Journal of Medicinal Chemistry

Article

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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.5b01715 • Publication Date (Web): 22 Feb 2016

Design and synthesis of benzimidazoles as novel corticotropin-releasing factor 1 receptor antagonists

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Abstract

Benzazole derivatives with a flexible aryl group bonded through a one-atom linker as a new scaffold for a corticotropin-releasing factor 1 (CRF_1) receptor antagonist were designed, synthesized and evaluated. We expected that structural diversity could be expanded beyond that of reported CRF_1 receptor antagonists. In a structure–activity relationship study, 4-chloro-N^2-(4-chloro-2-methoxy-6-methylphenyl)-1-methyl-N^7,N^7-dipropyl-1*H*-benzimidazole-2,7-diamine **29g** had the most potent binding activity against a human CRF_1 receptor and the antagonistic activity (IC_{50} = 9.5 nM and 88 nM, respectively) without concerns regarding cytotoxicity at 30 μM. Potent CRF_1 receptor-binding activity in brain in an ex vivo test and supression of stress-induced activation of the hypothalamus–pituitary–adrenocortical (HPA) axis were also observed

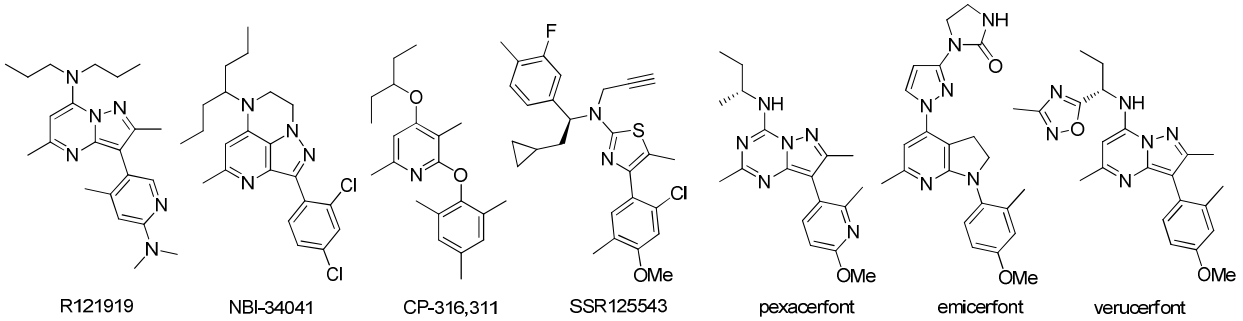
at 138 μmol/kg of compound **29g** after oral administration in mice. Thus, the newly designed benzimidazole **29g** showed in vivo CRF_1 receptor antagonistic activity and good brain penetration, indicating that it is a promising lead for CRF_1 receptor antagonist drug discovery research.

Introduction

Corticotropin-releasing factor (CRF) is a 41-amino-acid neuropeptide that mediates its actions through two G_s-coupled G protein-coupled receptor subtypes, CRF_1 and CRF_2.[1,2] CRF is believed to be the main regulator of the hypothalamus–pituitary–adrenocortical (HPA) axis via CRF_1 receptors and has an important role as a neurotransmitter in the mediation of stress-related behaviors.[3,4] After exposure to stress, secretion of CRF increases in the neurons of the paraventricular nucleus of the hypothalamus and stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland.[5,6] ACTH subsequently induces the secretion of cortisol from adrenal glands. In a healthy individual, cortisol stimulates and controls the hypothalamic secretion of CRF, which indicates that a negative feedback system against the activation of the HPA axis is operating. On the other hand, the negative feedback system collapses in patients with stress-related disorders. CRF also activates CRF_1 receptors in the brain, and such activation appears to be directly related to disease symptoms. In fact, mice lacking CRF_1 receptors exhibit decreased anxiety-like behavior and impaired stress responses, and neutralization of CRF_1 receptor expression by central administration of CRF antisense oligonucleotides attenuates stress responses in rats.[7] Moreover, intracerebroventricular (icv) infusion of CRF induces anxiety-like behavior, but behavioral responses to CRF or to a stressor can be ameliorated by CRF_1 receptor antagonists.[7]

Over the last 20 years, numerous non-peptides and small-molecule CRF_1 receptor antagonists have been reported to demonstrate their effectiveness in animal models for stress-related disorders. However, no drugs have been launched. The structures in clinical trials in the past were exhibited in Figure 1. In fact, **1a** (R121919)[8, 9] significantly decreased Hamilton Depression and Anxiety Rating Scale (HAM-D and HAM-A) scores after 10 days of oral administration in patients with major depression in an open-label, small-scale, phase IIa clinical trial.[8] Another trial of **1a** showed liver enzyme elevation,[9] which has been reported to depend on the structure of **1a** but not on the mechanism of action (CRF_1 antagonism).[9] Subchronic treatment with 2-(2,4-dichlorophenyl)-6-(heptan-4-yl)-4-methyl-7,8-dihydro-6*H*-1,3,6,8a-tetraazaacenaphthylene (NBI-34041)[10] also attenuated the neuroendocrine response to psychosocial stress in a placebo-controlled clinical study.[10] The results of both compounds suggest that non-peptide CRF_1 receptor antagonists could be useful therapeutics in the treatment of stress-related disorders such as depression and anxiety. However, **1b** (CP-316,311)[11], 4-(2-chloro-4-methoxy-5-methylphenyl)-*N*-((1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl)-5-methyl-*N*-(prop-2-yn-1-yl)-1,3-thiazol-2-amine(SSR125543)[12], and **1c** (verucerfont) failed to demonstrate efficacy in the treatment of major depression.[11–13] Pexacerfont[14,15] and emicerfont[16] also failed in clinical trials for major depression, general anxiety disorder, and irritable bowel syndrome. On the other hand, a clinical trial of **1c** has been conducted by an Emory University group[17] for post-traumatic stress disorder, and the National Institute on Alcohol Abuse and Alcoholism[18] plans to conduct a trial for alcohol dependence. In addition, a CRF_1 receptor antagonist is considered to be a relevant target for other stress-related diseases, such as pain and anorexia,[19,20] resulting from elevated CRF levels. Therefore, CRF_1 receptor antagonists are an attractive drug discovery target for the treatment of stress-related diseases.

Figure 1. Reported CRF_1 receptor antagonists.



A variety of typical non-peptide CRF_1 receptor antagonists reported by many companies have common structural features, as shown in **I** in Figure 2.[1,2,21] Generally, A *mono*-, *di*-, or *tri*-cyclic hetero aromatic core containing a ring A with an sp^2 basic nitrogen as a hydrogen-bonding acceptor (HBA) is substituted with a pendant aromatic group (Ar) at the bottom that has an orthogonal relationship to the core. The heteroaromatic core also has alkyl moieties, R^x, R^y, and R^z. R^x and R^y are located at the top region of the core and appear to fit into a large lipophilic pocket of a CRF_1 receptor, and R^z is a small alkyl group adjacent to the sp^2 nitrogen. Numerous reported CRF_1 receptor antagonists can be classified into two subgroups: one in which the pendant aryl group (Ar) directly bonds to the heteroaromatic core and the other in which the aryl group bonds through a one-atom linker Z without a ring B, such as **1b**.[22] Historically, CRF_1 receptor antagonists have been associated with less optimal physicochemical drug properties, and it has been assumed that these properties are a major obstacle in developing CRF_1 receptor antagonists in the clinic.[2] We focused on designing the latter type of scaffolds with a more flexible Ar group, with the expectation that the resulting improved physicochemical properties, such as solubility, would provide better biological profiles[23,24] than those of the former CRF_1

receptor antagonist subgroup. We designed a 6-5 fused type of scaffold with ring C rather than ring D or E, which was unique among the numerous reported CRF_1 receptor antagonists. Requisite lipophilic groups, such as R^x and R^y, were placed on ring C. Identification of a new type of scaffold would allow a wider design space range for future CRF_1 antagonists. Cytotoxicity was also closely monitored during our discovery efforts because **1a** showed both liver enzyme elevation in the clinical trial described above and cytotoxicity (*vide infra*). Although it is unclear if the liver enzyme elevation by **1a** was due to cytotoxicity, identification of a new scaffold with reduced cytotoxicity is one of the goals of our continuing research.

The unique structure **II** has been never reported as a CRF_1 receptor antagonist, and we proposed new benzoxazole, benzothiazole, and benzimidazole central cores. Flexible alignment of 7-dipropylaminobenzoxazole analog **5** to a typical type of reported CRF_1 receptor antagonist **1a** was performed using MOE.[25] It was found that the key functional groups, an HBA, a dialkylamino group, and pendant aryl groups, of benzoxazole analog **5** overlapped well with the corresponding key groups of **1a**, as illustrated in Figure 3. This superimposition study suggested that the new compound designs should exhibit potent CRF_1 receptor antagonism.

Figure 2. Design of a benzazole core for a novel type of CRF_1 receptor antagonists.

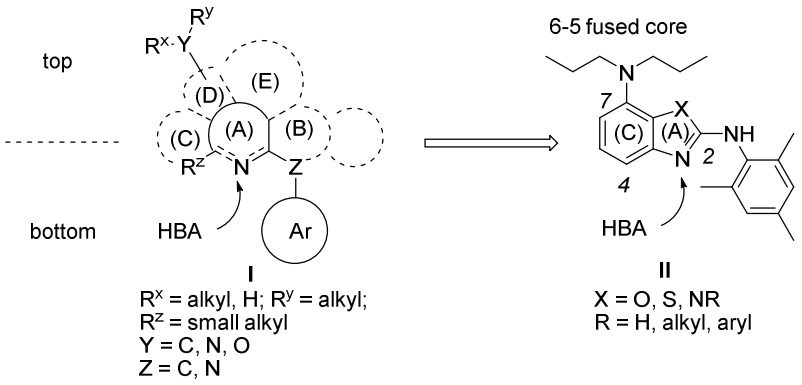
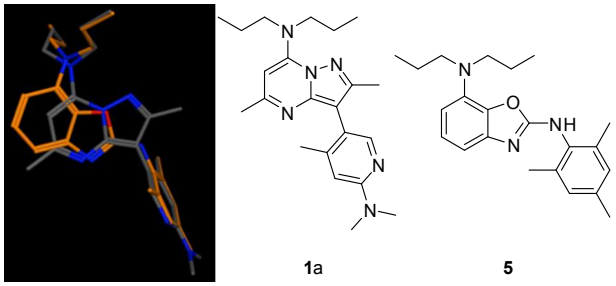


Figure 3. Superimposition of **1a** (gray) on benzoxazole analog **5** (orange).

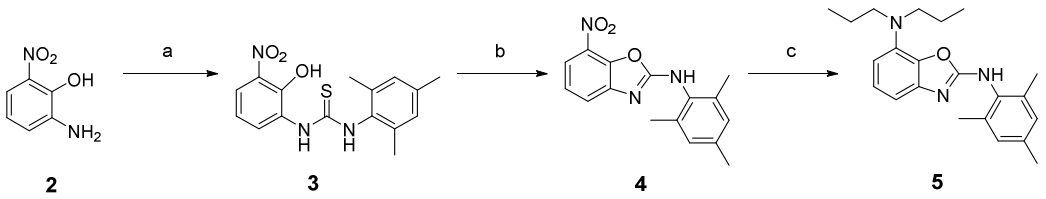


In this report, we describe the synthesis and structure–activity relationships (SARs) as well as the biological activities of a novel series of benzazoles as CRF_1 receptor antagonists.

Results and Discussion

Chemistry. Syntheses of the 7-alkylamino-2-anilinobenzazole series are illustrated in Schemes 1–5. A benzoxazole derivative **5** was synthesized according to Scheme 1. Thiourea **3** was prepared from commercially available aminophenol **2** and (2,4,6-trimethylphenyl)isothiocyanate. Cyclization of thiourea **3** with mercury(II) chloride provided 7-nitrobenzoxazole **4**, and subsequent hydrogenation and reductive alkylation with propionadehyde afforded the target compound **5**.

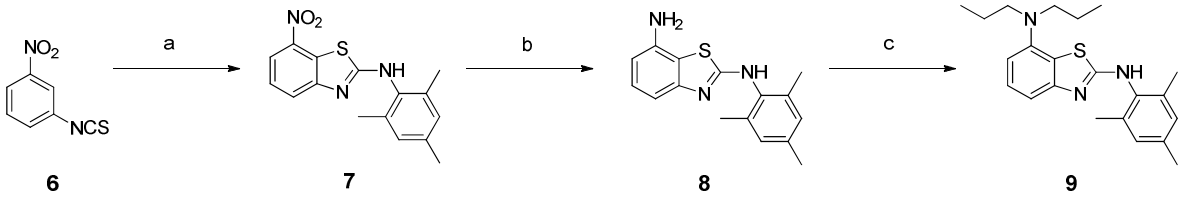
Scheme 1. Synthesis of a benzoxazole analog **5**[a]



[a] Reagents and conditions: (a) (2,4,6-trimethylphenyl)isothiocyanate, Na_2CO_3, EtOH, reflux, 80%; (b) $HgCl_2$, MeCN, rt, 90%; (c) (i) H_2, Pd/C, MeOH, rt; (ii) EtCHO, $NaBH_3CN$, AcOH, rt, 90%.

A benzothiazole analog **9** was synthesized from (3-nitrophenyl)isothiocyanate **6**. Condensation of compound **6** and mesitylamine was followed by cyclization with bromine to give 7-nitrobenzothiazole **7**. After reduction of the nitro group of **7** with iron to give aminobenzothiazole **8**, reductive alkylation of **8** afforded the target benzothiazole **9** (Scheme 2).

Scheme 2. Synthesis of a benzothiazole analog **9**[a]

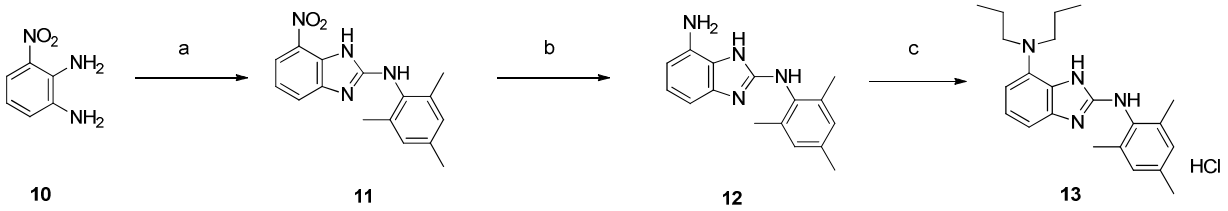


[a] Reagents and conditions: (a) (i) mesitylamine, MeOH, rt; (ii) Br_2, AcOH, reflux, 11%; (b) Fe, AcOH, EtOH, reflux, 55%; (c) EtCHO, $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, 50°C, 10%.

