small molecule CRF_1 receptor antagonist **1** (CP-154,526),[12] many research groups have reported preclinical studies that demonstrate the efficacy of their own CRF_1 receptor antagonists in animal models of anxiety and depression.[13−20] However, the clinical effectiveness of CRF_1 receptor antagonists remains to be confirmed. Evidence of antidepressant/anxiolytic activity of **2** (R121919) in patients with depression has been shown in a small open-label phase IIa study,[21,22] while **3** (CP-316,311) failed to show efficacy in a double-blind, placebo-controlled study[23] (Figure 1). It is tempting to speculate that the main reason for these equivocal results lies with the compound itself (e.g., suboptimal drug-like properties such as high lipophilicity or low water solubility and inadequate target engagement) and not with the mechanism of action. Therefore, we hypothesized that a CRF_1 receptor antagonist with appropriate drug-like characteristics may function as a robust, novel antidepressant in clinical practice.

1 (CP-154,526) **2** (R121919) **3** (CP-316,311) **4** **5** X = Me **6a** X = OMe

Figure 1. Reported CRF_1 receptor antagonists and designed compounds **5** and **6a**.

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Figure 2. Comparison of dihedral angles in the most stable conformer of **2** (left) and the designed compound **5** (right).

Scheme 1[a]

[a]Reagents and conditions: (a) CS_2, 2-bromo-1,1-diethoxyethane, Cs_2CO_3, cat. NaI, DMF, 60 °C; (b) $H_2NNH_2 \cdot H_2O$, EtOH, rt; (c) 5 M HCl, 1,4-dioxane, 60 °C, 32%, 3 steps; (d) MeI, Cs_2CO_3, DMF, rt, 70%; (e) (i) 5 M NaOH, EtOH, 80 °C, (ii) conc HCl, 1,4-dioxane, 60 °C, 75%, 2 steps; (f) (i) $NaNO_2$, 5 M HCl, H_2O, rt, (ii) Pd/C, EtOH, THF, rt; (g) Boc_2O, Et_3N, CH_2Cl_2, rt, 59%, 3 steps; (h) BrF_2CCF_2Br, *n*-BuLi/hexane, THF, −78 °C–rt, 67%; (i) Ar–$B(OH)_2$, $Pd(OAc)_2$, PPh_3, K_2CO_3, DME, H_2O, reflux, or Ar–$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O, reflux, 52–95%.

Scheme 2[a]

[a]Reagents and conditions: (a) (i) *O*-mesitylene sulfonylhydroxylamine, CH_2Cl_2, rt, (ii) AcONa, Ac_2O, reflux, 51%, 2 steps; (b) (i) $NaNO_2$, 5 M HCl, H_2O, rt (ii) Zn, 2 M HCl, rt, (iii) Boc_2O, Et_3N, CH_2Cl_2, rt, 56%, 3 steps; (c) BrF_2CCF_2Br, *n*-BuLi/hexane, THF, −78 °C–rt, 88%; (d) Ar–$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O, reflux, 99%.

As described in our previous article,[24] we focused on modifying the central core, particularly the 5,6-fused bicyclic heteroaromatic template, with the aim of conferring appropriate drug-like characteristics, and identified the potent CRF_1 receptor antagonist **4** (Figure 1). As part of our efforts to identify CRF_1 receptor antagonists, we investigated 5,5-fused bicyclic systems, which have not yet been explored for their potential as CRF_1 receptor antagonists. In this report, we

describe the investigation of pyrazolo[5,1-*b*]thiazole as a novel core structure. The dihedral angles are related to a key pharmacophore feature of CRF_1 receptor antagonists.[25] For a known CRF_1 receptor antagonist **2** and some 5,5-fused derivatives, the dihedral angles between the bicyclic core and the pendant aryl rings in the most stable conformation were calculated.[26] The results indicated that pyrazolo[5,1-*b*]thiazole **5** exhibits a conformation similar to **2** (59.2° for **5** and 59.0° for **2**) (Figure 2). Then, we established efficient, synthetic routes for investigating pyrazolo[5,1-*b*]thiazole derivatives.

RESULTS AND DISCUSSION

Chemistry. Key intermediate **16**, which is amenable to versatile modifications at the amine moiety in the 6-methoxy pyrazolo[5,1-*b*]thiazole core structure, was prepared according to Scheme 1. The one-pot reaction of diethyl malonate **7** with carbon disulfide and bromoacetaldehyde diethyl acetal in the presence of cesium carbonate and catalytic sodium iodide yielded **8**, which was treated with hydrazine hydrate to yield pyrazolone **9**. Intramolecular cyclization of **9** by treatment with hydrochloric acid followed by methylation of the resulting bicyclic compound **10** yielded 6-methoxy pyrazolo[5,1-*b*]-thiazole core **11**. Hydrolysis of **11** followed by decarboxylation under acidic conditions led to the formation of **12**. Subsequent nitrosation and reduction yielded aniline **13**, which was protected to give *tert*-butyl carbamate **14a**. Bromination with the desired regioselectivity by using *n*-butyllithium followed by Suzuki−Miyaura coupling of **15a** with various arylboronic acids produced key intermediate **16**.

Scheme 2 shows the synthesis of a 6-methyl analogue. *N*-Amination of **17** with *O*-mesitylene sulfonylhydroxylamine, followed by cyclization in the presence of sodium acetate in acetic anhydride, produced a 6-methyl pyrazolo[5,1-*b*]thiazole core **18**.[27] One-pot deacetylation and nitrosation of **18**, followed by reduction and subsequent conversion to *tert*-butyl carbamate, yielded **14b**. Similar to the 6-methoxy intermediate **16** described in Scheme 1, **14b** was converted to **19** via formation of **15b**.

The final compounds **6** and **5** were prepared according to Scheme 3. Alkylation of **16** or **19** with alkyl halides or alkyl mesylates under basic conditions followed by removal of the Boc group under acidic conditions and subsequent reductive amination yielded the desired products **6** and **5**.

Scheme 3[a]

16 R_1 = MeO; **19** R_1 = Me → (a) → **20** R_1 = MeO; **21** R_1 = Me → (b) → **6** R_1 = MeO; **5** R_1 = Me

[a]Reagents and conditions: (a) R_2-Br or R_2-I, NaH, DMF, rt, or R_2-OMs, NaOH powder, DMSO, rt−70 °C; (b) (i) TFA, CH_2Cl_2, rt, (ii) aldehyde or ketone, $NaBH(OAc)_3$, THF, rt, or $NaBH(OAc)_3$, THF, AcOH, rt, or *α*-picoline-borane, MeOH, AcOH, rt, 7−88%, 3 steps, (iii) for **6c**; DAST, CH_2Cl_2, 0 °C, 14%.

2,6-Dimethyl-substituted pyrazolo[5,1-*b*]thiazole analogue **23** was prepared by bromination of **5**, followed by palladium-catalyzed alkylation of **22** with dimethylzinc (Scheme 4).

Scheme 4[a]

5 → (a) → **22** → (b) → **23**

[a]Reagents and conditions: (a) BrF_2CCF_2Br, *n*-BuLi/hexane, THF, −78 °C−rt; (b) Me_2Zn/hexane, $Pd[P(t\text{-}Bu)_3]_2$, 1,4-dioxane, 65 °C, 49%, 2 steps.

Pharmacology. The affinity for human CRF_1 receptors was determined on the basis of competition with ^{125}I-CRF by using cell membranes prepared from human CRF_1 receptors expressed in HEK293 cells.[5]

Initial investigation of the pyrazolo[5,1-*b*]thiazole core is summarized in Table 1. The 5,5-fused compound **5**, which was first designed, showed affinity comparable to those of the representative compounds **4** and **24** from our previous compound series.[24] Replacement of the 6-methyl group in **5** with the methoxy group resulted in retention of potent binding affinity while showing a lower clogP[28] value than that shown by **5**. 2-Methyl compound **23** also showed binding affinity comparable to that of **6a**. Among these pyrazolo[5,1-*b*]thiazole compounds, **6a**, which had a lower clogP value, was considered to be a promising starting point for further optimization.

Table 1. Initial Investigation of the Binding Affinity of Pyrazolo[5,1-*b*]thiazole Derivatives

compd	A	X	Y	binding IC_{50} (nM)[a]	clogP
4	HC=CH	Et	H	50	4.7
24	HC=CH	Me	H	120	4.2
5	S	Me	H	46	4.2
6a	S	OMe	H	69	3.6
23	S	Me	Me	76	4.4

[a]All values are the averages of two measurements.

The 6-methoxy pyrazolo[5,1-*b*]thiazole compound **6a** showed relatively high human intrinsic clearance in vitro (hCLint; 0.46 mL/min/mg). Therefore, **6a** was modified mainly with a focus on improving its metabolic stability. Because it was presumed from our previous study[24] that *N*-dealkylation of the 7-dialkylamine moiety was the main metabolic pathway, modification of the dialkylamine moiety in **6a** was first investigated (Table 2). Introduction of a polar OH group in the 4-THP moiety to reduce lipophilicity resulted in a decrease in the activity (**6b**). Introduction of the F atom to the same carbon on the THP ring to block a potential metabolically fragile side chain maintained the binding affinity but increased its hCLint slightly (**6c**). Moreover, replacement of the 4-THP ring with the regioisomer of the THP ring (**6d** and **6e**), 3- or 2-THF compounds (**6f** and **6g**), and acyclic

Table 2. Effects of Dialkylamino Side Chain at 7-Position of Pyrazolo[5,1-*b*]thiazole Derivatives

compd	R	binding IC_{50} (nM)[a]	hCLint (mL/min/mg)[b]	solubility (μM)[c]	clogP
6a		69	0.46 ± 0.01	6.8	3.6
6b		223	NT[d]	58	2.2
6c		61	0.55 ± 0.03	0.9	3.8
6d		50	0.55 ± 0.02	15	3.6
6e		75	0.87 ± 0.02	6.4	4.3
6f		48	0.53 ± 0.02	18	3.2
6g		43	1.02 ± 0.03	18	3.8
6h		48	0.96 ± 0.02	48	3.5
6i		101	0.18 ± 0.01	30	3.0

[a]All values are the averages of two measurements. . [b]Rate of human intrinsic clearance in vitro. Each value represents the mean ± SEM of six measurements. [c]Kinetic solubility in phosphate buffer saline using 10 mM DMSO stock solution. [d]NT = not tested.

methoxyethyl ether (**6h**) did not improve hCLint. Because the replacement of the 4-THP ring in **6a** with the above cyclic or acyclic ethers did not result in improvement of hCLint, we speculate that the methylene moiety with the attached nitrogen might be susceptible to metabolism regardless of the size of the ether ring or the position of the oxygen atom. With the aim of blocking the metabolically fragile carbon by steric hindrance, the directly linked THP compound (**6i**) was synthesized, resulting in a significant improvement in metabolic stability. Reduction of lipophilicity could also improve metabolic stability. Although the compound showed a slight decrease in binding affinity, the excellent drug-like property of **6i** prompted further optimization.

Modification of another substituent at 7-position of **6i** was examined with the aim of enhancing binding affinity (Table 3). Ethyl **6j** and the *i*-propyl compound **6n** showed an almost 1.5-fold decrease in the binding affinity against **6i,** and *n*-propyl **6k** and *i*-butyl compound **6o** showed binding affinity comparable to that of **6i**. Meanwhile, cyclopropylethyl **6l** and *n*-butyl compound **6m** improved activity while maintaining preferable hCLint and solubility,[29] although their lipophilicity increased. The compounds with branched alkyl groups (**6n**, **6o**) showed relatively high hCLint, contrary to our expectations that *N*-dealkylation might be prevented by steric bulkiness. It is speculated that blocking dealkylation of the aniline moiety by

Table 3. Effects of the 7-Dialkylamino Side Chain of Pyrazolo[5,1-*b*]thiazole Derivatives

compd	R	binding IC_{50} (nM)[a]	hCLint (mL/min/mg)[b]	solubility (μM)[c]	clogP
6i	*c*PrCH_2	101	0.18 ± 0.01	30	3.0
6j	Et	147	0.20 ± 0.01	38	2.5
6k	*n*-Pr	98	0.22 ± 0.01	25	3.1
6l	*c*PrCH_2CH_2	83	0.18 ± 0.03	12	3.5
6m	*n*-Bu	52	0.20 ± 0.01	13	3.6
6n	*i*-Pr	136	0.67 ± 0.03	53	2.9
6o	*i*-Bu	91	0.36 ± 0.02	0	3.5

[a]All values are the averages of two measurements. [b]Rate of human intrinsic clearance in vitro. Each value represents the mean ± SEM of six measurements. [c]Kinetic solubility in phosphate buffer saline using 10 mM DMSO stock solution.

