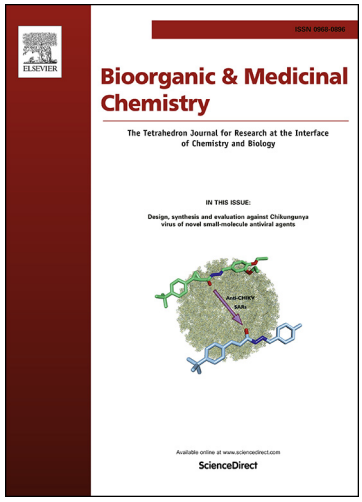
Accepted Manuscript



Discovery of 1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazoles as novel class of corticotropin releasing factor 1 receptor antagonists

Takuto Kojima, Michiyo Mochizuki, Takafumi Takai, Yasutaka Hoashi, Sachie Morimoto, Masaki Seto, Minoru Nakamura, Katsumi Kobayashi, Yuu Sako, Maiko Tanaka, Naoyuki Kanzaki, Yohei Kosugi, Takahiko Yano, Kazuyoshi Aso

PII: S0968-0896(17)32337-4
DOI: https://doi.org/10.1016/j.bmc.2018.01.020
Reference: BMC 14174

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 26 December 2017
Revised Date: 22 January 2018
Accepted Date: 24 January 2018

Please cite this article as: Kojima, T., Mochizuki, M., Takai, T., Hoashi, Y., Morimoto, S., Seto, M., Nakamura, M., Kobayashi, K., Sako, Y., Tanaka, M., Kanzaki, N., Kosugi, Y., Yano, T., Aso, K., Discovery of 1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazoles as novel class of corticotropin releasing factor 1 receptor antagonists, *Bioorganic & Medicinal Chemistry* (2018), doi: https://doi.org/10.1016/j.bmc.2018.01.020

Discovery of 1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazoles as novel class of corticotropin releasing factor 1 receptor antagonists

Takuto Kojima,* Michiyo Mochizuki, Takafumi Takai, Yasutaka Hoashi, Sachie Morimoto, Masaki Seto, Minoru Nakamura, Katsumi Kobayashi,[a] Yuu Sako, Maiko Tanaka, Naoyuki Kanzaki,[b] Yohei Kosugi, Takahiko Yano,[c] and Kazuyoshi Aso[d]

Research Division, Takeda Pharmaceutical Company Ltd., 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan

* Corresponding author
Tel: +81 466 32 1116; Fax: +81 466 29 4449; E-mail: takuto.kojima@takeda.com (T. Kojima)

[a]Present address: *ChromaJean Ltd. 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan*

[b]Present address: *Seedsupply Ltd. 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan*

[c]Present address: *Taisho Pharmaceutical Company Ltd. Taisho Pharmaceutical Company Ltd., 403, Yoshino-cho 1-chome, Kita-ku, Saitama-shi, Saitama 331-9530, Japan*

[d]Present address: *Axcelead Drug Discovery Partners, Inc. 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan*

Abstract

A new class of corticotropin releasing factor 1 (CRF_1) receptor antagonists characterized by a tricyclic core ring was designed and synthesized. Novel tricyclic derivatives **2a–e** were designed as CRF_1 receptor antagonists based on conformation analysis of our original 2-anilinobenzimidazole CRF_1 receptor antagonist. The synthesized tricyclic derivatives **2a–e** showed CRF_1 receptor binding activity with IC_{50} values of less than 400 nM, and the 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole derivative **2e** was selected as a lead compound with potent in vitro CRF_1 receptor binding activity (IC_{50} = 7.1 nM). To optimize the pharmacokinetic profiles of lead compound **2e**, we explored suitable substituents on the 1-position and 6-position, leading to the identification of compound **42c-*R***, which exhibited potent CRF_1 receptor binding activity (IC_{50} = 58 nM) with good oral bioavailability (F = 68% in rats). Compound **42c-*R*** exhibited dose-dependent inhibition of [^{125}I]-CRF binding in the frontal cortex (5 and 10 mg/kg, p.o.) as well as suppression of locomotor activation induced by intracerebroventricular administration of CRF in rats (10 mg/kg, p.o.). These results suggest that compound **42c-*R*** successfully binds CRF_1 receptors in the brain and exhibits the potential to be further examined for clinical studies.

Key words: CRF_1 receptor antagonists, 1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole, tricyclic ring system, stress-related disorders

Introduction

Corticotropin releasing factor (CRF), a 41-amino acid neuropeptide secreted in the hypothalamic paraventricular nucleus,[1] acts as a primary regulator of the hypothalamus-pituitary-adrenocortical axis and stimulates the secretion of adrenocorticotropic hormone from the pituitary.[2,3] CRF exerts its physiological functions via three subtypes (CRF_1, $CRF_{2\alpha}$, and $CRF_{2\beta}$) belonging to the class B subfamily of Gs-coupled G-protein coupled receptors.[4-6] CRF_1 receptor knockout mice exhibit decreased anxiety-like behavior and impaired stress responses.[7] Furthermore, intracerebroventricular (i.c.v.) administration of CRF induces anxiety-like behavior in rats, while the behavioral responses to CRF can be reversed by CRF_1 receptor antagonists.[8,9] These findings indicate that CRF_1 receptor antagonists are promising targets for stress-related disorders.

Small-molecule CRF_1 receptor antagonists, shown in Figure 1, have been evaluated in several clinical studies in patients with anxiety, depression, irritable bowel syndrome, post-traumatic stress disorder, and alcohol dependence;[10-16] however, these studies were not conclusive in defining the clinical utility of CRF_1 receptor antagonists. We hypothesized that these ambiguous results are attributable to the properties of the new investigational drugs rather than the mechanism of action. The reported CRF_1 receptor antagonists displayed in Figure 1 show higher cLogP values (3.4–8.2). We planned to optimize our compounds with considering logD which would be a key parameter not only for lipophilicity of the molecule but also for improvement in absorption, distribution, metabolism, and elimination (ADME) profiles.[17] Therefore, identification of novel drug candidates with potent binding activity and desirable ADME properties is still an attractive area of drug discovery research. In this article, we describe the design and synthesis of a new class of CRF_1 receptor antagonists characterized by a tricyclic core ring, as well as its optimization toward acquiring drug-like properties.

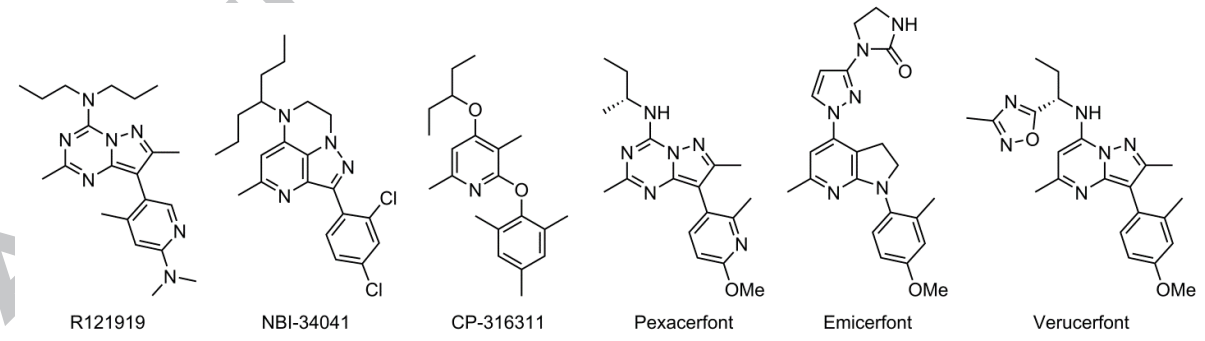


Figure 1. Reported CRF_1 receptor antagonists.

We previously reported the discovery of 2-anilinobenzimidazole derivatives, which have a unique structure among the numerous reported CRF_1 receptor antagonists. These benzimidazole CRF_1 receptor antagonists are

characterized by a nitrogen linker between the benzimidazole core and the pendant aryl group, which allows flexibility of the pendant aryl group. In contrast, most reported compounds contain a rigid pendant aryl moiety. The representative compound **1a** demonstrated potent CRF_1 receptor binding activities in vitro and in vivo.[18] In the benzimidazole series, there was a remarkable difference in the binding activity between compound **1b**, a 2,4,6-trisubstituted phenyl derivative (IC_{50} = 18 nM), and that of compound **1c**, a 2,4-disubstituted phenyl derivative (IC_{50} = 200 nM) (Figure 2). To understand the reason for the difference in binding activity, we examined the dihedral angle φ as a key parameter controlling torsional conformation between the benzimidazole core and the pendant aryl group (Figure 2(B). The calculated dihedral angle φ[19] of compound **1b**, with potent binding activity, exhibited an appropriate torsional angle (φ = 45), while compound **1c**, with a moderate binding activity, had a nearly planar relation between the benzimidazole core and the pendant aryl group (φ = 3). The relationship between binding activity and dihedral angle φ indicates that an active conformation of the benzimidazole derivatives requires a certain range in dihedral angle. Further, the 2,4,6-trisubstituted phenyl group induces enough steric repulsion of ortho-substituents to maintain the torsional active conformation. Following this rationale, we expected that stabilizing the torsional conformation of benzimidazole derivatives could lead to a novel chemotype. Since we expected that ring-closing at the 1-position and 2-position of the benzimidazole derivative could fix the restrained conformation by inducing a rotation barrier, we designed compound **2a**, a novel tricyclic, which predictably caused a torsional conformation with a dihedral angle (φ) of 48 degrees between the tricyclic core and the 2,4-dichlorophenyl group to exhibit potent binding activity with the CRF_1 receptor.

As described above, the dihedral angle φ is a key parameter for determining binding activity. Furthermore, we examined the electron density (*E* value[20]) of the hydrogen bond acceptor (HBA) nitrogen atom because its importance has been reported in the general pharmacophore.[21] Based on these considerations, we designed four tricyclic derivatives, **2a–d**, which stabilized the torsional conformation ($46 < \varphi < 83$), with the various electron densities (*E*). Compounds **2c** and **2d,** with a C1 sp^3 carbon instead of a sp^2 nitrogen, were designed to investigate the diversity of a ring system. During this work, we explored a novel class of CRF_1 receptor antagonists, focusing on the torsional conformation (φ value) and electron density (*E* value) of the HBA nitrogen.

During the scaffold hopping process, target compounds without a substituent at the 9-position were designed, attributable to chemical accessibility; the chlorine atom was important for the binding activity as previously described for benzimidazole CRF_1 antagonists.[18] In addition, substituents at the 1- and 6-position were also fixed by a 2,4-dichlorophenyl and diethylamino group, respectively. After selecting the best ring system, we optimized the 1- and 6-position with a chlorine atom at the 9-position. Our optimization strategy included decreasing the lipophilicity through pursuing polar and metabolically stable substituents at the 1- and 6-position, while maintaining potent binding activity.

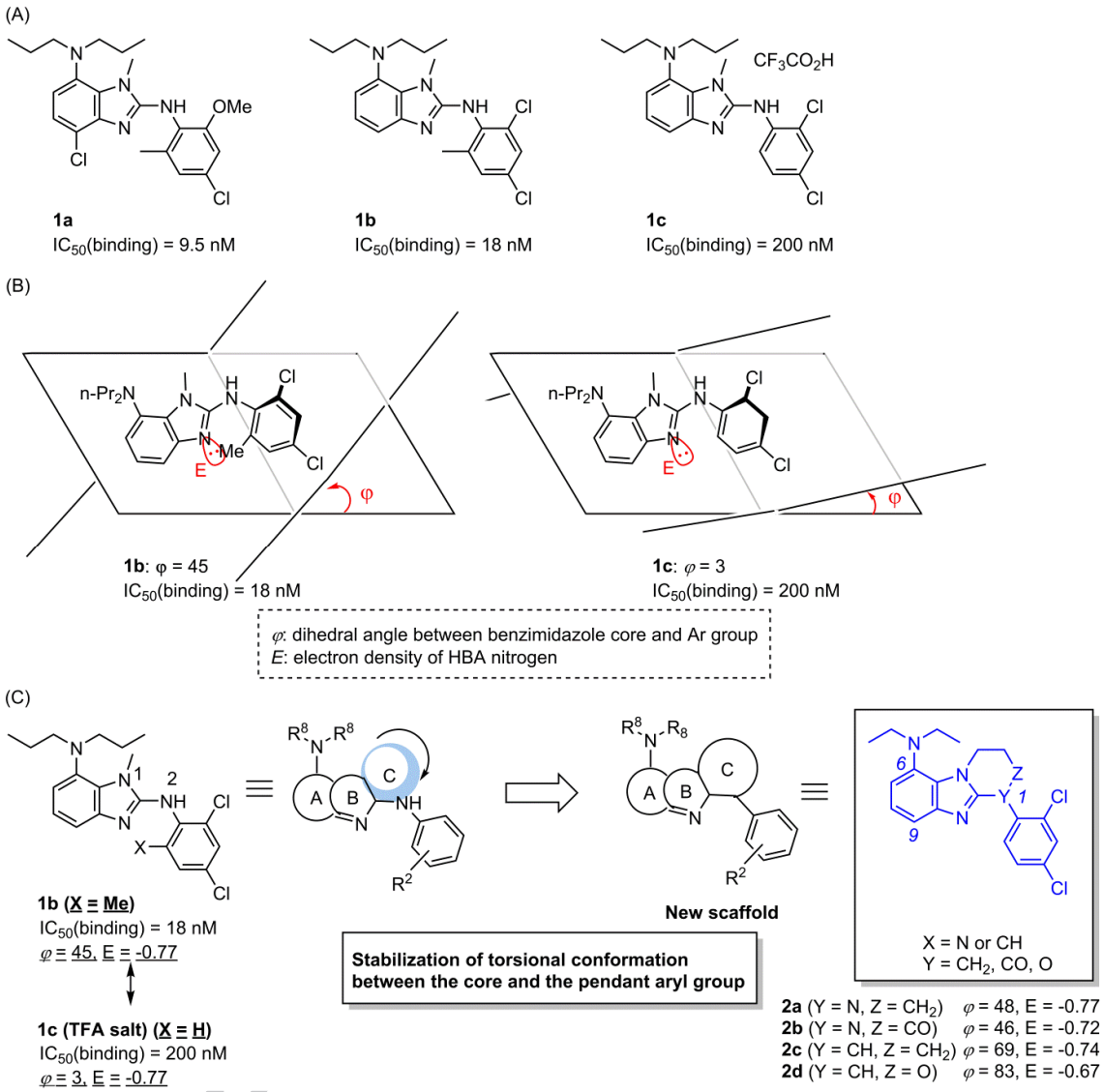


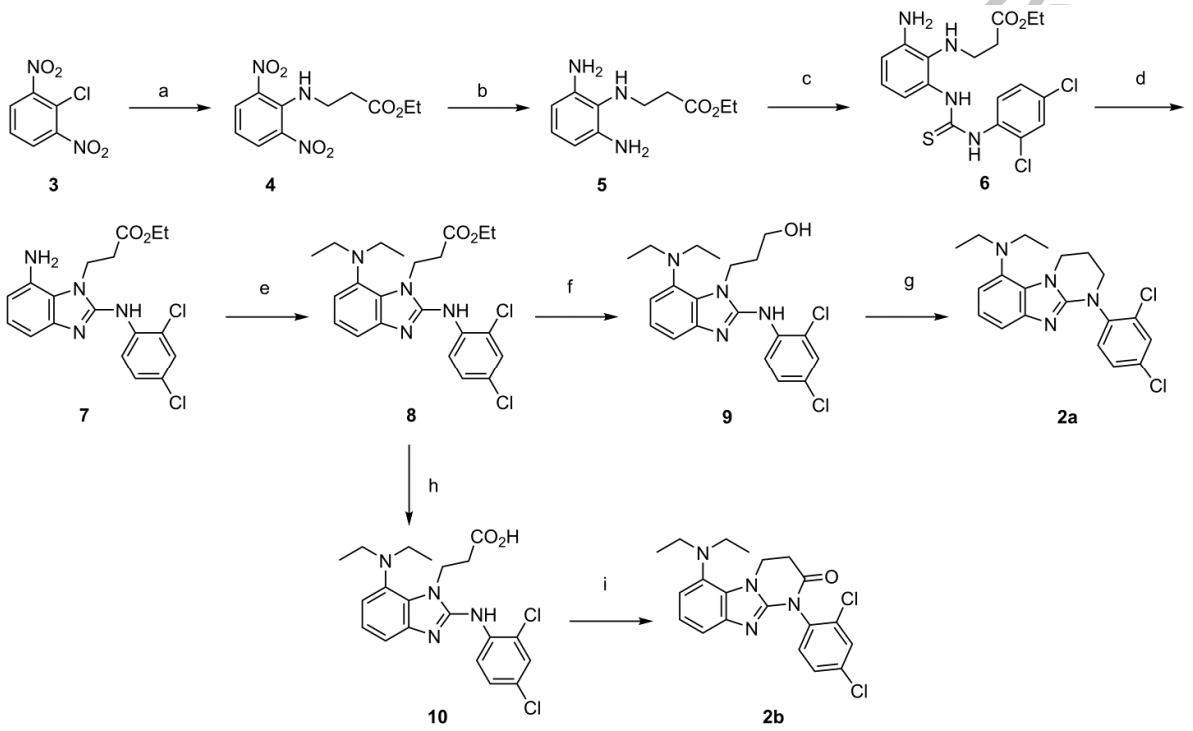
Figure 2. (A) Representative benzimidazole derivative for compounds **1a–c** and (B) φ value indicating the dihedral angle between planes, including the benzimidazole core and a plane with the pendant aryl ring. *E* values show electron density of the hydrogen bond acceptor (HBA) nitrogen. (C) Design of the novel tricyclic ring system **2a–d** to stabilize torsional conformation.

Chemistry

Synthesis of the newly designed tricyclic compounds **2a** and **2b** is described in Scheme 1. Amination of 2-chloro-1,3-dinitrobenzene **3** with β-alanine ethyl ester hydrochloride and the subsequent hydrogenation of nitro groups of compound **4** afforded triaminophenyl derivative **5**. Thiourea derivative **6** was prepared by reacting compound **5** with phenylisothiocyanate, and then benzimidazole ring was constructed by intramolecular cyclization in the presence of 1-(3-dimethylaminopropyl)3-ethylcarbodimide (EDCI). Sequential reductive alkylation of compound **7** with acetaldehyde provided a diethylaminobenzimidazole **8**, which was converted to hydroxypropylbenzimidazole derivative

9 via reduction of the ester group with lithium borohydride. After mesylation of the hydroxyl group of compound **9**, intramolecular cyclization afforded the desired 1,2,3,4-tetrahydropyrimido-[1,2-a]benzimidazole derivative **2a**. The 3,4-dihydropyrimido[1,2-a]benzimidazol-2(1H)-one derivative **2b** was synthesized by hydrolysis of the ester **8** and subsequent intramolecular amidation.