The synthetic route of the benzimidazole analog **13** is similar to that of benzoxazole **5** and benzothiazole **9** described in Schemes 1 and 2, respectively (Scheme 3). 3-Nitro-1,2-

phenylenediamine **10** was treated with (2,4,6-trimethylphenyl)isothiocyanate and subsequent DIC to give 7-nitro benzimidazole **11**. The nitro group of **11** was hydrogenated with a palladium catalyst, followed by reductive alkylation to give the target compound **13**.

Scheme 3. Synthesis of a benzimidazole analog **13**[a]



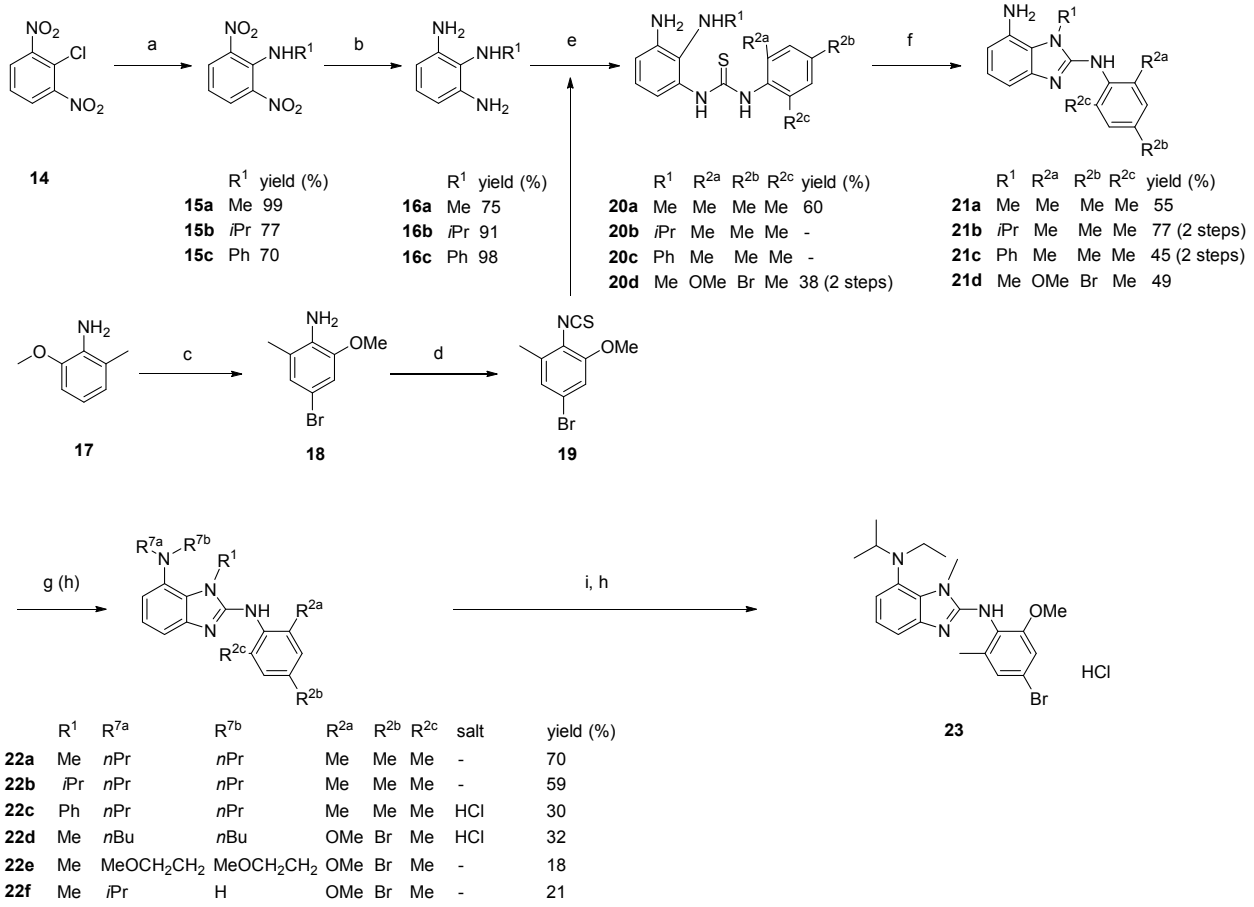
10 11 12 13

[a] Reagents and conditions: (a) (i) (2,4,6-trimethylphenyl)isothiocyanate, Na_2CO_3, EtOH, reflux; (ii) DIC, reflux, 54%; (b) H_2, Pd-C, MeOH, rt, 86%; (c) (i) EtCHO, $MPBH_3CN$, AcOH, MeOH, rt; (ii) conversion to HCl salt, 16%.

The synthesis of 7-dialkylaminobenzimidazoles with a substituent at the 1-position, **22a–e** and **23**, is described in Scheme 4. The intermediates **21a**–**d** were prepared from commercially available 2-chloro-1,3-dinitrobenzene **14** by substitution of the chloro group with a corresponding amine, hydrogenation, condensation of the corresponding isothiocyanate, and cyclization, respectively, in good yields. Reductive alkylation of the intermediates **21a**–**d** with propionaldehyde or n-butylaldehyde was performed to give the 7-dipropyl or 7-dibutylaminobenzimidazoles **22a**–**d**. The 7-isopropylamino analog **22f** was also prepared by reductive alkylation of **21d** with acetone. Preparation of methoxyethylamino analog **22e** was performed by reductive alkylation of **21d** with methoxyacetaldehyde prepared from 1,1,2-trimethoxyethane in the presence of iron(III) chloride. The ethyl(isopropyl)amino analog **23** was

obtained by additional reductive alkylation of **22f** with acetaldehyde. The isothiocyanate **19** for reaction with triamine **16a** was prepared by bromination of aniline **17** and subsequent treatment with carbon disulfide to convert the amino group of (4-bromo-2-methoxy-6-methyl)aniline **18** to isothiocyanate.

Scheme 4. Synthesis of benzimidazole analogs with various substituents at the 1- and 7-positions, **22a**–**e** and **23**[a]



14

	R^1	yield (%)
15a	Me	99
15b	*i*Pr	77
15c	Ph	70

	R^1	yield (%)
16a	Me	75
16b	*i*Pr	91
16c	Ph	98

	R^1	R^{2a}	R^{2b}	R^{2c}	yield (%)
20a	Me	Me	Me	Me	60
20b	*i*Pr	Me	Me	Me	-
20c	Ph	Me	Me	Me	-
20d	Me	OMe	Br	Me	38 (2 steps)

	R^1	R^{2a}	R^{2b}	R^{2c}	yield (%)
21a	Me	Me	Me	Me	55
21b	*i*Pr	Me	Me	Me	77 (2 steps)
21c	Ph	Me	Me	Me	45 (2 steps)
21d	Me	OMe	Br	Me	49

17 **18** **19**

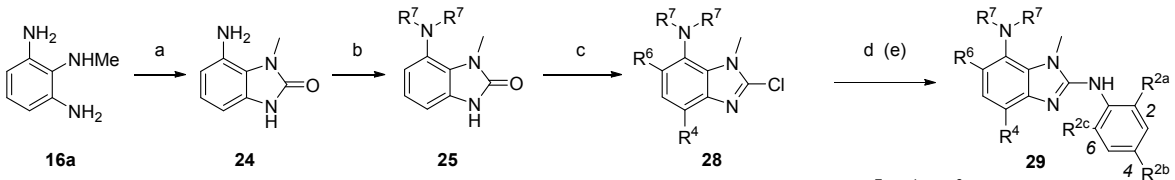
	R^1	R^{7a}	R^{7b}	R^{2a}	R^{2b}	R^{2c}	salt	yield (%)
22a	Me	*n*Pr	*n*Pr	Me	Me	Me	-	70
22b	*i*Pr	*n*Pr	*n*Pr	Me	Me	Me	-	59
22c	Ph	*n*Pr	*n*Pr	Me	Me	Me	HCl	30
22d	Me	*n*Bu	*n*Bu	OMe	Br	Me	HCl	32
22e	Me	$MeOCH_2CH_2$	$MeOCH_2CH_2$	OMe	Br	Me	-	18
22f	Me	*i*Pr	H	OMe	Br	Me	-	21

23

[a] Reagents and conditions: (a) R^1NH_2, MeOH or THF, rt or reflux; (b) H_2 or cyclohexene, Pd-C, MeOH, rt or reflux; (c) Br_2, AcOH, MeOH, rt, 53%; (d) CS_2, Et_3N, DCC, pyridine, −10°C, >99%; (e) (2,4,6-trimethylphenyl)isothiocyanate or (4-bromo-2-methoxy-6-methylphenyl)isothiocyanate **19**, (Na_2CO_3), EtOH or MeOH, reflux; (f) $HgCl_2$, (Et_3N), MeCN, rt or DIC, EtOH reflux; (then, $PSBH_3CN$, MeOH, rt); (g) EtCHO or *n*-PrCHO or acetone, $NaBH_3CN$ or $NaBH(OAc)_3$ or $MPBH_3CN$, (AcOH), MeOH or CH_2Cl_2, rt to 55°C; or $MeOCH_2CH(OMe)_2$, $FeCl_3$, $MPBH_3CN$, AcOH, MeOH, rt; (h) 2N HCl in Et_2O; (i) acetaldehyde, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, 50°C, 98%.

Syntheses of compounds with and without various substituents at the 2- and 4-positions **29a**–**o** are described in Scheme 5. An alternative route without using phenyl thiourea was effective for introducing 2-anilino groups at the final step. The benzimidazole core **24** was constructed by condensation of CDI and triamine **16a** in good yield. The common intermediates **25a** and **25b** were obtained by reductive alkylation of the prepared 7-aminobenzimidazol-2-one **24**. Target compounds without substituents at the 4-position **29a**–**f** were provided by chlorination of **25a** or **25b** using phosphorus oxychloride and subsequent treatment with various anilines. Chlorination of **25a** was performed using NCS to afford a mixture of 4-chloro (**26a**) and 4,6-dichloro (**26b**) derivatives and a single isomer of 6-chloro analog **26c**. 4-Chloro (**29g**), 4,6-dichloro (**29h**), and 6-chloro (**29i**) were obtained from **26a**–**c** via 2-chlorobenzimidazoles **28c–e** using a method similar to those used for the non-substituted targets **29a**–**f**. The next two steps from the mixture of 4-chloro (**26a**) and 4,6-dichlorobenzimidazol-2-one (**26b**) derivatives were carried out via compounds **28c** and **28d**, and the targets **29g** and **29h,** respectively, were separated by silica gel column chromatography in the final step. On the other hand, 4-chloro-7-diethylaminobenzimidazol-2-one **26d** was isolated as a single isomer after chlorination of the diethylamino derivative **25b** and converted to the target compound **29k** using methods similar to those described above. Bromination of **25a** and **25b** and subsequent substitution of the bromo group with a cyano group using copper cyanide afforded the 4-cyanobenzimidazol-2-ones **27a** and **27b**. The 4-methyl **27c** and 4-phenyl **27d** derivatives were synthesized using a coupling reaction of the 4-bromo analog **26f** with a corresponding tin reagent. A coupling reaction of the 4-bromo analog **26f** with sodium methoxide in the presence of copper iodide also afforded 4-methoxybenzimidazol-2-one **27e**. The targets **29j** and **29l-o** were obtained using methods similar to those described above.

Scheme 5. Synthesis of benzimidazole analogs with various substituents at the 4- and 6-positions, **29a–o**[a]



	R^7	yield (%)
25a	*n*Pr	73
25b	Et	91

	R^7	R^4	R^6	yield (%)
28a	*n*Pr	H	H	69
28b	Et	H	H	47
28c	*n*Pr	Cl	H	-
28d	*n*Pr	Cl	Cl	-
28e	*n*Pr	H	Cl	34
28f	*n*Pr	CN	H	62
28g	Et	Cl	H	>99
28h	Et	CN	H	43
28i	Et	Me	H	-
28j	Et	Ph	H	67 (2 steps)
28k	Et	OMe	H	98

	R^7	R^4	R^6	yield (%)
26a	*n*Pr	Cl	H	-
26b	*n*Pr	Cl	Cl	-
26c	*n*Pr	H	Cl	33
26d	Et	Cl	H	28
26e	*n*Pr	Br	H	33
26f	Et	Br	H	67

	R^7	R^4	yield (%)
27a	*n*Pr	CN	75
27b	Et	CN	82
27c	Et	Me	51
27d	Et	Ph	-
27e	Et	OMe	74

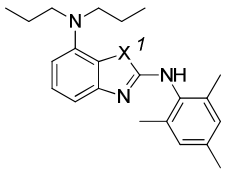
	R^7	R^4	R^6	R^{2a}	R^{2b}	R^{2c}	salt	yield (%)
29a	*n*Pr	H	H	Me	Me	H	TFA	60
29b	*n*Pr	H	H	Me	H	Me	TFA	45
29c	*n*Pr	H	H	OMe	Cl	Me	-	9.9
29d	*n*Pr	H	H	OMe	Br	Me	HCl	42
29e	*n*Pr	H	H	*i*Pr	Cl	Me	HCl	21
29f	Et	H	H	OMe	Br	Me	HCl	35
29g	*n*Pr	Cl	H	OMe	Cl	Me	-	7 (3steps)
29h	*n*Pr	Cl	Cl	OMe	Cl	Me	-	1.8 (3 steps)
29i	*n*Pr	H	Cl	OMe	Cl	Me	-	31
29j	*n*Pr	CN	H	OMe	Cl	Me	-	4.3
29k	Et	Cl	H	OMe	Cl	Me	-	52
29l	Et	CN	H	OMe	Cl	Me	-	48
29m	Et	Me	H	OMe	Cl	Me	-	36 (2 steps)
29n	Et	Ph	H	OMe	Cl	Me	-	57
29o	Et	OMe	H	OMe	Cl	Me	-	45

[a] Reagents and conditions: (a) CDI, THF, rt, 81%; (b) EtCHO or MeCHO, $NaBH_3CN$ or $NaBH(OAc)_3$, AcOH, MeOH or CH_2Cl_2, rt; (c) $POCl_3$, ($C_6H_5NMe_2$), 100°C–120°C; (d) $ArNH_2$, (NMP), 70°C–130°C; (e) conversion to HCl salt; (f) NCS or NBS, (AIBN,) CCl_4 or MeCN, rt to reflux; (g) CuCN, NMP, 180°C or 170°C, microwave; or Me_4Sn or Ph_4Sn, $Pd(PPh_3)_4$, HMPA, reflux; or NaOMe, CuI, MeOH, DMF, 100°C.

