Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

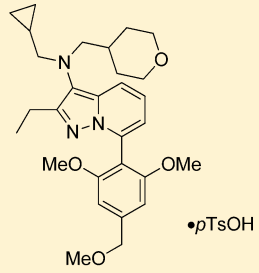
Synthesis and Structure–Activity Relationships of Pyrazolo[1,5-*a*]pyridine Derivatives: Potent and Orally Active Antagonists of Corticotropin-Releasing Factor 1 Receptor

Yoshinori Takahashi,*[,†] Shigeki Hibi,[†] Yorihisa Hoshino,[†] Koichi Kikuchi,[†] Kogyoku Shin,[†] Kaoru Murata-Tai,[‡] Masae Fujisawa,[§] Mitsuhiro Ino,[∥] Hisashi Shibata,[∥] and Masahiro Yonaga[†]

[†]Medicinal Chemistry, [∥]Biopharmacology, [‡]Physical Chemistry, and [§]Drug Metabolism and Pharmacokinetics, Tsukuba Research Laboratories, Eisai Co., Ltd., 5-1-3 Tokodai, Tsukuba-shi, Ibaraki 300-2635, Japan

Supporting Information

ABSTRACT: Design, synthesis, and structure–activity relationships of a series of 3-dialkylamino-7-phenyl pyrazolo[1,5-*a*]pyridines (**I**) as selective antagonists of the corticotropin-releasing factor 1 (CRF_1) receptor are described. The most prominent compound to emerge from this work, **46** (E2508), exhibits potent *in vitro* activity, excellent drug-like properties, and robust oral efficacy in animal models of stress-related disorders. It has advanced into clinical trials.



46 $hCRF_1$ K*i* = 11 nM

INTRODUCTION

Tricyclic antidepressants and serotonin and/or norepinephrine transporter reuptake inhibitors are widely used in the treatment of depression, anxiety, and stress-related disorders. These medicines, although clinically important, are not ideal owing to their insufficient efficacy, slow onset of action, or adverse events such as nausea and sexual dysfunction. Thus, new drugs with novel mechanisms of action, which may address some or all of the above unmet medical needs, have been long awaited in this area.

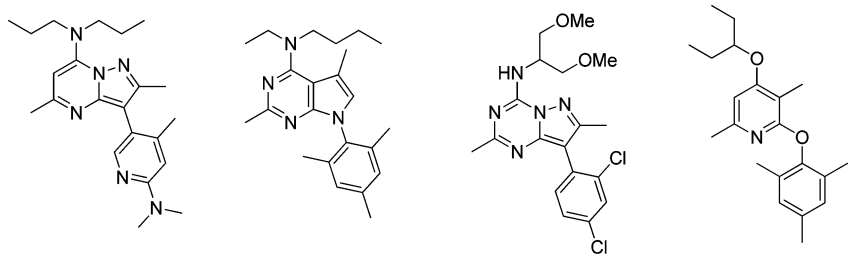
Corticotropin-releasing factor (CRF), first isolated by Vale from ovine brain extract in 1981,[1] is a 41-amino-acid neuropeptide secreted from the hypothalamus. It is closely linked to the release of adrenocorticotropic hormone from the pituitary and plays an important role in the regulation of the hypothalamic–pituitary–adrenal axis.[2] CRF exerts its effects through two receptor subtypes (CRF_1 and CRF_2) that belong to the class B subfamily of G-protein-coupled receptors,[3−6] which are widely distributed throughout the central and peripheral nervous systems. Preclinical and clinical evidence implicate CRF_1 receptors in stress-related diseases. For instance, CRF_1 receptor knockout mice show less anxious behavior,[7,8] and intracerebroventricular administration of CRF to rats induces anxiety and depression-like behavioral changes.[9] Moreover, in humans, high CRF levels in cerebrospinal fluid have been found in patients with depression.[10,11] By contrast, the role of CRF_2 as a target for stress-related disorders has not been fully established. Thus, it is hypothesized that selective CRF_1 receptor antagonists would be useful in the treatment of stress-related diseases such as depression, anxiety, and, possibly, irritable bowel syndrome. Indeed, the efficacy of CRF_1 receptor antagonists has been widely shown in preclinical animal models of anxiety and depression.[12−19]

However, the clinical utility of CRF_1 receptor antagonists has not yet been fully demonstrated. Compound **1** (R121919) exhibited antidepressant/anxiolytic activity in patients with depression in a small open-label phase IIa study,[20,21] whereas **4** (CP-316,311) failed to show efficacy in a double-blind, placebo-controlled study[22] (Figure 1). It is tempting to speculate that the main reason for these equivocal results lies with the compound itself (e.g., suboptimal drug-like properties such as high lipophilicity or low water solubility, and inadequate target engagement) and not with the mechanism of action. Therefore, we hypothesized that a CRF_1 receptor antagonist having the appropriate drug-like characteristics may show robust efficacy as a novel antidepressant in clinical practice.