Scheme 1. Synthesis of 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole derivative **2a** and 3,4-dihydropyrimido[1,2-*a*]benzimidazol-2(1*H*)-one derivative **2b**[a]

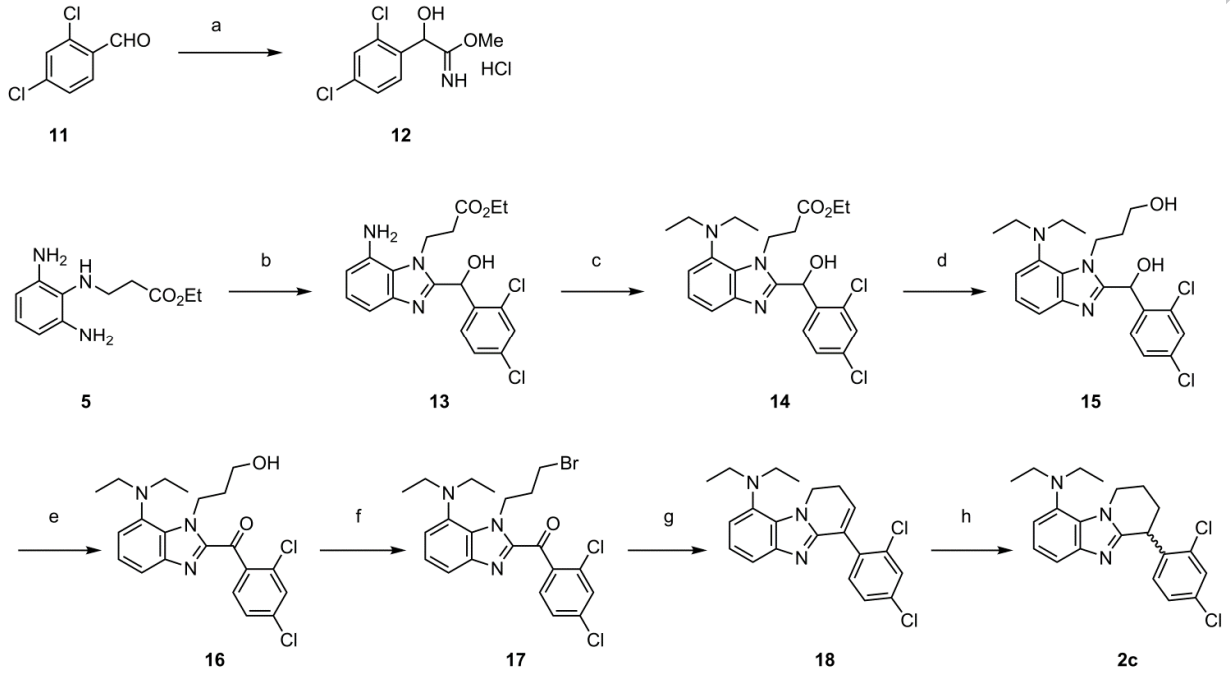


[a]Reagents and conditions: (a) β-alanine ethylester hydrochloride, Et_3N, THF, rt, quant; (b) H_2, Pd/C, THF, rt, 91%; (c) 2,4-dichlorophenyl isothiocyanate, THF, rt, 56%; (d) EDCI, THF, 50 °C, 83%; (e) MeCHO, $NaBH(OAc)_3$, AcOH, THF, 0 °C to rt, quant; (f) $LiBH_4$, THF, 0 °C to rt, 25%; (g) MsCl, pyridine, 0 °C to rt, 86%; (h) NaOH, H_2O, MeOH, THF, 0 °C to rt, 78% (i) EDCI, Et_3N, DMF, rt, 82%.

Synthesis of a C-linked 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole derivative **2c** started from triaminophenyl derivative **5** (Scheme 2). Condensation of compound **5** with methyl 2-(2,4-dichlorophenyl)-2-hydroxyethanimidate hydrochloride **12**, which was easily prepared from 2,4-dichlorobenzaldehyde **11**, directly afforded benzimidazole derivative **13**. Reductive alkylation of the 7-amino group of compound **13**, followed by reduction of the ester group with lithium aluminum hydride provided a diol derivative **15**. Selective oxidation of the secondary alcohol of compound **15** using manganese dioxide afforded 1-(3-hydroxypropyl)bezimidazole **16**, which was converted to bromide **17** by reaction with triphenylphosphine and

tetrabromomethane. After preparation of ylide intermediate from bromide **17**, intramolecular Wittig reaction under basic condition constructed 1,2-dihydropyrido[1,2-*a*]benzimidazole **18**, and following hydrogenation gave the desired 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole derivative **2c**.

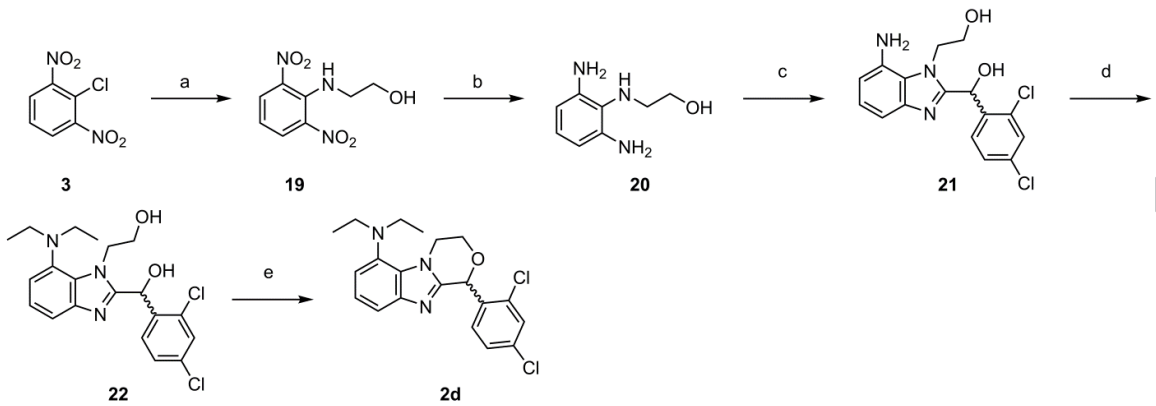
Scheme 2. Synthesis of 1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazole derivative **2c**[a]



[a]Reagents and conditions: (a) (i) TMSCN, DMAP, MeCN, rt; (ii) AcCl, MeOH, 0 °C to rt, 90% in 2 steps. (b) **12**, EtOH, rt, 90%; (c) MeCHO, $NaBH(OAc)_3$, AcOH, THF, 0 °C, 92%; (d) $LiAlH_4$, THF, -5 °C, 78%; (e) MnO_2, THF, rt, 99%; (f) PPh_3, CBr_4, MeCN, 0 °C, 88%; (g) PPh_3, MeCN, 80 °C then KO*t*-Bu, tolene, THF, rt, 23% in 2 steps; (h) H_2, Pd/C, MeOH, rt, 47%.

Synthesis of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivative **2d** is shown in Scheme 3. Amination of 2-chloro-1,3-dinitrobenzene **3** with ethanolamine, and the subsequent reduction of nitro groups of compound **19** gave 2-[(2,6-diaminophenyl)amino]ethanol derivative **20**. After key intermediate **21** was prepared by condensation of the compound **20** with the amidate **12**, the following reductive alkylation provided compound **22**. Intramolecular cyclization of diol **22** under Mitsunobu reaction condition afforded the desired 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivative **2d**.

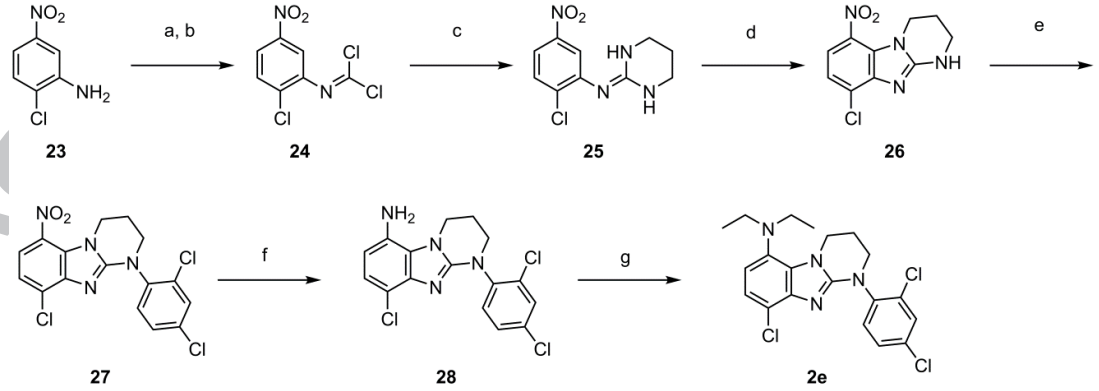
Scheme 3. Synthesis of 3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazole derivative **2d**[a]



[a]Reagents and conditions: (a) ethanol amine, Et_3N, THF, rt, 99%; (b) H_2, Pd/C, rt, THF, 89%; (c) **12**, EtOH, rt, 99%; (d) MeCHO, $NaBH(OAc)_3$, AcOH, THF, 0 °C, 99%; (e) PPh_3, DEAD, THF, rt, 59%.

Since 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole **2a** was identified as the best ring system among the designed compound **2a–d**, we subsequently introduced a chlorine atom on the 9-position. Synthesis of 9-chloro substituted 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole derivative **2e** is shown in Scheme 4. Condensation of 2-chloro-5-nitroaniline **23** with formic acid, and following chlorination under mixture of sulfuryl chloride and thionyl chloride provided dichloride derivative **24**, which was treated with 1,3-propandiamine to yield guanidine derivative **25**. Intramolecular cyclization of the compound **25** in the presence of potassium *tert*-butoxide in dimethylsulfoxide successfully afforded tricyclic derivative **26**. Arylation of amine **26** with 1,3-dichloro-4-iodobenzene gave the desired compound **27**. Reduction of the nitro group of compound **27** gave aniline derivative **28**, which was converted to the target compound **2e** by reductive alkylation.

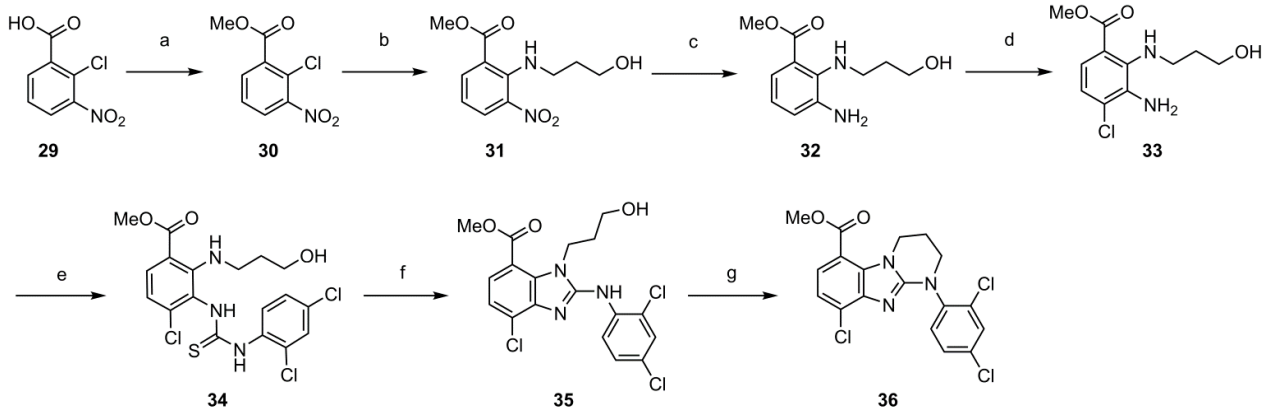
Scheme 4. Synthesis of 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole derivative with Cl atom on the 4-position **2e**[a]



[a]Reagents and conditions: (a) HCO_2H, rt; (b) SO_2Cl_2, $SOCl_2$, 55 °C, 67% in 2 steps; (c) 1,3-propandiamine, THF, 5-10 °C, 98%; (d) KO*t*-Bu, DMSO, 60 °C, 73%; (e) CuI, 2,2'-bipyridyl, 1,3-dichloro- 4-iodobenzene, 150 °C, 5%; (f) H_2, Pd/C, AcOH, rt, 45%; (g) MeCHO, $NaBH(OAc)_3$, AcOH, MeOH, 0 °C to rt, 98%.

Next, we focused on the optimization of the new lead 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole **2e** with chlorine atom on the 9-position. The synthesis of key intermediate **36** for derivative synthesis is illustrated in Scheme 5. Methylation of 2-chloro-3-nitrobenzoic acid **16**, substituent reaction with 3-hydroxylpropylamine, and reduction of the nitro group yielded compound **32**. Sequential chlorination by NCS gave tetrasubstituted benzene derivative **33** regioselectively. The reaction of compound **33** with 2,4-dichlorothioisocyanate yielded thiourea **34**, which was treated with water soluble condensing agent (WSC) to provide benzimidazole derivative **35**. Finally, the tricyclic structure was constructed by mesylation of the alcohol of the compound **35** and successive intramolecular cyclization under basic condition to afford the key intermediate **36**.

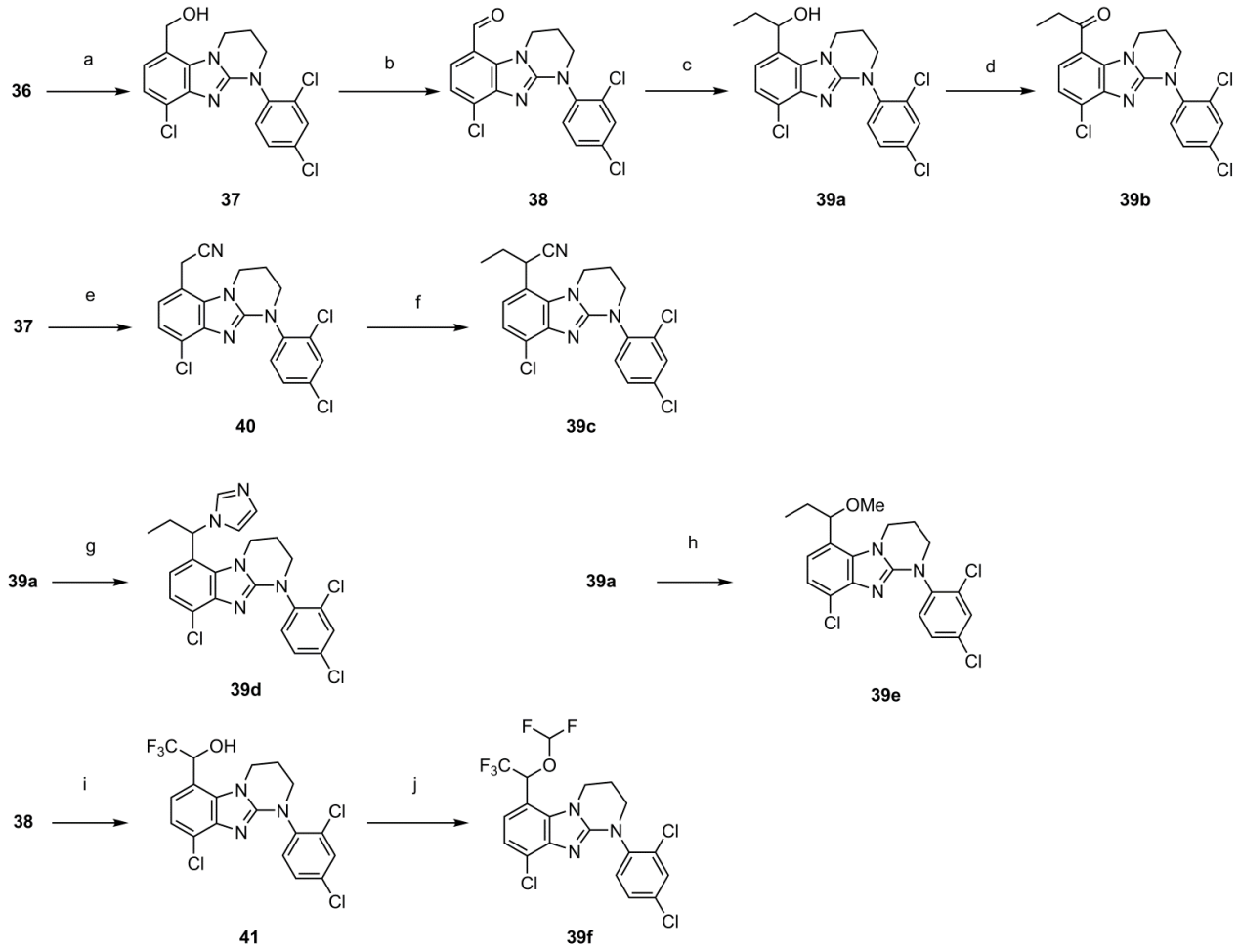
Scheme 5. Synthesis of the key intermediate **36**[a]



[a]Reagents and conditions: (a) $(COCl)_2$, THF, 0 °C, then MeOH, rt, 96%; (b) 3-hydroxypropyllamine, NEt_3, THF, 50 °C, 74%; (c) Pd/C, H_2, rt, THF, 78%; (d) NCS, CH_3CN, rt, 16%; (e) 2,4-dichloro-1-isothiocyanatobenzene, THF, rt; (f) WSC, NEt_3, THF, 50 °C, 71% in 2 steps; (g) 1) MsCl, pyridine, THF, 0 °C; 2) K_2CO_3, DMF, 70 °C, 87% in 2 steps.

Synthesis of compounds with variety of substituents at the 6-position is shown in Scheme 6. Reduction of ester of compound **36** by lithium borohydride was followed by oxidation of the alcohol by SO_3-pyridine to give aldehyde derivative **38**. Addition of ethylmagnesium bromide to aldehyde **38** provided 1-propanol derivative **39a**, which was converted to ketone derivative **39b** by oxidation with Dess-Martin reagent. After chlorination of compound **24** with $SOCl_2$ and successive substitution reaction by potassium cyanide to give compound **40**, treatment of ethyl iodide in the presence of potassium *tert*-butoxide afforded compound **39c**. Compound **39a** was converted to compound **39d** by the reaction with 1,1'-carbonyldiimidazole (CDI) to install imidazole and also converted to compound **39e** by methylation with iodomethane. Addition of trifluoromethyl anion generated by (trimethylsilyl)trifuluoromethane and tri*n*-butylammonium fluoride (TBAF) to aldehyde derivative **38** provided 2,2,2-trifluoroethaol derivative **41**, which was converted to compound **39f** by alkylation with difluoromehyl chloride gas under basic condition.