Table 4. Optimization of Pyrazolo[5,1-*b*]thiazole Derivatives

compd	R_1	R_2	R_3	binding IC_{50} (nM)[a]	hCLint (mL/min/mg)[b]	solubility (μM)[c]	clogP
6k	*n*-Pr	MeO	$MeOCH_2$	98	0.22 ± 0.01	25	3.1
6p	*n*-Pr	MeO	$EtOCH_2$	65	0.34 ± 0.02	21	3.5
6q	*n*-Pr	MeO	CN	126	0.15 ± 0.02	16	3.0
6r	*n*-Pr	Cl	$MeOCH_2$	63	0.37 ± 0.02	6.3	4.1
6s	*n*-Pr	H	$MeOCH_2$	254	NT[d]	7.9	3.6
6m	*n*-Bu	MeO	$MeOCH_2$	52	0.20 ± 0.01	13	3.6
6t	*n*-Bu	MeO	$EtOCH_2$	70	0.22 ± 0.01	7.1	4.0
6u	*n*-Bu	MeO	CN	46	0.15 ± 0.01	3.9	3.5

[a]All values are the averages of two measurements. [b]Rate of human intrinsic clearance in vitro. Each value represents the mean ± SEM of six measurements. [c]Kinetic solubility in phosphate buffer saline using 10 mM DMSO stock solution. [d]NT = not tested.

introducing a branched alkyl group might induce a new metabolically fragile site on the branched alkyl substituent itself.

Finally, the substituent effect on the 3-phenyl ring was examined using **6k** and **6m** (Table 4). Replacement of *para* methoxymethyl on the 3-phenyl ring in **6k** with an ethoxymethyl moiety improved the binding affinity while maintaining solubility (**6p**). Formation of the nitrile compound **6q**, which was aimed at avoiding demethylation of the *para* methoxymethyl moiety in **6k**, ameliorated hCLint as expected but decreased the binding affinity. Similar to **6p**, *ortho* chloro compound **6r** increased the in vitro activity but decreased hCLint. The decrease in the binding affinity on **6s** indicates that an appropriate conformation induced by the *ortho* disubstituent might be important for high affinity in this series. Subsequently, the preferable aryl group was introduced in **6m**, similar to the modification used for **6k**. The compounds **6t** and **6u** showed comparable binding affinity to **6m** with preferable hCLint values.

The compounds selected through the above-mentioned process were evaluated in a rat defecation model,[30] which is a possible IBS model, in order to confirm the in vivo antagonism of the CRF_1 receptor[31] (Table 5). It is well-known that exogenously administered CRF increases fecal pellet output in conscious rats. Compounds (10 mg/kg, po) were orally administered to rats 60 min before IV injection of CRF (10 μg/kg). Stool weight was measured for 4 h after CRF injection. The compounds **6r** and **6t** significantly inhibited CRF-induced defecation.

These two compounds were subjected to various in vitro assays in order to evaluate drug-likeness such as CYP inhibition, CYP induction, P-gp substratability, and hERG inhibition. There were few differences between these compounds, and they did not have major problems (data not shown). On the basis of the results, especially those related to hCLint, compound **6t** was chosen as the most promising candidate. The functional antagonism of **6t** was confirmed in a cAMP assay by using the human neuroblastoma cell line IMR-32 expressing human CRF_1 receptors. The IC_{50} value of **6t** was 7.1 nM.[32] Compound **2**, which was used as a positive control, had an IC_{50} value of 4.0 nM in the assay. Compound **6t** exhibited selectivity over CRF_2 receptors (CRF_2 IC_{50}, >10 μM).[33]

The effects of **6t** on the CRF-induced elevation of plasma ACTH concentration were evaluated in F344 rats[34] to confirm oral antagonism for the CRF_1 receptor (Figure 3).[35] Plasma concentration of ACTH was significantly increased on

Table 5. Effects on IV Injected CRF-Induced Fecal Pellet Output in Rats[a]

	stool output (g)		
compd	CRF control	compound-treated	inhibition of stool output (%)[b]
6m	1.48 ± 0.30	0.91 ± 0.24	39
6p	0.95 ± 0.19	0.44 ± 0.22	54
6r	1.65 ± 0.20	0.70 ± 0.31*[c]	58
6t	1.15 ± 0.20	0.40 ± 0.15*[c]	65
6u	1.15 ± 0.20	0.73 ± 0.20	36

[a]Compounds (10 mg/kg) were orally administered 60 min before intravenous injection of CRF (10 lag/kg). [b]Inhibition ratio (%) of stool output (g) of compounds treated groups compared to output (g) of CRF control groups. Each value represents the mean ± SEM of 6 rats. [c]*$P < 0.05$ vs CRF control groups (unpaired *t* test).

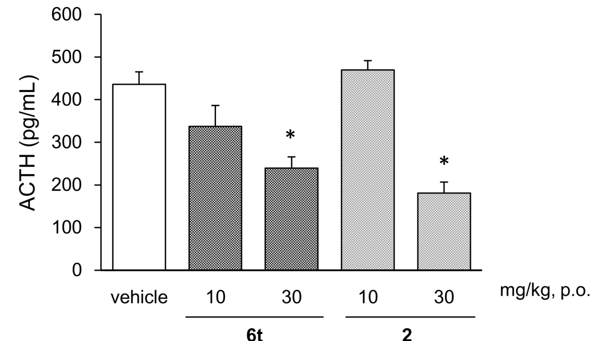

Figure 3. Effects of **6t** on the CRF-induced increase of plasma ACTH concentration in rats with **2** as a positive control. Each value represents the mean ± SEM of 8 rats. *$p < 0.05$ vs vehicle (CRF control) (one-way analysis of variance, followed by Dunnett's multiple comparison test).

subcutaneous injection of CRF (10 μg/kg). Further, **6t** (30 mg/kg, po) significantly inhibited the CRF-induced increase in plasma ACTH concentration at 30 min after CRF injection, suggesting that **6t** has an antagonistic effect on the CRF_1 receptor in vivo.

The anxiolytic efficacy of **6t** was investigated by performing a light/dark test in male BALB/c mice, which is commonly used to evaluate anxiolytics (Figure 4).[36] The compound increased the time spent in the light box in a dose-dependent manner, and a statistically significant effect was observed at 30 mg/kg po, suggesting that **6t** may have the potential to alleviate anxiety in patients.

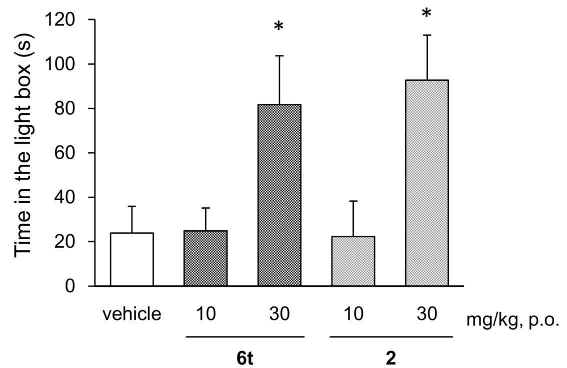

Figure 4. Anxiolytic effects of **6t** in mice light/dark test with **2** as a positive control. Each value represents the mean ± SEM of 12 mice. $^*p < 0.05$ vs vehicle (Dunnett's multiple comparison test).

The pharmacokinetic properties of **6t** were evaluated in male SD rats after iv and oral (po) administration at doses of 3 mg/kg iv and 10 mg/kg po (n = 3), respectively (Table 6). The results indicated a half-life of 5.2 h, plasma clearance of 1.9 L/h/kg, and an oral bioavailability of 23%. The hepatic clearances calculated from in vitro intrinsic clearance measured by performing a liver microsome assay were almost comparable to the in vivo total clearances in preclinical studies in other animals, including rat, mouse, dog, and monkey. These results indicated that the main elimination route of **6t** in animals is probably hepatic metabolism. Therefore, hepatic metabolism was considered the main elimination route in humans.

Table 6. Pharmacokinetic Parameters for 6t (iv and po) in Male Rats[a]

iv (3 mg/kg)		po (10 mg/kg)	
CL (L/h/kg)	1.93 ± 0.16	C_{max} (μg/mL)	0.54 ± 0.11
V_{dss} (L/kg)	6.32 ± 1.23	T_{max} (h)	0.5−1.0
AUC (μg/mL·h)	1.58 ± 0.15	AUC (μg/mL·h)	1.24 ± 0.36
$T_{1/2}$ (h)	5.2 ± 1.3	BA (%)	23

[a]Each value represents the mean ± SEM of three animals.

Screening for salt and crystal forms of **6t** by using various acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, methanesulfonic acid, or phosphoric acid revealed that diphosphate **24** was the most suitable crystalline form. The apparent solubility of **24** in Fasted State Simulated Intestinal Fluid was 50 times higher than that of the free base, suggesting that the salt form is better for human absorption.

■ CONCLUSIONS

Investigation of a CRF_1 receptor antagonist with a 5,5-fused ring core structure resulted in identification of novel pyrazolo[5,1-*b*]thiazole compounds as potent CRF_1 receptor antagonists with physicochemical characteristics preferable for PO administration; further, we established a synthetic route that was adequate for derivatization and enabled efficient optimization. The most promising compound, **6t**, demonstrated high affinity (IC_{50} = 70 nM) and functional antagonism (IC_{50} = 7.1 nM) for the human CRF_1 receptor; it significantly reduced CRF-induced elevation of ACTH levels at 30 mg/kg (po) and exhibited anxiolytic activity in the light/dark test in mice at 30 mg/kg (po). These results not only exhibited a promising profile of pyrazolo[5,1-*b*]thiazole derivatives as CRF_1 receptor antagonists but could also expand the possibility of discovering structurally diverse antagonists. The phosphate of **6t** (E2009) was selected as a candidate for further investigation to prove the clinical usefulness of CRF_1 receptor antagonists in the treatment of stress-related disorders such as depression and anxiety.

■ EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded on a Bruker Avance spectrometer (600 MHz) or Varian Mercury 400 spectrometer (400 MHz). ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (150 MHz) or JEOL JNM α400 spectrometer (100 MHz). Chemical shifts were calculated in ppm (δ) from the residual $CHCl_3$ signal at (δ_H) 7.26 ppm and (δ_C) 77.0 ppm in $CDCl_3$ or the residual C_5HD_4N signal at (δ_H) 8.71 ppm and (δ_C) 123.5 ppm in C_5D_5N, or the residual CD_3SOCD_2H signal at (δ_H) 2.49 ppm and (δ_C) 40.0 ppm in CD_3SOCD_3. High resolution mass spectra (HRMS) were recorded on a ThermoFisherScientific LTQ-Orbitrap XL spectrometer (using electrospray ionization). Compounds were purified by column chromatography on silica gel using the solvent systems indicated below, or by preparative HPLC separations by Waters system equipped with a Shiseido CAPCELL PAK C18 ACR (20 mm × 50 mm, 5 μm), eluting with a linear gradient of 10−90% MeOH in water containing 0.1% TFA at a flow rate of 30 mL/min over 10 min and monitored using a photodiodo array detector.