Biology. The synthesized compounds **5**, **9**, **13**, **22a**–**e**, **23,** and **29a**–**o** were screened for their inhibitory activity against ovine ^{125}I-CRF binding to human CRF_1 receptors expressed on Chinese hamster ovary (CHO) cellular membranes, and cytotoxicity was indicated by the ATP content in HepG2 cells. Compound **1a** was selected as a reference compound because it was used in clinical and the results were reported. Initial efforts were focused on the investigation of core scaffolds. The results of the benzazole series are listed in Table 1. Benzoxazole **5** showed submicromolar CRF_1 receptor-binding inhibition activity as expected by superimposition with a reported CRF_1 receptor antagonist **1** as described in Figure 3. Benzothiazole **9** also exhibited activity with an IC_{50} value of 77 nM, whereas benzimidazole **13** showed 30-fold less potent activity than benzothiazole **9**. These results suggested that hetero atoms at the 1-position of the core affected CRF_1 receptor-binding activity. In fact, flexible alignment of three compounds (**5**, **9,** and **13**) with MOE[25] indicated that the sulfur atom of benzothiazole **9** was possibly out of alignment with the oxygen and nitrogen atoms in the benzoxazole (**5**) and benzimidazole (**13**) rings, respectively (Figure 4). Therefore, introduction of a substituent on the 1-*N*-position of the benzimidazole core should be effective for occupying the space of a CRF_1 receptor surrounding 1-position. As expected, *N*-methylbenzimidazole **22a** markedly improved the binding activity, with an IC_{50} value of 15 nM, which was as potent as that of **1a**. Compound **22a** also showed less cytotoxicity than benzoxazole **5**, benzothiazole **9**, de-methyl benzimidazole **13,** and **1a**. Thus, we selected *N*-substituted benzimidazoles for research as pharmacophores of a 6-5 fused scaffold with a one-atom linker Z between the core and pendant aryl group. The analogs **22b** and **22c** with a bulkier group at the 1-*N*-position (isopropyl and phenyl, respectively), resulted in considerable reduction of the binding activity relative to that of the *N*-methyl analog **22a** as well

as severe cytotoxicity at 30 μM. The isopropyl and phenyl groups appeared to be too bulky to accommodate a CRF_1 receptor and too lipophilic to exhibit low cytotoxicity for this series. The compounds **22a**, **22b,** and **22c** had log D values[26] at pH 7.4 of 5.23, 5.90, and 6.31, respectively.

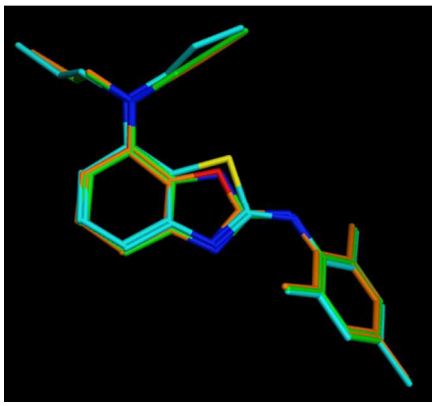
Table 1. $hCRF_1$ receptor-binding activities and cytotoxicity of the benzazole derivatives



Compound No.	X	Salt	Binding (IC_{50}, nM)[a]	Cytotoxicity (%@30 μM)[b]
5	O	-	990 (710–1400)	65
9	S	-	77 (61–98)	75
13	NH	HCl	2500 (1500–4200)	41
22a	NMe	-	15 (11–21)	89
22b	N*i*-Pr	-	97 (77–120)	10
22c	NPh	HCl	2200 (1500–3200)	32
1a	-	-	8.5 (5.7–13)	51

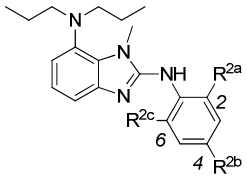
[a] IC_{50} values and 95% confidential intervals were calculated from the concentration–response curves (n = 1). [b] The values are rates of ATP content relative to that for 100% with only DMSO and no compound (n = 3).

Figure 4. Superimposition of **5** (orange), **9** (aqua), and **13** (green).



Anilino groups of the 1-*N*-methylbenzimidazole series were investigated (Table 2). Disubstituted phenyl analogs at the 2,4- and 2,6-positions (**29a** and **29b**, respectively) showed reduced CRF_1 receptor-binding activity relative to that of the 2,4,6-trisubstituted lead compound **22a**. As a result of the binding assay for various anilino groups and in consideration of diversity, a 4-chloro-2-methoxy-6-methylphenyl group in **29c** was found to bring activity equal to that of the trimethylphenyl group in **22a**. Replacement of the 4-chloro group of **29c** with a bromo group (**29d**) maintained potent activity. A bulkier isopropyl analog **29e** was also as potent as a 2-methoxy analog **29c**, suggesting that a branched substituent is acceptable at the ortho-position of a CRF_1 receptor. Unfortunately, the cytotoxicity of the isopropyl analog **29e** was worse than that of the methoxy analog **29d**. The lipophilicity of compound **29e** was higher than that of **29d**, with logDs at pH 7.4 of 5.96 and 5.39, respectively.

Table 2. Effects of anilino groups at the 2-position of 1-*N*-methylbenzimidazole derivatives on $hCRF_1$ receptor-binding activity and cytotoxicity

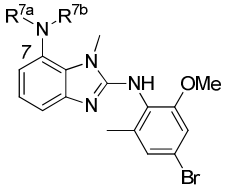


Compound No.	R^{2a}	R^{2b}	R^{2c}	Salt	Binding (IC_{50}, nM)[a]	Cytotoxicity (%@30 μM)[b]
22a	Me	Me	Me	-	15 (11–21)	89
29a	Me	Me	H	TFA	84 (67–100)	79
29b	Me	H	Me	TFA	140 (100–200)	72
29c	OMe	Cl	Me	-	14 (10–19)	ND[c]
29d	OMe	Br	Me	HCl	12 (7.7–17)	82
29e	*i*-Pr	Cl	Me	HCl	22 (16–31)	28
1a	-	-	-	-	8.5 (5.7–13)	51

[a] IC_{50} values and 95% confidential intervals are calculated from the concentration–response curves (n =1). [b] The values are rates of ATP content relative to that for 100% with only DMSO and no compound (n = 3).

The effects on conversion of dialkylamino groups at the 7-position are summarized in Table 3. The diethylamino (**29f**) and dibutylamino (**22d**) analogs were as potent as the dipropylamino analog **29d**. Furthermore, the (isopropyl)ethylamino analog **23** also had potent activity. For CRF_1 receptor-binding activity, these results suggested that a CRF_1 receptor loosely recognizes their length and size of the alkyl groups at this position On the other hand, conversion of the butyl group of **22d** to a methoxyethyl group (**22e**) was found to reduce activity, which indicated that oxygen at this position was less preferable for a CRF_1 receptor because of the lower lipophilicity. These results suggested that this site of CRF_1 receptors is lipophilic and the alkyl groups R^{7a} and R^{7b} are required for lipophilicity, as reported in previous SAR studies.[27] These structural changes in Table 3 had no effect on cytotoxicity.

Table 3. Effects of dialkylamino groups at the 7-position of 1-*N*-methylbenzimidazole derivatives on $hCRF_1$ receptor-binding activity and cytotoxicity



Compound No.	R^{7a}	R^{7b}	Salt	Binding (IC_{50}, nM)[a]	Cytotoxicity (%@30 μM)[b]
29d	*n*-Pr	*n*-Pr	HCl	12 (7.7–17)	82
29f	Et	Et	HCl	13 (11–17)	93
22d	*n*-Bu	*n*-Bu	HCl	14 (11–19)	90

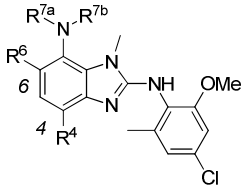
23	*i*-Pr	Et	HCl	11 (8.4–15)	87
22e	$MeOCH_2CH_2$	$MeOCH_2CH_2$	-	65 (53–79)	92
1a	-	-	-	8.5 (5.7–13)	51

[a] IC_{50} values and 95% confidential intervals are calculated from the concentration–response curves (n = 1). [b] The values are rates of ATP content relative to that for 100% with only DMSO and no compound (n = 3).

Substituents at the 4- and 6-positions of benzimidazole were investigated (Table 4). Introduction of a chloro group at the 4-position maintained the binding activity similar to that of **29g**. The 4,6-dichloro analog **29h** showed diminished activity, and the 6-chloro analog **29i** was inactive at 10 μM. Based on these results, we considered that a substituent at the 6-position of the benzimidazole core prevented good binding to the pocket of a CRF_1 receptor. The area of the 6-position of the core is outside the **1a** molecule (**1**), as shown in Figure 3. In addition, introduction of a chloro group at the 6-position may change the conformation of alkyl groups at the 7-position. SAR studies that led to **1b** have been reported to give similar results.[23] Replacement of a chloro group at the 4-position in **29g** with a more electron-withdrawing cyano group (**29j**) maintained CRF_1 receptor-binding activity. The methyl (**29m**) and methoxy (**29o**) analogs demonstrated activity comparable to that of the cyano analog **29l**; however, the phenyl analog **29n** was much less potent than was **29m**. On the other hand, the methoxy analog **29o** was shown to be highly cytotoxic. These results indicated that both electron-withdrawing and electron-donating properties did not greatly influence the binding activity but had a significant

effect upon cytotoxicity[28,29] and that a large substituent at the 4-position reduced CRF_1 receptor-binding affinity.

Table 4. $hCRF_1$ receptor-binding activities and cytotoxicity of 1-*N*-methylbenzimidazole derivatives substituted at the 4 and 6 positions



Compound No.	R^{7a}	R^{7b}	R^4	R^6	Binding (IC_{50}, nM)[a]	Cytotoxicity (%@30 μM)[b]
29c	*n*-Pr	*n*-Pr	H	H	14 (10–19)	ND[c]
29g	*n*-Pr	*n*-Pr	Cl	H	9.5 (6.0–15)	83
29h	*n*-Pr	*n*-Pr	Cl	Cl	170 (110–280)	72
29i	*n*-Pr	*n*-Pr	H	Cl	>10000	86
29j	*n*-Pr	*n*-Pr	CN	H	14 (11–18)	83
29k	Et	Et	Cl	H	24 (20–30)	76
29l	Et	Et	CN	H	13 (8.4–19)	81
29 m	Et	Et	Me	H	7.6 (5.4–11)	72
29n	Et	Et	Ph	H	>10000	92

29o	Et	Et	OMe	H	13 (8.3–20)	5
1a	-	-	-	-	8.5 (5.7–13)	51

[a] IC_{50} values and 95% confidential intervals are calculated from the concentration–response curves (n = 1). [b] The values are rates of ATP content relative to that for 100% with only DMSO and no compound (n = 3). [c] ND: no data.

The SAR study showed that this new type of benzazole series had potent CRF_1 receptor-binding activity and the pharmacophore was similar to that of reported CRF_1 receptor antagonists. Removal of ring B and addition of the flexible aryl group provided analogs with acceptable binding activity when a methyl group at the 1-position of the benzimidazole core was introduced. It was also found that the binding activity was undisturbed by incorporation of ring C, the benzene ring of the benzimidazole core. Compound **29g** exhibited potent CRF_1 receptor-binding inhibition activity and no cytotoxicity issues at 30 μM and was therefore selected for further evaluation.

Compound **29g** inhibited human CRF-stimulated cAMP accumulation in CHO cells expressing human CRF_1 receptors, with an IC_{50} value of 88 nM (95% confidential interval : 39–198 nM). In addition, no binding of compound **29g** to human $CRF_{2\alpha}$ and $CRF_{2\beta}$ was observed up to 10 μM (data not shown). This compound showed acceptable physicochemical properties, such as solubility for oral administration (33 μg/ml with bile acid in pH 6.8 phosphate buffer). Therefore, brain penetration and CRF_1 receptor-binding activity of compound **29g** after oral administration were also evaluated by ex vivo testing in mice (Table 5). Compound **29g** almost entirely inhibited the ^{125}I-CRF binding in the frontal cortex, olfactory bulb, and pituitary gland in mice

after 1 h of oral administration at 138 μmol/kg (60 mg/kg). This result indicated that compound **29g** was well absorbed orally and penetrated the blood–brain barrier in mice as well as **1a** at 53 μmol/kg (20 mg/kg).

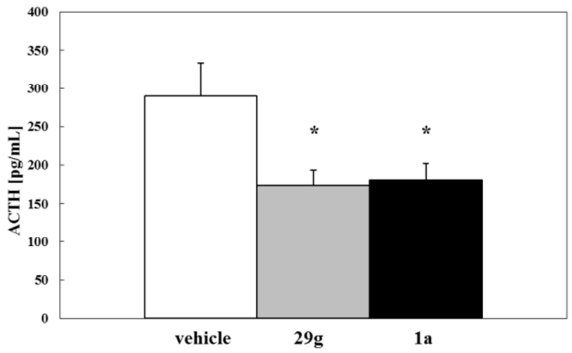
Table 5. Ex vivo ^{125}I-CRF binding inhibitory activity[a]

Compound No.	Frontal cortex	Olfactory bulb	Pituitary
29g	86	83	101
1a	96	95	98

[a] The values are % inhibition of ovine ^{125}I-CRF binding to mouse frontal cortex, olfactory bulb, and pituitary homogenates. Tissues were collected by decapitation 1 h after oral administration of 138 μg/kg (60 mg/kg) or 53 μg/kg (20 mg/kg) of compound **29g** or **1a** (n = 5), respectively. Homogenates of each brain area were prepared from 5 brains for each compound.

Compound **29g** was also evaluated against HPA axis activation in mice by measuring increased plasma ACTH levels in response to stress. As shown in Figure 5, significant reduction of HPA axis activation was observed after 1 h of oral administration at 138 μmol/kg (60 mg/kg), which was a dose that was effective in the ex vivo test described in Table 5. These results revealed that compound **29g** also antagonized CRF-related responses in mice.