Scheme 6. Synthesis 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole analogs with various substituents at the 6-position [a]

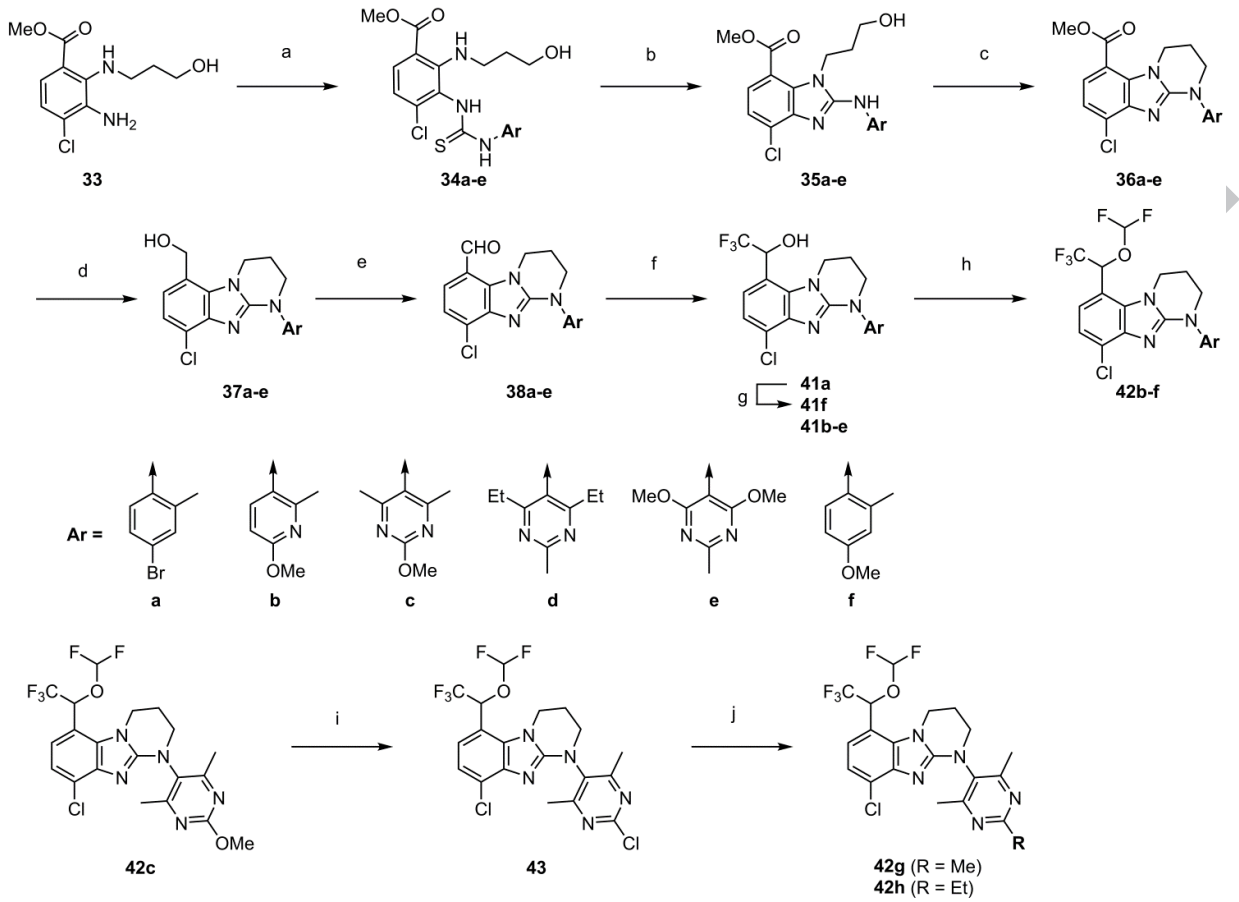


[a]Reagents and conditions: (a) $LiBH_4$, THF, rt, 89%; (b) SO_3-py, NEt_3, DMSO, CH_2Cl_2, rt, 82%; (c) EtMgBr, THF, 0 °C, 92%; (d) Dess-Martin reagent, CH_3CN, DMSO, 0 °C to rt, 88%; (e) 1) $SOCl_2$, pyridine, THF, 0 °C to rt; 2) KCN, DMSO, H_2O, rt, 96% in 2 steps; (f) KO*t*-Bu, EtI, THF, 0 °C, 38%; (g) CDI, THF, 50 °C, 94%; (h) NaH, MeI, DMF, rt°C, 76%; (i) $TMSCF_3$, TBAF, THF then HCl aq., 0 °C, 99%; (j) CHF_2Cl, $BnEt_3N$, THF, NaOH aq., 50 °C, 44%.

Synthesis of the compounds with variety of substituents on the 1-position are shown in Scheme 7. Starting from the compound **33**, 2,2,2-trifluoroethaol derivative **41a–e** were obtained by the same procedure described in Scheme 5 and 6. The compound **41a** with 4-bromo-2-methylphenyl group at 1-position was converted to compound **41f** by Ullmann reaction to replace bromo atom with methoxy group. Then, 2,2,2-trifluoroethaol derivative **41b–f** were treated with difluoromehyl chloride gas to synthesize the target compounds **42b–f**.

Scheme 7. Synthesis 1,2,3,4-tetrahydropyrimido-[1,2-*a*]benzimidazole analogs with various substituents at the 1-position

a



[a]Reagents and conditions: (a) ArNCS, THF, 65–70 °C; (b) WSC, NEt_3, THF, 50–60 °C, 6-64% in 2 steps; (c) 1) MsCl, NEt_3, THF, 0 °C to rt; 2) K_2CO_3, DMF, 70–80 °C, 76–90%; (d) $LiBH_4$, THF, 0 °C to 40–50 °C, 98–quant; (e) Dess-Martin reagent, DMSO, CH_3CN, 0 °C-rt, 79–93%; (f) $TMSCF_3$, TBAF, THF, 0 °C, then HCl, 0 °C-rt, 61–96%; (g) NaOMe, CuI, DMF, 100 °C, 94%; (h) CHF_2Cl, $BnEt_3NCl$, THF, NaOH aq., rt to 50 °C, 30–51%; (i) $POCl_3$, 100 °C, 42%; (j) for **42g**, MeMgBr, $NiCl_2$(dppp), THF, 0 °C to rt, 32%; for **42h**, EtMgBr, $NiCl_2$(dppp), THF, 0 °C to rt, 9%.

Results and discussion

Novel tricyclic compounds with a torsional conformation between the core ring and the pendant aryl group were synthesized and evaluated for their inhibitory activity against the binding of ovine [^{125}I]-CRF to human CRF_1 receptors expressed on Chinese hamster ovary (CHO) cellular membrane.

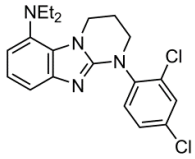
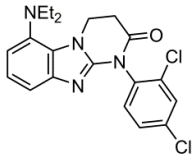
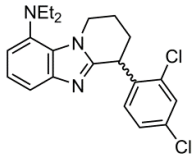
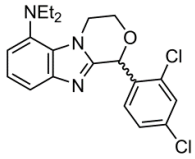
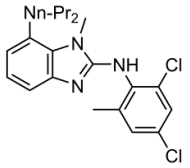
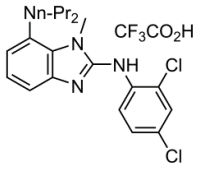
The relationship between binding activity and each parameter is discussed below. The most active tetrahydropyrimidobenzimidazole derivative **2a** (IC_{50} = 11 nM) was as potent as the benzimidazole derivative **1b** with a 2,4,6-trisubstituted phenyl group (IC_{50} = 18 nM), even though compound **2a** had a 2,4-disubstituted phenyl group. Comparisons between the tetrahydropyrimidobenzimidazole derivative **2a** and the benzimidazole derivative **1c** with a 2,4-disubstituted phenyl group provided useful information regarding the relationship between torsional angle and

binding activity. The binding activity of compound **2**a (IC_{50} = 11 nM) was 20-fold more potent than that of compound **1c** (IC_{50} = 200 nM). *E* values of compound **2a** (*E* = -0.77) and compound **1c** (*E* = -0.77) were equivalent, while there was large difference in the dihedral angles between compound **2a** (φ = 48) and compound **1c** (φ = 3). These data indicate that a torsional conformation between the core and pendant aryl group is better than a planar conformation to afford compounds exhibiting potent binding activity with similar *E* value ranges.

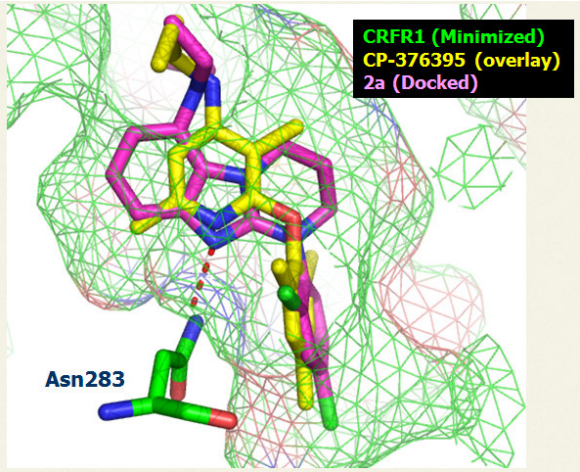
The dihydropyrimidonebenzimidazole derivative **2b** (IC_{50} = 39 nM) was 4-fold less active than compound **2a** (IC_{50} = 11 nM), while compound **2b** possessed similar φ value (φ = 46) to that of compound **2a** (φ = 48). This is likely attributable to the decreased electron density of the HBA by an inductive effect of the carbonyl group (**2b**; *E* = -0.72, **2a**; *E* = -0.77), which consequently resulted in the binding activity of **2b** being lower than that of compound **2a**. Compounds **2c** and **2d**, with a C1 sp^3 benzyl carbon instead of a sp^2 nitrogen, were synthesized and evaluated as racemate. The pendant aryl group conformation of compounds **2c** and **2d** was nearly orthogonal because their dihedral angles were more than 69 degrees. However, the tetrahydropyridobenzimidazole derivative **2c** (IC_{50} = 36 nM) was as potent as compound **2b** (IC_{50} = 39 nM). Since the electron density of compounds **2b** and **2c** was nearly equivalent (**2b**; E = -0.72, **2c**; E = -0.74), differences in their dihedral angles (**2b**; φ = 46, **2c**; φ = 69) did not impact binding activity. In other words, only torsional conformation was required to critically impact binding activity, and there were no differences between torsional conformation and orthogonal conformation. The dihydrooxazinobenzimidazole derivative **2d** (IC_{50} = 270 nM) had a 24-fold lower binding activity than that of compound **2a**. We attributed this marked reduction in the binding activity of compound **2d** to the low electron density of its HBA nitrogen atom (**2d**; E = -0.67), although the dihedral angle appeared to be sufficient. These results indicate that both the torsional conformation and electron density of the HBA nitrogen atom affect binding activity.

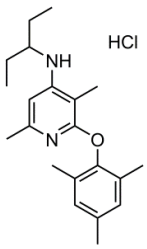
To confirm our hypothesis, a docking study was conducted using GOLD software and MOE[22] based on the reported crystal structure of the CRF_1 receptor with CP-376395.[23] The dihedral angle of compound **2a** in the model was 74 degrees, revealing that the torsional conformation was required for compounds to fit in the receptor pocket. In addition, the electron density of the HBA nitrogen atom impacted binding activity, attributable to the HBA nitrogen of compound **2a** in the model having an obvious interaction with Asn283 of the receptor. This model also showed that compounds **2c** and **2d,** with a C1 sp^3 benzyl carbon, had decreased activity, attributable to a loss of energy resulting from folding the pendant aryl group into a suitable position from their most stable conformation, as well as a decrease in activity of the HBA nitrogen atom. These analyses matched and supported our hypothesis and results.

Table 1. $hCRF_1$ receptor binding activities of novel tricyclic ring derivatives

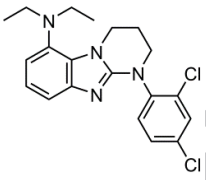
compound	structure[a]	binding[b] (IC_{50}, nM)	φ	*E*
2a		11 (5.2–23)	48	-0.77
2b		39 (26–58)	46	-0.72
2c		36 (32–40)	69	-0.74
2d		270 (200–370)	83	-0.67
1b		18 (13–25)	57	-0.77
1c		200 (150–280)	3	-0.77

[a]Ar is 2,4-dichlorophenyl group. [b]IC_{50} values and 95% confidential intervals were calculated from the concentration-response curve (n = 1).





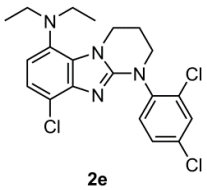
CP-376395 (yellow)



2a (magenta)

Figure 3. Docking pose for compound **2a** (magenta) in the CRF_1 receptor; its crystal structure was identified with CP376395 (yellow).[23] This docking study indicates that the torsional conformation was necessary to occupy the binding pocket in a suitable manner and the HBA nitrogen on the tricyclic ring clearly interacted with Asn283.

Eventually, our efforts to explore novel tricyclic ring systems resulted in identification of pyrimidobenzimidazole derivative **2a**, as the best scaffold to show the most potent binding activity among the designed compounds **2a–2d**. Our previous study indicated that the introduction of a small substituent, especially a chlorine atom, enhances the activity.[18] Further, the docking model suggested the presence of a small space around the 9-position. Therefore, we introduced a chlorine atom to the 9-position to identify compound **2e** with the most potent binding activity (IC_{50} = 7.1 nM). Compound **2e** also inhibited human CRF-stimulated cAMP accumulation with an IC_{50} value of 4.4 nM in CHO cells expressing $hCRF_1$ receptors, indicating that it was a promising lead compound for further optimization.



2e

binding[a] (IC_{50}, nM)	$logD_{pH7.4}$	solubility[b] (μg/mL)	metabolic stability[c] (human, rat, μL/min/mg)	F(rats)[d] (%)
7.1 (3.6–14)	5.25	<0.06	184, 182	4.1

Figure 4. Profiles of compound **2e**.

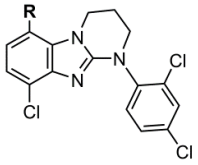
[a]IC_{50} values and 95% confidential intervals were calculated from the concentration-response curve (n = 1). [b]10 mmol/L solution of the compound in DMSO was evaluated using JP2 buffer (pH 6.8), as described in the Japanese Pharmacopoeia. [c]Metabolic stability was determined based on the disappearance of parent compound after incubation with human or rat liver microsmes for 20 min. [d]Results from the rat cassette dosing test (mean, n = 3, 0.1 mg/kg, i.v., 1

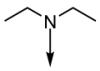
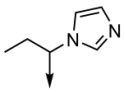
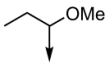
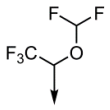
mg/kg, p.o.) are shown.

Although lead compound **2e** exhibited potent binding activity (IC_{50} = 7.1 nM), its bioavailability (BA) was poor (cassette dosing in rats, BA = 4.1%, 1 mg/kg, p.o. and 0.1 mg/kg, i.v.), attributable to low metabolic stability and low solubility resulting from its high lipophilicity (cLogP = 8.1, $logD_{pH7.4}$ = 5.25). Therefore, we aimed to reduce lipophilicity by replacing the diethylamino group on the 6-position and the 2,4-dichlorophenyl group on the 1-position with polar substituents. Since it was expected that the diethylamino group would be easily metabolized, we further explored which substituents provided resistance against metabolism.

First, we explored substituents at the 6-position, which was expected to maintain binding activity and reduce lipophilicity. The synthesized compounds, along with binding activities, logD measured at pH 7.4, and in vitro metabolic clearance in human hepatic microsomes are shown in Table 2. According to the docking model and our previous structure-activity relationship (SAR) studies of the benzimidazole derivatives,[18] we intensively designed compounds with a 2-forked chain structure at the 6-position. IC_{50} values in the order of 10^{-8} M were maintained after introducing a hydroxyl group (**39a**, IC_{50} = 89 nM), cyano group (**39c**, IC_{50} = 66 nM), and methoxy group (**39e**, IC_{50} = 20 nM), while the introduction of an oxo group (**39b**, IC_{50} = 210 nM) and imidazolyl group (**39d**, IC_{50} = 130 nM) resulted in the binding activity being 18–30 fold lower than that of compound **2e**. Compound **39f**, a 2,2,2-trifluoroethyl-1-difluoromethoxy derivative (IC_{50} = 50 nM) that was designed to complement the metabolic stability of compound **39e**, exhibited potent binding activity with excellent metabolic stability. Compound **39f** was more stable in human hepatic microsomes than compound **39d**, which possessed the lowest logD among test compounds **39a–39f**. These data suggest that drastic improvements in the metabolic stability of compound **39f** were dependent on decreasing logD values as well as blockage of the metabolic site by fluoride atoms. While modifying the 6-position, decreasing the logD by installing several polar substituents tended to improve metabolic stability; compound **39f** was identified as the next lead compound, with potent binding activity and magnificent metabolic stability.

Table 2. Effect of substituents at the 6-position of compound **2e** on $hCRF_1$ receptor binding activities, logDpH7.4, and metabolic stability in human



compound	R	binding[a] (IC_{50}, nM)	$logD_{pH7.4}$	metabolic stability[b] (human, (μL/min/mg)
2e		7.1 (3.6–14)	5.25	184
39a		89 (65–120)	3.57	29
39b		210 (170–270)	4.01	72
39c		66 (44–99)	3.69	16
39d		130 (87–180)	3.53	14
39e		20 (14–30)	4.38	166
39f		50 (40–64)	4.30	<1

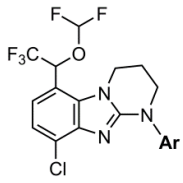
[a]IC_{50} values and 95% confidential intervals were calculated from the concentration-response curve (n = 1). [b]Metabolic stability was determined based on the disappearance of parent compound after incubation with human liver microsmes for 20 min.

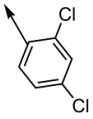
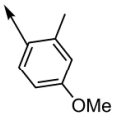
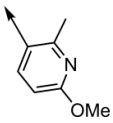
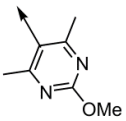
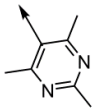
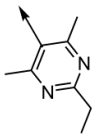
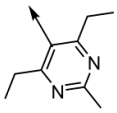
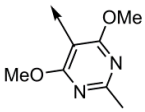
Since the solubility of compound **39f** was poor (< 0.09 μg/ml), further reduction of its lipophilicity was required. We aimed to improve the solubility of compound **39f** by optimizing the lipophilic 2,4-dichlorophenyl group on the 1-position. Introduction of a methoxy on the phenyl group at the 2-position decreased the logD and increased solubility, while maintaining binding activity (**42f**; IC_{50} = 48 nM, $logD_{pH7.4}$ = 3.76, and solubility = 1.5 μg/ml). Replacement of the phenyl group compound **42f** with a pyridine group further improved solubility and resulted in potent binding activity (**42b**; IC_{50} = 48 nM, $logD_{pH7.4}$ = 3.43, and solubility = 17 μg/ml), indicating that a pyridine group nitrogen atom at the 1-position successfully reduced lipophilicity and had ignorable impact on CRF_1 receptor binding.

These results encouraged us to introduce a pyrimidine group at the 1-position, and pyrmidine derivative **42c** exhibited moderate binding activity and excellent solubility (**42c**; IC_{50} = 110 nM, $logD_{pH7.4}$ = 3.08, and solubility = 54 μg/ml).

Pyrimidine derivatives with compact substituents were synthesized and evaluated, as our previous SAR studies for benzimidazole derivatives revealed that only small substituents were tolerated on the aryl group at the 1-position.[21] The binding activity of compound **42g** (IC_{50} = 320 nM) was 3-fold lower than that of compound **42c**. Compound **42h** (IC_{50} = 210 nM) was also 2-fold less potent than compound **42c**, suggesting that the methoxy group oxygen atom on the pyrimidine group affected binding activity. However, based on results of the docking model, we did not expect interactions with the CRF_1 receptor as shown in Figure 3. The effect of ortho substituents on binding activity was investigated using compounds **42d** (IC_{50} = 100 nM) and **42e** (IC_{50} = 46 nM) relative to compound **42g** (IC_{50} = 320 nM). These results show that larger substituents are preferable on the point of binding activity, which was consistent with our torsion conformation hypothesis. However, the solubility of compounds **42d** and **42e** was lower and the metabolic stability was less optimal than those of compound **42c** due to increased lipophilicity. From these results, we concluded that lipophilicity could be successfully reduced without a significant loss in binding activity. Further, compound **42c** was identified as the best compound with potent binding activity, excellent solubility and metabolic stability.

Table 3. Effect of substituens at the 1-position of compound **39f** on $hCRF_1$ receptor binding activities, logDpH7.4, metabolic stability in human, and solubility



compound	Ar	binding[a] (IC_{50}, nM)	$logD_{pH7.4}$	metabolic stability[b] (human, (μL/min/mg)	solubility[c] (μg/mL)
39f		50 (40–64)	4.30	<1	<0.09
42f		48 (36–62)	3.76	ND	1.5
42b		48 (43–53)	3.43	26	17
42c		110 (99–130)	3.08	<1	54
42g		320 (240–430)	2.87	9	71
42h		210 (190–240)	3.21	53	37
42d		100 (85–120)	3.52	82	46
42e		46 (35–59)	3.71	82	14

[a]IC_{50} values and 95% confidential intervals were calculated from the concentration-response curve (n = 1). All compounds shown in this table were synthesized and evaluated as racemates. [b]Metabolic stability was determined based on the disappearance of parent compound after incubation with human liver microsmes for 20 min. [c]10 mmol/L solution of the compound in DMSO was evaluated using JP2 buffer (pH 6.8), as described in the Japanese Pharmacopoeia.