The purity of the biological tested compounds was determined by an analytical HPLC method and was found to be greater than or equal to 95% for all compounds. The parameters of the HPLC method were as follows: Accucore RP-MS column (2.1 mm × 50 mm, 2.6 μm); mobile phase: A = H_2O with 0.1% HCO_2H, B = acetonitrile with 0.1% HCO_2H, 0−1 min, 0% B; 1−4 min, 0% B → 100% B; 4−8 min, 100% B; 8−11 min, 0% B; flow rate =0.4 mL/min; detector: UV 254 nm; run time = 11 min.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methyl-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyrazolo[5,1-*b*][1,3]thiazol-7-amine (5).** Compound **5** was prepared according to the procedure described for the synthesis of **6a** using **19**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:4), followed by column chromatography on NH silica gel (*n*-heptane:EtOAc = 1:1) to afford **5** (57%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01−0.05 (m, 2H), 0.38−0.44 (m, 2H), 0.81−0.92 (m, 1H), 1.22−1.34 (m, 2H), 1.49−1.61 (m, 1H), 1.71−1.78 (m, 2H), 2.28 (s, 3H), 2.74 (d, J = 6.8 Hz, 2H), 2.88 (d, J = 7.2 Hz, 2H), 3.36 (br dd, J = 10.4, 11.6 Hz, 2H), 3.45 (s, 3H), 3.76 (s, 6H), 3.93 (br dd, J = 2.8, 11.2 Hz, 2H), 6.52 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 150.0, 142.4, 131.9, 128.3, 123.4, 108.8, 105.6, 103.3, 74.7, 67.9, 61.9, 60.8, 58.4, 56.1, 33.8, 31.5, 12.4, 9.7, 3.6. HRMS calcd for ($C_{26}H_{36}N_3O_4S$) $[M + H]^+$ 486.2421; found 486.2422. HPLC purity: > 99%.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[5,1-*b*][1,3]thiazol-7-amine (6a).** To a solution of **16a** (100 mg, 0.22 mmol) in DMF (2.5 mL) was added

NaH (60% dispersion in oil: 12 mg, 0.30 mmol) while cooling on a water bath. After stirring for 10 min, cyclopropylmethyl bromide (26 μL, 0.27 mmol) was added, and the mixture was stirred for 1 h. Water was added to the reaction mixture while cooling on an ice bath, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in next step without purification.

To the obtained crude product was added CH_2Cl_2 (3 mL), followed by TFA (1 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixute was concentrated under reduced pressure.

To a solution of the obtained residue in THF (2 mL) were added tetrahydro-2*H*-pyran-4-carbaldehyde (51 mg, 0.45 mmol), followed by $NaBH(OAc)_3$ (94 mg, 0.45 mmol), and the mixture was stirred at room temperature for 1 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to afford **6a** (90 mg, 81%, 3 steps) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.33−0.48 (m, 2H), 0.85−0.98 (m, 1H), 1.22−1.38 (m, 2H), 1.55−1.69 (m, 1H), 1.72−1.83 (m, 2H), 2.78−2.85 (m, 2H), 2.87−2.94 (m, 2H), 3.30−3.39 (m, 2H), 3.48 (s, 3H), 3.80 (s, 6H), 3.88 (s, 3H), 3.91−3.98 (m, 2H), 4.52 (s, 2H), 6.42 (s, 1H), 6.66 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.7, 159.4, 142.0, 133.4, 128.2, 110.3, 105.9, 105.7, 103.4, 74.8, 68.0, 61.4, 60.5, 58.5, 56.3, 56.1, 33.9, 31.5, 9.6, 3.4. HRMS calcd for ($C_{26}H_{35}N_3O_5S$) $[M + H]^+$ 502.2370; found 502.2379. HPLC purity: 98.5%.

4-{[(Cyclopropylmethyl){3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxypyrazolo[5,1-*b*][1,3]-thiazol-7-yl}amino]methyl}tetrahydro-2*H*-pyran-4-ol (6b). To a solution of **16a** (100 mg, 0.22 mmol) in DMF (6 mL) was added NaH (60% dispersion in oil: 11.6 mg, 0.29 mmol) at room temperature. After stirring for 30 min, cyclopropylmethyl bromide (28 μL, 0.29 mmol) was added, and the mixture was stirred for 1 h. Water was added to the reaction mixture while cooling on ice, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in next step without purification.

To the obtained crude product was added CH_2Cl_2 (5 mL), followed by TFA (1 mL), and the mixture was stirred at room temperature overnight. The reaction mixute was concentrated under reduced pressure.

To a solution of the obtained residue in MeOH (8 mL) and AcOH (1 mL) were added 4-hydroxytetrahydro-2*H*-pyran-4-carbaldehyde (289 mg, 2.2 mmol), followed by α-picolineborane (238 mg, 2.2 mmol), and the mixture was stirred at room temperature for 14 h. An aqueous 5 M NaOH was added and the mixture was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:2) to afford **6b** (70 mg, 61%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02−0.10 (m, 2H), 0.38−0.48 (m, 1H), 0.84−0.98 (m, 1H), 1.38−1.62 (m, 4H), 2.84 (d, *J* = 6.8 Hz, 2H), 3.02 (s, 2H), 3.47 (s, 3H), 3.77 (s, 6H), 3.64−3.83 (m, 4H), 3.88 (s, 3H), 4.50 (s, 2H), 6.44 (s, 1H), 6.64 (s, 2H). HRMS calcd for ($C_{26}H_{36}N_3O_6S$) $[M + H]^+$ 518.2319; found 518.2314. HPLC purity: > 99%.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-*N*-[(4-fluorotetrahydro-2*H*-pyran-4-yl)methyl]-6-methoxypyrazolo[5,1-*b*][1,3]thiazol-7-amine (6c).** To a solution of **6b** (65 mg, 0.12 mmol) in CH_2Cl_2 (4 mL) was added diethylaminosulfur trifluoride (25 μL, 0.19 mmol) at 0 °C. After stirring for 5 min, a saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by HPLC to afford **6c** (8.9 mg, 14%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01−0.10 (m, 2H), 0.36−0.44 (m, 2H), 0.83−0.98 (m, 1H), 2.16−2.25 (m, 2H), 2.80 (d, *J* = 6.8 Hz, 2H), 3.47 (s, 3H), 3.59 (s, 2H), 3.74 (t, *J* = 5.6 Hz, 2H), 3.77 (s, 6H), 3.87 (s, 3H), 4.04−4.10 (m, 2H), 4.50 (s, 2H), 5.58−5.64 (m,1H), 6.40 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 159.5, 142.0, 134.2, 128.3, 111.6, 105.9, 105.6, 103.4, 94.6 (d, *J* = 172.8 Hz), 74.8, 63.7 (d, *J* = 1.3 Hz), 62.5 (d, *J* = 23.0 Hz), 61.9, 58.5, 56.2, 56.1, 33.6 (d, *J* = 21.3 Hz), 10.1, 3.4. HRMS calcd for ($C_{26}H_{35}FN_3O_5S$) $[M + H]^+$ 520.2276; found 520.2272. HPLC purity: > 99%.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-*N*-[(tetrahydro-2*H*-pyran-3-yl)methyl)pyrazolo[5,1-*b*][1,3]thiazol-7-amine (6d).** Compound **6d** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by HPLC to give **6d** (7.5 mg, 7%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00−0.06 (m, 2H), 0.35−0.45 (m, 2H), 0.83−0.95 (m, 1H), 1.48−1.68 (m, 2H), 1.68−1.81 (m, 2H), 1.81−1.92 (m, 1H), 2.76 (dd, *J* = 6.4, 13.6 Hz, 1H), 2.81 (m, 2H), 2.93 (dd, *J* = 6.4, 12.4 Hz, 1H), 3.17 (dd, *J* = 9.6, 11.6 Hz, 1H), 3.35−3.43 (m, 1H), 3.47 (s, 3H), 3.79 (s, 6H), 3.80−3.86 (m, 1H), 3.86 (s, 3H), 4.01−4.07 (m, 1H), 4.50 (s, 2H), 6.39 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.7, 159.5, 142.0, 133.5, 128.2, 110.0, 105.9, 105.8, 103.4, 74.8, 72.5, 68.7, 61.3, 58.5, 56.7, 56.4, 56.1, 34.8, 28.0, 25.5, 9.5, 3.5, 3.5. HRMS calcd for ($C_{26}H_{35}N_3O_5S$) $[M + H]^+$ 502.2370; found 502.2372. HPLC purity: 96.0%.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-*N*-[(tetrahydro-2*H*-pyran-2-yl)methyl)pyrazolo[5,1-*b*][1,3]thiazol-7-amine (6e).** To a solution of **16a** (90 mg, 0.20 mmol) and (tetrahydro-2*H*-pyran-2-yl)methyl methanesulfonate (51 mg, 0.26 mmol) in DMSO (3 mL) was added NaOH (10 mg, 0.26 mmol) at room temperature, and the mixture was stirred at 70 °C for 2 h. Water was added to the reaction mixture under ice cooling, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in next step without purification.

To the obtained crude product was added CH_2Cl_2 (2 mL), followed by TFA (1 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixute was concentrated under reduced pressure.

To a solution of the obtained residue in THF (10 mL) and AcOH (1 mL) was added cyclopropanecarboxaldehyde (30 μL, 0.40 mmol), followed by $NaBH(OAc)_3$ (85 mg, 0.40 mmol), and the mixture was stirred at room temperature for 2 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to afford **6e** (41 mg, 41%, 3 steps) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02−0.08 (m, 2H), 0.34−0.42 (m, 2H), 0.86−0.94 (m, 1H), 1.38−1.49 (m, 1H), 1.49−1.61 (m, 4H), 1.70−1.83 (m, 1H), 2.81−2.93 (m, 2H), 2.95 (dd, *J* = 5.6, 12.8 Hz, 1H), 3.17 (dd, *J* = 6.4, 12.8 Hz, 1H), 3.29−3.37 (m, 1H), 3.38 (dt, *J* = 2.4, 11.6 Hz, 1H), 3.47 (s, 3H), 3.79 (s, 3H), 3.87 (s, 6H), 3.92−4.00 (m, 1H), 4.50 (s, 2H), 6.41 (s, 1H), 6.65(s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.6, 159.5, 142.0, 133.6, 128.1, 110.3, 106.0, 105.8, 103.4, 76.4, 74.8, 68.4, 61.1, 59.9, 58.5, 56.3, 56.1, 30.2, 26.3, 23.4, 9.7, 3.6, 3.2. HRMS calcd for ($C_{26}H_{35}N_3O_5S$) $[M + H]^+$ 502.2370; found 502.2372. HPLC purity: 98.3%.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-*N*-[(tetrahydrofuran-3-yl)methyl]pyrazolo[5,1-*b*][1,3]thiazol-7-amine (6f).** Compound **6f** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6f** (83%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02−0.10 (m, 2H), 0.36−0.46 (m, 2H), 0.84−0.98 (m, 1H), 1.56−1.71 (m, 1H), 1.90−2.02 (m, 1H), 2.26−2.42 (m, 1H), 2.81 (d, *J* = 6.4 Hz, 2H), 2.95 (dd, *J* = 8.4, 12.0 Hz, 1H), 3.06 (dd, *J* = 6.8, 12.0 Hz, 1H), 3.47 (s, 3H), 3.56 (dd, *J* = 6.0, 8.4 Hz, 1H), 3.64−3.74 (m, 1H), 3.78 (s, 6H), 3.75−3.90 (m, 2H), 3.87 (s, 3H), 4.50 (s, 2H), 6.41 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.9, 159.4, 142.0, 133.9, 128.3, 109.6, 105.8, 105.7, 103.4, 74.8, 72.4, 67.7, 61.2, 58.5, 57.5, 56.3, 56.1, 38.4, 30.6, 9.7, 3.5, 3.5. HRMS calcd for ($C_{25}H_{33}N_3O_5S$) $[M + H]^+$ 488.2214; found 488.2223. HPLC purity: > 99%.