Figure 5. Effect of compound **29g** on the suppression of ACTH secretion[a]



[a] ACTH concentration in blood was measured 1 h after oral administration of mice with 138 μmol/kg (60 mg/kg) of compound **29g** or 53 μmol/kg (20 mg/kg) of compound **1a**. Data are indicated as the mean ± standard error of the mean (SEM) (n = 10). *p < 0.05, parametric Dunnett's test compared with vehicle.

Conclusion

We designed a novel type of CRF_1 receptor antagonist using a 6-5 fused benzazole series with an anilino group substituted on the scaffold via one atom. Ring A, which includes HBA, is the core structure and is fused with ring C, which contains a small alkyl group R^z, and the compound does not have a ring B, which distinguishes this novel type of CRF_1 receptor antagonist from numerous others that have been reported. Among the designed benzazole series consisting of benzoxazole **5**, benzothiazole **9,** and benzimidazole **13** derivatives, benzothiazole **9** had the most potent CRF_1 receptor-binding activity. We believe that the outermost sulfur atom on the benzothiazole ring illustrated in Figure 4 contributed to the CRF_1 receptor-binding activity. As expected, the binding activity of the 1-*N*-methylbenzimidazole analog **22a** was improved relative

to that of benzothiazole **9**. The results of SAR studies at the other positions on the 1-*N*-methylbenzimidazole core suggested that the newly designed 6-5 fused benzazole series was quite effective at binding to a CRF_1 receptor. Identification of new types of scaffolds will allow a wide variety of CRF_1 receptor antagonist designs in the future. Furthermore, our benzimidazole series has greater structural flexibility than series with scaffolds directly bonding to the heteroaromatic core and is expected to have better physicochemical properties after further optimization to develop a clinical candidate. Among the synthesized compounds in this series, 4-chloro-N^2-(4-chloro-2-methoxy-6-methylphenyl)-1-methyl-N^7,N^7-dipropyl-1*H*-benzimidazole-2,7-diamine **29g** showed CRF_1 receptor-binding inhibition activity as potent as compound **1**, and it was less cytotoxic at 30 μM than compound **1**. Compound **29g** also exhibited inhibitory activity of human CRF-stimulated cAMP accumulation and ex vivo CRF binding inhibitory activity in the brain after oral administration in mice. In addition, oral treatment of compound **29g** demonstrated efficacy for suppression of stress-induced HPA axis activation in mice. These results suggested that compound **29g** is a promising lead compound for the development of a novel CRF_1 receptor antagonist. Additional optimization studies for this benzimidazole series continue and will be reported in due course.

Experimental section

All reactions were performed using commercially available starting materials, reagents, and solvents without further purification. In the following experimental, reaction progress was determined by thin layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck) or by liquid chromatography–mass spectrometry (LC/MS) analysis. LC/MS analysis was performed using five methods. The first method was performed on a HP-1100 (Agilent Technologies)

separations module [CAPCELL PAK UG-120 ODS (2.0 × 50 mm I.D., Shiseido Co., Ltd., Japan); 0.1% TFA in distilled H_2O/MeCN gradient; UV detection at 220 nm or 254 nm]. MS spectra were recorded using a Micromass ZMD with electrospray ionization. The second method was performed on a Thermo Surveyor high-performance liquid chromatography (HPLC) separations module [YMC ODS-AQ (4.6 × 50 mm I.D.); 1% isopropyl alcohol and 0.01% heptafluorobutyric acid in distilled H_2O/MeCN gradient; UV detection at 220 nm or 254 nm]. MS spectra were recorded using a Finnigan LCQ Duo Ion Trap MS with atmospheric pressure chemical ionization in the positive mode. The third method was performed on a Thermo Surveyor HPLC separations module [YMC ODS-AQ (4.6 × 50 mm I.D.); 1% isopropyl alcohol and 10 mM NH_4OAc in a distilled H_2O/MeCN gradient; UV detection at 220 nm or 254 nm]. MS spectra were recorded using a Finnigan LCQ Duo Ion Trap MS with atmospheric pressure chemical ionization in the negative mode. The fourth method was performed on a Shimadzu LC-20AD separations module [L-column2 ODS (3.0 × 50 mm I.D., CERI, Japan); 5 mM $AcONH_4$ in an ultrapure H_2O/MeCN gradient; UV detection at 220 nm or 254 nm]. MS spectra were recorded using a Shimadzu LCMS-2020 system with electrospray ionization. The fifth method was performed on a Waters 2795 separations module [L-column2 ODS (3.0 × 50 mm I.D., CERI, Japan); 0.05% TFA in an ultrapure H_2O/MeCN gradient; UV detection at 220 nm or 254 nm]. MS spectra were recorded using a Waters ZQ2000 with electrospray ionization. Magnesium sulfate or sodium sulfate was used for desiccants of organic extracts. Chromatographic purification was performed on a silica gel column (Kieselgel 60, 0.063–0.22 mm, Merck) or on Purif-Pack (SI 60 μm or NH 60 μm, Fuji Silysia, Ltd.). Preparative HPLC was performed on a Gilson pumping system used with a photodiode array detector (Hewlett Packard 1100 series) [YMC ODS-A(20 × 50 mm I.D.); 0.1% TFA in a distilled H_2O/MeCN gradient; UV detection at

220 nm]. Hydrochlorides were prepared from a corresponding free form using 4 M hydrogen chloride in EtOAc, 10% in MeOH, 2 M in Et_2O, or 4 M in 1,4-dioxane unless noted otherwise. Data of synthesized compounds were measured and shown as described below. LC/MS analysis for the detection of mass ion peaks was performed as described above. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Mercury-300 (300 MHz), Bruker DPX-300 (300 MHz), or Varian INOVA-400 (400 MHz). Chemical shifts are given in parts per million (ppm), with tetramethylsilane used as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, sxt = sextet, m = multiplet, dd = doublets of doublet, br s = broad singlet, br = broad. Coupling constants (*J* values) are given in hertz (Hz). The acidic protons of diketones, carboxylic acids, alcohols, or anilines were not frequently observed in the ^{1}H NMR spectra. Carbon nuclear magnetic resonance (^{13}C-NMR) spectra was recorded on a Bruker Avance III 400 (400 MHz). Chemical shifts are given in ppm, with tetramethylsilane used as an internal standard. The purities of all compounds tested in the biological systems were assessed by elemental analyses, analytical HPLC, or LC/MS. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd. HPLC analyses were performed on a Varian ProStar [YMC ODS-AQ (4.6 × 150 mm I.D.); 1% *i*-PrOH and 10 mM NH_4OAc in a distilled H_2O/MeCN gradient; UV detection at 220 nm or 254 nm] or a Shimadzu UFLC instrument [L-column2 ODS (3.0 × 50 mm, I.D.); 0.1% of TFA in a distilled H_2O/MeCN gradient; UV detection at 220 nm]. LC/MS analyses were performed as described above. All compounds were ≥95% pure as determined by HPLC and LC/MS analyses, unless noted otherwise.

***N*-Mesityl-7-nitro-1,3-benzoxazol-2-amine 4**. To a mixture containing compound **2** (0.10 g, 0.65 mmol) and sodium carbonate (0.14 g, 1.3 mmol) in EtOH was added (2,4,6-

trimethylphenyl)isothiocyanate (0.14 g, 0.78 mmol), and the mixture was refluxed overnight. After cooling, the reaction mixture was filtered. The filtrate was concentrated *in vacuo*. Purification of the residue via Biotage chromatography eluting with 20% EtOAc/CH_2Cl_2 gave 1-(2-hydroxy-3-nitrophenyl)-3-mesitylthiourea **3** (0.17 g, 80%). MS Calcd.: 331; Found: 332 (M+H). To a solution containing compound **3** (0.06 g, 0.18 mmol) in MeCN was added mercury (II) chloride (0.10 g, 0.36 mmol), and the mixture was then stirred for 1 h. The reaction mixture was diluted with EtOAc (2 mL) and filtered through a prepacked celite column. The filtrate was concentrated *in vacuo*. The residue was purified via Biotage chromatography eluting with 20% EtOAc/CH_2Cl_2 to afford the title compound (0.047 g, 90%). MS Calcd.: 297; Found: 298 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.29 (6H, s), 2.32 (3H, s), 6.99 (2H, s), 7.30 (1H, t, J = 8.2 Hz), 7.77 (1H, d, J = 8.1 Hz), 7.78 (1H, d, J = 8.6 Hz).

***N*^{2}-Mesityl-*N*^{7},*N*^{7}-dipropyl-1,3-benzoxazole-2,7-diamine 5**. To a flask was added compound **4** (0.10 g, 0.34 mmol) and MeOH (40 mL). The flask was purged with nitrogen followed by the addition of palladium on carbon (10%, 0.01 g). The flask was evacuated and pressurized to 2-3 psig hydrogen and stirred for 1 h. After completion as determined by HPLC, the reaction was filtered. To the filtrate were added propionaldehyde (0.1 mL, 1.7 mmol), $NaBH_3CN$ (0.1 g, 1.7 mmol) and AcOH (1 mL). The mixture was stirred overnight, then diluted with EtOAc and washed with water. The organic layer was dried and concentrated *in vacuo*. The residue was purified by Biotage chromatography eluting with 5% MeOH/CH_2Cl_2 gave the title compound (0.11 g, 90%). MS Calcd.: 351; Found: 352 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.74 (6H, t, J = 7.2 Hz), 1.47–1.53 (4H, m), 2.27 (6H, s), 2.29 (3H, s), 3.18 (4H, t, J = 7.8 Hz), 6.34 (1H, d, J = 8.1 Hz), 6.70 (1H, d, J = 7.0 Hz), 6.93 (2H, s), 6.98 (1H, t, J = 8.1 Hz).

N^2-Mesityl-N^7,N^7-dipropyl-1,3-benzothiazole-2,7-diamine 9. A mixture of 3-nitrophenylisothiocyanate **6** (2.25 g, 12.5 mmol) and mesityl amine (1.4 mL, 10 mmol) in MeOH (10 mL) was stirred at rt for 2 h. The resulting precipitate was collected by filtration to give 1-(mesityl)-3-(3-nitrophenyl)thiourea. To the suspension of the thiourea (1.26 g, 4.0 mmol) in AcOH (20 mL) was added bromine (0.22 mL, 4.2 mmol), and the mixture was refluxed for 1 h. After cooling, the reaction mixture was concentrated *in vacuo*. The resulting solid was solved in MeOH and the insoluble material was filtered off. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with 25% EtOAC/hexane to give N^2-mesityl-7-nitro-1,3-benzothiazol-2-amine **7** (0.14 g, 11%). MS Calcd.: 313; Found: 314 (M+H). To a solution of compound **7** (1.8 g, 5.7 mmol) in glacial AcOH (7.2 mL) and EtOH (25 mL) was added iron powder (1.8 g, 32 mmol). The resulting solution was refluxed for 18 h. After cooling, the slurry was filtered and the filtrate was concentrated *in vacuo* to give a brown solid. The solid was slurried in water, collected by filtration and purified by flash chromatography eluting with a 33% hexane/EtOAc mixture to give N^2-mesityl-1,3-benzothiazole-2,7-diamine **8** (0.9 g, 55%) as a tan powder. MS Calcd.: 283; Found: 284 (M+H). To compound **8** (0.125 g, 0.44 mmol) and propionaldehyde (0.16 mL, 2.2 mmol) in 1,2-dichloroethane (5 mL) was added glacial AcOH (one drop) and $NaBH(OAc)_3$ (0.28 g. 1.3 mmol). The mixture was stirred at 50°C for 1 h and concentrated *in vacuo*. The residue was purified by flash chromatography eluting with a 2% MeOH/CH_2Cl_2 mixture to give the title compound (0.016 g, 10%) as a tan powder. MS Calcd.: 367; Found: 368 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.73 (6H, t, J = 7.4 Hz), 1.31–1.40 (4H, m), 2.23 (6H, s), 2.26 (3H, s), 2.94–2.98 (4H, m), 6.67 (1H, t, J = 2.7 Hz), 6.92 (2H, s), 7.14–7.17 (2H, m).

***N*^{2}-Mesityl-*N*^{7},*N*^{7}-dipropyl-1*H*-benzimidazole-2,7-diamine hydrochloride 13**. To a mixture containing 3-nitro-*o*-phenylenediamine **10** (5.0 g, 33 mmol) and sodium carbonate (10.0 g, 98 mmol) in EtOH was added (2,4,6-trimethylphenyl)isothiocyanate (5.79 g, 32.6 mmol), and the mixture was refluxed for 12 h. DIC was added at this temperature, and the reaction mixture was refluxed for 48 h. After cooling, the solvent was removed *in vacuo*. Purification of the residue via Biotage chromatography eluting with 30% EtOAc/CH_2Cl_2 gave *N*-mesityl-7-nitro-1*H*-benzimidazole-7-amine **11** (5 g, 54 %). MS Calcd.: 296; Found: 297 (M+H). Compound **11** (0.078 g, 0.263 mmol) was dissolved in MeOH and hydrogenated with palladium on carbon (0.028 g) at rt under balloon pressure for 0.5 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to give N^2-mesityl-1*H*-benzimidazole-2,7-diamine **12** (0.06 g, 86%). MS Calcd.: 266; Found: 267 (M+H). A mixture of compound **12** (0.089 g, 0.334 mmol), propionaldehyde (0.097 g, 1.70 mmol) and 5% AcOH/MeOH was stirred at rt for 10 min. $MPBH_3CN$ was added to the mixture, followed by stirring at rt for 2 h. The reaction mixture was filtrated, and the filtrate was washed with saturated aqueous $NaHCO_3$ and concentrated *in vacuo*. The residue was purified by flash column chromatography to give the free form of the title compound (0.05 g, 43%). The residue was transformed to the hydrogen chloride (0.02 g, 16%). MS Calcd.: 350; Found: 351 (M+H). ^{1}H NMR (DMSO-d_6, 00 MHz) δ 0.82 (6H, t, J = 7.4 Hz), 1.42 (4H, d, J = 7.6 Hz), 2.02–2.23 (6H, m), 2.23–2.38 (3H, m), 3.25 (4 H, br s), 6.97 (2H, br s), 7.02–7.25 (3H, m), 9.70 (1H, br s), 12.30 (1H, br s).

***N*-Methyl-2,6-dinitroaniline 15a.** To a suspension of 2-chloro-1,3-dinitrobenzene **14** (200 g, 987 mmol) in MeOH (300 mL) was added methylamine (40% solution in MeOH; 314 mL, 2.96 mol), and the mixture was stirred at rt for 3 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in EtOAc and saturated aqueous $NaHCO_3$. The aqueous layer was

separated and extracted with EtOAc. The organic layer was washed with water, brine, passed through Celite, dried and concentrated *in vacuo* to give the title compound (192 g, 99%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.89 (3H, d, J = 5.4 Hz), 6.76 (1H, t, J = 8.1 Hz), 8.18 (2H, d, J = 8.1 Hz), 8.49 (1H, br s).