Optical resolution of compound **42c** was carried out to identify compound **42c-*R*** as a eutomer whose absolute configuration was determined by X-ray crystal analysis. Compound **42c-*R*** (IC_{50} = 58 nM) was approximately 2-fold more potent than its racemate **42c**. In contrast, compound **42c-*S***, the distomer (IC_{50} = 350 nM), was 3-fold less potent than its racemate **42c**. Since some reported CRF_1 receptor antagonists, such as pexacerfont and vercerfont, also have a chiral center on the superposition of the 6-position of compound **42c-*R***, the CRF_1 receptor should strictly recognize ligand conformation around this position. Further, we asymmetrically synthesized the **41c-*R*** precursor (Scheme 8), which was converted to the **42c-*R*** eutomer by the same procedure described above. After preparing ketone **44c** from a racemic alcohol **41c** by oxidation with etrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine *N*-oxide (NMO), asymmetric hydrogenation with ruthenium (II) complexes[24] under 0.7 MPa of hydrogen pressure successfully afforded target *R*-configurations with excellent enantioselectivity (99.4%ee after recrystallization).

Compound **42c-*R*** also inhibited human CRF-stimulated cAMP accumulation with an IC_{50} value of 55 nM in CHO cells expressing $hCRF_1$ receptors. In addition, compound **42c-*R*** was inactive against $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptors (IC_{50} > 10 μM). Therefore, we selected compound **42c-*R*** for further evaluation of its pharmacological properties.

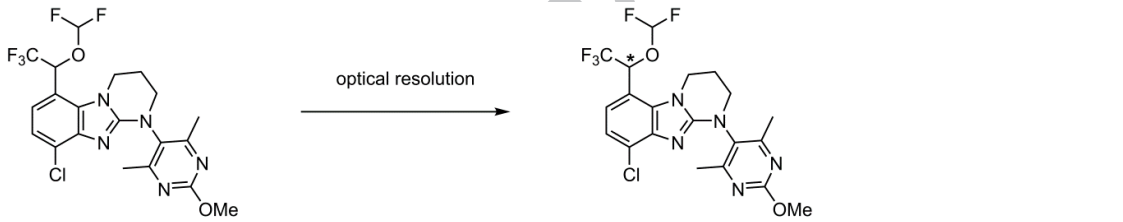
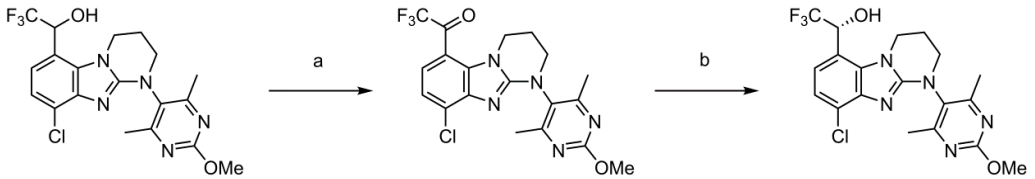


Figure 5. Profiles of chiral **42c-*R*** and **42c-*S***

[a]IC_{50} values and 95% confidential intervals were calculated from the concentration-response curve (n = 1).

Scheme 8. Asymmetric synthesis alcohol derivative **41c-*R***[a]



[a]Reagents and conditions: (a) TPAP, NMO, MS4A, CH_3CN, 0 °C–rt, 86%; (b) H_2 (0.7 MPa), KO*t*-Bu, [$RuCl_2$\{(R)-xylbinap\}\{(R)-daipen\}], 2-propanol, toluene, rt, 75%, 99.4%ee.

The oral bioavailability of compound **42c-*R*** was 68% in rats (i.v. 1 mg/kg, p.o. 5 mg/kg), which was drastically improved from the initial lead compound, **2e**. The oral bioavailability of **42c-*R*** is likely attributable to its metabolic stability, which enabled it to withstand oxidative metabolism in hepatic microsomes (rat: 24 μl/min/mg, human: < 1 μl/min/mg) and improved its kinetic solubility in phosphate buffer saline (55 μg/ml, logD = 3.18). To confirm brain penetration, we conducted an ex vivo binding test in rats. After orally administering compound **42c-*R*** (5 and 10 mg/kg) to rats, [^{125}I]-CRF binding in the frontal cortex was measured over time. Compound **42c-*R*** inhibited [^{125}I]-CRF binding in the frontal cortex in a dose-dependent manner. In addition, maximum inhibition of [^{125}I]-CRF binding was observed 2 h after oral administration, and the inhibition diminished 24 h after administration. These results indicate that compound **42c-*R*** penetrates the brain in rats. Finally, we examined CRF_1 receptor antagonist activity in rats. After oral administration of compound **42c-*R*** (2.5, 5, and 10 mg/kg) followed by i.c.v. administration of CRF (1 μg) 2 h later, we monitored locomotor activity for 1 h. Results show that administration of compound **42c-*R*** suppresses CRF-induced increases in locomotor activity in a dose-dependent manner, suggesting that compound **42c-*R*** exerts in vivo CRF_1 receptor antagonist activity.

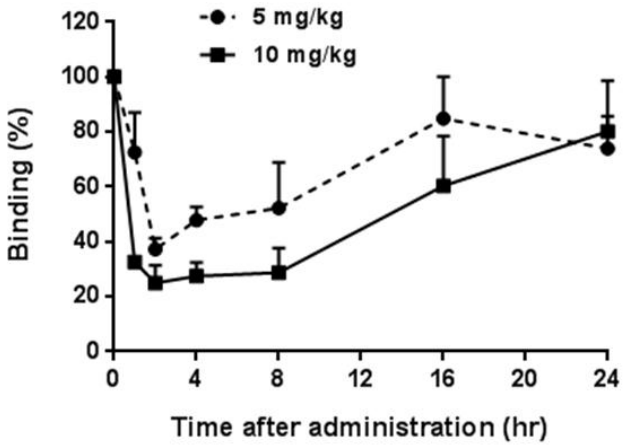


Figure 6. [^{125}I]-Corticotropin-releasing factor (CRF) binding in membranes of the frontal cortex 1, 2, 4, 8, 16, and 24 h after oral administration of 5 and 10 mg/kg compound **42c-*R***. Binding of [^{125}I]-CRF in rat brain tissues under drug-free conditions was defined as 100% binding. Data are expressed as means + S.E.M (n = 3).

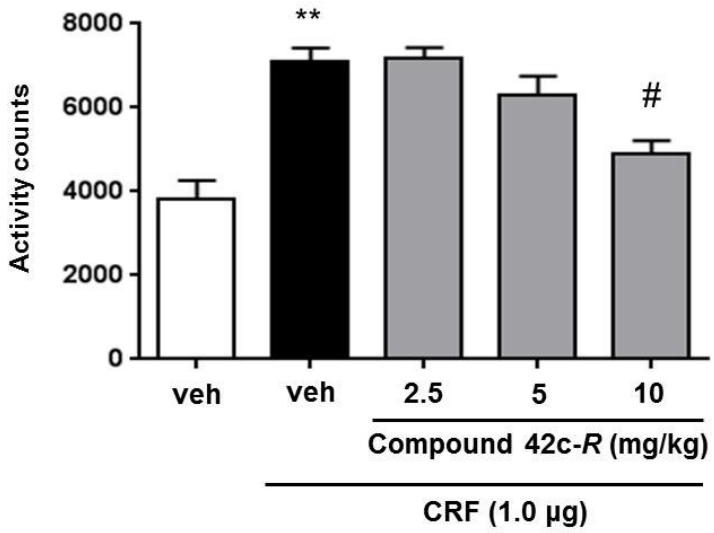


Figure 7. Effect of orally administering 2.5, 5, and 10 mg/kg compound **42c-R** on corticotropin-releasing factor (CRF)-induced locomotor activation by i.c.v. administration in rats. Locomotor activity was measured as the mean number of counts for 60 min after i.c.v. administration of CRF. Data are expressed as means + S.E.M. (n = 9–17). $^{**}p < 0.001$ vs vehicle group via the Aspin-Welchtest; # $p < 0.025$ vs vehicle-h/rCRF group via the Shirley-Williams-test.

Conclusion

In the course of exploration of a novel class of CRF_1 receptor antagonists, we hypothesized that stabilization of torsional conformation between the core ring and pendant aryl group is important to determine binding activity based on analyses of the 2-anilinobenzimidazole derivative pharmacophore. The relationship between binding activity and electron density of a HBA nitrogen atom was also examined. Based on these considerations, the tricyclic compounds **2a–e** with torsional conformations were designed and synthesized. The results suggested that both the dihedral angle and electron density played important roles for determining binding activity. The torsional conformation between the core and pendant aryl group was better than a planar conformation for exhibiting potent binding activity. Moreover, the binding activity critically depended on the electron density of a HBA nitrogen atom. These considerations were supported by a docking study using reported CRF_1 receptor crystal structure information. Among the synthesized derivatives (**2a–e**), 1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazole derivative **2e**, was identified as the most potent ring system, and then we moved to optimization to improve its metabolic stability and solubility. The exploration for a suitable substituent at the 6-position resulted in the identification of 2,2,2-trifluoroethyl-1-difluoromethoxy derivative **39f**, with excellent metabolic stability. We further aimed to reduce lipophilicity by optimizing the 1-position. Consequently, 2,6-dimethyl-4-methoxypyrimidine derivative **42c**, was identified and determined to be compatible with potent binding activity and good solubility. Finally, optical resolution of compound **42c** led to the discovery of compound **42c-*R***, with

potent binding activity and a good pharmacokinetic profile. Compound **42c-*R*** dose-dependently inhibited [^{125}I]-CRF binding in the frontal cortex (5 and 10 mg/kg, p.o.) and suppressed locomotor activation induced by i.c.v. administration of CRF in rats (10 mg/kg, p.o.), suggesting that compound **42c-*R*** successfully binds CRF_1 receptors in the brain. Compound **42c-*R*** was selected as a candidate for further investigation to examine the clinical usefulness of CRF_1 receptor antagonists.

Acknowledgements

The authors thank Dr. Yoshiro Tomimatsu, Mr. Katsuya Sakimura, and Dr. Yoshikazu Ohtani for their assistance with pharmacological tests and helpful discussions during the preparation of the manuscript; Mr. Akihiro Yokota for calculating the φ and E values, performing the docking study, and helpful discussions during preparation of the manuscript; Mr. Yuji Shimizu and Mr. Kenichi Kuroshima for performing the in vitro CRF_1 receptor binding assays, human CRF-stimulated cAMP accumulation assays, and reviewing the data; Mr. Mitsuyoshi Nishitani for X-ray structure analysis; Ms. Miki Hara for conducting optical resolution of compound **42**, measuring the optical purity of compound **42c-*R***, and performing chiral resolution and analysis of enantiomer excess rate; and Mr. Masayuki Yamashita for conducting the asymmetric hydrogenation of **44c**.

Experimental section

Chemistry

General. In the following experimental, reactions were run using the commercially available starting materials and solvents without further purification. Melting points were determined on a Yanaco micro melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Varian Mercury-300 (300 MHz). Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = doublets of doublet, brs = broad singlet. Coupling constants (J values) are given in hertz (Hz). Mass spectra (MS) were acquired using an Agilent LC/MS system (Agilent1200SL/Agilent6130MS), Shimadzu LC/MS system (LC-10ADvp high pressure gradient system/LCMS-2010A) or Shimadzu UFLC/MS (Prominence UFLC high pressure gradient system/LCMS-2020) operating in electron spray ionization mode (ESI+). The column used was an L-column 2 ODS (3.0 x 50 mm I.D., 3 μm, CERI, Japan) with a temperature of 40 °C and a flow rate of 1.2 or 1.5 mL/min. Mobile phase A was 0.05% TFA in ultrapure water. Mobile phase B was 0.05% TFA in acetonitrile which was increased linearly from 5% to 90% over 2 minutes, 90% over the next 1.5 minutes, after which the column was equilibrated to 5% for 0.5 minutes. Elemental analyses and Optical rotation analysis were carried out by Takeda Analytical Research Laboratories, Ltd. Chromatographic purification was carried out on silica gel columns (Kieselgel 60, 0.063-0.22 mm, Merck) or on Purif-Pack (SI 60 μm or NH 60 μm, Fuji Silysia, Ltd.). Each compound was confirmed to be ≥95% purity by either LC/MS or elemental analysis. Yields were not optimized.

Ethyl *N*-(2,6-dinitrophenyl)-*β*-alaninate (4). A mixture of *β*-alanine ethyl ester hydrochloride (911 mg, 5.39 mmol), 2-chloro-1,3-dinitrobenzene (1.00 g, 4.94 mmol), triethylamine (2.07 mL, 14.9 mmol) and THF (50 mL) was stirred at room temperature for 2 h. To the reaction mixture were added *β*-alanine ethyl ester hydrochloride (455 mg, 2.96 mmol) and triethylamine (1.03 mL, 7.39 mmol) at room temperature and the resultant mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 0-20% EtOAc/*n*-hexane gradient mixture to give **4** as an oil (1.39 g, 4.92 mmol, quant). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, *J* = 7.1 Hz, 3H), 2.66 (t, *J* = 5.8 Hz, 2H), 3.23 - 3.31 (m, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 6.78 (t, *J* = 8.2 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 2H), 8.53 (brs, 1H).

Ethyl *N*-(2,6-diaminophenyl)-*β*-alaninate (5). Under hydrogen gas atmosphere, a mixture of compound **4** (1.39 g, 4.92 mmol), 10% palladium on carbon (50% wet, 280 mg) and THF (50 mL) was stirred at room temperature for 6 h. The reaction mixture was filtered and concentrated *in vacuo* to give **5** as an oil (1.00 g, 4.50 mmol, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (t, *J* = 7.2 Hz, 3H), 2.56 - 2.62 (m, 2H), 3.12 - 3.17 (m, 2H), 3.95 (brs, 4H), 4.21 (q, *J* = 7.2 Hz, 2H), 6.19 (d, *J* = 7.8 Hz, 2H), 6.75 (t, *J* = 7.8 Hz, 1H).

Ethyl *N*-[2-amino-6-({[(2,4-dichlorophenyl)amino]carbonothioyl}amino)phenyl]-*β*-alaninate (6). To a solution of compound **5** (500 mg, 2.24 mmol) in THF (22 mL) was added 2,4-dichlorophenyl isothiocyanate (503 mg, 2.46 mmol) at room temperature. The resultant mixture was stirred at room temperature for 1 h and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 10-40% EtOAc/*n*-hexane gradient mixture and NH-silica gel eluting with a 10-90% EtOAc/*n*-hexane gradient mixture to give **6** as a solid (533 mg, 1.25 mmol, 56%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, *J* = 7.1 Hz, 3H), 2.66 (t, *J* = 5.8 Hz, 2H), 3.23 - 3.31 (m, 2H), 4.17 (q, *J* = 7.1 Hz, 2H), 6.78 (t, *J* = 8.2 Hz, 1H), 8.17 (d, *J* = 8.2 Hz, 2H), 8.53 (brs, 1H).

Ethyl 3-{7-amino-2-[(2,4-dichlorophenyl)amino]-1*H*-benzimidazol-1-yl}propanoate (7). To a solution of compound **6** (159 mg, 0.372 mmol) in THF (4 mL) was added EDCI (214 mg, 1.12 mmol) at room temperature. The resultant mixture was stirred at 50 °C for 2 h. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over Na2SO4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc to give **7** as an oil (122 mg, 0.309 mmol, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.21 (t, *J* = 7.2 Hz, 3H), 3.06 - 3.14 (m, 2H), 3.70 (brs, 2H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.48 - 4.53 (m, 2H), 6.53 (d, *J* = 7.8 Hz, 1H), 6.98 (t, *J* = 7.8 Hz, 1H), 7.12 - 7.23 (m, 2H), 7.36 (d, *J* = 2.2 Hz, 1H), 8.08 (brs, 1H), 8.16 - 8.27 (m, 1H).

Ethyl 3-[2-[(2,4-dichlorophenyl)amino]-7-(diethylamino)-1*H*-benzimidazol-1-yl]propanoate (8). To a solution of the compound **7** (214 mg, 0.544 mmol) in MeOH (5.5 mL) and AcOH (0.11 mL) was added acetoaldehyde (0.204 mL, 3.27 mmol) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min. To the reaction mixture was added sodium triacetoxyborohydride (692 mg, 3.27 mmol) at 0 °C. After the resultant mixture was stirred at room temperature for 12 h, the mixture was diluted with $NaHCO_3$ aq. and extracted with EtOAc. The combined organic layer was washed with brine,

dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on NH-silica gel eluting with EtOAc to give **8** as a solid (255 mg, 0.567 mmol, quant). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.06 (t, *J* = 7.1 Hz, 6H), 1.21 (t, *J* = 7.2 Hz, 3H), 3.00 (t, *J* = 5.9 Hz, 2H), 3.07 (q, *J* = 7.1 Hz, 4H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.62 (t, *J* = 5.9 Hz, 2H), 7.01 (dd, *J* = 1.1, 7.8 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 7.20 - 7.25 (m, 1H), 7.37 - 7.42 (m, 2H), 8.16 (s, 1H), 8.30 (d, *J* = 9.1 Hz, 1H).

3-[2-[(2,4-Dichlorophenyl)amino]-7-(diethylamino)-1*H*-benzimidazol-1-yl]propan-1-ol (9). To a suspension of lithium borohydride (22.5 mg, 1.03 mmol) in THF (3.5 mL) was added compound **8** (155 mg, 0.345 mmol) at 0 °C. After the resultant mixture was stirred at room temperature for 20 h, the mixture was diluted with NH_4Cl aq. at 0 °C and stirred at 0 °C for 30 min. The resultant mixture was diluted with $NaHCO_3$ aq. and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 25% EtOAc/*n*-hexane mixture to give **9** as a solid (35.7 mg, 0.0876 mmol, 25%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.06 (t, *J* = 7.2 Hz, 6H), 1.25 (s, 1H), 2.03 - 2.14 (m, 2H), 3.09 (q, *J* = 7.2 Hz, 4H), 3.56 (t, *J* = 5.5 Hz, 2H), 4.56 (t, *J* = 6.2 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 8.0 Hz, 1H), 7.22 - 7.28 (m, 2H), 7.32 - 7.40 (m, 2H), 8.40 (brs, 1H).

1-(2,4-Dichlorophenyl)-*N,N*-diethyl-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-amine (2a). To a solution of compound **9** (35.7 mg, 0.0876 mmol) in pyridine (1 mL) was added methanesulfonyl chloride (0.0339 mL, 0.438 mmol) at 0 °C. After the resultant mixture was stirred at room temperature for 2 h, the mixture was diluted with $NaHCO_3$ aq. and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 10-40% EtOAc/*n*-hexane gradient mixture to give **2a** as a solid (29.2 mg, 0.0750 mmol, 86%). Analytically pure material was obtained by recrystallization from EtOAc/*n*-hexane. mp 131-133 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.01 (t, *J* = 7.0 Hz, 6H), 2.27 - 2.38 (m, 2H), 3.01 - 3.12 (m, 4H), 3.63 - 3.75 (m, 2H), 4.59 - 4.67 (m, 2H), 6.86 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.01 (t, *J* = 7.9 Hz, 1H), 7.21 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.31 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.48 (d, *J* = 8.5 Hz, 1H), 7.51 (d, *J* = 2.5 Hz, 1H). LC-MS (ESI): m/z Calcd.: 388.1; Found: 388.9 (M+H). Anal. Calcd for $C_{20}H_{22}N_4Cl_2$: C,61.70; H,5.70; N,14.39. Found: C,61.81; H,5.68; N,14.43.