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N-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-N-[(tetrahydrofuran-2-yl)methyl]pyrazolo[5,1-b][1,3]thiazol-7-amine (6g). Compound **6g** was prepared according to the procedure described for the synthesis of **6a** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to give **6g** (64%, 3 steps) as a pale-brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.02−0.10 (m, 2H), 0.33−0.45 (m, 2H), 0.85−0.94 (m, 1H), 1.56−1.68 (m, 1H), 1.74−1.92 (m, 2H), 1.89−2.01 (m, 1H), 2.85 (dd, *J* = 6.8, 13.3 Hz, 1H), 2.92 (dd, *J* = 6.4, 13.3 Hz, 1H), 3.00 (dd, *J* = 6.8, 12.8 Hz, 1H), 3.25 (dd, *J* = 6.0, 12.8 Hz, 1H), 3.46 (s, 3H), 3.68−3.74 (m, 1H), 3.78 (s, 6H), 3.81−3.87 (m, 1H), 3.87 (s, 3H), 3.89−3.97 (m, 1H), 4.50 (s, 2H), 6.40 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.6, 159.5, 142.0, 133.6, 128.2, 110.1, 105.9, 105.8, 103.4, 77.8, 74.8, 67.9, 61.1, 58.7, 58.5, 56.3, 56.1, 30.0, 25.5, 9.6, 3.6, 3.2. HRMS calcd for ($C_{25}H_{33}N_3O_5S$) $[M + H]^+$ 488.2214; found 488.2210. HPLC purity: > 99%.

N-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-N-(2-methoxyethyl)-pyrazolo[5,1-b][1,3]thiazol-7-amine (6h). To a solution of **16a** (100 mg, 0.22 mmol) and 2-bromoethyl methyl ether (27 μL, 0.29 mmol) in DMF (2 mL) was added NaH (60% dispersion in oil: 14 mg, 0.29 mmol) at room temperature, and then the mixture was stirred for 4.5 h. Water was added to the reaction mixture under ice cooling, and the residue was extracted with Et_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in next step without purification.

To the obtained crude product was added CH_2Cl_2 (5 mL), followed by TFA (2 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixute was concentrated under reduced pressure.

To a solution of the obtained residue in THF (10 mL) and AcOH (1 mL) were added cyclopropanecarbaldehyde (33 μL, 0.44 mmol), followed by $NaBH(OAc)_3$ (94 mg, 0.44 mmol), and the mixture was stirred at room temperature for 1 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to afford **6h** (77 mg, 75%, 3 steps) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.03−0.10 (m, 2H), 0.36−0.45 (m, 2H), 0.89−0.98 (m, 1H), 2.87 (d, *J* = 6.8 Hz, 2H), 3.25 (t, *J* = 6.4 Hz, 2H), 3.33 (s, 3H), 3.41−3.50 (m, 5H), 3.78 (s, 6H), 3.87 (s, 3H), 4.50 (s, 2H), 6.41 (s, 1H), 6.65 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.6, 159.5, 142.0, 133.3, 128.2, 109.7, 105.9, 105.8, 103.4, 74.8, 71.3, 60.9, 58.9, 58.5, 56.3, 56.1, 53.6, 9.5, 3.4. HRMS calcd for ($C_{23}H_{31}N_3O_5S$) $[M + H]^+$ 462.2057; found 462.2065. HPLC purity: 96.3%.

N-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]thiazole-7-amine (6i). Compound **6i** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:2) to give **6i** (77%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 2H), 0.29−0.40 (m, 2H), 0.78−0.92 (m, 1H), 1.50−1.66 (m, 2H), 1.78−1.88 (m, 2H), 2.88 (d, *J* = 6.8 Hz, 2H), 3.10−3.22 (m, 1H), 3.39 (td, *J* = 1.6, 11.6 Hz, 2H), 3.47 (s, 3H), 3.79 (s, 6H), 3.87 (s, 3H), 3.92−4.03 (m, 2H), 4.50 (s, 2H), 6.41 (s, 1H), 6.65 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 159.5, 142.0, 135.5, 128.3, 107.3, 105.9, 105.6, 103.4, 74.8, 67.5, 59.6, 58.5, 56.3, 56.1, 55.8, 31.3, 10.2, 3.4. HRMS calcd for ($C_{25}H_{33}N_3O_5S$) $[M + H]^+$ 488.2214; found 488.2216. HPLC purity: 98.3%.

3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-N-ethyl-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]-thiazol-7-amine (6j). Compound **6j** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6j** (59%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, *J* = 7.2 Hz, 3H), 1.53−1.67 (m, 2H), 1.78−1.88 (m, 2H), 3.00−3.14 (m, 3H, involving a quartet at 3.05, *J* = 7.2 Hz), 3.32−3.45 (m, 2H), 3.47 (s, 3H), 3.79 (s, 6H), 3.86 (s, 3H), 3.94−4.03 (m, 2H), 4.51 (s, 2H), 6.42 (s, 1H), 6.65 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.1, 159.5, 142.1, 134.8, 128.3, 106.1, 105.8, 105.8, 103.3, 74.8, 67.5, 60.4, 58.5, 56.3, 56.1, 44.9, 31.1, 13.6. HRMS calcd for ($C_{23}H_{31}N_3O_5S$) $[M + H]^+$ 462.2057; found 462.2058. HPLC purity: > 99%.

3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-N-propyl-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]-thiazole-7-amine (6k). Compound **6k** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6k** (68%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.32−1.45 (m, 2H), 1.53−1.66 (m, 2H), 1.78−1.88 (m, 2H), 2.90−2.99 (m, 2H), 3.00−3.12 (m, 1H), 3.38 (td, *J* = 2.0, 12.0 Hz, 2H), 3.47 (s, 3H), 3.79 (s, 6H), 3.86 (s, 3H), 3.94−4.03 (m, 2H), 4.50 (s, 2H), 6.42 (s, 1H), 6.65 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 159.5, 142.0, 135.0, 128.2, 106.6, 105.8, 105.7, 103.3, 74.8, 67.5, 60.7, 58.5, 56.3, 56.1, 52.8, 31.1, 21.8, 11.7. HRMS calcd for ($C_{24}H_{33}N_3O_5S$) $[M + H]^+$ 476.2214; found 476.2209. HPLC purity: > 99%.

N-(2-Cyclopropylethyl)-3-[4-(ethoxymethyl)-2,6-dimethoxy-phenyl]-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]thiazole-7-amine (6l). Compound **6l** was prepared according to the procedure described for the synthesis of **6e** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to give **6l** (83%, 3 steps) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03−0.04 (m, 2H), 0.34−0.43 (m, 2H), 0.63−0.74 (m, 1H), 1.20−1.37 (m, 2H), 1.54−1.69 (m, 2H), 1.86 (d, *J* = 12.0 Hz, 2H), 3.02−3.15 (m, 3H), 3.40 (t, *J* = 12.0 Hz, 2H), 3.49 (s, 3H), 3.81 (s, 6H), 3.87 (s, 3H), 3.96−4.04 (m, 2H), 4.52 (s, 2H), 6.44 (s, 1H), 6.67 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 159.5, 142.0, 134.9, 128.2, 106.6, 105.8, 105.8, 103.3, 74.8, 67.6, 61.0, 58.5, 56.3, 56.1, 50.8, 33.9, 31.0, 8.9, 4.2. HRMS calcd for ($C_{26}H_{35}N_3O_5S$) $[M + H]^+$ 502.2370; found 502.2373. HPLC purity: 98.6%.

N-Butyl-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]-thiazole-7-amine (6m). Compound **6m** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2:3) to give **6m** (80%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.2 Hz, 3H), 1.22−1.42 (m, 4H), 1.56−1.68 (m, 2H), 1.77−1.88 (m, 2H), 2.97 (t, *J* = 7.2 Hz, 2H), 3.00−3.11 (m, 1H), 3.33 (td, *J* = 1.6, 11.6 Hz, 2H), 3.47 (s, 3H), 3.79 (s, 6H), 3.86 (s, 3H), 3.92−4.03 (m, 2H), 4.50(s, 2H), 6.42 (s, 1H), 6.65(s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.0, 159.5, 142.0, 135.0, 128.2, 106.7, 105.8, 105.8, 103.3, 74.8, 67.5, 60.7, 58.5, 56.3, 56.1, 50.6, 31.1, 30.7, 20.4, 14.0. HRMS calcd for ($C_{25}H_{35}N_3O_5S$) $[M + H]^+$ 490.2370; found 490.2363. HPLC purity: > 99%.

3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-N-isopropyl-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b][1,3]-thiazol-7-amine (6n). Compound **6n** was prepared according to the procedure described for the synthesis of **6a** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 50:1) to give **6n** (59%, 3 steps) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (d, *J* = 6.0 Hz, 6H), 1.80−1.98 (m, 4H), 3.39−3.47 (m, 2H), 3.48 (s, 3H), 3.78 (s, 6H), 3.90 (s, 3H), 3.97−4.03 (m, 1H), 3.99−4.07 (m, 2H), 4.11−4.21 (m, 1H), 4.51 (s, 2H), 6.58 (s, 1H), 6.65 (s, 2H). HRMS calcd for ($C_{24}H_{33}N_3O_5S$) $[M + H]^+$ 476.2214; found 476.2213. HPLC purity: > 98.0%.

3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-N-isobutyl-6-methoxy-N-(tetrahydro-2H-pyran-4-yl)pyrazolo[5,1-b]thiazol-7-amine (6o). Compound **6o** was prepared according to the procedure described for the synthesis of **6b** using **16a**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6o** (88%, 3 steps) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (d, *J* = 6.8 Hz, 3H), 1.44−1.67 (m, 3H), 1.75−1.86 (m, 2H), 2.74 (d, *J* = 7.2 Hz, 2H), 2.90−3.06 (m, 1H), 3.36 (td, *J* = 2.0, 12.0 Hz, 2H), 3.47 (s, 3H), 3.79 (s, 6H), 3.85 (s, 3H), 3.92−4.02 (m, 2H), 4.50 (s, 2H), 6.41 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 159.5, 142.0, 135.6, 128.2, 107.1, 105.9, 105.7, 103.3, 74.8, 67.7, 61.2, 58.8, 58.5, 56.3, 56.1, 31.2, 27.3,

20.6. HRMS calcd for ($C_{25}H_{35}N_3O_5S$) $[M + H]^+$ 490.2370; found 490.2369. HPLC purity: > 99%.

3-[4-(Ethoxymethyl)-2,6-dimethoxyphenyl]-6-methoxy-*N*-propyl-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[5,1-*b*][1,3]-thiazol-7-amine (6p). Compound **6p** was prepared according to the procedure described for the synthesis of **6h** using **16b**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6p** (82%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 7.6 Hz, 3H), 1.30 (t, *J* = 6.8 Hz, 3H), 1.30−1.44 (m, 2H), 1.52−1.66 (m, 2H), 1.78−1.88 (m, 2H), 2.94 (dd, *J* = 7.2, 7.6 Hz, 2H), 3.00−3.11 (m, 1H), 3.38 (td, *J* = 2.0, 12.0 Hz, 2H), 3.62 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 6H), 3.86 (s, 3H), 3.93−4.03 (m, 2H), 4.54 (s, 2H), 6.40 (s, 1H), 6.66 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.9, 159.5, 142.4, 135.0, 128.3, 106.6, 105.8, 105.7, 103.4, 72.9, 67.5, 66.1, 60.7, 56.3, 56.1, 52.8, 31.1, 21.8, 15.3, 11.7. HRMS calcd for ($C_{25}H_{36}N_3O_5S$) $[M + H]^+$ 490.2370; found 490.2367. HPLC purity: > 99%.