***N*^{2}-Methylbenzene-1,2,3-triamine 16a.** A mixture of compound **15a** (12.2 g, 0.122 mmol) and palladium on carbon (10%, 4.30 g) in MeOH (450 mL) was stirred at rt under hydrogen atmosphere for 2 h. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with 50–100% EtOAc/hexane gradient mixture to give the title compound (12.6 g, 75%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.71 (3H, br s), 3.73 (5H, br s), 6.22 (2H, d, J = 7.8 Hz), 6.75 (1H, d, J = 7.8 Hz).

4-Bromo-2-methoxy-6-methylaniline 18. To a solution of 2-methoxy-6-methylaniline **17** (25.0 g, 182 mmol) in AcOH (30 mL) and MeOH (60 mL) was added a solution of bromine (9.34 mL, 182 mmol) in AcOH (60 mL) at 0°C, and the mixture was stirred at rt for 2 h. The precipitate was collected by filtration, washed with Et_2O and dissolved in EtOAc and saturated aqueous $NaHCO_3$. The organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried and concentrated *in vacuo* to give the title compound (20.7 g, 53%) as a brown solid. MS Calcd.: 215; Found: 216 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.14 (3H, s), 3.83 (3H, s), 6.80 (1H, s), 6.84 (1H, s).

(4-Bromo-2-methoxy-6-methylphenyl)isothiocyanate 19. To a solution of Et_3N (0.65 mL, 4.63 mmol) in pyridine (3 mL) were added dropwise carbon disulfide (0.70 mL 11.6 mmol) and a solution of compound **18** (1.00 g, 4.63 mmol) in pyridine (5 mL) at −10^{o}C, and the mixture was stirred at −10^{o}C for 1 h. DCC (0.955 g, 4.63 mmol) was added to the mixture at -10 ^{o}C, followed by stirring at −10^{o}C for 3 h and at rt overnight. The reaction mixture was concentrated

in vacuo. The residue was diluted with hexane and sonicated. The resulting solid was removed by filtration. The filtrate was concentrated *in vacuo* to give the title compound (1.20 g, >99%) as an orange solid. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.32 (3H, s), 3.89 (3H, s), 6.88 (1H, s), 6.95 (1H, s).

1-(3-Amino-2-methylaminophenyl) 3-mesitylthiourea 20a. To a mixture containing compound **16a** (0.25 g, 1.82 mmol) and sodium carbonate (0.40 g, 3.7 mmol) in EtOH was added (2,4,6-trimethylphenyl)isothiocyanate (0.32g, 1.86 mmol), and the mixture was refluxed. After cooling, the solvent was removed *in vacuo*. Purification of the residue via Biotage chromatography eluting with 20% EtOAc/CH_2Cl_2 gave the title compound (0.34 g, 60 %). MS Calcd.: 314; Found: 315 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.19 (6H, s), 2.26 (3H, s), 3.68 (3H, s), 3.85 (4H, s), 6.20 (2H, d, J = 8.1 Hz), 6.87 (2H, s), 6.95 (1H, t, J = 8.1 Hz), 7.07 (1H, s).

1-(3-Amino-2-methylaminophenyl) 3-(4-bromo-2-methoxy-6-methylphenyl)thiourea 20d. To a solution of compound **15a** (1.00 g, 5.07 mmol) in MeOH (40 mL) were added cyclohexene (5 mL, 30.4 mmol) and palladium on carbon (10%, 1.35 g), and the mixture was refluxed for 3 h. Palladium on carbon (10%, 0.300 g) was added to the mixture, followed by being refluxed for 1.5 h. The catalyst was removed by filtration. Na_2CO_3 (0.645 g, 6.09 mmol) and compound **19** (1.30 g, 5.07 mmol) were added to the filtrate. The mixture was refluxed for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with EtOAc and the resulting solid was removed by filtration through silica gel eluting with EtOAc. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a 35% EtOAc/hexane mixture to give the title compound (0.76 g, 38%) as a solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.12 (3H, s), 3.42 (3H, s), 3.71 (3H, s), 4.68 (4H, br s), 6.05 (2H, d, J = 7.8 Hz), 6.76 (1H, t, J = 7.9 Hz), 6.99 (2H, s), 7.62 (1H, br s).

***N*²-Mesityl-1-methyl-1*H*-benzimidazole-2,7-diamine 21a**. To a solution containing compound **20a** (0.25 g, 0.79 mmol) in MeCN was added mercury (II) chloride (0.52 g, 1.6 mmol), and the mixture stirred for 1h. The reaction mixture was diluted with EtOAc and filtered through a prepacked celite column. The filtrate was concentrated *in vacuo*. The residue was purified via Biotage chromatography eluting with 20% EtOAc/CH_2Cl_2 to afford the title compound (0.12 g, 55 %). MS Calcd.: 280; Found: 281 (M+H). ^{1}H NMR (CD_3OD, 400 MHz) δ 2.27 (6H, s), 2.36 (3H, s), 4.13 (3H, s), 7.13 (2H, s), 7.24-7.26 (2H, m), 7.33 (1H, t, *J* = 8.1 Hz).

***N*²-Mesityl-1-methyl-*N*⁷,*N*⁷-dipropyl-1*H*-benzimidazole-2,7-diamine 22a**. To a solution containing compound **21a** (0.05 g, 0.18 mmol) in MeOH (5 mL) was added propionaldehyde (0.03 mL, 0.54 mmol), $NaBH_3CN$ (0.03 g, 0.54 mmol) and AcOH (0.1 mL), and the mixture was stirred overnight. The reaction mixture was diluted with EtOAc. The organic layer was washed with water, dried and concentrated *in vacuo*. The residue was purified via Biotage chromatography eluting with 5% MeOH/CH_2Cl_2 gave the title compound (0.04 g, 70 %). MS Calcd.: 364; Found: 365 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (6H, t, *J* = 7.3 Hz), 1.46-1.53 (4H, m), 2.22 (6H, s), 2.28 (3H, s), 2.98 (4H, s), 3.94 (3H, s), 6.86 (1H, d, *J* = 7.8 Hz), 6.92 (2H, s), 6.99 (1H, t, *J* = 8.1 Hz), 7.20 (1H, s).

1-Isopropyl-*N*²-mesityl-*N*⁷,*N*⁷-dipropyl-1*H*-benzimidazole-2,7-diamine 22b. To a solution containing compound **14** (1.0 g, 4.90 mmol) in THF was added isopropyl amine (0.58 g, 9.90 mmol), and the mixture was stirred for 12 h. The reaction mixture was diluted with CH_2Cl_2 and filtered through a glass filter. The filtrate was concentrated *in vacuo*. The residue was purified via Biotage chromatography eluting with 10% EtOAc/CH_2Cl_2 to afford *N*-isopropyl-2,6-dinitroaniline **15b** (0.85 g, 77%). A solution of compound **15b** (0.90 g, 4.00 mmol) in MeOH was hydrogenated with palladium on carbon (10%, 0.043 g) for 30 min at rt. The catalyst was

filtered off, and the filtrate was concentrated *in vacuo* to give N^2-isopropylbenzene-1,2,3-triamine **16b** (0.60 g, 91%). MS Calcd.: 165.1; Found: 166.1 (M+H). (2,4,6-Trimethylphenyl)isothiocyanate (0.75 g, 4.2 mmol) was added to the suspension of compound **16b** (0.70 g, 4.2 mmol) and Na_2CO_3 (1.10 g, 11 mmol) in EtOH (20 mL). After the mixture was refluxed for 2 h, DIC was added to the mixture, followed by being refluxed for 12 h. The reaction mixture was cooled to rt and concentrated *in vacuo*. The residue was purified by flash column chromatography to give 1-isopropyl-N^2-mesityl-1*H*-benzimidazole-2,7-diamine **21b** (1.0 g, 77%). MS Calcd.: 308.2; Found: 309.2 (M+H). 8M Solution of compound **21b** (0.2 g, 0.65 mmol) and propionaldehyde (0.15 g, 2.6 mmol) in 5% AcOH/MeOH was stirred at rt for 10 min. $MPBH_3CN$ was added to the mixture, followed by being stirred for 12 h. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography to give the title compound (0.15 g, 59%). MS Calcd.: 392; Found: 393 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (6H, t, J = 7.4 Hz), 1.45–1.55 (4H, m), 1.64 (6H, d, J = 7.0 Hz), 2.24 (6H, s), 2.28 (3H, s), 2.90–3.05 (4H, m), 6.57-6.65 (1H, m), 6.89 (1H, d, J = 7.8 Hz), 6.93 (2H, s), 6.98 (1H, t, J = 7.8 Hz), 7.23 (1H, d, J = 7.8 Hz).

***N*^{2}-Mesityl-1-phenyl-*N*^{7},*N*^{7}-dipropyl-1*H*-benzimidazole-2,7-diamine hydrochloride 22c**. To a solution containing compound **14** (1.0 g, 4.9 mmol) in THF was added aniline (0.69 g, 7.4 mmol), and the mixture was refluxed for 48 h. After cooling, the reaction mixture was concentrated *in vacuo*. The residue was purified via Biotage chromatography eluting with 10% EtOAc/CH_2Cl_2 to afford *N*-phenyl-2,6-dinitroaniline **15c** (0.9 g, 70%). A solution of compound **15c** (0.2 g, 0.77 mmol) in MeOH was hydrogenated with palladium on carbon (0.0082 g) for 30 min at rt. The catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give N^2-phenylbenzene-1,2,3-triamine **16c** (0.15 g, 98%). MS Calcd.: 199; Found: 200 (M+H). (2,4,6-

Trimethylphenyl)isothiocyanate (0.20 g, 1.00 mmol) was added to the suspension of compound **16c** (0.178 g, 1.00 mmol) and Na_2CO_3 (0.27 g, 2.5 mmol) in EtOH (20 mL). The mixture was refluxed for 2 h. DIC (0.127 g, 1.00 mmol) was added and refluxed for 48 h. After cooling, the reaction mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography to give N^2-mesityl-1-phenyl-1*H*-benzimidazole-2,7-diamine **21c** (0.17 g, 45%). To 8M solution of compound **21c** (0.05 g, 0.146 mmol) in 5% AcOH/MeOH was added propionaldehyde (0.034 g, 0.58 mmol), and the mixture was stirred at rt for 10 min. $NaBH_3CN$ (0.028 g, 0.44 mmol) was added, and the mixture was stirred for 12 h. The reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography, and the desired fraction was concentrated *in vacuo* to give the free form of the title compound. The free form was transformed to the hydrogen chloride (0.020 g, 30%). MS Calcd.: 426; Found: 427 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.56–0.76 (6H, m), 0.99–1.19 (4H, m), 1.96–2.13 (6H, m), 2.17 (3H, s), 2.45 (4H, br s), 6.61 (2H, br s), 6.81–7.04 (2H, m), 7.09 (2H, br s), 7.19–7.30 (4H, m), 7.32 (1H, br s), 13.79 (1H, br s).

N^2-(4-Bromo-2-methoxy-6-methylphenyl)-N^7,N^7-dibutyl-1-methyl-1*H*-benzimidazole-2,7-diamine hydrochloride 22d. To a solution of compound **20d** (0.760 g, 1.92 mmol) in MeCN (150 mL) were added Et_3N (2.41 mL, 17.3 mmol) and mercury(II) chloride (0.522 g, 1.92 mmol), and the mixture was stirred at rt for 1 h. Mercury(II) chloride (1.06 g, 3.90 mmol) was added to the mixture, followed by stirring at rt for 1 h. Additional mercury(II) chloride (1.33 g, 4.90 mmol) was added, the mixture was stirred at rt for 1 h, further mercury(II) chloride (0.620 g, 2.28 mmol) was added, and the mixture was stirred at rt overnight. The reaction mixture was concentrated *in vacuo*. The residue was diluted with water and extracted with EtOAc. The organic layer was passed through a silica gel pad, and the filtrate was concentrated *in vacuo*. The

residue was dissolved in MeOH, and the solution was treated with $PSBH_3CN$. The resin was removed by celite filtration. The filtrate was concentrated *in vacuo* to give N^2-(4-bromo-2-methoxy-6-methylphenyl)-1-methyl-1*H*-benzimidazole-2,7-diamine **21d** (0.340 g, 49%). To a solution of compound **21d** (0.100 g, 0.277 mmol) in CH_2Cl_2 (10 mL) were added AcOH (0.1 mL), *n*-butylaldehyde (0.0399 g, 0.554 mmol) and $NaBH(OAc)_3$ (0.176 g, 0.830 mmol), and the mixture was stirred at 55 ^oC for 3 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with water, basified with potassium carbonate, and extracted with EtOAc. The organic layer was washed with water, dried and concentrated *in vacuo*. The residue was converted to hydrogen chloride to give the title compound (0.045 g, 32%). mp 116–117^oC. MS Calcd.: 472; Found: 473 (M+H). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (6H, t, J = 7.3 Hz), 1.13–1.37 (4H, m), 1.37–1.53 (4H, m), 2.19 (3H, s), 3.01 (4H, br s), 3.83 (3H, s), 4.04 (3H, s), 6.81–6.97 (2H, m), 6.97–7.13 (2H, m), 7.26 (2H, s).

N^2-(4-Bromo-2-methoxy-6-methylphenyl)-N^7,N^7-bis(2-methoxyethyl)-1-methyl-1*H*-benzimidazole-2,7-diamine 22e. To a solution of 1,1,2-trimethoxyethane (0.209 mL, 1.66 mmol) in $CDCl_3$ was added $FeCl_3$ (5%, 5.39 g, 1.66 mmol), and the mixture was stirred at rt for several hours. The insoluble material was removed by filtration through silica gel pad. The filtrate was concentrated *in vacuo* until the rest of the filtrate was about 5 mL. To a solution of compound **21d** (0.200 g, 0.554 mmol) in MeOH (10 mL) and AcOH (1 mL) were added $MPBH_3CN$ (1.15 g, 2.27 mmol) and the aldehyde prepared above, and the mixture was stirred at rt overnight. The aldehyde prepared again and $MPBH_3CN$ were added to the reaction mixture, followed by stirring at rt overnight. The handling was carried out again, and the mixture was stirred at rt overnight. The reaction mixture was purified by silica gel column chromatography eluting with a 40% EtOAc/hexane with 2% of ammonium hydroxide to give the title compound

(0.048 g, 18%). mp 206–208°C. MS Calcd.: 476; Found: 477 (M+H).^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.19 (3H, s), 3.15–3.30 (6H, m), 3.33 (4H, br s), 3.41 (4H, br s), 3.82 (3H, s), 4.06 (3H, s), 5.85 (1H, s), 6.77–7.00 (2H, m), 7.00–7.09 (2H, m), 7.30 (1H, d, *J* = 7.8 Hz).