3-[2-[(2,4-Dichlorophenyl)amino]-7-(diethylamino)-1*H*-benzimidazol-1-yl]propanoic acid (10). To a solution of compound **8** (100 mg, 0.223 mmol) in a mixture of THF (2 mL) and MeOH (1 mL) was added 1N NaOH (0.446 mL, 0.446 mmol) at 0 °C. After the resultant mixture was stirred at room temperature for 1 h, the mixture was neutralized with 1N HCl and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc to give **10** as a solid (73.3 mg, 0.174 mmol, 78%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.98 (t, *J* = 7.0 Hz, 6 H), 2.70 - 2.84 (m, 2 H), 3.01 (q, *J* = 7.0 Hz, 4 H), 4.51 - 4.67 (m, 2 H), 6.89 - 7.29 (m, 3 H), 7.31 - 7.47 (m, 1 H), 7.60 (brs, 1 H), 8.07 (brs, 1 H), 8.74 (brs, 1 H), 12.57 (brs, 1 H).

1-(2,4-Dichlorophenyl)-6-(diethylamino)-3,4-dihydropyrimido[1,2-*a*]benzimidazol-2(1*H*)-one (2b). A mixture of compound **10** (57.0 mg, 0.135 mmol), EDCI hydrochlroide (31.1 mg, 0.162 mmol), triethylamine (0.0226 mL, 0.162 mmol) and DMF (3 mL) was stirred at room temperature for 1 day. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 20-50% EtOAc/*n*-hexane gradient mixture to give **2b** as a solid (44.8 mg, 0.111 mmol, 82%). Analytically pure material was obtained by recrystallization from EtOAc/*n*-hexane. mp 113-114 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02 (t, *J* = 7.1 Hz, 6H), 3.05 - 3.22 (m, 6H), 4.73 - 5.01 (m, 2H), 7.03 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.13 (t, *J* = 7.9 Hz, 1H), 7.36 (dd, *J* = 7.9, 0.9 Hz, 1H), 7.39 - 7.46 (m, 2H), 7.59 (d, *J* = 1.9 Hz, 1H). LC-MS (ESI): m/z Calcd.: 402.1; Found: 403.0 (M+H). Anal. Calcd for $C_{20}H_{20}N_4OCl_2$: C,59.56; H,5.00; N,13.89. Found: C,59.62; H,4.98; N,13.93.

Methyl 2-(2,4-dichlorophenyl)-2-hydroxyethanimidoate hydrochloride (12). To a stirred solution of 2,4-dichlorobenzaldehyde (**11**) (18.3 g, 105 mmol) and 4-dimethylaminopyridine (128 mg, 1.05 mmol) in CH_3CN (200 mL) was added trimethylsilyl cyanide (13.7 mL, 110 mmol) at room temperature. After 2 h, the reaction mixture was concentrated *in vacuo*. To a stirred MeOH (150 mL) was added acetyl chloride (100 mL) at 0 °C, and the mixture was warmed up to room temperature. After 30 min, the residue was added. After 2 h, the reaction mixture was concentrated *in vacuo*. The resulting solid was washed with diethyl ether to afford **12** as colorless solid (25.5 g, 94.3 mmol, 90%), which was used next step without further purification.

Ethyl 3-{7-amino-2-[(2,4-dichlorophenyl)(hydroxy)methyl]-1*H*-benzimidazol-1-yl}propanoate (13). To a stirred solution of **5** (4.11 g, 18.4 mmol) in EtOH (36.8 mL) was added **12** (5.59 g, 20.7 mmol) at room temperature. After 12 h, the reaction mixture was poured into water (120 mL). The resulting solid was collected by filtration and washed with EtOAc to afford **13** as a colorless solid (6.77 g, 16.6 mmol, 90 %). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.20 (t, *J* = 7.2 Hz, 3H), 2.55 - 2.70 (m, 1H), 2.76 - 2.90 (m, 1H), 3.94 (brs, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 4.36 - 4.61 (m, 2H), 4.93 (brs, 1H), 6.36 (s, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 7.05 (t, *J* = 7.8 Hz, 1H), 7.19 - 7.30 (m, 2H), 7.34 - 7.46 (m, 2H).

Ethyl 3-{2-[(2,4-dichlorophenyl)(hydroxy)methyl]-7-(diethylamino)-1*H*-benzimidazol-1-yl}propanoate (14). To a suspension of **13** (6.00 g, 14.7 mmol) and AcOH (7.4 mL) in MeOH (147 mL) was added acetaldehyde (4.95 mL, 88.2 mmol) at 0 °C. After 30 min, sodium triacetoxyborohydride (18.7 g, 88.2 mmol) was added. After 2 h, the reaction mixture was quenched with water, concentrated *in vacuo*, diluted with EtOAc, washed with 1N NaOH and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 10-30% EtOAc/*n*-hexane gradient mixture to give **14** as a colorless amorphous (6.30 g, 13.6 mmol, 92%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 - 1.11 (m, 6H), 1.23 (t, *J* = 7.1 Hz, 3H), 2.24 - 2.41 (m, 1H), 2.65 - 2.84 (m, 1H), 2.87 - 3.17 (m, 4H), 4.11 (q, *J* = 7.1 Hz, 2H), 4.44 - 4.68 (m, 2H), 5.13 (brs, 1H), 6.42 (s, 1H), 7.05 (d, *J* = 7.7 Hz, 1H), 7.13 - 7.30 (m, 2H), 7.33 - 7.54 (m, 3H).

3-{2-[(2,4-Dichlorophenyl)(hydroxy)methyl]-7-(diethylamino)-1*H*-benzimidazol-1-yl}propan-1-ol (15). To a suspension of lithium aluminum hydride (327 mg, 8.61 mmol) in THF (38 mL) was added a solution of **14** (2.00 g, 4.31 mmol) in THF (5 mL) at -5 °C. After the addition, Na_2SO_4-$10H_2O$ (3.3 g) was added. After 1 h, the resultant mixture was filtrated and concentrated *in vacuo.* The residue was purified by column chromatography on silica gel eluting with a 30-70% EtOAc/*n*-hexane gradient mixture to give **15** as a colorless solid (1.42 g, 3.36 mmol, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 - 1.15 (m, 6H), 1.71 - 1.85 (m, 1H), 1.87 - 2.01 (m, 1H), 2.98 - 3.19 (m, 4H), 3.31 - 3.56 (m, 2H), 4.23 - 4.39 (m, 1H), 4.49 - 4.65 (m, 1H), 4.92 (brs, 1H), 6.41 (s, 1H), 7.04 (d, *J* = 7.4 Hz, 1H), 7.15 - 7.25 (m, 2H), 7.38 - 7.47 (m, 2H), 7.51 (d, *J* = 8.0 Hz, 1H).

(2,4-Dichlorophenyl)[7-(diethylamino)-1-(3-hydroxypropyl)-1*H*-benzimidazol-2-yl]methanone (16). A suspension of **15** (1.18 g, 2.79 mmol) and manganese dioxide (2.43 g, 27.9 mmol) in THF (14 mL) was stirred for 1.5 h at room temperature. The reaction mixture was filtrated, and the filtrate was concentrated *in vacuo* to afford **16** as a yellow solid (1.16 g, 2.76 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.10 (t, *J* = 7.0 Hz, 6H), 2.02 - 2.14 (m, 2H), 2.55 (t, *J* = 5.9 Hz, 1H), 3.07 - 3.28 (m, 4H), 3.57 (q, *J* = 5.9 Hz, 2H), 5.21 (t, *J* = 6.9 Hz, 2H), 7.18 (dd, *J* = 7.7, 1.1 Hz, 1H), 7.23 - 7.31 (m, 1H), 7.38 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.56 - 7.65 (m, 2H).

[1-(3-Bromopropyl)-7-(diethylamino)-1*H*-benzimidazol-2-yl](2,4-dichlorophenyl)methanone (17). To a stirred suspension of **16** (404 mg, 0.961 mmol) and tetrabromomethane (637 mg, 1.92 mmol) in CH_3CN was added triphenylphosphine (504 mg, 1.92 mmol). After 15 min, the reaction mixture was diluted with EtOAc, washed with $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 0-30% EtOAc/*n*-hexane gradient mixture to afford **17** as yellow solid (407 mg, 0.842 mmol, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.09 (t, *J* = 7.1 Hz, 6 H), 2.33 - 2.46 (m, 2H), 3.02 - 3.26 (m, 4H), 3.47 (t, *J* = 6.6 Hz, 2H), 5.19 - 5.28 (m, 2H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.28 (t, *J* = 7.8 Hz, 1H), 7.38 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.49 (d, *J* = 1.9 Hz, 1H), 7.56 - 7.64 (m, 2H).

4-(2,4-Dichlorophenyl)-*N,N*-diethyl-1,2-dihydropyrido[1,2-*a*]benzimidazol-9-amine (18). A solution of **17** (360 mg, 0.745 mmol) and triphenylphosphine (586 mg, 2.23 mmol) in CH_3CN (5 mL) was stirred for 60 h at 80 °C. The reaction mixture was concentrated *in vacuo*. Toluene (5 mL), THF (1 mL), and KO*t*-Bu (85%, 83.6 mg, 0.745 mmol) was added at room temperature. After 1 h, the reaction mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, filtrated, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 5-25% EtOAc/*n*-hexane gradient mixture to afford **18** as pale yellow solid (65.5 mg, 0.170 mmol, 23%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, *J* = 7.0 Hz, 6H), 2.72 - 2.87 (m, 2H), 2.94 - 3.24 (m, 4H), 4.81 (t, *J* = 7.3 Hz, 2H), 6.37 (t, *J* = 4.7 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.27 - 7.33 (m, 1H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.43 - 7.56 (m, 2H).

4-(2,4-Dichlorophenyl)-*N,N*-diethyl-1,2,3,4-tetrahydropyrido[1,2-*a*]benzimidazol-9-amine (2c). A solution of compound **18** (53.0 mg, 0.137 mml) in MeOH was stirred in the presence of 1.9% palladium on fibroin (10 mg) under

hydrogen atmosphere at room temperature for 7 days. The catalyst was removed and the filtrate was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with a 0-30% EtOAc/*n*-hexane gradient mixture to afford **2c** as pale yellow oil (24.8 mg, 0.0640 mmol, 47%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, *J* = 6.9 Hz, 6H), 1.92 - 2.20 (m, 3H), 2.28 - 2.41 (m, 1H), 2.96 - 3.21 (m, 4H), 4.45 - 4.55 (m, 1H), 4.70 - 4.90 (m, 2H), 6.90 (d, *J* = 8.2 Hz, 1H), 7.05 (dd, *J* = 7.7, 1.0 Hz, 1H), 7.09 - 7.21 (m, 2H), 7.41 (d, *J* = 1.9 Hz, 1H), 7.46 (dd, *J* = 7.7, 1.0 Hz, 1H). LC-MS (ESI): m/z Calcd.: 387.1; Found: 387.9 (M+H).

2-[(2,6-Dinitrophenyl)amino]ethanol (19). A mixture of ethanolamine (5.96 mL, 49.4 mmol) and 2-chloro-1,3-dinitrobenzene (**3**) (5.00 g, 24.7 mmol) in THF (50 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc, washed with aqueous sodium hydrogen carbonate and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give **19** as a brown solid (5.54 g, 24.4 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.12 - 3.19 (m, 2 H), 3.85 - 3.90 (m, 2 H), 6.77 (t, J=8.2 Hz, 1 H), 8.19 (d, J=8.2 Hz, 2 H), 8.71 (brs., 1 H).

2-[(2,6-Diaminophenyl)amino]ethanol (20). A mixture of **19** (5.54 g, 24.4 mmol) and 10% palladium on carbon (50% wet, 1.1 g) in THF (240 mL) was stirred at room temperature under H_2 atmosphere for 2 h. The reaction mixture was filtered and concentrated *in vacuo* to give **20** as a brown oil (3.63 g, 21.7 mmol, 89%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.09 - 3.15 (m, 2 H), 3.55 - 3.60 (m, 2 H), 3.83 (brs., 4 H), 6.24 (d, J=7.8 Hz, 2 H), 6.75 (t, J=7.8 Hz, 1 H).

2-{7-Amino-2-[(2,4-dichlorophenyl)(hydroxy)methyl]-1*H*-benzimidazol-1-yl}ethanol (21). To a stirred solution of **20** (3.63 g, 21.7 mmol) in EtOH (43 mL) was added **12** (7.04 g, 26.0 mmol) at room temperature. After 12 h, the reaction mixture was diluted with $NaHCO_3$ aq.. The resultant mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc to give **21** as an orange amorphous solid (7.58 g, 21.5 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.55 - 3.71 (m, 2 H), 4.10 - 4.21 (m, 1 H), 4.23 (brs., 2 H), 4.43 - 4.54 (m, 1 H), 6.01 (s, 1 H), 6.48 (dd, J=6.5, 2.2 Hz, 1 H), 6.89 - 6.98 (m, 2 H), 7.00 (dd, J=8.5, 2.2 Hz, 1 H), 7.25 - 7.32 (m, 2 H).

2-{2-[(2,4-Dichlorophenyl)(hydroxy)methyl]-7-(diethylamino)-1*H*-benzimidazol-1-yl}ethanol (22). To a solution of **21** (7.58 g, 21.5 mmol) in MeOH (220 mL) and AcOH (11 mL) was added acetoaldehyde (8.04 mL, 129 mmol) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min. To the reaction mixture was added sodium triacetoxyborohydride (27.3 g, 129 mmol) at 0 °C. After the resultant mixture was stirred at room temperature for 2 h, the mixture was diluted with $NaHCO_3$ aq. and water, and extracted with EtOAc. The combined organic layer was washed with 1N NaOH and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc to afford a crude solid, which was washed with diisopropylether to give **22** as a colorless solid (8.70 g, 21.3 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 - 1.09 (6 H, m), 2.91 - 3.13 (4 H, m), 3.62 - 3.82 (2 H, m), 4.38 - 4.56 (2 H, m), 4.71 - 4.81 (1 H, m), 5.61 (1 H, brs.), 6.31 (1 H, s), 6.98 (1 H, dd, J=7.8, 0.8 Hz), 7.08 (1 H, t, J=7.8 Hz), 7.25 (1 H, dd, J=8.3, 2.1 Hz), 7.32 - 7.37 (2 H, m), 7.63 (1 H, d, J=8.3 Hz).

1-(2,4-Dichlorophenyl)-*N,N*-diethyl-3,4-dihydro-1*H*-[1,4]oxazino[4,3-*a*]benzimidazol-6-amine (2d). To a stirred

solution of compound **22** (200 mg, 0.490 mmol) and triphenylphosphine (193 mg, 0.736 mmol) in THF (5 mL) was added a solution of diethyl azodicarboxylate in toluene (40%, 0.335 mL, 0.736 mmol) at room temperature. The mixture was stirred at room temperature for 12 h, concentrated *in vacuo*, and purified by column chromatography on silica gel eluting with a 5-15% EtOAc/*n*-hexane gradient mixture to give **2d** as a colorless solid (112 mg, 0.287 mmol, 59%). Analytically pure material was obtained by recrystallization from *n*-hexane. mp 130-131 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, J=7.1 Hz, 6 H), 3.11 (q, J=7.1 Hz, 4 H), 4.08 - 4.20 (m, 1 H), 4.29 - 4.38 (m, 1 H), 4.53 - 4.65 (m, 1 H), 4.82 - 4.92 (m, 1 H), 6.34 (s, 1 H), 7.07 (dd, J=7.7, 0.8 Hz, 1 H), 7.18 (t, J=7.7 Hz, 1 H), 7.22 - 7.23 (m, 2 H), 7.45 - 7.50 (m, 2 H). LC-MS (ESI): m/z Calcd.: 389.1; Found: 389.9 (M+H). Anal. Calcd for $C_{20}H_{21}N_3OCl_2$: C,61.55; H,5.42; N,10.77. Found: C,61.65; H,5.48; N,10.80.

(2-Chloro-5-nitrophenyl)carbonimidic dichloride (24). 2-Chloro-5-nitroaniline (**23**) (43.1 g, 250 mmol), formic acid (250 mL) was stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo*. The solid residue was taken up in water (350 mL), filtered, washed with water. The solid residue was dried at 50 °C under vacuum to give a crude formanilide as a light brown amorphous. A mixture of the crude formanilide, thionyl chloride (164 mL) and sulfuryl chloride (58 mL) was stirred at 55 °C for 48 h. The mixture was concentrated *in vacuo*. The residue was dissolved in petroleum ether, decanted from a precipitate and the clear solution was evaporated *in vacuo* to give **24** as a light brown amorphous (42.2 g, 166 mmol, 67%). ^{1}H-NMR($CDCl_3$, 300 MHz) δ 7.62 (1H, d, *J* = 8.7 Hz), 7.83 (1H, d, *J* = 2.7 Hz), 8.04 (1H, dd, *J* = 2.7, 8.7 Hz).

2-Chloro-5-nitro-*N*-(tetrahydropyrimidin-2(1*H*)-ylidene)aniline (25). A solution of **24** (35.5 g, 140 mmol) in THF (135 mL) was added dropwise over 30 min to a solution of 1,3-propanediamine (58 mL, 700 mmol) in THF (335 mL) with ice bath cooling. The reaction mixture was stirred for 1 h at 5-10 °C, and stirred 2 hr at room temperature. The insoluble material was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was washed with water and diisopropyl ether. After drying at 50 °C under vacuum, **25** was obtained as yellow crystals (35.0 g, 137.4 mmol, 98%). ^{1}H-NMR($CDCl_3$, 300 MHz) δ 1.95-2.03 (2H, m), 3.35 (4H, t, *J* = 6.0 Hz), 4.67 (2H, brs), 7.47 (1H, d, *J* = 8.7 Hz), 7.68 (1H, dd, *J* = 2.7, 8.7 Hz), 7.85 (1H, d, *J* = 8.7 Hz).

9-Chloro-6-nitro-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (26). A mixture of **25** (5.0 g, 19.6 mmol) and KO*t*-Bu (440 mg, 3.93 mmol) in DMSO (100 mL) was stirred at 60 °C for 24 h. After cooling to room temperature, to the reaction mixture was directly purified by silica gel column chromatography eluting with a 50-100% EtOAc/*n*-hexane gradient mixture to give **26** as an yellow amorphous (3.63 g, 14.3 mmol, 73 %). ^{1}H-NMR(DMSO-d_6, 300 MHz) δ 1.91-2.01 (2H, m), 3.03-3.40 (2H, m), 4.12 (2H, t, *J* = 6.0 Hz), 7.16 (1H, d, *J* = 9.0 Hz), 7.47 (1H, d, *J* = 9.0 Hz), 8.13 (1H, brs).