3,5-Dimethoxy-4-{6-methoxy-7-[propyl(tetrahydro-2*H*-pyran-4-yl)amino]pyrazolo[5,1-*b*][1,3]thiazol-3-yl}benzonitrile (6q). Compound **6q** was prepared according to the procedure described for the synthesis of **6h** using **16c**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2:1) to give **6q** (54%, 3 steps) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.30−1.44 (m, 2H), 1.50−1.66 (m, 2H), 1.78−1.88 (m, 2H), 2.94 (t, *J* = 7.2 Hz, 2H), 3.00−3.12 (m, 1H), 3.38 (t, *J* = 12.0 Hz, 2H), 3.82 (s, 6H), 3.85 (s, 3H), 3.94−4.03 (m, 2H), 6.49 (s, 1H), 6.93 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.2, 159.6, 135.2, 126.3, 118.6, 114.4, 111.8, 108.0, 107.0, 106.9, 67.5, 60.7, 56.4, 56.3, 52.8, 31.1, 21.8, 11.7. HRMS calcd for ($C_{23}H_{29}N_4O_4S$) $[M + H]^+$ 457.1904; found 457.1898. HPLC purity: 96.9%.

3-[2-Chloro-6-methoxy-4-(methoxymethyl)phenyl]-6-methoxy-*N*-propyl-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[5,1-*b*]-[1,3]thiazole-7-amine (6r). Compound **6r** was prepared according to the procedure described for the synthesis of **6a** using **16d**. The product was purified by column chromatography on NH silica gel (*n*-heptane:EtOAc = 3:1) to give **6r** (61%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.2 Hz, 3H), 1.32−1.44 (m, 2H), 1.52−1.66 (m, 2H), 1.79−1.86 (m, 2H), 2.92−2.97 (m, 2H), 3.02−3.11 (m, 1H), 3.34−3.41 (m, 2H), 3.47 (s, 3H), 3.80 (s, 3H), 3.85 (s, 3H), 3.96−4.02 (m, 2H), 4.49 (s, 2H), 6.46 (s, 1H), 6.93 (br s, 1H), 7.09 (br s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.3, 159.9, 142.3, 136.1, 135.1, 128.8, 120.6, 116.5, 108.6, 107.0, 106.4, 73.9, 67.5, 67.5, 60.6, 58.6, 56.4, 56.3, 52.7, 31.2, 31.1, 21.8, 11.7. HRMS calcd for ($C_{23}H_{31}ClN_3O_4S$) $[M + H]^+$ 480.1718; found 480.1715. HPLC purity: > 99%.

6-Methoxy-3-[2-methoxy-4-(methoxymethyl)phenyl]-*N*-propyl-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[5,1-*b*][1,3]-thiazol-7-amine (6s). Compound **6s** was prepared according to the procedure described for the synthesis of **6b** using **16e**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:1), followed by column chromatography on NH silica gel (*n*-heptane:EtOAc = 2:1) to give **6s** (66%, 3 steps) as a pale-yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88−0.92 (m, 3H), 1.29−1.43 (m, 2H), 1.49−1.66 (m, 2H), 1.77−1.88 (m, 2H), 2.89−3.13 (m, 3H), 3.30−3.42 (m, 2H), 3.44 (s, 3H), 3.90−4.04 (m, 8H, involving a singlet at 3.93 and a singlet at 3.97), 4.52 (s, 2H), 6.99 (s, 1H), 7.04 (s, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.8, 157.2, 140.4, 136.0, 130.3, 129.5, 119.5, 116.9, 110.2, 106.7, 106.6, 74.4, 67.4, 60.6, 58.3, 56.3, 55.7, 52.6, 31.2, 21.7, 11.7. HRMS calcd for ($C_{23}H_{32}N_3O_4S$) $[M + H]^+$ 446.2108; found 446.2101. HPLC purity: > 99%.

***N*-Butyl-3-[4-(ethoxymethyl)-2,6-dimethoxyphenyl]-6-methoxy-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[5,1-*b*][1,3]-thiazole-7-amine (6t).** Compound **6t** was prepared according to the procedure described for the synthesis of **6h** using **16b**. The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give **6t** (75%, 3 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.30 (t, *J* = 6.8 Hz, 3H), 1.24−1.40 (m, 4H), 1.52−1.67 (m, 2H), 1.78−1.87 (m, 2H), 2.97 (t, *J* = 6.8 Hz, 2H), 2.99−3.11 (m, 1H), 3.38 (td, *J* = 1.6, 11.6 Hz, 2H), 3.63 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 6H), 3.86 (s, 3H), 3.94−4.03 (m, 2H), 4.55 (s, 2H), 6.41 (s, 1H), 6.66 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.0, 159.5, 142.4, 135.0, 128.3, 106.7, 105.8, 105.7, 103.4, 72.9, 67.5, 66.1, 60.7, 56.3, 56.1, 50.6, 31.1, 30.7, 20.4, 15.3, 14.0. HRMS calcd for ($C_{26}H_{37}N_3O_5S$) $[M + H]^+$ 504.2527; found 504.2519. HPLC purity: > 99%.

4-{7-[Butyl(tetrahydro-2*H*-pyran-4-yl)amino]-6-methoxypyrazolo[5,1-*b*]thiazol-3-yl}-3,5-dimethoxybenzonitrile (6u). To a solution of **16c** (90 mg, 0.21 mmol) and 1-iodobutane (36 μL, 0.32 mmol) in DMSO (0.8 mL) was added NaOH (17 mg, 0.42 mmol) at room temperature, and the mixture was stirred for 1 h. A saturated aqueous solution of NH_4Cl was added, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in next step without purification.

To the obtained crude product was added CH_2Cl_2 (0.6 mL), followed by TFA (0.2 mL), and the mixture was stirred at room temperature for 2 h. The reaction mixute was concentrated under reduced pressure.

To a solution of the obtained residue in THF (1 mL) were added tetrahydro-4*H*-pyran-4-one (36 mg, 0.32 mmol), followed by $NaBH(OAc)_3$ (67 mg, 0.32 mmol), and the mixture was stirred at room temperature for 2 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 3:2) to afford **6u** (81 mg, 82%, 3 steps) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82−0.92 (m, 3H), 1.22−1.38 (m, 4H), 1.50−1.64 (m, 2H), 1.78−1.86 (m, 2H), 2.94−3.01 (m, 2H), 3.06 (tt, *J* = 4.0, 11.2 Hz, 1H), 3.38 (dt, *J* = 2.0, 12.0 Hz, 2H), 3.82 (s, 6H), 3.85 (s, 3H), 3.95−4.02 (m, 2H), 6.49 (s,1H), 6.93 (s,2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.2, 159.6, 135.2, 126.3, 118.6, 114.4, 111.8, 108.0, 107.1, 106.9, 67.5, 60.8, 56.4, 56.3, 50.6, 31.1, 30.7, 20.4, 14.0. HRMS calcd for ($C_{24}H_{30}N_4O_4S$) $[M + H]^+$ 471.2061; found 471.2058. HPLC purity: > 99%.

Ethyl 6-Oxo-5,6-dihydropyrazolo[5,1-*b*][1,3]thiazole-7-carboxylate (10). To a solution of diethyl malonate 7 (100 g, 624 mmol), cesium carbonate (488 g, 1.5 mol), and carbon disulfide (45.3 mL, 749 mmol) in DMF (900 mL) was added dropwise bromoacetaldehyde diethylacetal (290 mL, 1.87 mol), followed by sodium iodide (9.34 g, 62.4 mmol) at room temperature. The reaction mixture was heated to 60 °C for 8 h and then cooled to ambient temperature. The reaction mixture was filtered, and the filtrate was partitioned between water and Et_2O. The organic layer was separated, and the aqueous layer was extracted with Et_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a crude product, which was used in the next step without further purification.

To a solution of the crude product from the previous step in EtOH (900 mL) was added hydrazine hydrate (60.7 mL, 1.25 mol) while stirring on a water bath, and the mixture was stirred for 13 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to give crude compound, which was used in the next step without further purification.

To the obtained crude product were added 1,4-dioxane (1 L) and aqueous 5 M HCl (200 mL) in that order, and the mixture was stirred at 60 °C for 4 h. The mixture was cooled to room temperature, and the solvent was distilled off under reduced pressure. Water was added to the resulting residue, and the resulting solid was filtered, washed with water, and dried under reduced pressure to afford **10** (42.5 g, 32%, 3 steps) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (t, *J* = 7.0 Hz, 3H), 4.40 (q, *J* = 7.0 Hz, 2H), 6.89 (d, *J* = 4.0 Hz, 1H), 7.69 (d, *J* = 4.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.0, 164.6, 141.8, 123.0, 111.2, 89.9, 60.7, 14.5. HRMS calcd for ($C_8H_8N_2O_3S$) $[M + H]^+$ 213.0328; found 213.0332.

Ethyl 6-Methoxypyrazolo[5,1-*b*][1,3]thiazole-7-carboxylate (11). To a solution of **10** (41.3 g, 195 mmol) in DMF (624 mL) was added cesium carbonate (127 g, 389 mmol), followed by iodomethane (24.2 mL, 389 mmol) at room temperature. The reaction mixture was stirred at ambient temperature for 1 h, and then

water and a mixed solvent of EtOAc/Et_2O (1/1) were added. The organic layer was separated, washed with brine, dried over $MgSO_4$ and filtered. The solvent was distilled off under reduced pressure. The residue was purified by silica gel column chromatography on silica gel (*n*-heptane:EtoAc = 1:2.3) to afford **11** (30.7 g, 70%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (t, *J* = 7.0 Hz, 3H), 4.08 (s, 3H), 4.35 (q, *J* = 7.0 Hz, 2H), 6.87 (d, *J* = 4.4 Hz, 1H), 7.66 (d, *J* = 4.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.1, 161.9, 145.1, 122.6, 111.3, 92.0, 60.1, 56.8, 14.5. HRMS calcd for ($C_9H_{10}N_2O_3S$) [M + H]$^+$ 227.0485; found 227.0488.

6-Methoxypyrazolo[5,1-*b*][1,3]thiazole (12). To a solution of **11** (30.7 g, 136 mmol) in EtOH (407 mL) was added aqueous 5 M NaOH (136 mL, 680 mmol) at room temperature. The mixture was stirred at 80 °C for 2 h and then cooled to ambient temperature. The reaction mixture was adjusted to neutral pH with aqueous 5 M HCl at 0 °C. The resulting mixture was concentrated to remove EtOH, and then it was filtered. Solid cake was washed with H_2O and dried under vacuum to afford a crude product, which was used in the next step.

To the obtained crude product were added 1,4-dioxane (400 mL) and concentrated HCl (200 mL) in that order, and the mixture was stirred at 60 °C for 1.5 h. The reaction mixute was concentrated under reduced pressure, and then was adjusted to weakly acidic pH with solid NaOH under ice cooling. The residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered, and then the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2.3:1) to afford **12** (15.8 g, 75%, 2 steps) as brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.95 (s, 3H), 5.81 (d, *J* = 0.8 Hz, 1H), 6.60 (d, *J* = 4.0 Hz, 1H), 7.58 (dd, *J* = 0.8, 4.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.7, 140.5, 122.2, 107.8, 81.7, 56.5. HRMS calcd for ($C_6H_6N_2OS$) [M + H]$^+$ 155.0274; found 155.0276.

***tert*-Butyl (6-Methoxypyrazolo[5,1-*b*][1,3]thiazol-7-yl)-carbamate (14a).** To a solution of **12** (15.8 g, 103 mmol) in aqueous 5 M HCl (350 mL) was added a mixture of sodium nitrite (10.6 g, 154 mmol) and water (115 mL) under ice cooling. The mixture was stirred at room temperature for 0.5 h and then adjusted to neutral pH with aqueous 5 M NaOH under ice cooling. The precipitate was collected by filtration and washed with water.

To the obtained crude product were added EtOH (200 mL), THF (300 mL), and 10% palladium−carbon (50% wet: 16 g) in that order, and the mixture was stirred at room temperature for 5 h at an atmospheric pressure under a hydrogen atmosphere. The mixture was filtered with Celite and concentrated under reduced pressure.

To a solution of the obtained crude product in CH_2Cl_2 (425 mL) were added triethylamine (17.8 mL, 128 mmol) and Boc_2O (24.1 g, 111 mmol) at room temperature. The mixture was stirred at room temperature for 11 h and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2:1) to afford **14a** (16.5 g, 59%, 3 steps) as light-pink solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H), 3.98 (s, 3H), 6.12 (br s, 1H), 6.54 (d, *J* = 4.0 Hz, 1H), 7.48 (d, *J* = 4.4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 153.1, 131.5, 122.1, 108.9, 98.9, 80.7, 56.5, 28.3. HRMS calcd for ($C_{11}H_{15}N_3O_3S$) [M + H]$^+$ 270.0907; found 270.0910.