***N*^{2}-(4-Bromo-2-methoxy-6-methylphenyl)-*N*^{7}-isopropyl-1-methyl-1*H*-benzimidazole-2,7-diamine 22f**. A mixture of compound **21d** (1.00 g, 2.77 mmol), acetone (2.03 mL, 1.61 mmol), $NaBH(OAc)_3$ (2.35 g, 11.1 mmol) and CH_2Cl_2 (20 mL) was stirred at 55°C for 2 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried and concentrated *in vacuo*. The residue was purified by column chromatography eluting with a 50% EtOAc/hexane with 3% MeOH to give the title compound (0.233 g, 21%). MS Calcd.: 402; Found: 403 (M+H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.19–1.28 (6H, m), 1.95–2.10 (1H, m), 2.16 (3H, s), 3.45–3.61 (1H, m), 3.79 (3H, s), 3.89 (3H, d, *J* = 0.8 Hz), 6.51 (1H, d, *J* = 8.0 Hz), 6.91 (1H, s), 6.93–7.10 (3 H, m).

***N*^{2}-(4-Bromo-2-methoxy-6-methylphenyl)-*N*^{7}-ethyl-*N*^{7}-isopropyl-1-methyl-1*H*-benzimidazole-2,7-diamine hydrochloride 23**. A mixture of compound **22f** (0.066 g, 0.164 mmol), acetaldehyde (0.101 mL, 1.80 mmol), $NaBH(OAc)_3$ (0.10 g, 0.49 mmol), AcOH (1 drop) and CH_2Cl_2 (5 mL) was stirred at 50°C for 2 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried and concentrated *in vacuo*. The free form of the title compound was converted the hydrochloride to give the title compound (0.075 g, 98%). mp 177–178°C. MS Calcd.: 430; Found: 431 (M+H). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.78–0.98 (3H, m), 0.98–1.06 (3H, m), 1.06–1.35 (3H, m), 2.18–2.39 (3H, m), 3.09 (2H, br s), 3.29–3.43 (1H, m), 3.74–3.93 (3H, m), 4.11 (3H, s), 6.95–7.10 (1H, m), 7.10–7.25 (2H, m), 7.30 (2H, dd, *J* = 17.8, 1.8 Hz), 10.21 (1H, s), 12.61 (1H, s).

7-Amino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 24. CDI (15.6 g, 96.2 mmol) was added to a solution of compound **16a** (12.6 g, 91.9 mmol) in THF (260 mL) at rt, and the mixture was stirred at rt for 16 h. The mixture was concentrated *in vacuo*, and the residue was washed with CH_2Cl_2 to give the title compound as a colorless powder (12.2 g, 81%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.50 (3H, br s), 4.84 (2H, s), 6.29 (1H, dd, J = 7.8, 0.9 Hz), 6.34 (1H, dd, J = 7.8, 0.9 Hz), 6.67 (1H, t, J = 8.1 Hz).

7-Dipropylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 25a. Propionaldehyde (21.6 mL, 300 mmol) was added to a solution of compound **24** (4.90 g, 30.0 mmol) in MeOH (200 mL). After the mixture was stirred for 1 h at rt, $NaBH_3CN$ (18.9 g, 300 mmol) and AcOH (5.15 mL) were added. The mixture was stirred for 48 h at rt and diluted saturated aqueous $NaHCO_3$. The aqueous solution was extracted with EtOAc. The organic layer was washed with brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with 20–60% EtOAc/hexane gradient mixture to give the title compound (5.42 g, 73%). MS Calcd.: 247; Found: 248 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.84 (6H, t, J = 7.5 Hz), 1.35–1.50 (4H, m), 2.93 (4H, t, J = 7.5 Hz), 3.75 (3H, s), 6.85-7.05 (3H, m), 9.95 (1H, s).

7-Diethylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 25b. Acetaldehyde (30.4 mL, 621 mmol) was added to a mixture of compound **24** (15.7 g, 96.4 mmol) and AcOH (22 mL) in CH_2Cl_2 (450 mL) at 0^{o}C. $NaBH(OAc)_3$ (102 g, 482 mmol) was added to the mixture at 0^{o}C, and the mixture was stirred at rt for 2 h. The mixture was poured into cold water, neutralized with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was separated, washed with brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluted with 75–100% EtOAc/hexane gradient mixture to give the title

compound as a colorless solid (19.2 g, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (6H, t, *J* = 7.2 Hz), 3.03 (4H, q, *J* = 7.2 Hz), 3.58 (3H, br s), 6.88–7.02 (3H, m), 9.96 (1H, s).

4-Chloro-7-dipropylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 26a, 4,6-dichloro-7-dipropylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 26b and **6-chloro-7-dipropylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 26c.** A mixture of compound **25a** (5.00 g, 20.2 mmol), NCS (3.29 g, 24.2 mmol) and AIBN (0.166 g, 1.01 mmol) in CCl_4 (400 mL) was refluxed for 18 h. The mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with 10–60% EtOAc/hexane gradient mixture to give a mixture (1.64 g) of the title compounds, **26a** and **26b,** and another title compound **26c** (1.89 g, 33%). The ratio of the mixture of **26a** and **26b** was 2 to 1 by LCMS analysis. Compound **26a**: MS Calcd.: 281; Found: 282 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (6H, t, *J* = 7.5 Hz), 1.35–1.50 (4H, m), 2.90 (4H, t, *J* = 7.5 Hz), 3.72 (3H, s), 6.85 (1H, d, *J* = 8.7 Hz), 7.00 (1H, d, *J* = 8.7 Hz), 8.91 (1H, br s). Compound **26b**: MS Calcd.: 315; Found: 316 (M+H). Compound **26c**: MS Calcd.: 281; Found: 282 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.84 (6H, t, *J* = 7.5 Hz), 1.30–1.60 (4H, m), 3.00-3.10 (2H, m), 3.01–3.20 (2H, m), 3.74 (3H, s), 6.86 (1H, d, *J* = 8.1 Hz), 7.00 (1H, d, *J* = 8.1 Hz), 10.07 (1H, br s).

4-Chloro-7-diethylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 26d. NCS (8.30 g, 62.2 mmol) was added to a solution of compound **25b** (13.0 g, 59.2 mmol) in MeCN (450 mL) at rt, and the mixture was stirred at 60^{o}C for 12 h. The mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a 25–60% EtOAc/hexane gradient mixture to give the title compound (4.15 g, 28%). ^{1}H

NMR ($CDCl_3$, 300 MHz) δ 0.98 (6H, t, *J* = 7.2 Hz), 3.00 (4H, q, *J* = 7.2 Hz), 3.72 (3H, br s), 6.85 (1H, d, *J* = 8.4 Hz), 6.97 (1H, d, *J* = 8.4 Hz), 9.18 (1H, br s).

4-Bromo-7-diethylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 26f. NBS (30.4 g, 171 mmol) was added to a solution of compound **25b** (35.8 g, 163 mmol) in MeCN (1400 mL) at 0 ^oC, and the mixture was stirred at rt for 40 h. The mixture was diluted with saturated aqueous $NaHCO_3$. The precipitate was collected by filtration and washed with water and EtOAc to give the product (32.5 g, 67%). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.98 (6H, t, *J* = 7.2 Hz), 3.00 (4H, q, *J* = 7.2 Hz), 3.72 (3H, s), 6.80 (1H, dd, *J* = 8.7, 1.5 Hz), 7.09 (1H, d, *J* = 8.7 Hz), 9.85 (1H, br s).

7-(Dipropylamino)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbonitrile 27a. NBS (1.62 g, 9.09 mmol) was added to a solution of compound **25a** (1.50 g, 6.06 mmol) in CCl_4 (100 mL) at rt, and the mixture was refluxed for 48 h. After cooling, the mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a 10–50% EtOAc/hexane gradient mixture to give 4-bromo-7-dipropylamino-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one **26e** (0.660 g, 33%). MS Calcd.: 325; Found: 326 (M+H). Copper(I) cyanide (0.461 g, 5.14 mmol) was added to a solution of compound **26e** (0.840 g, 2.57 mmol) in NMP (20 mL) at rt, and the mixture was stirred at 180^oC for 18 h. After cooling, the solution was diluted with EtOAc. The solution was washed with water, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a 5–50% EtOAc/hexane gradient mixture to give the title compound (0.522 g, 75%). MS Calcd.: 272; Found: 273 (M+H). NMR ($CDCl_3$, 300 MHz) δ 0.85 (6H, t, *J* = 7.4 Hz), 1.40–1.55 (4H, m), 2.98–3.02 (4H, m), 3.70 (3H, s), 6.88 (1H, d, *J* = 8.4 Hz), 7.21 (1H, d, *J* = 8.4 Hz), 9.39 (1H, br s).

7-(Diethylamino)-1-methyl-2-oxo-2,3-dihydro-1*H*-benzimidazole-4-carbonitrile 27b. Copper(I) cyanide (0.910 g, 10.2 mmol) was added to a solution of compound **26f** (2.02 g, 6.77 mmol) in NMP (10 mL) in a microwave vessel. The vessel was sealed and subjected to microwave irradiation at 170°C (150W) for 10 min. After cooling, the reaction mixture was purified by silica gel column chromatography to give the title compound (1.35 g, 82%). MS Calcd.: 244; Found: 245 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02 (6H, t, J = 7.2 Hz), 3.09 (4H, q, J = 7.2 Hz), 3.71 (3H, s), 6.90 (1H, d, J = 8.4 Hz), 7.23 (1H, d, J = 8.4 Hz), 9.68 (1H, br s).

7-(Diethylamino)-1,4-dimethyl-1,3-dihydro-2*H*-benzimidazol-2-one 27c. A solution of compound **26f** (0.100 g, 0.34 mmol), tetrakis(triphenylphosphine)palladium (0.0774 g, 0.067 mmol), tetramethyltin (0.5 mL, 3.60 mmol) in HMPA (2 mL) was refluxed overnight. After cooling, water was added, and the mixture was extracted with CH_2Cl_2. The organic layer was dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with EtOAc/hexane to give the title compound (0.040 g, 51%). MS Calcd.: 233; Found: 234 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.98 (6H, t, J = 6.9 Hz), 2.40 (3H, s), 2.99 (4H, q, J = 6.9 Hz), 3.75 (3H, s), 6.79–6.86 (2H, m), 11.01 (1H, br s).

7-(Diethylamino)-4-methoxy-1-methyl-1,3-dihydro-2*H*-benzimidazol-2-one 27e. A solution of compound **26f** (0.400 g, 1.34 mmol), anhydrous copper(I) iodide (0.306 g, 1.60 mmol), sodium methoxide (28% in MeOH; 10 mL) in DMF (10 mL) was heated at 100°C for 1 h. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was dried and concentrated *in vacuo*. The residue was crystallized from MeOH and water to give the title compound (0.246 g, 74%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.97 (6H, t, J =

6.9 Hz), 2.97 (4H, q, *J* = 6.9 Hz), 3.71 (3H, s), 3.87 (3H, s), 6.56 (1H, d, *J* = 8.7 Hz), 6.85 (1H, d, *J* = 8.7 Hz), 7.77 (1H, br s).

2-Chloro-7-dipropylamino-1-methyl-1*H*-benzimidazole 28a. A mixture of compound **25a** (3.55 g, 14.4 mmol), *N,N*-dimethylaniline (2.7 mL, 21.5 mmol) and $POCl_3$ (44 mL) was stirred at 100°C for 4.5 h. After cooling to rt, $POCl_3$ was removed *in vacuo*. Ice water was slowly added to the residue and extracted with EtOAc. The organic layer was concentrated *in vacuo*. The residue was diluted with EtOAc, and the resulting solid was filtered off through a pad of silica gel. The filtrate was concentrated *in vacuo* to give the product as an oil (3.62 g, 69%). MS Calcd.: 265; Found: 266 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.79 (6H, t, *J* = 7.3 Hz), 1.39 (4H, sxt, *J* = 7.4 Hz), 2.97 (4H, br s), 4.04–4.15 (3H, m), 7.02–7.22 (2H, m), 7.31 (1H, dd, *J* = 7.8, 1.2 Hz).

2-Chloro-7-diethylamino-1-methyl-1*H*-benzimidazole 28b. A mixture of compound **25b** (2.73 g, 12.4 mmol) and $POCl_3$ (23 mL) was stirred at 100°C for 4.5 h. After cooling to rt, $POCl_3$ was removed *in vacuo*. The residue was dissolved in saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with 10% EtOAc/hexane to give the title compound as a colorless solid (1.40 g, 47%). MS Calcd.: 237; Found: 238 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87–1.10 (6H, m), 3.08 (4H, q, *J* = 6.9 Hz), 3.96–4.20 (3H, m), 7.07 (1H, dd, *J* = 7.8, 1.0 Hz), 7.18 (1H, td, *J* = 7.9, 1.6 Hz), 7.38–7.52 (1H, m).

2,4-Dichloro-1-methyl-*N,N*-dipropyl-1*H*-benzimidazol-7-amine 28c and 2,4,6-trichloro-1-methyl-*N,N*-dipropyl-1*H*-benzimidazol-7-amine 28d. A mixture of compound **28c** and **28d** (1.21 g, the ratio: 62 to 37 by LCMS analysis) was prepared from a mixture of compound **26a** and **26b** (1.64 g, the ratio: 2 to 1 by LCMS analysis) in a manner similar to that described in compound **28b**. Compound **28c**: MS Calcd.: 299; Found: 300 (M+H). ^{1}H NMR ($CDCl_3$, 300

MHz) δ 0.84 (6H, t, *J* = 7.5 Hz), 1.35–1.48 (4H, m), 2.97 (4H, m), 4.13 (3H, s), 7.00 (1H, d, *J* = 8.7 Hz), 7.18 (1H, d, *J* = 8.7 Hz). Compound **28d**: MS Calcd.: 333; Found: 334 (M+H).