9-Chloro-1-(2,4-dichlorophenyl)-6-nitro-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (27). A mixture of **26** (10.0 g, 39.6 mmol), 2,4-dichloro-1-iodobenzene (54.0g, 197.9 mmol), copper(I) iodide (7.57 g, 39.6 mmol), 2,2'-bipyridyl (12.3 g, 79.2 mmol) and Cs_2CO_3 (25.8 g, 79.2 mmol) in DMF (600 mL) was stirred at 150 °C for 12 h. The

mixture was duluted with EtOAc, washed with water, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with a 10-25% EtOAc/*n*-hexane gradient mixture to give **27** as a light yellow amorphous (750 mg, 1.88 mmol, 5%). ^{1}H-NMR($CDCl_3$, 300 MHz) δ 2.30-2.42 (2H, m), 3.70-3.85 (2H, m), 4.36 (2H, t, *J* = 6.0 Hz), 7.14 (1H, d, *J* = 9.0 Hz), 7.34 (1H, dd, *J* = 2.4, 8.4 Hz), 7.47 (1H, d, *J* = 8.4 Hz), 7.53 (1H, d, *J* = 2.4 Hz), 7.61 (1H, d, *J* = 9.0 Hz).

9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-amine (28). A mixture of **27** (123 mg, 0.308 mmol) and 10% palladium on carbon (12 mg) in AcOH (5.0 mL) was stirred under H_2 atmosphere at room temperature for 8 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel eluting with a 50-100% EtOAc/*n*-hexane gradient mixture to give **28** (51 mg, 0.139 mmol, 45%) as an oil. ^{1}H-NMR(CDCl, 300 MHz) δ 2.33-2.42 (2H, m), 3.56 (2H, brs), 3.62-3.75 (2H, m), 4.58 (2H, t, *J* = 6.0 Hz), 6.32 (1H, d, *J* = 9.0 Hz), 6.86 (1H, d, *J* = 9.0 Hz), 7.29 (1H, dd, *J* = 2.4, 8.4 Hz), 7.47 (1H, d, *J* = 2.4 Hz), 7.52 (1H, d, *J* = 8.4 Hz).

9-Chloro-1-(2,4-dichlorophenyl)-*N*,*N*-diethyl-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-amine (2e). To a solution of **28** (45 mg, 0.122 mmol) in MeOH (5.0 mL) and AcOH (1.0 mL) was added acetaldehyde (0.10 mL, 1.64 mmol) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min. To the reaction mixture was added sodium triacetoxyborohydride (346 mg, 1.64 mmol) at 0 °C. After stirring at room temperature for 1 h, the mixture was concentrated *in vacuo*, diluted with water, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on a silica gel eluting with a 10-50% EtOAc/*n*-hexane gradient mixture to give **2e** (50 mg, 0.120 mmol, 98%) as a colorless amorphous. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.00 (6H, t, *J* = 7.2 Hz), 2.28-2.35 (2H, m), 3.02 (4H, q, *J* = 7.2 Hz), 3.69 (2H, brs), 4.61 (2H, t, *J* = 6.0 Hz), 6.78 (1H, d, *J* = 8.4 Hz), 7.01 (1H, d, *J* = 8.4 Hz), 7.30 (1H, dd, *J* = 2.4, 8.7 Hz), 7.49 (1H, d, *J* = 2.4 Hz), 7.52 (1H, d, *J* = 8.7 Hz). LC-MS (ESI): m/z Calcd.: 422.1; Found: 423.0 (M+H).

Methyl 2-chloro-3-nitrobenzoate (30). To a solution of 2-chloro-3-nitrobenzoic acid (**29**) (100 g, 0.496 mol) in THF (1000 mL) was added dropwise oxalyl chloride (46.8 mL, 0.546 mol) at 0 °C, and the mixture was stirred at room temperature for 3 h. To the mixture was added dropwise MeOH (300 mL) at 0 °C, and the mixture was stirred at room temperature for 14 h. The mixture was concentrated *in vacuo* and the residue was neutralized with $NaHCO_3$ aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the solid, which was washed with *n*-hexane to give **30** as an yellow powder (103 g, 0.478 mol, 96%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.98 (s, 3H), 7.47 (t, *J* = 8.7 Hz, 1H), 7.83 (dd, *J* = 1.8 Hz, 8.7 Hz, 1H), 7.94 (dd, *J* = 1.8 Hz, 8.7 Hz, 1H).

Methyl 2-[(3-hydroxypropyl)amino]-3-nitrobenzoate (31). To a solution of **30** (100 g, 0.463 mol) in MeOH (800 mL) and triethylamine (129 mL) was added 3-amino-1-propanol (52.2 g, 0.696 mol), and the mixture was stirred at 50 °C for 6 h. The mixture was concentrated *in vacuo* and the residue was diluted with NH_4Cl aq., and extracted with EtOAc. The

combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 50% EtOAc/*n*-hexane mixture to give **31** as a brown oil (86.6 g, 0.341 mol, 74%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.61 (s, 1H), 1.86-1.95 (m, 2H), 3.04-3.12 (m, 2H), 3.77 (q, *J* = 6.0 Hz, 2H), 3.91 (s, 3H), 6.66 (t, *J* = 7.8 Hz, 1H), 7.95 (dd, *J* = 2.4 Hz, 8.7 Hz, 1H), 8.04 (dd, *J* = 2.4 Hz, 8.7 Hz, 1H), 8.52 (s, 1H).

Methyl 3-amino-2-[(3-hydroxypropyl)amino]benzoate (32). To a solution of **31** (86.6 g, 0.341 mol) in THF (1000 mL) was added 10% palladium on carbon (50% wet; 8.70 g), and the mixture was purged with H_2 and stirred under balloon pressure hydrogen at room temperature for 23 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give **32** as a brown oil (59.8 g, 0.267 mol, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.75 (s, 1H), 1.81-1.86 (m, 2H), 3.15 (t, *J* = 6.3 Hz, 2H), 3.86 (t, *J* = 6.3 Hz, 2H), 3.87 (s, 3H), 3.99 (s, 2H), 6.10 (s, 1H), 6.84-6.86 (m, 2H), 7.35-7.39 (m, 1H).

Methyl 3-amino-4-chloro-2-[(3-hydroxypropyl)amino]benzoate (33). *N*-Chlorosuccinimide (74.1 g, 555 mmol) was added to a stirred solution of **32** (81.9 g, 370 mmol) in CH_3CN (1480 mL) at room temperature, and the mixture was stirred at room temperature for 16 h. Additional *N*-chlorosuccinimide (9.88 g, 74.0 mmol) was added to the mixture, and the mixture was stirred at room temperature for 90 min. The mixture was diluted with $NaHCO_3$ aq., concentrated *in vacuo,* and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 20-45% EtOAc/*n*-hexane gradient mixture to give **33** as a brown oil (15.7 g, 60.8 mmol, 16%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.79-1.87 (m, 2H), 2.33 (s, 1H), 3.15 (t, *J* = 6.3 Hz, 2H), 3.81-3.93 (m, 2H), 3.86 (s, 3H), 4.41 (s, 2H), 6.22 (s, 1H), 6.95 (d, *J* = 9.0 Hz, 1H), 7.29 (d, *J* = 9.0 Hz, 1H).

Methyl 4-chloro-2-[(2,4-dichlorophenyl)amino]-1-(3-hydroxypropyl)-1*H*-benzimidazole-7-carboxylate (35). A mixture of **33** (13.1 g, 50.6 mmol) and 2,4-dichloro-1-isothiocyanatobenzene (13.4 g, 65.8 mmol) in THF (150 mL) was stirred at room temperature for 3 days. The mixture was diluted with $NaHCO_3$ aq., concentrated *in vacuo,* and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was washed with diisopropyl ether to give **34** as a colorless powder (19.6 g). A mixture of **34** (19.6 g), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (8.12 g, 42.3 mmol), and triethylamine (6.5 mL) in THF (200 mL) was stirred at 50°C for 3 h. The mixture was concentrated *in vacuo,* diluted with $NaHCO_3$ aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was washed with diisopropyl ether to give **35** as a colorless powder (15.4 g, 35.9 mmol, 71% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.00-2.11 (m, 3H), 3.54-3.61 (m, 2H), 3.95 (s, 3H), 4.64 (t, *J* = 6.3 Hz, 2H), 7.22 (d, *J* = 8.7 Hz, 1H), 7.28 (dd, *J* = 2.4 Hz, 9.0 Hz, 1H), 7.35 (d, *J* = 2.4 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 1H), 8.00 (s, 1H), 8.52 (d, *J* = 9.0 Hz, 1H).

Methyl 9-chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36).

Methanesulfonyl chloride (11.3 mL, 143 mmol) was added to a stirred solution of **35** (15.3 g, 35.7 mmol), pyridine (50 mL) and triethylamine (25 mL) in THF (100 mL) at 0 °C. The mixture was stirred at 0 °C for 13 h, and diluted with $NaHCO_3$ aq. and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. A mixture of the resulting mesylate, K_2CO_3 (14.7 g, 106 mmol) in DMF (60 mL) was stirred at 70 °C for 4 h. The mixture was diluted with $NaHCO_3$ aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was washed with diisopropyl ether to give **36** as a colorless powder (12.8 g, 31.2 mmol, 87% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27-2.35 (m, 2H), 3.69-3.77 (m, 2H), 3.94 (s, 3H), 4.42 (t, *J* = 6.0 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.32 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 7.43-7.50 (m, 3H).

[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]methanol (37). Lithium borohydride (2.12 g, 97.4 mmol) was added to a stirred solution of **36** (10.0 g, 24.3 mmol) in THF (80 mL) at room temperature, and the mixture was stirred at room temperature for 16 h. The reaction was quenched by NH_4Cl aq. at 0 °C, and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the solid, which was washed with EtOAc/diisopropyl ether to give **37** as a colorless powder (8.31 g, 21.7 mmol, 89%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.25-2.33 (m, 2H), 3.63-3.71 (m, 2H), 4.49-4.60 (m, 2H), 4.74 (d, *J* = 5.1 Hz, 2H), 5.38 (t, *J* = 5.1 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 6.96 (d, *J* = 8.1 Hz, 1H), 7.54 (dd, *J* = 2.4 Hz, 8.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.81 (d, *J* = 2.4 Hz, 1H).

9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carbaldehyde (38). Sulfur trioxide-pyridine complex (24.1 g, 152 mmol) was added to a stirred solution of **37** (8.30 g, 21.7 mmol), triethylamine (24 mL), and dimethyl sulfoxide (150 mL) in CH_2Cl_2 (100 mL) at room temperature, and the mixture was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo,* diluted with $NaHCO_3$ aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 15-50% EtOAc/*n*-hexane gradient mixture to give the solid, which was washed with EtOAc/diisopropyl ether to give **38** as a pale yellow powder (6.82 g, 17.9 mmol, 82%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.31-2.41 (m, 2H), 3.67-3.80 (m, 2H), 4.69 (t, *J* = 6.0 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.25-7.37 (m, 2H), 7.47-7.51 (m, 2H), 9.98 (s, 1H).

1-[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]propan-1-ol (39a). Ethylmagnesium bromide (3.0 M solution in diethyl ether, 1.1 mL, 3.3 mmol) was added to a stirred solution of **39a** (1.00 g, 2.63 mmol) in THF (13 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction was quenched by NH_4Cl aq., and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was recrystallized from EtOH to give **39a** as a colorless crystal (992 mg, 2.42 mmol, 92%). mp 244-245 °C (EtOH). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.94 (t, *J* = 7.5 Hz, 3H), 1.72-1.85 (m, 2H), 2.23-2.35 (m, 2H), 3.60-3.71 (m, 2H), 4.43-4.55 (m, 2H), 4.91-5.03 (m, 1H), 5.30 (d, *J* = 4.5 Hz, 1H),

6.96 (d, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 7.54 (dd, *J* = 2.4 Hz, 8.7 Hz, 1H), 7.63 (d, *J* = 8.7 Hz, 1H), 7.80 (d, *J* = 2.4 Hz, 1H). LC-MS (ESI): m/z Calcd.: 409.1; Found: 410.0 (M+H). Anal. Calcd for $C_{19}H_{18}N_3OCl_3$: C,55.56; H,4.42; N,10.23; Cl,25.90. Found: C,55.41; H,4.32; N,10.21; Cl,25.89.

1-[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]propan-1-one (39b). Dess-Martin reagent (1.27 g, 3.00 mmol) was added to a stirred suspension of **39a** (1.12 g, 2.73 mmol) in CH_3CN (23 mL) at 0 °C, and the mixture was stirred at room temperature for 1 hr. The mixture was diluted with $NaHCO_3$ aq., concentrated *in vacuo,* and extracted with EtOAc/THF. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel eluting with a 15-50% EtOAc/*n*-hexane gradient mixture to give the solid, which was washed with diisopropyl ether to give **39b** as a colorless powder (980 mg, 2.40 mmol, 88%). mp 156-157 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, *J* = 7.5 Hz, 3H), 2.24-2.33 (m, 2H), 3.05 (q, *J* = 7.5 Hz, 2H), 3.67-3.77 (m, 2H), 4.16 (t, *J* = 6.0 Hz, 2H), 7.11 (d, *J* = 8.1 Hz, 1H), 7.26 (d, *J* = 8.1 Hz, 1H), 7.29-7.33 (m, 1H), 7.47-7.50 (m, 2H). LC-MS (ESI): m/z Calcd.: 407.0; Found: 408.0 (M+H).

[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]acetonitrile (40). Thionyl chloride (0.53 mL, 7.22 mmol) was added to a stirred solution of **37** (1.38 g, 3.61 mmol) and pyridine (0.10 mL) in THF (30 mL) at 0 °C, and the mixture was stirred at room temperature for 90 min. The mixture was diluted with $NaHCO_3$ aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over MgSO4, filtered, and concentrated *in vacuo.* A solution of sodium cyanide (354 mg, 7.22 mmol) in water (1.5 mL) was added to the resulting benzylchloride in DMSO (12 mL), and the mixture was stirred at room temperature for 14 h. The mixture was diluted with $NaHCO_3$ aq., and the precipitate was collected by filtration, washed with diisopropyl ether to give **40** as a colorless powder (1.36 g, 3.47 mmol, 96% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39-2.49 (m, 2H), 3.68-3.78 (m, 2H), 4.00 (s, 2H), 4.54 (t, *J* = 6.0 Hz, 2H), 6.82 (d, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 7.22 (dd, *J* = 2.1 Hz, 8.7 Hz, 1H), 7.47-7.50 (m, 2H).

2-[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]butanenitrile (39c). KO*t*-Bu (315 mg, 2.81 mmol) was added to a stirred solution of **40** (500 mg, 1.28 mmol) and ethyliodide (499 mg, 3.20 mmol) in THF (13 mL) at 0 °C, and the mixture was stirred at 0 °C for 1 h. The mixture was diluted with NH_4Cl aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo.* The residue was purified by flash column chromatography on silica gel eluting with a 20-50% EtOAc/*n*-hexane gradient mixture to give the solid, which was recrystallized from EtOH/EtOAc to give **39c** as a colorless crystal (202 mg, 0.481 mmol, 38%). mp 220-221 °C (EtOH/EtOAc). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18 (t, *J* = 7.5 Hz, 3H), 2.00-2.10 (m, 2H), 2.39-2.46 (m, 2H), 3.68-3.76 (m, 2H), 4.27-4.37 (m, 2H), 4.44-4.50 (m, 1H), 7.01 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.31 (dd, *J* = 2.4 Hz, 8.7 Hz, 1H), 7.48-7.51 (m, 2H). LC-MS (ESI): m/z Calcd.: 418.1; Found: 419.0 (M+H).

9-Chloro-1-(2,4-dichlorophenyl)-6-[1-(1*H*-imidazol-1-yl)propyl]-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (39d). A mixture of **39a** (150 mg, 0.326 mmol) and *N,N'*-carbonyldiimidazole (119 mg, 0.734 mmol) in CH_2Cl_2 (2.0 mL) was stirred at 50 °C for 22 h. The mixture was concentrated *in vacuo,* diluted with 1N HCl and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 50-100% EtOAc/*n*-hexane gradient mixture to give the solid, which was recrystallized from EtOAc/*n*-hexane to give **39d** as a colorless crystal (141 mg, 0.306 mmol, 94%). mp 197-199 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.04 (t, *J* = 7.5 Hz, 3H), 2.25-2.39 (m, 4H), 3.62-3.74 (m, 2H), 3.99-4.09 (m, 1H), 4.30-4.40 (m, 1H), 5.65 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 1.2 Hz, 1H), 6.94 (d, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 1.2 Hz, 1H), 7.13 (d, *J* = 8.1 Hz, 1H), 7.31 (dd, *J* = 2.4 Hz, 8.7 Hz, 1H), 7.46-7.52 (m, 3H). LC-MS (ESI): m/z Calcd.: 459.1; Found: 460.1 (M+H). Anal. Calcd for $C_{22}H_{20}N_5OCl_3$: C,57.34; H,4.37; N,15.20; Cl,23.08. Found: C,57.10; H,4.34; N,15.25; Cl,22.93.

9-Chloro-1-(2,4-dichlorophenyl)-6-(1-methoxypropyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (39e). A mixture of **39a** (460 mg, 1.12 mmol), K_2CO_3 (201 mg, 1.46 mmol) and methyl iodide (207 mg, 1.46 mmol) in DMF (4.5 mL) was stirred at room temperature for 9 h. The mixture was diluted with NH_4Cl aq., and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 5-40% EtOAc/*n*-hexane gradient mixture to give the solid, which was recrystallized from EtOAc/diisopropyl ether to give **39e** as a colorless crystal (363 mg, 0.855 mmol, 76%). mp 136-137 °C (EtOAc/diisopropyl ether). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.97 (t, *J* = 7.5 Hz, 3H), 1.75-1.90 (m, 1H), 1.91-2.06 (m, 1H), 2.32-2.40 (m, 2H), 3.24 (s, 3H), 3.65-3.78 (m, 2H), 4.30-4.43 (m, 1H), 4.50-4.63 (m, 1H), 4.52 (t, *J* = 6.9 Hz, 1H), 6.88 (d, *J* = 8.1 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 7.31 (dd, *J* = 2.1 Hz, 8.4 Hz, 1H), 7.49 (d, *J* = 2.1 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H). LC-MS (ESI): m/z Calcd.: 423.1; Found: 424.0 (M+H). Anal. Calcd for $C_{20}H_{20}N_3OCl_3$: C,56.55; H,4.75; N,9.89; Cl,25.04. Found: C,56.58; H,4.64; N,9.96; Cl,25.16.

1-[9-Chloro-1-(2,4-dichlorophenyl)-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazol-6-yl]-2,2,2-trifluoroethanol (41). To a stirred solution of **38** (500 mg, 1.31 mmol) and trimethyl(trifluoromethyl)silane (582 µl, 3.94 mmol) in THF (6.5 mL) was added a solution of tetrabutylammonium fluoride in THF (1.0 M, 131 µl, 0.131 mmol) at 0 °C. After 15 min, 1N HCl (2.0 mL) was added. After 30 min, the reaction mixture was diluted with EtOAc, washed with $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was washed with diisopropyl ether to give **41** as a colorless powder (590 mg, 1.30 mmol, 99%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.16 - 2.49 (m, 2H), 3.49 - 3.67 (m, 1H), 3.77 - 3.98 (m, 1H), 4.16 - 4.29 (m, 2H), 4.64 (brs, 1H), 5.43 (q, *J* = 6.2 Hz, 1H), 7.11 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 8.3 Hz, 1H), 7.30 - 7.40 (m, 1H), 7.45 - 7.60 (m, 2H).

9-Chloro-1-(2,4-dichlorophenyl)-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazole (39f). A solution of **41** (75.1 mg, 0.167 mmol) in THF (0.5 mL) and 8N NaOH (0.2 mL, 1.6 mmol) was stirred for 3 h at room temperature under chlorodifluoromethane atmosphere. The reaction mixture was diluted with

EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 0-30% EtOAc/*n*-hexane gradient mixture to give **39f** as a colorless solid (38.7 mg, 0.0734 mmol, 44%). Analytically pure material was obtained by recrystallization from EtOAc/*n*-hexane. mp 200-202 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 - 2.55 (m, 2H), 3.62 - 3.85 (m, 2H), 4.27 - 4.43 (m, 2H), 6.02 (q, *J* = 5.9 Hz, 1H), 6.42 (t, *J* =72.2 Hz, 1H), 7.17 (s, 2H), 7.34 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.48 - 7.56 (m, 2H). LC-MS (ESI): m/z Calcd.: 499.0; Found: 500.0 (M+H). Anal. Calcd for $C_{19}H_{13}N_3OF_5Cl_3$: C,45.58; H,2.62; N,8.39. Found: C,45.61; H,2.80; N,8.40.