***tert*-Butyl (6-Methylpyrazolo[5,1-*b*][1,3]thiazol-7-yl)-carbamate (14b).** To a solution of **18** (1.0 g, 5.7 mmol) in aqueous 5 M HCl (24 mL) was added a mixture of sodium nitrite (0.78 g, 11.3 mmol) and water (1.5 mL) under ice cooling. The mixture was stirred at 0 °C for 2 h and then warmed to room temperature. After stirring overnight, the mixture was adjusted to alkalic pH with aqueous 5 M NaOH under ice cooling. The precipitate was collected by filtration and washed with water, which was used in the next step without further purification.

To the obtained crude product were added 2 M HCl (22 mL) and Zn powder (0.37 g, 5.7 mmol) in that order, and the mixture was stirred at room temperature for 15 min at room temperature. Additional Zn powder (0.37 g, 5.7 mmol) was added, and then the mixture was stirred at room temperature for 1 h at room temperature. The mixture was filtered with Celite. The mixture was adjusted to neutral pH with aqueous 5 M NaOH and then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered, and then the solvent was distilled off under reduced pressure, which was used in the next step without further purification.

To a solution of the obtained crude product in CH_2Cl_2 (20 mL) were added triethylamine (0.83 mL, 5.9 mmol) and Boc_2O (0.95 g, 4.4 mmol) at room temperature. After stirring at room temperature overnight, water was added and the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, and filtered, and then the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to afford **14b** (0.81 g, 56%, 3 steps) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (s, 9H), 2.33 (s, 3H), 6.03 (br s, 2H), 6.71 (d, *J* = 4.0 Hz, 1H), 7.59 (d, *J* = 4.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.2, 144.6, 132.0, 121.9, 112.0, 110.6, 80.8, 28.3, 11.7. HRMS calcd for ($C_{11}H_{16}N_3O_2S$) [M + H]$^+$ 254.0958; found 254.0957.

***tert*-Butyl (3-Bromo-6-methoxypyrazolo[5,1-*b*][1,3]thiazol-7-yl)carbamate (15a).** To a solution of **14a** (16.5 g, 61.4 mmol) in THF (410 mL) was added *n*-butyllithium (2.77 M solution in *n*-hexane: 62.1 mL, 172 mmol) at −78 °C. After stirring the mixture at −78 °C for 40 min, 1,2-dibromotetrafluoroethane (10.2 mL, 86 mmol) was added and then the mixture was allowed to warm to room temperature over 2 h while stirring. To the reaction mixture were added a saturated aqueous solution of NH_4Cl and EtOAc, followed by AcOH to adjust to a weakly acidic pH. The mixture was separated and the organic layer was washed with brine, dried over $MgSO_4$, and filtered, and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 4:1) to afford **15a** (14.3 g, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 9H), 4.04 (s, 3H), 6.16 (br s, 1H), 6.50 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.2, 152.9, 129.6, 106.6, 103.6, 101.0, 81.0, 56.8, 28.3. HRMS calcd for ($C_{11}H_{14}BrN_3O_3S$) [M + H]$^+$ 348.0012; found 348.0020.

***tert*-Butyl (3-Bromo-6-methylpyrazolo[5,1-*b*][1,3]thiazol-7-yl)carbamate (15b).** Compound **15b** was prepared according to the procedure described for the synthesis of **15a** using **14b** (1.2 g, 4.7 mmol) in THF (40 mL), 1,2-dibromotetrafluoroethane (0.62 mL, 5.2 mmol), and *n*-butyllithium (1.57 M solution of *n*-hexane: 6.6 mL, 10.4 mmol). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:2) to afford **15b** (1.4 g, 88%) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.37 (s, 3H), 6.07 (br s, 2H), 6.68 (s,1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.0, 144.1, 130.1, 112.7, 110.0, 103.2, 81.2, 28.3, 11.8. HRMS calcd for ($C_{11}H_{15}BrN_3O_2S$) [M + H]$^+$ 332.0063; found 332.0058.

***tert*-Butyl {3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-6-methoxypyrazolo[5,1-*b*][1,3]thiazol-7-yl}carbamate (16a).** To a solution of **15a** (998 mg, 2.87 mmol) in DME (107 mL) and water (36 mL) were added [2,6-dimethoxy-4-(methoxymethyl)phenyl]-boronic acid (973 mg, 4.31 mmol), K_2CO_3 (791 mg, 5.74 mmol), triphenylphosphine (374 mg, 1.43 mmol), and palladium acetate (64.5 mg, 0.285 mmol). The mixture was stirred at 90 °C for 1.5 h under a nitrogen stream. The reaction mixture was cooled, and then water was added. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and filtered, and then the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to afford **16a** (1.22 g, 95%) as a beige solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 3.46 (s, 3H), 3.75 (s, 6H), 3.88 (s, 3H), 4.49 (s, 2H), 6.08 (br s, 1H), 6.43 (s, 1H), 6.63 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 157.1, 153.2, 142.0, 131.0, 127.9, 107.7, 106.1, 103.3, 98.3, 80.4, 74.8, 58.4, 56.8, 56.0, 28.3. HRMS calcd for ($C_{21}H_{27}N_3O_6S$) [M + H]$^+$ 450.1693; found 450.1703.

***tert*-Butyl {3-[4-(Ethoxymethyl)-2,6-dimethoxyphenyl]-6-methoxypyrazolo[5,1-*b*][1,3]thiazol-7-yl}carbamate (16b).** Compound **16b** was prepared according to the procedure described for the synthesis of **16a** using **15a** (2.0 g, 5.7 mmol) and [4-(ethoxymethyl)-2,6-dimethoxyphenyl]boronic acid (2.1 g, 8.6 mmol). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to afford **16b** (2.5 g, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (t, *J* = 7.2 Hz, 3H), 1.52 (s, 9H), 3.61

(q, J = 7.2 Hz, 2H), 3.75 (s, 6H), 3.87 (s, 3H), 4.53 (s, 2H), 6.09 (br s, 1H), 6.42 (s, 1H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 157.2, 153.2, 142.4, 131.0, 127.9, 107.7, 106.0, 103.4, 98.3, 80.4, 72.9, 66.1, 56.8, 56.0, 28.3, 15.2. HRMS calcd for ($C_{22}H_{29}N_3O_6S$) [M + H]$^+$ 464.1850; found 464.1851.

***tert*-Butyl [3-(4-Cyano-2,6-dimethoxyphenyl)-6-methoxypyrazolo[5,1-*b*][1,3]thiazol-7-yl]carbamate (16c).** Compound **16c** was prepared according to the procedure described for the synthesis of **16a** using **15a** (461 mg, 1.3 mmol) and (4-cyano-2,6-dimethoxyphenyl)boronic acid (412 mg, 2.0 mmol). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2:1) to afford **16c** (296 mg, 52%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 3.78 (s, 6H), 3.87 (s, 3H), 6.11 (br s, 1H), 6.50 (s, 1H), 6.91 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.6, 157.1, 153.2, 130.7, 125.9, 118.7, 114.3, 112.0, 109.0, 108.0, 98.7, 80.6, 56.8, 56.3, 28.3. HRMS calcd for ($C_{20}H_{23}N_4O_5S$) [M + H]$^+$ 431.1384; found 431.1381.

***tert*-Butyl {3-[2-Chloro-6-methoxy-4-(methoxymethyl)-phenyl]-6-methoxy-pyrazolo[5,1-*b*][1,3]thiazol-7-yl}carbamate (16d).** To a solution of **15a** (250 mg, 0.72 mmol) in toluene (5 mL) and EtOH (2.5 mL) was added [2-chloro-6-methoxy-4-(methoxymethyl)phenyl]boronic acid (248 mg, 1.1 mmol), $Pd(PPh_3)_4$ (83 mg, 0.072 mmol), and a 1 M aqueous solution of Na_2CO_3 (1.4 mL, 1.4 mmol). After refluxing for 3 h under a nitrogen stream, the reaction mixture was cooled and then water was added. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and filtered, and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to afford **16d** (281 mg, 86%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 3.46 (s, 3H), 3.76 (s, 3H), 3.87 (s, 3H), 4.48 (s, 2H), 6.13 (br s, 1H), 6.48 (s, 1H), 6.91 (s, 1H), 7.08 (s, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.9, 157.2, 153.2, 142.3, 136.1, 130.6, 128.4, 120.5, 116.8, 108.6, 108.5, 98.9, 80.5, 73.9, 58.6, 56.9, 56.2, 28.3. HRMS calcd for ($C_{20}H_{24}ClN_3O_5S$) [M + H]$^+$ 454.1198; found 454.1196.

***tert*-Butyl {6-Methoxy-3-[2-methoxy-4-(methoxymethyl)-phenyl]pyrazolo[5,1-*b*][1,3]thiazol-7-yl}carbamate (16e).** Compound **16e** was prepared according to the procedure described for the synthesis of **16d** using **15a** (150 mg, 0.43 mmol) and [2-methoxy-4-(methoxymethyl)phenyl]boronic acid (127 mg, 0.65 mmol). The product was purified by column chromatography on NH silica gel (*n*-heptane:EtOAc = 5:1) to afford **16e** (150 mg, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 3.43 (s, 3H), 3.90 (s, 3H), 3.98 (s, 3H), 4.51 (s, 2H), 6.12 (br s, 1H), 6.96 (s, 1H), 7.02 (d, J = 7.6 Hz, 1H), 7.03 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.3, 156.9, 153.1, 140.5, 131.5, 130.3, 129.5, 119.5, 117.3, 110.3, 108.4, 98.4, 80.5, 74.4, 58.3, 56.7, 55.7, 28.3. HRMS calcd for ($C_{20}H_{25}N_3O_5S$) [M + H]$^+$ 420.1588; found 420.1582.

1-(6-Methylpyrazolo[5,1-*b*][1,3]thiazol-7-yl)ethanone (18). To a solution of 2-methylthiazole (5.8 g, 58.2 mmol) in CH_2Cl_2 (35 mL) were added *O*-mesitylene sulfonylhydroxylamine (12.5 g, 58.2 mmol, CAUTION!) dissolved with CH_2Cl_2 (35 mL) at 0 °C, and then reaction mixture was stirred at room temperature for 9.5 h. The reaction mixture was concentrated and dried under reduced pressure overnight to afford a crude product, which was used in the next step without further purification.

To the obtained crude product (15.6 g) were added acetic anhydride (125 mL, 1.32 mol) and sodium acetate (6.1 g, 74.4 mmol) in that order at room temperature, and the mixture was refluxed for 5 h. The mixture was cooled to room temperature, and the solvent was distilled off under reduced pressure. Water was added to the resulting residue, and then a saturated aqueous solution of K_2CO_3 was added at 0 °C. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and filtered, and then the solvent was distilled off under reduced pressure. The resulting solid was sonicated in diisopropylether (40 mL), and filtered to afford **18** (5.3 g, 51%, 2 steps) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.51 (s, 3H), 2.65 (s, 3H), 7.00 (d, J = 4.0 Hz, 1H), 7.76 (d, J = 4.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 190.2, 155.3, 145.2, 122.1, 114.2, 114.0, 28.9, 15.5. HRMS calcd for ($C_8H_9N_2OS$) [M + H]$^+$ 181.043; found 181.0428.

***tert*-Butyl {3-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-6-methylpyrazolo[5,1-*b*]thiazol-7-yl}carbamate (19).** Compound **19** was prepared according to the procedure described for the synthesis of **16d** using **15b** (1.8 g, 5.5 mmol) and [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (1.9 g, 8.2 mmol). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:9) to afford **19** (2.4 g, 99%) as a pale-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.27 (s, 3H), 3.44 (s, 3H), 3.73 (s, 6H), 4.48 (s, 2H), 5.99 (br s, 1H), 6.55 (s, 1H), 6.61 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.5, 153.4, 144.3, 142.4, 132.0, 127.9, 110.4, 110.0, 105.6, 103.2, 80.5, 74.7, 58.4, 56.0, 28.3, 11.9. HRMS calcd for ($C_{21}H_{27}N_3O_5S$) [M + H]$^+$ 434.1744; found 434.1742.