2,6-Dichloro-1-methyl-*N,N*-dipropyl-1*H*-benzimidazol-7-amine 28e. Compound **28e** (0.55 g, 34%) was prepared from compound **26c** (0.148 g, 0.525 mmol) in a manner similar to that described in compound **28b**. MS Calcd.: 299; Found: 300 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.84 (6H, t, *J* = 7.5 Hz), 1.30–1.55 (4H, m), 3.00–3.15 (2H, m), 3.18–3.30 (2H, m), 4.12 (3H, s), 7.18 (1H, d, *J* = 8.7 Hz), 7.41 (1H, d, *J* = 8.7 Hz).

2-Chloro-4-cyano-1-methyl-*N,N*-dipropyl-1*H*-benzimidazol-7-amine 28f. A mixture of compound **27a** (0.520 g, 1.91 mmol) and $POCl_3$ (5.3 mL) was stirred at 110^{o}C for 18 h. After cooling, the mixture was poured into ice-cooled water and extracted with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a 5–50% EtOAc/hexane gradient mixture to give the title compound (0.343 g, 62%) as a colorless solid. MS Calcd.: 290; Found: 291 (M+H). ^{1}NMR ($CDCl_3$, 300 MHz) δ 0.85 (6H, t, *J* = 7.5 Hz), 1.40–1.55 (4H, m), 3.00–3.10 (4H, m), 4.10 (3H, s), 7.01 (1H, d, *J* = 8.4 Hz), 7.49 (1H, d, *J* = 8.4 Hz).

2,4-Dichloro-7-(diethylamino)-1-methyl-1*H*-benzimidazole 28g. Compound **28g** (3.56 g, >99%) was prepared from compound **26d** (0.148 g, 0.525 mmol) in a manner similar to that described in compound **28f**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (6H, t, *J* = 6.9 Hz), 3.05 (4H, q, *J* = 6.9 Hz), 4.13 (3H, s), 6.99 (1H, d, *J* = 8.1 Hz), 7.19 (1H, d, *J* = 8.1 Hz).

2-Chloro-7-(diethylamino)-1-methyl-1*H*-benzimidazole-4-carbonitrile 28h. Compound **28h** (0.220 g, 43%) was prepared from compound **27b** (0.472 g, 1.93 mmol) in a manner similar to that described in compound **28f**. MS Calcd.: 262; Found: 263 (M+H). ^{1}H NMR ($CDCl_3$, 300

MHz) δ 1.04 (6H, t, *J* = 7.2 Hz), 3.17 (4H, q, *J* = 7.2 Hz), 4.11 (3H, s), 7.02 (1H, d, *J* = 8.4 Hz), 7.52 (1H, d, *J* = 8.4 Hz).

2-Chloro-*N*,*N*-diethyl-1-methyl-4-phenyl-1*H*-benzimidazol-7-amine 28j. 7-(Diethylamino)-1-methyl-4-phenyl-1,3-dihydro-2*H*-benzimidazol-2-one **27d**, which was used for the next step without further purification, was prepared from compound **26f** (0.100 g, 0.335 mmol) in a manner similar to that described in compound **27c**. Compound **28j** (0.071 g, 67%) was prepared from compound **27d** described above in a manner similar to that described in compound **28f**. MS Calcd.: 341; Found: 342 (M+H). ^{1}H NMR($CDCl_3$, 300 MHz) δ 1.04 (6H, t, *J* = 7.2 Hz), 3.11 (4H, q, *J* = 7.2 Hz), 4.16 (3H, s), 7.13 (1H, d, *J* = 8.1 Hz), 7.30–7.35 (2H, m), 7.43–7.48 (2H, m), 7.88–7.91 (2H, m).

2-Chloro-*N*,*N*-diethyl-4-methoxy-1-methyl-1*H*-benzimidazol-7-amine 28k. Compound **28k** (0.210 g, 98%) was prepared from compound **27e** (0.200 g, 0.803 mmol) in a manner similar to that described in compound **28f**. MS Calcd.: 267; Found: 268 (M+H). ^{1}H NMR($CDCl_3$, 300 MHz) δ 0.96 (6H, t, *J* = 6.9 Hz), 3.01 (4H, q, *J* = 6.9 Hz), 3.97 (3H, s), 4.11 (3H, s), 6.62 (1H, d, *J* = 8.7 Hz), 7.09 (1H, d, *J* = 8.7 Hz).

N^2-(2,4-Dimethylphenyl)-1-methyl-N^7,$N^{7`}$-dipropyl-1*H*-benzimidazole-2,7-diamine trifluoroacetate 29a. A mixture of compound **28a** (0.050 g, 0.188 mmol) and 2,4-dimethylaniline (0.0702 mL, 0.564 mmol) was stirred at 100°C overnight. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was concentrated *in vacuo*. The residue was purified by HPLC to give the title compound (0.052 g, 60%). mp 90–91 °C. MS Calcd.: 350; Found: 351 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.57–0.93 (6H, m), 1.20–1.55 (4H, m), 2.26 (3H, s), 2.36 (3H, s), 3.01 (4H, br s), 4.08 (3H, s), 7.03 (1H, dd, *J* = 7.2, 1.9 Hz), 7.08-7.26 (3H, m), 7.27 (1H, s), 7.34 (1H, d, *J* = 7.9 Hz), 10.23

(1H, br s), 12.49 (1 H, br s). Anal. Calcd for $C_{22}H_{30}N_4 \cdot CF_3CO_2H \cdot 0.5H_2O$: C, 60.87; H, 6.81; N, 11.83. Found: C, 60.58; H, 6.65; N, 11.60.

***N*²-(2,6-Dimethylphenyl)-1-methyl-*N*⁷,*N*⁷`-dipropyl-1*H*-benzimidazole-2,7-diamine trifluoroacetate 29b.** Compound **29b** (0.039 g, 45%) was prepared from compound **28a** (0.050 g, 0.188 mmol) and 2,6-dimethylaniline (0.070 g, 0.579 mmol) in a manner similar to that described in compound **29a**. mp 100–102°C. MS Calcd.: 350; Found: 351 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.58–0.97 (6H, m), 1.19–1.58 (4H, m), 2.25 (6H, s), 3.01 (4H, br s), 4.12 (3H, s), 7.01 (1H, dd, J = 7.2, 1.5 Hz), 7.10–7.24 (2H, m), 7.24–7.44 (3H, m), 10.24 (1H, br s), 12.55 (1H, br s). HPLC: 94.5% purity.

***N*²-(4-Chloro-2-methoxy-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷`-dipropyl-1*H*-benzimidazole-2,7-diamine 29c.** A mixture of compound **28a** (1.00 g, 3.76 mmol) and 4-chloro-2-methoxy-6-methylaniline (1.29 g, 7.52 mmol) was stirred at 70°C overweekend. After cooling, the reaction mixture was diluted with hexane and the insoluble material was collected by filtration. The filter cake was purified by silica gel column chromatography eluting with a 2% MeOH/CH_2Cl_2 to give the title compound (0.150 g, 9.9%). MS Calcd.: 400; Found: 401 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.82 (6H, t, J = 7.3 Hz), 1.31–1.57 (4H, m), 2.10 (3H, s), 2.95 (4H, br s), 3.75 (3H, s), 3.96 (3H, s), 6.79 (1H, d, J = 3.9 Hz), 6.87 (2H, d, J = 4.7 Hz), 6.95 (1H, s), 7.00 (1H, s), 7.85 (1H, s).

***N*²-(4-Bromo-2-methoxy-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷`-dipropyl-1*H*-benzimidazole-2,7-diamine hydrochloride 29d.** Compound **29d** (0.250 g, 42%) was prepared from compound **28a** (0.328 g, 1.23 mmol) and compound **22** (0.797 g, 3.69 mmol) in a manner similar to that described in compound **29a**. mp 133–135°C. MS Calcd.: 444; Found: 445 (M+H). ^{1}H NMR

(CD_3OD, 300 MHz) δ 0.90 (6H, t, J = 7.4 Hz), 1.43–1.64 (4H, m), 2.34 (3H, s), 3.08 (4H, br s), 3.84 (3H, s), 4.22 (3H, s), 7.05 (1H, dd, J = 6.5, 2.2 Hz), 7.17–7.41 (4H, m).

***N*²-(4-Chloro-2-isopropyl-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷`-dipropyl-1*H*-benzimidazole-2,7-diamine hydrochloride 29e.** Compound **29e** (0.052 g, 21%) was prepared from compound **28a** (0.150 g, 0.564 mmol) and 4-chloro-2-isopropyl-6-methylaniline- (0.311 g, 1.69 mmol) in a manner similar to that described in compound **29a**. mp 137–139°C. MS Calcd.: 412; Found: 413 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.84 (6H, t, J = 7.4 Hz), 1.13 (3H, d, J = 6.8 Hz), 1.19 (3H, d, J = 6.8 Hz), 1.31–1.67 (4H, m), 2.22 (3H, s), 3.01 (4H, br s), 3.05-3.36 (1H, m), 4.17 (3H, s), 7.01 (1H, dd, J = 7.2, 1.9 Hz), 7.09–7.33 (2H, m), 7.41 (2H, s), 10.72 (1H, s), 12.55 (1H, s).

***N*²-(4-Bromo-2-methoxy-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷`-diethyl-1*H*-benzimidazole-2,7-diamine hydrochloride 29f.** Compound **29f** (0.620 g, 35%) was prepared from compound **28b** (1.0 g, 4.21 mmol) and compound **22** (1.82 g, 8.41 mmol) in a manner similar to that described in compound **29a**. mp 196–197°C. MS Calcd.: 416; Found: 417 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.96 (6H, t, J = 7.0 Hz), 2.09 (3H, s), 3.03 (4H, q, J = 6.7 Hz), 3.69 (1H, d, J = 3.5 Hz), 3.75 (3H, s), 3.95 (3H, s), 6.71–6.83 (1H, m), 6.83–6.94 (2H, m), 7.09 (2H, d, J = 8.4 Hz), 7.84 (1H, s).

4-Chloro-*N*²-(4-chloro-2-methoxy-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷-dipropyl-1*H*-benzimidazole-2,7-diamine 29g and **4,6-dichloro-*N*²-(4-chloro-2-methoxy-6-methylphenyl)-1-methyl-*N*⁷,*N*⁷-dipropyl-1*H*-benzimidazole-2,7-diamine 29h.** To a solution of a mixture of **28c** and **28d** (1.20 g) in NMP (5 mL) was added compound **32** (2.05 g, 11.9 mmol), and the mixture was stirred at 120°C for 48 h. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and

brine, dried and concentrated *in vacuo*. The residue was purified by preparative HPLC, and the desired fraction was concentrated *in vacuo*. The residue was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, dried and concentrated *in vacuo*. The resulting solid was recrystallized from EtOAc to give the title compound **29g** (0.619 g, 7.0% in 3 steps). Another title compound **29h** (0.174 g, 1.8% in 3 steps) was also isolated in the same manner. Compound **29g**: mp 204–205°C. MS Calcd.: 434; Found: 435 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.82 (6H, t, J = 7.2 Hz), 1.35–1.50 (4H, m), 2.13 (3H, s), 2.87–3.05 (4H, br), 3.77 (3H, s), 3.96 (3H, s), 6.79 (1H, d, J = 8.4 Hz), 6.93 (1H, d, J = 8.4 Hz), 6.98 (1H, s), 7.03 (1H, s), 8.09 (1H, br s). ^{13}C NMR (DMSO-*d*6, 101 MHz) δ 155.5, 154.0, 140.3, 138.1), 134.8 (C-8), 130.4, 130.1, 126.1, 121.8, 120.1, 115.2, 114.6, 109.8, 56.0, 55.9, 30.6, 19.3, 17.8, 11.6. Anal. Calcd for $C_{22}H_{28}Cl_2N_4O$: C, 60.69; H, 6.58; N, 12.87; Cl, 16.29. Found: C, 60.35, H, 6.35; N, 12.89; Cl, 16.13. Compound **29h**: mp 217–219°C. MS Calcd.: 468; Found: 469 (M+H). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.85 (6H, t, J = 7.5 Hz), 1.35–1.55 (4H, m), 2.21 (3H, s), 3.00–3.11 (2H, m), 3.15–3.25 (2H, m), 3.80 (3H, s), 3.97 (3H, s), 6.00–6.10 (1H, m), 6.77 (1H, s), 6.89 (1H, s), 7.09 (1H, s). Anal. Calcd for $C_{22}H_{27}Cl_3N_4O{\cdot}0.3H_2O$: C, 55.60; H, 5.85; N, 11.79. Found: C, 55.62; H, 5.88; N, 11.65.

6-Chloro-N^2-(4-chloro-2-methoxy-6-methylphenyl)-1-methyl-N^7,N^7-dipropyl-1*H*-benzimidazole-2,7-diamine 29i. Compound **29i** (0.0245 g, 31%) was prepared from compound **28e** (0.055 g, 0.183 mmol) in a manner similar to that described in compounds **29g** and **29h**. mp: 234–235°C. MS Calcd.: 434; Found: 435 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (6H, t, J = 7.5 Hz), 1.30–1.55 (4H, m), 2.19 (3H, s), 3.00–3.15 (2H, m), 3.15–3.25 (2H, m), 3.82 (3H, s), 4.06 (3H, s), 5.95–6.10 (1H, m), 6.78 (1H, d, J = 1.6 Hz), 6.89 (1H, d, J = 1.6 Hz), 7.02 (1H, d, J

= 8.7 Hz), 7.22 (1H, d, *J* = 8.7 Hz). Anal. Calcd for $C_{22}H_{28}Cl_2N_4O \cdot 0.5H_2O$: C, 59.46; H, 6.58; N, 12.61. Found: C, 59.73; H, 6.46; N, 12.57.

***N*^{2}-(4-Chloro-2-methoxy-6-methylphenyl)-4-cyano-1-methyl-*N*^{7},*N*^{7}-dipropyl-1*H*-benzimidazole-2,7-diamine 29j.** Compound **29j** (0.012 g, 4.3%) was prepared from compound **28f** (0.020 g, 0.0688 mmol) in a manner similar to that described in compounds **29g** and **29h**. MS Calcd.: 425; Found: 426 (M+H). ^{1}NMR ($CDCl_3$, 300 MHz) δ 0.85 (6H, t, *J* = 7.5 Hz), 1.40–1.55 (4H, m), 2.25 (3H, s), 3.05 (4H, t, *J* = 7.5 Hz), 3.80 (3H, s), 3.90 (3H, s), 6.25–6.40 (1H, m), 6.75 (1H, s), 6.80 (1H, d, *J* = 8.0 Hz), 6.90 (1H, s), 7.30 (1H, d, *J* = 8.0 Hz).