Methyl 1-(4-bromo-2-methylphenyl)-9-chloro-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36a). The compound **36a** was prepared from **33** by the same methods as that described for **34**, **35**, and **36**. Pale yellow powder (66% in 3 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.26 (s, 3H), 2.30-2.33 (m, 2H), 3.71 (m, 2H), 3.96 (s, 3H), 4.43 (m, 2H), 7.10 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.38-7.46 (m, 3H).

Methyl 4-chloro-1-(3-hydroxypropyl)-2-[(6-methoxy-2-methylpyridin-3-yl)amino]-1*H*-benzimidazole-7-carboxylate (35b). The compound **35b** was prepared from **33** by the same methods as that described for **34** and **35**. Colorless powder (48% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.96-2.24 (m, 3H), 2.46 (s, 3H), 3.65-3.78 (m, 2H), 3.91 (s, 3H), 3.95 (s, 3H), 4.56 (t, *J* = 6.2 Hz, 2H), 6.64 (d, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 8.7 Hz, 1H), 7.44 (brs, 1 H), 7.53 (d, *J* = 8.7 Hz, 1H), 8.17 (d, *J* = 9.0 Hz, 1H).

Methyl 9-chloro-1-(6-methoxy-2-methylpyridin-3-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36b). The compound **36b** was prepared from **35b** by the same methods as that described for **36**. Colorless powder (90%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.25-2.37 (m, 2H), 2.40 (s, 3H), 3.53-3.78 (m, 2H), 3.94 (s, H), 3.95 (s, 3H), 4.26-4.57 (m, 2H), 6.65 (d, *J* = 8.7 Hz, 1H), 7.09 (d, *J* = 8.3 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H).

Methyl 4-chloro-2-[(3-hydroxypropyl)amino]-3-{[(2-methoxy-4,6-dimethylpyrimidin-5-yl)carbamothioyl]amino}benzoate (34c). The compound **34c** was prepared from **33** by the same methods as that described for **34**. Yellow amorphous (98%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.81-1.94 (m, 2H), 2.36 (s, 6H), 2.51-2.57 (brs, 1H), 3.60-3.80 (m, 4H), 3.89 (s, 3H), 3.95 (s, 3H), 6.75 (s, 1H), 6.88 (d, *J* = 9.0 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 8.15 (s, 1H), 8.26 (s, 1H).

Methyl 4-chloro-1-(3-hydroxypropyl)-2-[(2-methoxy-4,6-dimethylpyrimidin-5-yl)amino]-1*H*-benzimidazole-7-carboxylate (35c). The compound **35c** was prepared from **34c** by the same methods as that described for **35**. Pale yellow powder (98%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.73-1.81 (m, 2H), 2.31 (s, 6H), 3.31-3.42 (m, 2H), 3.91 (s, 3H), 3.92 (s, 3H), 4.46 (t, *J* = 7.2 Hz, 2H), 4.70-4.76 (m, 1H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 8.69 (s, 1H).

Methyl

9-chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36c). The compound **36c** was prepared from **35c** by the same methods as that described for **36**. Colorless powder (76%). H NMR ($CDCl_3$, 300 MHz) δ 2.27-2.37 (m, 2H), 2.37 (s, 6H), 3.59 (t, *J* = 5.7 Hz, 2H), 3.95 (s, 3H), 4.01 (s, 3H), 4.44 (t, *J* = 6.3 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H).

Methyl 4-chloro-2-[(4,6-diethyl-2-methylpyrimidin-5-yl)amino]-1-(3-hydroxypropyl)-1*H*-benzimidazole-7-carboxylate (35d). The compound **35d** was prepared from **33** by the same methods as that described for **34** and **35**. Colorless powder (52% in 3 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, *J* = 7.5 Hz, 6H), 2.15-2.25 (m, 2H), 2.31-2.37 (m, 1H), 2.68 (q, *J* = 7.5 Hz, 4H), 2.71 (s, 3H), 3.76-3.85 (m, 2H), 3.95 (m, 3H), 4.59 (t, *J* = 6.0 Hz, 2H), 7.12 (d, *J* = 8.7 Hz, 1H), 7.50 (s, 1H), 7.52 (d, *J* = 8.7 Hz, 1H).

Methyl 9-chloro-1-(4,6-diethyl-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36d). The compound **36d** was prepared from **35d** by the same methods as that described for **36**. Colorless solid (80%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, *J* = 7.2 Hz, 6H), 2.29-2.37 (m, 2H), 2.58-2.72 (m, 4H), 2.74 (s, 3H), 3.60 (t, *J* = 5.1 Hz, 2H), 3.95 (s, 3H), 4.46 (t, *J* = 6.3 Hz, 2H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H).

Methyl 4-chloro-2-[(4,6-dimethoxy-2-methylpyrimidin-5-yl)amino]-1-(3-hydroxypropyl)-1*H*-benzimidazole-7-carboxylate (35e). The compound **35e** was prepared from **33** by the same methods as that described for **34** and **35**. Pale yellow powder (6% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.99-2.08 (m, 2H), 2.19-2.24 (m, 1H), 2.54 (s, 3H), 3.57-3.64 (m, 2H), 3.94 (s, 6H), 3.95 (s, 3H), 4.59 (t, *J* = 6.0 Hz, 2H), 7.01 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H).

Methyl 9-chloro-1-(4,6-dimethoxy-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carboxylate (36e). The compound **36e** was prepared from **35e** by the same methods as that described for **36**. Colorless powder (80%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20-2.28 (m, 2H), 2.55 (s, 3H), 3.57 (t, *J* = 6.0 Hz, 2H), 3.93 (s, 9H), 4.35 (t, *J* = 6.0 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H).

[9-Chloro-1-(4-bromo-2-methylphenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]methanol (37a). The compound **36a** was prepared from **33** by the same methods as that described for **37**. Colorless powder (quant). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.19 (s, 3H), 2.27 (br, 2H), 3.60 (br, 2H), 4.48 (br, 2H), 4.72 (d, *J* = 3.9 Hz, 2H), 5.34 (t, *J* = 5.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 7.46 (dd, *J* = 2.1 Hz, 8.4 Hz, 1H), 7.56 (d, *J* = 1.8 Hz, 1H).

[9-Chloro-1-(6-methoxy-2-methylpyridin-3-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]methanol (37b). The compound **37b** was prepared from **36b** by the same methods as that described for **37**. Colorless solid (99%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.25-2.30 (m, 2H), 2.32 (s, 3H), 3.62 (brs, 2H), 3.88 (s, 3H), 4.33-4.65 (m, 2H), 4.74

(d, *J* = 5.3 Hz, 2H), 5.27-5.38 (m, 1H), 6.75 (d, *J* = 9.1 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 9.1 Hz, 1H).

[9-Chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]methanol (37c). The compound **36c** was prepared from **35c** by the same methods as that described for **37**. Colorless solid (98%). H NMR (DMSO-d_6, 300 MHz) δ 2.20-2.40 (m, 2H), 2.29 (s, 6H), 3.57-3.65 (m, 2H), 3.92 (s, 3H), 4.56 (t, *J* = 6.0 Hz, 2H), 4.74 (d, *J* = 5.1 Hz, 2H), 5.35 (t, *J* = 5.1 Hz, 1H), 6.83 (d, *J* = 8.1 Hz, 1H), 6.95 (d, *J* = 8.1 Hz, 1H).

9-Chloro-1-(4-bromo-2-methylphenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carbaldehyde (38a). To a solution of **37a** (4.68 g, 11.5 mmol) in dimethylsulfoxide (80 mL) was added Dess-Martin reagent (5.37 g, 12.7 mmol) , and the mixture was stirred at room temperature for 1 h. To the reaction mixture was added Dess-Martin reagent (0.49 g, 1.15 mmol), and the mixture was stirred at room temperature for 1 h. To the reaction mixture was added Dess-Martin reagent (1.46 g, 3.45 mmol) , and the mixture was stirred at room temperature for 1 h. The reaction mixture diluted with EtOAc, and the mixture was quenched by saturated aqueous sodium hydrogen carbonate. The insoluble matrial was removed by filtration, and the filtrate was extracted with EtOAc. The combined organic layer was washed with brine, dried over MgSO4, filtered and concentrated *in vacuo* to give the title compound as a pale yellow powder (4.54 g, 11.2 mmol, 97%). This product was used next reaction without purification. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.26 (s, 3H), 2.36 (m, 2H), 3.71 (br, 2H), 4.72 (br, 2H), 7.20 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 7.35-7.42 (m, 2H), 7.47 (s, 1H), 9.99 (s, 1H).

9-Chloro-1-(6-methoxy-2-methylpyridin-3-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carbaldehyde (38b). The compound **38b** was prepared from **37b** by the same methods as that described for **38a**. Pale yellow solid (93%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29-2.39 (m, 2H), 2.39 (s, 3H), 3.67 (t, *J* = 5.7 Hz, 2H), 3.95 (s, 3H), 4.70 (brs, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 9.99 (s, 1H).

9-Chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carbaldehyde (38c). The compound **38c** was prepared from **36c** by the same methods as that described for **37** and **38a**. Yellow solid (79%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.35-2.42 (m, 2H), 2.37 (s, 6H), 3.60 (t, *J* = 5.7 Hz, 2H), 4.01 (s, 3H), 4.72 (t, *J* = 5.7 Hz, 2H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 9.97 (s, 1H).

9-Chloro-1-(4,6-diethyl-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole-6-carbaldehyde (38d). The compound **38d** was prepared from **36d** by the same methods as that described for **37** and **38a**. Pale yellow solid (66%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.22-1.30 (m, 6H), 2.32-2.42 (m, 2H), 2.56-2.72 (m, 4H), 2.74 (s, 3H), 3.61 (t, *J* = 5.7 Hz, 2H), 4.73 (t, *J* = 6.3 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.35 (d, *J* = 8.4 Hz, 1H), 9.97 (s, 1H).

9-Chloro-1-(4,6-dimethoxy-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazole-6-carbaldehyde (38e). The compound **38e** was prepared from **36e** by the same methods as that described for **37** and **38a**. Pale yellow solid (75% in 2 steps). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.24 - 2.36 (m, 2H), 2.56 (s, 3H), 3.54 - 3.65 (m, 2H), 3.93 (s, 6H), 4.64 (t, *J* = 6.2 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 10.00 (s, 1H).

1-[1-(4-Bromo-2-methylphenyl)-9-chloro-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]-2,2,2-trifluoroethanol (41a). The compound **41a** was prepared from **38a** by the same methods as that described for **41**. Colorless powder (61%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.18 (s, 3H), 2.29 (br, 2H), 3.57-3.71 (br, 2H), 4.37 (br, 2H), 5.71 (t, *J* = 6.6 Hz, 1H), 6.99 (d, *J* = 4.8 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 7.11 (d, *J* = 8.4 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.47 (d, *J* = 8.7 Hz, 1H), 7.56 (s, 1H).

1-[9-Chloro-1-(6-methoxy-2-methylpyridin-3-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]-2,2,2-trifluoroethanol (41b). The compound **41b** was prepared from **38b** by the same methods as that described for **41**. Colorless powder (94%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.19-2.39 (m, 5H), 3.47-3.78 (m, 2H), 3.88 (s, 3H), 4.22-4.61 (m, 2H), 5.73 (brs, 1 H), 6.69-6.82 (m, 1H), 7.03 (brs, 1H), 7.05-7.17 (m, 2H), 7.71 (d, *J* = 8.3 Hz, 1H).

1-[9-Chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]-2,2,2-trifluoroethanol (41c). The compound **41c** was prepared from **38c** by the same methods as that described for **41**. Colorless powder (81%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.32 (s, 3H), 2.35-2.48 (m, 2H), 2.41 (s, 3H), 3.46 (d, *J* = 5.1 Hz, 1H), 3.54-3.62 (m, 2H), 4.01 (s, 3H), 4.37-4.46 (m, 2H), 5.50-5.58 (m, 1H), 7.08-7.12 (m, 2H).

1-[9-Chloro-1-(4,6-diethyl-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazol-6-yl]-2,2,2-trifluoroethanol (41d). The compound **41d** was prepared from **38d** by the same methods as that described for **41**. Colorless solid (96%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23-1.33 (m, 6H), 2.32-2.44 (m, 2H), 2.49-2.79 (m, 5H), 2.73 (s, 3H), 3.51-3.64 (m, 2H), 4.38 (t, *J* = 5.7 Hz, 2H), 5.46-5.53 (m, 1H), 7.05-7.09 (m, 2H).

1-[9-Chloro-1-(4-methoxy-2-methylphenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazo-6-yl]-2,2,2-trifluoroethanol (41f). Under N_2 atmosphere, to a solution of **41a** (0.40 g, 0.84 mmol) and NaOMe (28% solution in MeOH, 6.0 mL) in DMF (6.0 mL) was added copper (I) iodide (0.241 mg, 1.26 mmol), and the mixture was stirred at 100 °C for 3 h. The reaction mixture was diluted with NH_4Cl aq., and the mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give **41f** as a colorless powder (0.34 g, 0.80 mmol, 94%). ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.15 (s, 3H), 2.28 (br, 2H), 3.52-3.60 (br, 2H), 3.77 (s, 3H), 4.37 (br, 2H), 5.71 (br, 1H), 6.82 (dd, *J* = 2.7 Hz, 8.4 Hz, 1H), 6.89 (d, *J* = 2.7 Hz, 1H), 7.00 (br, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.23 (d, *J* = 8.4 Hz, 1H).

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(6-methoxy-2-methylpyridin-3-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42b). The compound **42b** was prepared from **41b** by the same methods as that described for **39f**. Colorless crystal (34%). mp 175-177 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 5H), 3.66 (d, *J* = 4.9 Hz, 2H), 3.95 (s, 3H), 4.36 (brs, 2H), 6.01 (q, *J* = 6.1 Hz, 1H), 6.13-6.65 (m, 1H), 6.64 (d, *J* = 8.7 Hz, 1H), 7.04-7.25 (m, 2H), 7.53 (d, *J* = 8.7 Hz, 1H). LC-MS (ESI): m/z Calcd.: 476.1; Found: 477.1 (M+H). Anal. Calcd for $C_{20}H_{18}N_4O_2ClF_5$: C,50.38; H,3.8; N,11.75; Cl, 7.44; F, 19.92. Found: C,50.55; H,3.79; N,11.72.

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42c). The compound **42c** was prepared from **41c** by the same methods as that

described for **39f**. Colorless crystal (30%). mp 169-170 °C (EtOAc/diisopropyl ether). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 6H), 2.41-2.49 (m, 2H), 3.54-3.67 (m, 2H), 4.01 (s, 3H), 4.33-4.41 (m, 2H), 5.99 (q, *J* = 5.7 Hz, 1H), 6.42 (t, *J* = 72.0 Hz, 1H), 7.12-7.17 (m, 2H). LC-MS (ESI): m/z Calcd.: 491.1; Found: 492.1 (M+H). Anal. Calcd for $C_{20}H_{19}N_5O_2ClF_5$: C,48.84; H,3.89; N,14.24; Cl,7.21; F,19.31. Found: C,48.70; H,3.98; N,14.18; Cl,7.23; F,19.31.

9-Chloro-1-(4,6-diethyl-2-methylpyrimidin-5-yl)-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42d). The compound **42d** was prepared from **41d** by the same methods as that described for **39f**. Colorless crystal (35%). mp 194-195 °C (EtOAc/diisopropyl ether). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25-1.31 (m, 6H), 2.41-2.49 (m, 2H), 2.57-2.73 (m, 4H), 2.74 (s, 3H), 3.55-3.67 (m, 2H), 4.33-4.44 (m, 2H), 5.99 (q, *J* = 6.0 Hz, 1H), 6.43 (t, *J* = 72.3 Hz, 1H), 7.12-7.14 (m, 2H). LC-MS (ESI): m/z Calcd.: 503.2; Found: 504.1 (M+H). Anal. Calcd for $C_{22}H_{23}N_5OClF_5$: C,52.44; H,4.60; N,13.90; Cl,7.04; F,18.85. Found: C,52.46; H,4.53; N,13.80; Cl,7.18; F,18.80.

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(4,6-dimethoxy-2-methylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazole (42e). The compound **42e** was prepared from **38e** by the same methods as that described for **41** and **39f**. Colorless crystal (44% in 2 steps). mp 190-192 °C (MeOH/diisopropyl ether). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29 - 2.45 (m, 2H), 2.56 (s, 3H), 3.49 - 3.68 (m, 2H), 3.95 (s, 6H), 4.22 - 4.38 (m, 2H), 6.01 (q, *J* = 6.2 Hz, 1H), 6.39 (t, *J* = 72.5 Hz, 1H), 7.06 - 7.16 (m, 2H). LC-MS (ESI): m/z Calcd.: 507.1; Found: 508.1 (M+H).

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(4-methoxy-2-methylphenyl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42f). The compound **42f** was prepared from **41f** by the same methods as that described for **39f**. Colorless crystal (33%). mp 178-182 °C (EtOAc/diisopropyl ether). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.28 (s, 3H), 2.43 (m, 2H), 3.60-3.70 (m, 2H), 3.84 (s, 3H), 4.36 (m, 2H), 6.03 (m, 1H), 6.41 (br, 1H), 6.79-6.85 (m, 2H), 7.13 (m, 2H), 7.23 (d, *J* = 8.4 Hz, 1H). LC-MS (ESI): m/z Calcd.: 475.1; Found: 476.1 (M+H).

9-Chloro-1-(2-chloro-4,6-dimethylpyrimidin-5-yl)-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (43). A mixture of **42c** (2.10 g, 4.27 mmol) and phosphoryl chloride (11 mL) was stirred at 100 °C for 5 h. The mixture was concentrated *in vacuo,* diluted with water, neutralized with $NaHCO_3$ aq. and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 50-100% EtOAc/*n*-hexane gradient mixture to give the the solid, which was washed with diisopropyl ether/*n*-hexane to give the title compound as a colorless powder (889 mg, 1.79 mmol, 42%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.45 (s, 6H), 2.45-2.55 (m, 2H), 3.57-3.70 (m, 2H), 4.36-4.45 (m, 2H), 5.98 (q, *J* = 6.0 Hz, 1H), 6.43 (t, *J* = 72.0 Hz, 1H), 7.15-7.19 (m, 2H).

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(2,4,6-trimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42g). Methylmagnesium bromide (1.0 M solution in THF, 0.49 mL, 0.490 mmol) was added dropwise to a stirred suspension of **43** (163 mg, 0.328 mmol) and [1,3-bis(diphenylphosphino)propane]dichloronickel (17.8 mg, 0.0328 mmol) in THF (1.3 mL) at 0 °C, and the mixture was stirred at room temperature for 7 h. The mixture was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$,

filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 30-100% EtOAc/*n*-hexane gradient mixture to give the solid, which was purified by preparative HPLC to give the title compound as the trifluoroacetic acid salt. The obtained compound was neutralized with $NaHCO_3$ aq. and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the solid, which was recrystallized from EtOAc/*n*-hexane to give **42g** as a colorless crystal (49.8 mg, 0.105 mmol, 32%). mp 168-170 °C (EtOAc/*n*-hexane). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.40-2.53 (m, 2H), 2.42 (s, 6H), 2.71 (s, 3H), 3.56-3.69 (m, 2H), 4.34-4.45 (m, 2H), 5.99 (q, *J* = 6.0 Hz, 1H), 6.43 (t, *J* = 72.3 Hz, 1H), 7.12-7.18 (m, 2H). LC-MS (ESI): m/z Calcd.: 475.1; Found: 476.1 (M+H). Anal. Calcd for $C_{20}H_{19}N_5OClF_5$: C,50.48; H,4.02; N,14.72; Cl,7.45; F,19.96. Found: C,50.09; H,4.01; N,14.42; Cl,7.42; F,19.80.