***N*-(Cyclopropylmethyl)-3-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2,6-dimethyl-*N*-(tetrahydro-2*H*-pyran-4-ylmethyl)pyrazolo[5,1-*b*][1,3]thiazol-7-amine (23).** To a solution of **5** (120 mg, 0.25 mmol) in THF (2 mL) was added *n*-butyllithium (2.69 M solution in *n*-hexane: 0.10 mL, 0.27 mmol) at −78 °C. After stirring the mixture at −78 °C for 20 min, 1,2-dibromotetrafluoroethane (0.034 mL, 0.28 mmol) was added and then the mixture was allowed to warm to room temperature over 1 h while stirring. A saturated aqueous solution of NH_4Cl was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered, and the solvent was distilled off under reduced pressure to afford a crude product, which was used in next step without purification.

To a solution of the obtained residue in 1,4-dioxane (1.5 mL) was added dimethylzinc (1.0 M solution in *n*-hexane: 0.49 mL, 0.49 mmol) and $Pd[P(t\text{-}Bu)_3]_2$ (5 mg, 0.010 mmol), and the mixture was stirred at 65 °C for 1 h under a nitrogen stream. The reaction mixture was cooled, and then a saturated aqueous solution of NH_4Cl was added. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered, and the solvent was distilled off under reduced pressure. The residue was purified by HPLC to afford **23** (60 mg, 49%, 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04−0.07 (m, 2H), 0.34−0.46 (m, 2H), 0.78−0.94 (m, 1H), 1.20−1.37 (m, 2H), 1.48−1.62 (m, 1H), 1.67−1.84 (m, 2H), 2.14 (s, 3H), 2.25 (s, 3H), 2.67−2.76 (m, 2H), 2.81−2.90 (m, 2H), 3.26−3.39 (m, 2H), 3.47 (s, 3H), 3.76 (s, 3H), 3.87−3.99 (m, 2H), 4.49 (s, 2H), 6.64 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.4, 148.3, 142.3, 130.3, 124.5, 122.9, 120.6, 105.1, 103.4, 74.9, 68.0, 62.0, 60.8, 58.5, 56.1, 33.8, 31.5, 13.3, 12.3, 9.6, 3.6. HRMS calcd for ($C_{27}H_{38}N_3O_4S$) [M + H]$^+$ 500.2578; found 500.2576. HPLC purity: 96.9%.

***N*-Butyl-3-[4-(ethoxymethyl)-2,6-dimethoxyphenyl]-6-methoxy-*N*-(tetrahydro-2*H*-pyran-4-yl)pyrazolo[5,1-*b*][1,3]-thiazol-7-amine bis(phosphate) (24).** To a solution of **6t** (3.0 g, 6.0 mmol) in EtOH (7 mL) was added 85% phosphoric acid (0.84 mL, 12.3 mmol) at 60 °C, and then the mixture was cooled to room temperature. To the mixture was added a seed crystal of **24**, followed by *n*-heptane (5 mL) over 10 min. An additional *n*-heptane (25 mL) was added, and then the mixture was stirred at room temperature for 3 h.

The precipitate was collected by suction filtration, washed with *n*-heptane (9 mL), and dried by heating at 40 °C under reduced pressure to afford **24** as a white solid (3.8 g, 90%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 0.82 (t, J = 7 Hz, 3H), 1.19 (t, J = 7 Hz, 3H), 1.23 (tt, J = 7, 7 Hz, 2H), 1.29 (tq, J = 7, 7 Hz, 2H), 1.34 (dddd, J = 4, 11, 11, 11 Hz, 1H), 1.70 (br d, J = 11 Hz, 2H), 2.91 (t, J = 7 Hz, 2H), 2.98 (tt, J = 4, 11 Hz, 1H), 3.25 (br dd, J = 11, 11 Hz, 2H), 3.56 (q, J = 7 Hz, 2H), 3.70 (s, 6H), 3.71 (s, 3H), 3.83 (br d, J = 11 Hz, 2H), 4.50 (s, 2H), 6.74 (s, 2H), 6.75 (s, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 162.4, 159.1, 143.1, 135.2, 127.8, 106.8, 106.4, 104.9, 103.5, 71.8, 66.5, 65.5, 59.8, 56.2, 56.1, 49.6, 31.2, 30.7, 19.8, 15.3, 14.0.

Biology. *Binding Assays.* HEK293 cells expressing human CRF_1 receptor were cloned using essentially the same method as that described in the literature.[5] CRF_1 receptor binding assay was performed using the homogeneous technique of scintillation proximity (SPA, Amersham Pharmacia, UK) with 96-well plates. Cell membrane (5 μg/well), wheat germ agglutinin-coated SPA beads (1 mg/well),

[^{125}I] human/rat CRF (0.1 nM), and diluted test compound solution were suspended in 150 μL of assay buffer (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 10 mM $MgCl_2$, 2 mM EGTA, 1.5% bovine serum albumin [BSA], pH 7.0). Total binding and nonspecific binding were measured in the absence and presence of 0.4 μM of unlabeled sauvagine, respectively. Plates were shaken gently and incubated for more than 2 h at room temperature. After plate centrifugation (260g, 5 min, room temperature), radioactivity was detected using TopCount (Packard, USA, MA) with a 1 min counting time per well. Each count was corrected by subtracting the value for nonspecific binding and was represented as a percentage of the total binding. The IC_{50} value of each compound was calculated using a concentration−response curve. The affinity for human $CRF_{2\alpha}$ receptors was evaluated using HEK293 cell membrane expressing human $CRF_{2\alpha}$ receptors, which were cloned as described in the literature.[33] The assay was conducted in similar fashion as the above-described CRF_1 receptor binding assay, except that cell membrane (5 μg/well) was incubated with [^{125}I] sauvagine (0.1 nM) instead of [^{125}I] human/rat CRF.

Functional Assay. To determine antagonistic activity, the effect of the test compound on CRF-stimulated intracellular cAMP accumulation was examined using IMR-32 cells, which is a human neuroblastoma cell line, as described in the literature[32] but with slight modification. cAMP production was measured using an enzyme immunoassay kit (Amersham Pharmacia, UK). IMR-32 cells (100000 cells/well) in MEM Earle's medium containing 10% fetal bovine serum were seeded in 96-well plates in the presence of 1 mM 3-isobutyl-1-methylxanthine, which is a phosphodiesterase inhibitor. After preincubation for 30 min, the diluted test compound was added and incubated for 30 min at 37 °C. Cells were stimulated with 1 nM of human/rat CRF for 30 min at 37 °C and collected by centrifugation (630g, 5 min, 4 °C). After aspiration of the medium, the cells were lysed with lysis buffer, and the amount of intracellular cAMP was measured according to the procedures detailed in the instruction manual of the kit. In each case, the basal amounts of cAMP (i.e., in the absence of CRF) were subtracted from the produced cAMP and were expressed as a percentage of the total amount produced. The IC_{50} value of the compound was calculated using a concentration−response curve.

Effects on CRF-Induced Defecation in Rats. Male Fischer 344 rats (Charles River Japan Inc. Kanagawa, Japan) weighing 192−223 g were used. Test compounds were orally administered to the nonfasted rats (n = 6) 1 h before the iv injection of CRF (10 μg/kg). Rats in the CRF control group were orally administered an equivalent volume of vehicle (dimethyl sulfoxide/cremophor/saline (5:5:90, v/v/v), 5 mL/kg; n = 6). Then 1 h after the po administration of tested compounds (10 mg/kg), the rats were anesthetized lightly with ether and CRF was injected intravenously via the lateral tail vein. The rats were placed in individual compartments of unit cages. The feces excreted by each rat were collected and weighed 4 h after injection of CRF.

Data are expressed as a percentage of the inhibition of stool output (g/4 h) of compound-treated groups compared to stool output of CRF control groups, using the mean ± standard error of the mean (SEM). The results were analyzed using an unpaired t test. A value of $p < 0.05$ (two-sided) was considered statistically significant.

Effects on the CRF-Induced Increase of Plasma ACTH Concentration in Rats. Male Fischer 344 rats (Charles River Japan Inc. Kanagawa, Japan) weighing 173−200 g were used. Test compounds (10, 30 mg/kg; n = 8) were orally administered to the rats 1 h before subcutaneous injection of CRF (10 μg/kg). The CRF control groups received an equivalent volume of vehicle (0.5% methyl cellulose, 5 mL/kg; n = 8). Then 30 min after the subcutaneous injection of CRF, blood samples obtained via the decapitated animals were stirred with 100 μL of EDTA-2Na (100 mg/mL) and kept on ice. The blood samples were centrifuged (1000g, 4 °C, 5 min), and plasma samples were prepared. Plasma ACTH concentrations were determined using an immunoradiometric assay kit (ACTH Irma Mitsubishi, Mitsubishi Chemical Medience). Radioactivity of the beads was measured using a scintillation counter (ARC-1000M, Aloka). The ACTH concentration was calculated from a standard curve that was prepared using the standard ACTH solution.

Data are expressed as the mean ± SEM. The differences between the CRF control and compound-treated groups were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. A value of $p < 0.05$ (two-sided) was considered statistically significant.

Light/Dark Test in Mice. Male BALB/c mice (Charles River Japan Inc., Kanagawa, Japan) weighing 18.9−25.6 g were used. Test compounds (10 and 30 mg/kg; n = 12) were orally administered to the mice 1 h before the test. A control group received an equivalent volume of vehicle (0.5% methyl cellulose, 10 mL/kg; n = 12). The test apparatus was a modified version of that described by Belzung et al.[37] It comprised two acrylic boxes, one of which was a black darkened box (dark box; 10 cm × 15 cm × 20 cm high) and the other was a white open-top box (light box; 20 cm × 15 cm × 20 cm high). A black acrylic tunnel (7 cm × 10 cm × 4.5 cm high) separated the dark box from the light box. To record the behavior of the animals, the front- and back sides of the light box (20 cm × 20 cm) were made of clear acrylic glass. The light intensity on the floor of the light box was fixed at 150 lx. At the beginning of the experiment, a mouse was placed in the dark box. Its behavior was recorded on videotape over a 5 min period, and the time spent in the light box was measured by an observer. A mouse whose four paws were in the light box was considered as being in the light box.

Data are expressed as the mean ± SEM. Differences between the vehicle control and the compound-treated groups were evaluated using one-way analysis of variance, followed by Dunnett's multiple comparison test. A value of $p < 0.05$ (two-sided) was considered statistically significant.

Determination of in Vitro Hepatic Clearance. The in vitro hepatic clearance data were obtained by measuring depletion in human liver microsomes. Pooled human liver microsomes (n = 150) were purchased from BD (MA, USA). A stability assay was conducted using 0.3 μmol/L substrate and 0.1 mg/mL microsomal protein in which the final concentration of organic solvent was 0.01% DMSO. A microsomal matrix contained 0.1 mmol/L EDTA, 100 mmol/L phosphate buffer (pH 7.4), an NADPH-generating system, liver microsomes, and substrates. The NADPH-generating system was prepared as a mixture containing 3.3 mmol/L β-NADP$^+$, 80 mmol/L G6P, 60 mmol/L $MgCl_2$ and 1 unit/mL G6PDH. The incubation was conducted at 37 °C for 0 and 15 min by adding the NADPH-generating system. After the incubation, the microsomal matrix was deproteinized by adding acetonitrile/methanol containing the internal standard. After centrifugation, the resulting supernatant was analyzed with LC/MS/MS.