4-Chloro-*N*^{2}-(4-chloro-2-methoxy-6-methylphenyl)-*N*^{7},*N*^{7}-diethyl-1-methyl-1*H*-benzimidazole-2,7-diamine 29k. A mixture of compound **28g** (0.101 g, 0.371 mmol), compound **32** (0.191 g, 1.11 mmol) and NMP (4 drops) was stirred at 110^{o}C overnight. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with a EtOAc/hexane mixture. The desired fraction was concentrated *in vacuo*. The residue was crystallized from MeOH and water to give the title compound (0.078 g, 52%). mp 208–209^{o}C. MS Calcd.: 406; Found: 407 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (6H, t, *J* = 6.9 Hz), 2.22 (3H, s), 3.04 (4H, q, *J* = 6.9 Hz), 3.79 (3H, s), 3.95 (3H, s), 6.03 (1H, br s), 6.76 (1H, d, *J* = 2.4 Hz), 6.79 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 2.4 Hz), 7.03 (1H, d, *J* = 8.4 Hz). Anal. Calcd for $C_{20}H_{24}N_4OCl_2$: C,58.97; H,5.94; N,13.75; Cl,17.41. Found: C,58.84; H,5.88; N,13.82; Cl,17.36.

2-[(4-Chloro-2-methoxy-6-methylphenyl)amino]-7-(diethylamino)-1-methyl-1*H*-benzimidazole-4-carbonitrile 29l A mixture of compound **28h** (0.220 g, 0.837 mmol) and hydrochloride of **32** (0.208 g, 1.00 mmol) in NMP (1 mL) was heated at 130^{o}C for 1 h. The

reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluting with EtOAc/hexane to give the title compound (0.160 g, 48%). mp 185–186°C. MS Calcd.: 397; Found: 398 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (6H, t, *J* = 7.2 Hz), 2.25 (3H, s), 3.14 (4H, q, *J* = 7.2 Hz), 3.81 (3H, s), 3.94 (3H, s), 6.11 (1H, br s), 6.78 (1H, d, *J* = 2.1 Hz), 6.82 (1H, d, *J* = 8.4 Hz), 6.91 (1H, d, *J* = 2.1 Hz), 7.32 (1H, d, *J* = 8.4 Hz). Anal. Calcd for $C_{21}H_{24}ClN_5O \cdot 0.5H_2O$: C, 61.99; H, 6.19; N, 17.21. Found: C, 62.13; H, 6.04; N, 17.08.

***N*^{2}-(4-Chloro-2-methoxy-6-methylphenyl)-*N*^{7},*N*^{7}-diethyl-1,4-dimethyl-1*H*-benzimidazol-2,7-diamine 29m.** 2-Chloro-*N,N*-diethyl-1,4-dimethyl-1*H*-benzimidazol-7-amine **28i**, which was used for the next step without further purification, was prepared from compound **27c** (0.200 g, 0.857 mmol) in a manner similar to that described in compound **28f**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (6H, t, *J* = 6.9 Hz), 2.57 (3H, s), 3.06 (4H, q, *J* = 6.9 Hz), 4.13 (3H, s), 6.99 (2H, s). Compound **29m** (0.119 g, 36%) was prepared from compound **28i** described above in a manner similar to that described in compounds **29k**. mp 200–201°C. MS Calcd.: 386; Found: 387 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.01 (6H, t, *J* = 7.2 Hz), 2.18 (3H, s), 2.44 (3H, s), 3.04 (4H, q, *J* = 7.2 Hz), 3.82 (3H, s), 3.97 (3H, s), 6.01 (1H, br s), 6.78 (1H, d, *J* = 2.4 Hz), 6.77-6.91 (3H, m). Anal. Calcd for $C_{21}H_{27}ClN_4O \cdot 0.5H_2O$: C, 63.71; H, 7.13; N, 14.15. Found: C, 63.85; H, 7.01; N, 14.12.

***N*^{2}-(4-Chloro-2-methoxy-6-methylphenyl)-*N*^{7},*N*^{7}-diethyl-1-methyl-4-phenyl-1*H*-benzimidazole-2,7-diamine 29n.** Compound **29n** (0.058 g, 57%) was prepared from compound **28j** (0.071 g, 0.227 mmol) in a manner similar to that described in compounds **29k**. mp 198–200°C. MS Calcd.: 448; Found: 449 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.06 (6H, t, *J* = 7.2 Hz), 2.28 (3H, s), 3.12 (4H, q, *J* = 7.2 Hz), 3.79 (3H, s), 4.02 (3H, s), 6.07 (1H, br s), 6.74 (1H,

d, J = 2.4 Hz), 6.88 (1H, d, J = 2.4 Hz), 6.96 (1H, d, J = 8.1 Hz), 7.22 (1H, d, J = 8.1 Hz), 7.23–7.39 (3H, m), 7.92–7.94 (2H, m). Anal. Calcd for $C_{26}H_{29}ClN_4O \cdot H_2O$: C, 66.87; H, 6.69; N, 12.00. Found: C, 67.02; H, 6.39; N, 11.88.

N^2-(4-Chloro-2-methoxy-6-methylphenyl)-N^7,N^7-diethyl-4-methoxy-1-methyl-1*H*-benzimidazole-2,7-diamine 29o. Compound **29o** (0.145 g, 45%) was prepared from compound **28k** (0.220 g, 0.803 mmol) in a manner similar to that described in compounds **29k**. mp: 186–188^{o}C. Calcd.: 402; Found: 403 (M+H). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.00 (6H, t, J = 7.2 Hz), 2.16 (3H, s), 3.03 (4H, q, J = 7.2 Hz), 3.80 (3H, s), 3.90 (3H, s), 4.01 (3H, s), 5.87 (1H, br s), 6.54 (1H, d, J = 6.0 Hz), 6.74 (1H, d, J = 1.8 Hz), 6.83 (1H, d, J = 6.0 Hz), 6.84 (1H, d, J = 1.8 Hz). Anal. Calcd for $C_{21}H_{27}N_4O_2Cl$: C, 62.60; H, 6.75; N, 13.91; Cl, 8.80. Found: C, 62.90; H, 6.80; N, 13.89; Cl, 8.77.

Measurement of the corticotropin-releasing factor 1 binding inhibitory rate. A receptor-binding experiment was performed using a human CRF_1 receptor expressing a CHO cellular membrane fraction and ovine CRF, ^{125}I-CRF. Various concentrations of a test compound were incubated with 1 μg of human CRF_1 receptor expressing a CHO cellular membrane fraction and 50 pM of ^{125}I-CRF in a binding assay buffer [50 mM Tris–HCl, 5 mM EDTA, 10 mM $MgCl_2$, 0.05% CHAPS, 0.1% BSA, 0.5 mM PMSF, 0.1 μg/ml pepstatin, and 20 μg/ml leupeptin (pH 7.5)]. In addition, for measuring non-specific binding (NSB), 0.1 μM unlabeled human urocortin was incubated with 1 μg of human CRF_1 receptor expressing a CHO cellular membrane fraction and 50 pM of ^{125}I-CRF in a binding assay buffer. After the binding reaction was performed at rt for 1.5 h, the membrane was entrapped on a glass filter (UniFilter plate GF-C/Perkin Elmer) by suction filtration using a cell harvester (Perkin Elmer) and washed with ice-cold 50 mM Tris–HCl (pH 7.5). After drying the glass filter, a liquid scintillation cocktail (Microscinti; Perkin

Elmer) was added, and the radioactivity of ^{125}I-CRF remaining on the glass filter was measured using Topcount (Perkin Elmer). The percent inhibition was determined by the following equation:

$$\% \text{ inhibition} = (\text{Bound} - \text{NSB})/(\text{TB} - \text{NSB}) \times 100,$$

where Bound is radioactivity when a compound is added, TB is the total binding radioactivity, and NSB is the non-specific binding radioactivity). The IC_{50} values and 95% confidential intervals were calculated using GraphPad Prism software.

CRF_1 antagonistic activity. CRF_1 antagonistic activity was obtained by measuring inhibition of adenylate cyclase using a CRE-luciferase reporter gene assay. Human CRF_1 receptor expressing CHO with a CRE-luciferase gene was inoculated on a 96-well plate at 40,000 cells/well and allowed to grow for 24 h. After cultivation, the culture medium was removed, and the cells were treated with various drug concentrations in 100 μL of assay buffer [20 mM HEPES, Ham F-12, and 0.1% BSA (pH 7.2)] containing 1 nM human CRF for 4 h. After exposure to the test compounds, the cells were lysed, and luciferase activity was measured using a Steady-Glo® Luciferase Assay System (Promega). Light output was detected using an ARVO-SX (Wallac). The IC_{50} values and 95% confidential intervals were calculated using GraphPad Prism software.

ATP Content Assay. HepG2 cells were cultured at 37°C and 5% CO_2 in DMEM supplemented with 10% fetal bovine serum, 50 IU/mL penicillin, and 50 μg/mL streptomycin. The cells were seeded at 2×10^4 cells/well in a 96-well plate and cultured with test compounds in DMEM supplemented with 0.5% fetal bovine serum, 1% L-glutamine, 1% sodium pyruvate, 50 IU/mL penicillin, and 50 μg/mL streptomycin for 1 d. The ATP content was measured using ATPLite™-M (PerkinElmer) according to the manufacturer's instructions. The ATP content (%)

was calculated (n = 3) as 100% of the control (addition of only DMSO).

Solubility. Small volume of compound in DMSO was added to an aqueous buffer. After incubation, precipitates were separated from by filtration through a filter plate. The filtrate was analyzed for a compound in solution by HPLC analysis.

Preparation of brain membrane homogenates. Mice were sacrificed by decapitation, and their brains were rapidly removed and homogenized at 4°C using a Physcotron homogenizer (setting, 10 s) in lysis buffer [50 mM Tris–HCl (pH 7.0), 10 mM $MgCl_2$, 2 mM EDTA, and 100 KU/mL aprotinin]. The frontal cortex was diluted at a final concentration of 5 mg wet tissue/mL by lysis buffer. The olfactory bulb was homogenized in 5 mL of lysis buffer and diluted 1/5 by lysis buffer. The pituitary was homogenized in 2.5 mL of lysis buffer and diluted to a final concentration of 5 mg wet tissue/mL in lysis buffer.

Animals were handled according to the procedures approved by animal experiment ethics committee of Takeda Pharmaceutical Company Ltd.

Drugs. The compounds were suspended in 0.5% methyl cellulose (MC; ShinEtsu) in water and administered orally in a volume of 10 mL/kg for use in the ex vivo binding assay and ACTH test described below.

Ex vivo binding assay in mice. Compound **29g**, **1a,** or the corresponding vehicle was orally administered to mice (5 per group) 1 h before decapitation and organ (frontal cortex, olfactory bulb, and pituitary) removal. The tissues were homogenized in ice-cold lysis buffer using a Physcotron homogenizer and diluted as described above. ^{125}I-CRF (ovine) binding was performed with membrane homogenates in the presence of 100 pM of ^{125}I-CRF (ovine) in lysis buffer containing 0.1% BSA, 0.5% DMSO, and 0.05% CHAPS in a final volume of 200 μL. After incubation at rt for 2 h, the incubation mixture was filtered on a Whatman GF/C filter

presoaked in 0.3% polyethylenimine. The filters were washed six times with ice-cold wash buffer (PBS containing 0.05% CHAPS and 0.01% Triton X-100) and dried. Radioactivity was determined using a gamma scintillation counter. The results were expressed as an inhibitory rate of ^{125}I-CRF (ovine) binding, with in vitro determination of NSB using 1 μM of the selective CRF_1 receptor antagonist **1a**.

ACTH secretion test in mice. The Compounds were administered orally 1 h before the test. Blood was sampled by decapitation 0.5 h after PBS icv administration (5 μL/mouse) and collected into 2-mL Eppendorf tubes. Plasma was separated from whole blood by centrifugation (10 min, 3,000–15,000 rpm at 4°C) and stored in 1.5-mL Eppendorf tubes at 20°C until measurement. Plasma ACTH concentration was measured using a commercially available immunoradiometric assay kit (ACTH: Mitsubishi Chemical Medience Corporation, Tokyo, Lot No. A521).

Statistical Analysis. Data and statistical analysis were performed using the computer programs Microsoft Excel and Preclinical C Package (PCP).. All results are presented as the mean ± SEM. Statistical analyses of two-group comparisons of independent samples were performed using Student's t-test or Welch's test, and statistical significance was accepted at $p < 0.05$. To examine the dose responses of CRF or compounds, statistical analyses were performed using the Williams test or Shirley–Williams test. Statistical significance was accepted at $p < 0.025$. To compare the effects between different compounds, statistical analyses were performed using the Welch test. Statistical significance was accepted at $p < 0.05$.

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ACKNOWLEDGMENTS

The authors thank Dr. Takanobu Kuroita for helpful discussions during the preparation of the manuscript. The authors also thank Mr. Ken-ichi Kuroshima and Mr. Yuuji Shimizu for performing in vitro CRF_1 receptor binding assays, human CRF-stimulated cAMP accumulation assays, and reviewing the data and Mr. Kiyoshi Noguchi for his assistance with the cytotoxicity testing. The authors extend gratitude to Dr. Timothy Turner, Dr. Steve Boyd, Dr. Christopher Siedem, and Dr. Kevin Condroski for their valuable discussions on structural modification.

ABBREVIATIONS

$MPBH_3CN$, macroporous polystyrene-bound cyanoborohydride; $PSBH_3CN$, polymer supported cyanoborohydride; DIC, *N,N'*-diisopropylcarbodiimide; CDI, *N,N'*-carbodiimiddazole; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; PMSF, phenylmethylsulfonyl fluoride; HEPES, 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; DMEM, Dulbecco's Modified Eagle's Medium.

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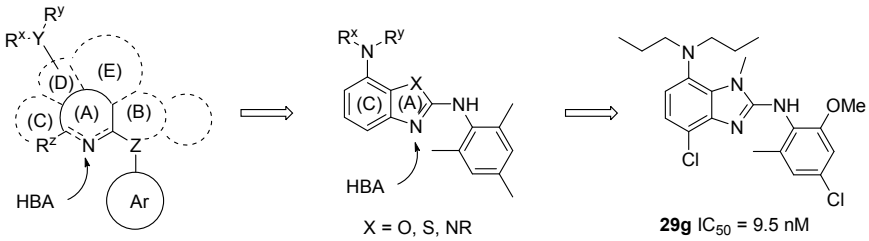
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