9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(2-ethyl-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42h). Ethylmagnesium chloride (2.0 M solution in THF, 0.37 mL, 0.740 mmol) was added dropwise to a stirred suspension of **43** (183 mg, 0.369 mmol) and [1,3-bis(diphenylphosphino)propane]dichloronickel (20.0 mg, 0.0369 mmol) in THF (1.5 mL) at 0 °C, and the mixture was stirred at room temperature for 14 h. The mixture was diluted with NH_4Cl aq. and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 70-100% EtOAc/*n*-hexane gradient mixture to give the solid, which was purified by preparative HPLC to give the title compound as the trifluoroacetic acid salt. The mixture was neutralized with $NaHCO_3$ aq. and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo* to give the solid, which was washed with EtOAc/*n*-hexane to give **42h** as a colorless amorphous (16.5 mg, 0.0337 mmol, 9%). Amorphous. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (t, *J* = 7.5 Hz, 3H), 2.40-2.51 (m, 2H), 2.42 (s, 6H), 2.94 (q, *J* = 7.5 Hz, 2H), 3.56-3.68 (m, 2H), 4.34-4.43 (m, 2H), 5.99 (q, *J* = 6.0 Hz, 1H), 6.43 (t, *J* = 72.3 Hz, 1H), 7.13-7.18 (m, 2H). LC-MS (ESI): m/z Calcd.: 489.1; Found: 490.1 (M+H).

(*R*)-9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42c-*R*) and **(*S*)-9-Chloro-6-[1-(difluoromethoxy)-2,2,2-trifluoroethyl]-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-*a*]benzimidazole (42c-*S*).** Racemate **42c** (2454 mg) was resolved by preparative HPLC, using CHIRALCEL OJ (5 cm i.d. × 50 cm, Daicel Chemical Industries, Ltd.) with the flow rate of 80 mL/min at 40 °C and hexane/ethanol (90/10) as the mobile phase, and obtaining the stereoisomer having a shorter retention time (1182 mg) in an enantiomer excess greater than 99.9% and the stereoisomer having a longer retention time (1212 mg) in an enantiomer excess of 99.7%. The obtained compounds were recrystallized from EtOAc/diisopropyl ether to give the optically active compounds as a colorless crystal respectively (**42-*R***: 958 mg, **42-*S***: 1.01 g). **42c-R:** >99.9% ee, $[\alpha]_D^{20}$ = - 40.5 (c = 0.4000, MeOH), mp 215-217 °C (EtOAc/diisopropyl ether). ^{1}H NMR and LCMS: same as **42c**, Anal. Calcd for $C_{20}H_{19}N_5O_2ClF_5$: C,48.84; H,3.89; N,14.24; Cl,7.21; F,19.31. Found: C,48.63; H,3.92; N,14.03; Cl,7.22; F,19.34., **42c-*S*:**

99.4% ee, $[\alpha]_D^{20}$ = + 39.6 (c = 0.4195, MeOH), mp 216-218 °C (EtOAc/diisopropyl ether). NMR and LCMS: same as **42c**, Anal. Calcd for $C_{20}H_{19}N_5O_2ClF_5$: C,48.84; H,3.89; N,14.24; Cl,7.21; F,19.31. Found: C,48.84; H,4.00; N,14.17; Cl,7.20; F,19.24.

1-(9-chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazol-6-yl)-2,2,2-trifluoroethanone (44c). Tetrapropylammonium perruthenate (7.06 g, 0.0201 mol) was added to a mixture of **41c** (177 g, 0.401 mol), 4-methylmorpholine 4-oxide (93.9 g, 0.802 mol), and MS4A (activated powder, 177 g) in CH_3CN (1600 ml) at 0 °C, and the mixture was stirred at room temperature for 5 h. The mixture was concentrated *in vacuo,* diluted with water, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with EtOAc, and the obtained solid was recrystalized from 2-propanol/water to give **44c** as an yellow crystal (152 g, 0.345 mol, 86% yield). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29-2.40 (m, 2H), 2.37 (s, 6H), 3.63 (t, 2H, J = 5.7 Hz), 4.02 (s, 3H), 4.24 (t, 2H, J = 5.7 Hz), 7.18 (d, 1H, J = 8.7 Hz), 7.48 (dd, 1H, J = 1.8 Hz, 8.7 Hz).

(*R*)-1-(9-chloro-1-(2-methoxy-4,6-dimethylpyrimidin-5-yl)-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazol-6-yl)-2,2,2-trifluoroethanol (41c-*R*). KO*t*-Bu (1.0 M in t-BuOH, 3.7 ml, 3.7 mmol) was added to the mixture of **44c** (80.0 g, 182 mmol), [$RuCl_2${(R)-xylbinap}{(R)-daipen}] (222 mg, 0.182 mmol), toluene (200 ml), and 2-propanol (400 ml) in glass autoclave containing magnetic stirring bar at room temperature under Ar atmosphere. The mixture was stirred under H_2 atmosphere (0.7 MPa) at room temperature for 5 h. After the reaction atmosphere was replaced with Ar atmosphere, 1N HCl (3.7 ml, 3.7 mmol) was added to the mixture, and the mixture was concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel eluting with a 0-10% MeOH/EtOAc gradient mixture to give the crude product (80.9 g, 97.1%ee). The crude product was recrystalized from EtOAc/*n*-heptane to give **41c-*R*** as a colorless crystal (60.5 g, 137 mmol, 75% yield, 99.4%ee). ^{1}H NMR: same as **41c**.

X-ray structure analysis

Crystal data for **42c-*R***: $C_{20}H_{19}ClF_5N_5O_2$, MW = 491.85; crystal size, 0.28 x 0.14 x 0.06 mm; colorless, platelet; monoclinic, space group $P2_1$, a = 8.07956(15) Å, b = 12.6103(3) Å, c = 20.3926(4) Å, $\alpha = \gamma = 90°$, β = 95.7360(10)°, V = 2067.31(7) Å^3, Z = 4, Dx = 1.580 g/cm^3, T = 100 K, μ = 2.326 mm^{-1}, λ = 1.54187 Å, R_1 = 0.058, wR_2 = 0.205, Flack Parameter[25] = 0.01(2). All measurements were made on a Rigaku R-AXIS RAPID diffractometer using graphite monochromated Cu-Kα radiation. The structure was solved by direct methods with SHELXS-97[26] and was refined using full-matrix least-squares on F^2 with SHELXL-97.[26] All non-H atoms were refined with anisotropic displacement parameters. CCDC 1528469 for compound **42c-*R*** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?.

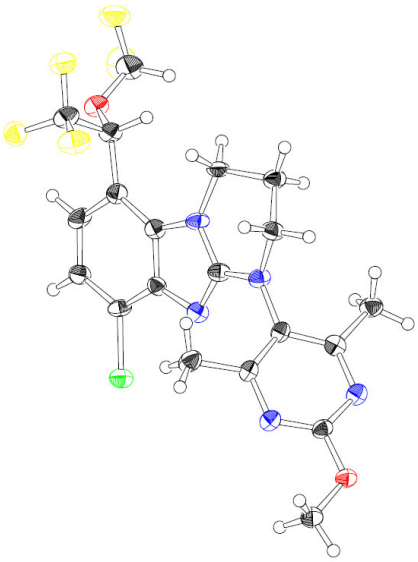


Figure 8. ORTEP of **42c-*R***, thermal ellipsoids are drawn at 50% probability.

Biology

Measurement of Corticotropin-Releasing Factor (CRF) binding inhibitory rate. A receptor binding experiment was carried out using a human CRF receptor expressing CHO cellular membrane fraction and ovine CRF, [^{125}I]-tyr^{0}(^{125}I-CRF). Various concentrations of a test compound was incubated with 1 μg of human CRF receptor expressing 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, 20 μg/ml leupeptin, pH 7.5). In addition, for measuring nonspecific binding (NSB), 0.1 μM unlabelled human Urocortin was incubated with 1 μg of human CRF receptor expressing CHO cellular membrane fraction and 50 pM of ^{125}I-CRF in a binding assay buffer. After a binding reaction was carried out at room temperature 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-cooled 50 mM Tris-HCl (pH 7.5). After drying the glass filter, a liquid scintillation cocktail (Microscinti 0, Perkin Elmer) was added, and the radioactivity of ^{125}I-CRF remaining on a glass filter was measured using Topcount (Perkin Elmer). (TB-SB)/(TB-NSB) × 100 (SB: radioactivity when a compound is added, TB: maximum binding radioactivity, NSB: nonspecific binding radioactivity) was calculated to obtain a binding inhibitory rate under the presence of 1,000 nM of each test substances. The IC_{50} values were calculated by using GraphPad Prism software.

CRF antagonistic activity. CRF antagonistic activity was obtained by measuring inhibition of Adenylate cyclase by the use of a CRE-luciferase reporter gene assay. Human CRF 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, 0.1% BSA, pH 7.2) containing 1 nM human CRF for 4 hr. Following drug exposures, the cells were lysed, and

luciferase activity was measured using a Steady-Glo® Luciferase Assay System (Promega). Light output was detected by ARVO-SX (Wallac). The IC_{50} values were calculated by using GraphPad Prism software.

Pharmacology

Animals. Adult male Wistar rats (Clea Japan, Tokyo, Japan) weighing 250-300 g were group housed in 5 rats in stainless cage. Animals could access water and food *ad libitum* and were maintained on a 12 hr light-dark cycle (light on 7:00 a.m.), with constant temperature (23±3℃) and humidity (55±15%). Animals were allowed to acclimate to these housing conditions for one week before experiments or operation.

Drugs. Compounds were suspended with 0.5% methylcellulose (Shin-Etsu Chemical, Japan) in distilled water. The concentration of the drug was 10 mg/kg, and administered orally in a volume of 2 mL/kg.

Ex vivo binding assay in rats. Rats were treated with compounds (5, 10 mg/kg) or the vehicle orally 60 minutes before decapitation. After that, the brain was rapidly removed and divided into the frontal cortex, olfactory bulb and pituitary. Tissues of two rats were collected in one tube, measured weight as wet tissue and stored at –80 °C until use. They were homogenized in Lysis buffer (50 mM Tris-HCl pH 7.0, 10 mM $MgCl_2$, 2 mM EDTA, 100 KU/mL aprotinin) at 4 ^{o}C with a PHYSCOTRON (setting, 10 sec). Frontal cortex was diluted into 5 mg wet tissue /mL by lysis buffer. Olfactory bulb was homogenized in 5 mL of lysis buffer and added 20 mL of lysis buffer. Pituitary was homogenized in 2.5 mL of lysis buffer and diluted into 5 mg wet tissue /mL by lysis buffer. After preparation of brain membrane homogenates, [^{125}I]-CRF (ovine) binding was performed 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 under a final volume of 200 mL. After incubation at room temperature for 2 hours, the incubation mixture was filtered on Whatman GF/C filter presoaked in 0.3% polyethyleneimine. The filters were washed sixth with ice-cld wash buffer (PBS containing 0.05% CHAPS, 0.01% Triton X-100) and dried. The radioactivity was determined with a gamma scintillation counter. Results were expressed as an inhibitory rate of [^{125}I]- CRF (ovine) binding, with in vitro determination of the nonspecific binding by using a 1 μM of the selective CRF_1 receptor antagonist R121919.

Surgery. Subjects were anethetized with pentobarbital (50 mg/kg, ip), mounted on a stereotaxic apparatus (David Kopf Instruments,CA, USA) and prepared with indwelling cannulae directed unilaterally at the lateral ventricle (AP –0.8 mm, L –1.6 mm from bregma, and DV –3.7 mm from the surface of the skull) for i.c.v. injection. A dummy cannula was used to maintain patency. Subjects were allowed to recover for at least one week.

Preparation of CRF. We prepared a stock solution of 10 μg/μL h/r CRF (Peptide institute, Inc.) in PBS and stored at –20 °C until use. On the experimental day, the stock solution was diluted into 1.0 μg/5 μL.

Measurement of spontaneous locomotor activity. After recovery period, rats were acclimated to the apparatus of spontaneous activity measurement system (Muromachi-kikai Co., Ltd.) using the apparatus consisting of 3 parts: plexiglas cage (38 X 25 X 32 cm), infrared sensor (Supermex) and software for analysis (CompACT AMS ver. 3). After overnight acclimation, the rats were administered compounds or vehicle orally between 9:30 and 10:00 a.m., followed by

icv-administration of CRF or vehicle (PBS) 2 hous later. The spontaneous locomotor activity was measured for 60 minutes after i.c.v.-administration. The data was calculated as the total spontaneous locomotor activity .

References

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

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

(3) Dzierba, C. D.; Hartz, R. A.; Bronson, J. J. Recent Advances in Corticotropin-Releasing Factor Receptor Antagonists. *Annu. Rep. Med. Chem*. **2008**, *43*, 3–23.

(4) Gilligan, P. J. Corticotropin-Releasing Factor Receptor Antagonists. *Expert Opin. Ther. Pat.* **2006**, *16*, 913–924.

(5) Williams, J. P. Corticotropin-Releasing Factor 1 Receptor Antagonists: A Patent Review. *Expert Opin. Ther. Pat.* **2013**, *23*, 1057–1068.

(6) Eric, P. Z.; George, F. K. The Therapeutic Potential of CRF_1 Antagonists for Anxiety. *Expert Opin. Invest. Drugs* **2004**, *13*, 799–828..

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

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

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

(10) Zobel, A. W.; Nickel, T.; Künzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects of the High-Affinity Corticotropin-Releasing Hormone Receptor 1 Antagonist R121919 in Major Depression: The First 20 Patients Treated. *J. Psychiatr. Res*. **2000**, *34*, 171–181.

(11) Ising, M.; Zimmermann, U. S.; Künzel, H. E.; Uhr, M. Clinical Phase 1 Proof of Concept Data of the High-Affinity CRF1 Receptor Antagonist NBI-34041 Suggest Efficacy in Attenuating Elevated Stress Response. Proceedings of the *46th Annual Meeting, American College of Neuropsychopharmacology,* Boca Raton, FL, December 9–13, 2007(Boca Raton); American College of Neuropsychopharmacology: Brentwood, TN, 2007.

(12) Binneman, B.; Feltner, D.; Kolluri, S.; Shi, Y.; Qiu, R.; Stiger, T. A 6-Week Randomized, Placebo-Controlled Trial of CP-316,311 (a Selective CRH1 Antagonist) in the Treatment of Major Depression. *Am. J. Psychiatry* **2008**, *165*,

617−620.

(13) Coric, V.; Feldman, H. H.; Oren, D. A.; Shekhar, A.; Pultz, J.; Dockens, R. C.; Wu, X.; Gentile, K. A.; Huang, S.-P.; Emison, E.; Delmonte, T.; D'Souza, B. B.; Zimbroff, D. L.; Grebb, J. A.; Goddard, A. W.; Stock, E. G. Multicenter, Randomized, Double-Blind, Active Comparator and Placebo-Controlled Trial of a Corticotropin-Releasing Factor Receptor-1 Antagonist in Generalized Anxiety Disorder. *Depression Anxiety* **2010**, *27*, 417−425.

(14) Sweetser, S. R.; Linker Nord, S. J.; Burton, D. D.; Grudell, A.; Eckert, D. J.; Manini, M. L.; Busciglio, I.; Tong, G.; Dockens, R. C.; Zinsmeister, A. R.; Camilleri, M. Effects of a Novel Corticotrophin Releasing Factor Receptor-1 Antagonist, BMS-562086, On Gastrointestinal and Colonic Transit and Bowel Habits in Patients with Diarrhea-Predominant Irritable Bowel Syndrome (D-IBS). *Gastroenterology* **2008**, *134*, A-548.

(15) Thoua, N. M.; Hobson, A. R.; Dukes, G. E.; Kelleher, D. L.; Hicks, K. J.; Boardley, R. L.; Raeburn, A. J.; Emmanuel, A. V. The Selective CRF-1 Receptor Antagonist GW876008 Attenuates Stress Induced Rectal Hypersensitivity in Patients with Irritable Bowel Syndrome (IBS). Proceedings from the Gastro 2009, London, U.K., November 21−25, 2009; OP097.

(16) ClinicalTrials.gov (A service of the U.S. National Institutes of Health). https://clinicaltrials.gov/ (as of March 31, 2017).

(17) Waring, M. J. Lipophilicity in Drug Discovery. *Expert Opin. Drug Discovery* **2010**, *5*, 235−248.

(18) Mochizuki, M.; Kori, M.; Kobayashi, K.; Yano, T.; Sako, Y.; Tanaka, M.; Kanzki, N.; Gyorkos, A. C.; Corrette, C. P.; Cho, S. Y.; Pratt, S. A.; Aso, K. Design and Synthesis of Benzimidazole as Novel Corticotropin-Releasing Factor 1 Receptor Antagonists. *J. Med. Chem.,* **2016**, *52*, 2551−2566.

(19) The edited molecules were optimized and their global minimum conformations in vacuum were obtained by stochastic search using MMFF94s force field and its default partial charges in Molecular Operating Environment (MOE), 2011.10; Chemical Computing Group Inc., 1010. Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2011.

(20) The atomic partioal charges fitted to the MNDO electrostatic potential were evaluated by MOPAC 7.0 program implemented in MOE 2010.10. During the MNDO electrostatic potential calculations, quantum mechanics optimizations were performed.

(21) Lanier M.; Williams, J. P. Small molecule corticotropin-releasing factor antagonist. *Expert Opin. Ther. Pat.* **2002**, *12*, 1619−1630.

(22) Docking studies were performed using GOLD software version 5.2, based on the complex crystal structure of 4K5Y, which has an antagonist CP-376395 in the transmembrane domain. The Protonate 3D module of MOE2013.08 was used to predict ion states, tautomers and positions of polar hydrogen atoms of the protein structure followed by energy minimization at the MMFF94s force field and the distant dependent dielectric constant (4r) using MOE2013.08. And only the protein structure was extracted for docking study. The conditions of GOLD software

were the standard default settings except for number of GA attempts (changed to 20), and defining binding site as the CP-376395 position. Docking mode with the highest score was adopted.

(23) Hollenstein, K.; Kean, J; Bortolato, A.; Robert, Cheng, R. K. Y.; Dore, A. S.; Jazayeri, A.; Cooke, R. M.; Weir, M.; Marshall,F. H. Structure of class B GPCR corticotropin-releasing factor receptor 1. *Nature* **2013**, *499*, 438−443.

(24) Sandoval, C. A.; Ohkuma, T.; Muniz, K.; Noyori, R. Mechanism of Asymmetric Hydrogenation of Ketones Catalyzed by BINAP/1,2-Diamine-Ruthenium(II) Complexes. *J. Am. Chem. Soc.,* **1998**, *120*, 13529−13530.

(25) Flack, H. D. On enantiomorph-polarity estimation. *Acta Cryst. A* **1983**, *39*, 876−881.

(26) Sheldrick, G.M. A short history of *SHELX*. *Acta Cryst. A* **2008**, *64*, 112−122.