Rat Pharmacokinetic Study. Pharmacokinetic parameters were estimated in fasted male Sprague−Dawley (SD) rats (Charles River Japan Inc., Kanagawa, Japan) after iv (3 mg/kg; n = 3) and po (10 mg/kg; n = 3) administration. The dosing solution for iv administration was prepared in 1% ethanol−0.1 mol/L HCl−glucose at a concentration of 3 mg/mL. The posing solutions for PO administration were prepared in 5% ethanol−0.1 mol/L HCl−-glucose at concentrations of 2 mg/mL. Blood samples were collected from the jugular vein at 0.083 (5 min; for IV), 0.25, 0.5, 1, 2, 4, 6, and 8 h after dosing (n = 3 at each time point). Plasma was separated by centrifugation and stored in a frozen state until analysis. Plasma was separated by centrifugation and stored frozen until analysis. Plasma concentrations were measured using the LC/MS/MS method. Plasma samples (100 μL) were deproteinized by adding 250 μL of acetonitrile containing the internal standard (imipramine). The samples were mixed and centrifuged. After centrifugation, the resulting supernatant (5 μL) was subjected to LC/MS/MS. Chromatography was performed in the reverse phase mode with a CAPCELL PAK C18 MGIII (2.0 mm i.d. × 150 mm, Shiseido Co. Ltd., Tokyo, Japan). Mobile phase A comprising distilled water containing 0.1% HCOOH and mobile phase B comprising acetonitrile containing 0.1% HCOOH were pumped at 0.5 mL/min, by using the linear gradient program [B%, 30% (0−1 min), 30−95% (1−3 min), 95% (3−5 min)]. Ionization was initiated using an electrospray (positive mode) along with monitoring of

parent−daughter peaks of 504.26 > 419.2 for 19ax and 281.1 > 86.1 for the internal standard.

Kinetic Solubility Assay. Water solubility was determined as follows: Sample solutions were prepared by dilution of 2.5 μL of 10 mM DMSO stock solution with 250 μL of Dulbecco's phosphate buffered saline in a 96-well filter plate. The plate was shaken for 15 min at room temperature to allow the compounds to equilibrate. The sample solutions were filtered into another 96-well plate by vacuum. Standard solutions were prepared by dilution of 2.5 μL of 10 mM stock DMSO solution with 250 μL of DMSO in a 96-well plate. The filtrated sample solutions and standard solutions were analyzed by HPLC to determine the solubility.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

CRF, corticotropin-releasing factor; hCLint, intrinsic clearance in human liver microsomes; THP, tetrahydropyranyl

REFERENCES

(1) Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* **1981**, *213*, 1394−1397.

(2) Owens, M. J.; Nemeroff, C. B. Physiology and Pharmacology of Corticotropin-Releasing Factor. *Pharmacol. Rev.* **1991**, *43*, 425−473.

(3) Spiess, J.; Dautzenberg, F. M.; Sydow, S.; Hauger, R. L.; Rühmann, A.; Blank, T.; Radulovic, J. Molecular Properties of the CRF Receptor. *Trends Endocrinol. Metab.* **1998**, *9*, 140−145.

(4) Kostich, W. A.; Chen, A.; Sperle, K.; Largent, B. L. Molecular Identification and Analysis of a Novel Human Corticotropin-Releasing Factor (CRF) Receptor: The $CRF_{2\gamma}$ Receptor. *Mol. Endocrinol.* **1998**, *12*, 1077−1085.

(5) Chen, R.; Lewis, K. A.; Perrin, M. H.; Vale, W. W. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 8967−8971.

(6) Perrin, M. H.; Grace, C. R.; Riek, R.; Vale, W. W. The Three-Dimensional Structure of the N-Terminal Domain of Corticotropin-Releasing Factor Receptors. Sushi Domains and the B1 Family of G Protein-Coupled Receptors. *Ann. N. Y. Acad. Sci.* **2006**, *1070*, 105−119.

(7) Smith, G. W.; Aubry, J.-M.; Dellu, F.; Contrarino, A.; Bilezikjian, L. M.; Gold, L. H.; Chen, R.; Marchuk, Y.; Hauser, C.; Bentley, C. A.; Sawchenko, P. E.; Koob, G. F.; Vale, W.; Lee, K.-F. Corticotropin Releasing Factor Receptor 1-Deficient Mice Display Decreased Anxiety, Impaired Stress Response, and Aberrant Neuroendocrine Development. *Neuron* **1998**, *20*, 1093−1102.

(8) Timpl, P.; Spanagel, R.; Sillaber, I.; Kresse, A.; Reul, J. M. H. M.; Stalla, G. K.; Blanquet, V.; Steckler, T.; Holsboer, F.; Wurst, W. Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nature Genet.* **1998**, *19*, 162−166.

(9) Dunn, A. J.; Berridge, C. W. Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? *Brain Res. Rev.* **1990**, *15*, 71−100.

(10) Nemeroff, C. B.; Widerlov, E.; Bissette, G.; Walleus, H.; Karlsson, I.; Eklund, K.; Kilts, C. D.; Loosen, P. T.; Vale, W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **1984**, *226*, 1342−1344.

(11) Arborelius, L.; Owens, M. J.; Plotsky, P. M.; Nemeroff, C. B. The role of corticotropin-releasing factor in depression and anxiety disorders. *J. Endocrinol.* **1999**, *160*, 1−12.

(12) Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. Synthesis and Oral Efficacy of a 4-(Butylethylamino)pyrrolo[2,3-*d*]pyrimidine: A Centrally Active Corticotropin-Releasing $Factor_1$ Receptor Antagonist. *J. Med. Chem.* **1997**, *40*, 1749−1754.

(13) Zorrilla, E. P.; Koob, G. F. Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discovery Today* **2010**, *15*, 371−383.

(14) Ising, M.; Zimmermann, U. S.; Künzel, H. E.; Uhr, M.; Foster, A. C.; Learned-Coughlin, S. M.; Holsboer, F.; Grigoriadis, D. E. High-Affinity CRF_1 Receptor Antagonist NBI-34041: Preclinical and Clinical Data Suggest Safety and Efficacy in Attenuating Elevated Stress Response. *Neuropsychopharmacology* **2007**, *32*, 1941−1949.

(15) Heinrichs, S. C.; De Souza, E. B.; Schulteis, G.; Lapsansky, J. L.; Grigoriadis, D. E. Brain Penetrance, Receptor Occupancy and Antistress In Vivo Efficacy of a Small Molecule Corticotropin Releasing Factor Type I Receptor Selective Antagonist. *Neuropsychopharmacology* **2002**, *27*, 194−202.

(16) Gutman, D. A.; Owens, M. J.; Skelton, K. H.; Thrivikraman, K. V.; Nemeroff, C. B. The Corticotropin-Releasing $Factor_1$ Receptor Antagonist R121919 Attenuates the Behavioral and Endocrine Responses to Stress. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 874−880.

(17) Mansbach, R. S.; Brooks, E. N.; Chen, Y. L. Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21−26.

(18) Arborelius, L.; Skelton, K. H.; Thrivikraman, K. V.; Plotsky, P. M.; Schulz, D. W.; Owens, M. J. Chronic Administration of the Selective Corticotropin-Releasing Factor 1 Receptor Antagonist CP-154,526: Behavioral, Endocrine and Neurochemical Effects in the Rat. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 588−597.

(19) Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; Schmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, F. D., III; Winston, E. N.; Chen, Y. L.; Heym, J. CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 10477−10482.

(20) Li, Y.-W.; Hill, G.; Wong, H.; Kelly, N.; Ward, K.; Pierdomenico, M.; Ren, S.; Gilligan, P.; Grossman, S.; Trainor, G.; Taub, R.; McElroy, J.; Zazcek, R. Receptor Occupancy of Nonpeptide Corticotropin-Releasing Factor 1 Antagonist DMP696: Correlation with Drug Exposure and Anxiolytic Efficacy. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 86−96.

(21) Chen, C.; Grigoriadis, D. E. NBI 30775 (R121919), An Orally Active Antagonist of the Corticotropin-Releasing Factor (CRF) Type-1 Receptor for the Treatment of Anxiety and Depression. *Drug Dev. Res.* **2005**, *65*, 216−226.

(22) Zobel, A. W.; Nickel, T.; Künzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatr. Res.* **2000**, *34*, 171−181.

(23) Binneman, B.; Feltner, D.; Kolluri, S.; Shi, Y.; Qiu, R.; Stiger, T. A 6-Week Randomized, Placebo-Controlled Trial of CP-316,311 (a Selective CRH_1 Antagonist) in the Treatment of Major Depression. *Amer. J. Psychiatry* **2008**, *165*, 617−620.

(24) Takahashi, Y.; Hibi, S.; Hoshino, Y.; Kikuchi, K.; Shin, K.; Murata-Tai, K.; Fujisawa, M.; Ino, M.; Shibata, H.; Yonaga, M. Synthesis and Structure−Activity Relationship of Pyrazolo[1,5-*a*]-pyridine Derivatives: Potent and Orally Active Antagonists of

Corticotropin-Releasing Factor 1 Receptor. *J. Med. Chem.* **2012**, *55*, 5255−5269.
(25) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. Corticotropin Releasing Factor (CRF) Receptor Modulators: Progress and Opportunities for New Therapeutic Agents. *J. Med. Chem.* **2000**, *43*, 1641−1660.
(26) The force field and DFT calculations were performed using the MACROMODEL and JAGUAR programs in Maestro 9.2 software package from Schrodinger. For the conformational search, OPLS2005 force field was used. Selected conformers generated from the force field were further optimized using the B3LYP hybrid density functional with the 6-31G(d, p) basis set.
(27) Koga, H.; Hirobe, M.; Okamoto, T. Syntheses of Novel Antimicrobial Compounds: Pyrazolo[5,1-*b*]thiazole, Imidazo[1,2-*b*]-pyrazole, and Thiazolo[3,2-*b*]dihydro-1,2-diazepine. *Chem. Pharm. Bull.* **1974**, *22*, 482−484.
(28) ClogP was calculated by software of Daylight Chemical Information Systems, Inc.
(29) Sugaya, Y.; Yoshiba, T.; Kajima, T.; Ishihama, Y. Development of Solubility Screening Methods in Drug Discovery. *Yakugaku Zasshi* **2002**, *122*, 237−246.
(30) Williams, C. L.; Peterson, J. M.; Villar, R. G.; Burks, T. F. Corticotropin-releasing factor directly mediates colonic responses to stress. *Am. J. Physiol.* **1987**, *253*, G582−G586.
(31) Saunders, P. R.; Maillot, C.; Million, M.; Tache, Y. Peripheral corticotropin-releasing factor induces diarrhea in rats: role of CRF_1 receptor in fecal watery excretion. *Eur. J. Pharmacol.* **2002**, *435*, 231−235.
(32) Dieterich, K. D.; De Souza, E. B. Functional corticotropin-releasing factor receptors in human neuroblastoma cells. *Brain Res.* **1996**, *733*, 113−118.
(33) Liaw, C. W.; Lovenberg, T. W.; Barry, G.; Oltersdorf, T.; Grigoriadis, D. E.; de Souza, E. B. Cloning and characterization of the human corticotropin-releasing factor-2 receptor complementary deoxyribonucleic acid. *Endocrinology* **1996**, *137*, 72−77.
(34) Rivier, C.; Brownstein, M.; Spiess, J.; Rivier, J.; Vale, W. In Vivo Corticotropin-Releasing Factor-Induced Secretion of Adrenocorticotropin, β-Endorphin, and Corticosterone. *Endocrinology* **1982**, *110*, 272−278.
(35) Pelleymounter, M. A.; Joppa, M.; Ling, N.; Foster, A. C. Pharmacological Evidence Supporting a Role for Central Corticotropin-Releasing $Factor_2$ Receptors in Behavioral, but not Endocrine, Response to Environmental Stress. *J. Pharmacol. Exp. Ther.* **2002**, *302*, 145−152.
(36) Belzung, C.; Misslin, R.; Vogel, E. Behavioural effects of the benzodiazepine receptor partial agonist RO 16−6028 in mice. *Psychopharmacology* **1989**, *97*, 388−391.

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