Analyses of CRF_1 receptor antagonists published so far, such as **1**,[23] **2** (CP-154,526),[24] **3** (DMP696),[25] and **4**,[26] indicate the following structural features:[27] (1) a 6,5-, or 6,6-fused heteroaromatic core or monocyclic core containing an sp^2-hybridized nitrogen atom, which is thought to be essential as a hydrogen acceptor; (2) a small alkyl or tertiary amine group attached to the core ring; (3) an aryl (or heteroaryl) ring appended to the core and mono- or di-*ortho*-substituted to maintain an appropriate orthogonal conformation (Figure 2).

According to the above-mentioned requirements, we focused on a 5,6-fused bicyclic heteroaromatic template (**I**) with both the sp^2-hybridized nitrogen atom and the dialkylamino group on the core's five-membered ring; this structural system is

Received: February 24, 2012
Published: May 15, 2012



1 (R121919) **2** (CP-154,526) **3** (DMP696) **4** (CP-316,311)

Figure 1. Reported 6,5-fused or monocyclic CRF_1 receptor antagonists.

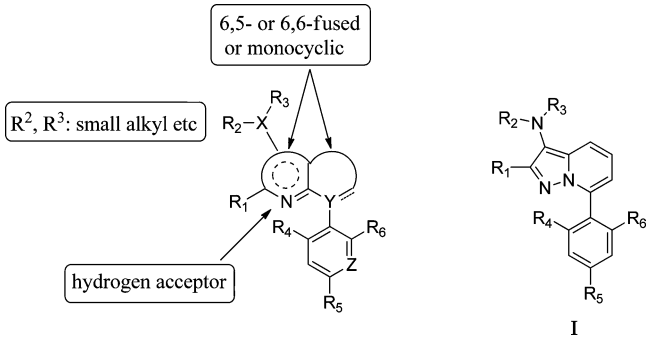


Figure 2. Designed template **I** based on the structural similarity of CRF_1 receptor antagonist.

unprecedented among CRF_1 receptor antagonists described so far. In this paper, we report the synthesis, structure–activity relationships, and *in vivo* efficacy in animal models for stress-related disorders for a series of pyrazolo[1,5-*a*]pyridine derivatives. Our research efforts in this area have led to the discovery of **46** as a promising CRF_1 receptor antagonist that has advanced into clinical trials.

RESULTS AND DISCUSSION

Chemistry. The syntheses of **7**, **10**, and **14**, which are intermediates to 2-alkyl pyrazolo[1,5-*a*]pyridine analogues, are described in Schemes 1 and 2. Palladium-catalyzed coupling of 2-bromopyridine **5** with 1-butyne afforded **6**. *N*-Amination of **6** with *O*-mesitylenesulfonylhydroxylamine,[28] followed by cyclization in the presence of sodium methoxide in methanol, generated the 2-ethyl pyrazolo[1,5-*a*]pyridine core **7** (Scheme 1). Bromination of **7** and the 2-methyl analogue **11**[29] gave **8** and **12**, respectively. Subsequent nitration and reduction produced the corresponding amines, which were protected to give *tert*-butyl carbamates **10** and **14**, respectively (Scheme 2).

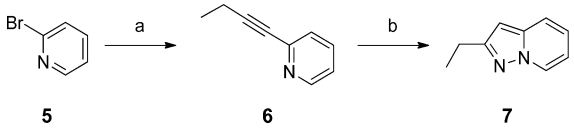
Schemes 3 and 4 show the syntheses of 2-methoxy- and 2-methylthio-substituted core rings, respectively, using procedures similar to those in Scheme 2. Thus, **15**[30] was converted to *tert*-butyl carbamate **18** in three steps via nitrosation by using $NaNO_2$/AcOH; note that NO_2BF_4 gave a complex reaction mixture, whereas $Cu(NO_3)_2 \cdot 3H_2O$ failed to promote the reaction. Similar to the 2-methoxy intermediates, reduction of the nitro group of **19**[31] followed by conversion to *tert*-butyl carbamate gave **20**, which was iodinated to **21**.

The preparation of pyrazolo[1,5-*a*]pyridine derivatives **24**, **27**, **30**, and **33** is described in Schemes 5 and 6. Alkylation of **10**, **18**, **21**, and **14** with alkyl bromides or alkyl tosylates in the presence of sodium hydride, followed by removal of the Boc group gave **22**, **25**, **28**, and **31**, respectively. Subsequent reductive amination afforded the corresponding tertiary amines. Suzuki coupling of the amines with various arylboronic acids gave the desired products (Scheme 5). Alternatively, the palladium-catalyzed reaction to introduce various aryl groups was carried out prior to elaboration of the dialkylamino moiety to afford the desired products **24**, **27**, and **30** (Scheme 6).

Suzuki coupling was also investigated as a method for introduction of the aryl group into the nitro compound **9** (Scheme 7). Reduction of the nitro group of **40** followed by reductive amination yielded the symmetrical dialkyl amine **24**.

The synthesis of 2-cyano analogue **45** is shown in Scheme 8. Oxidation of the methylthio group in **19** gave the sulfone **41**. Then, displacement of the methylsulfonyl group with cyano group gave **42**. Finally, introduction of the aryl group via iodide **44** afforded the desired product **45**.

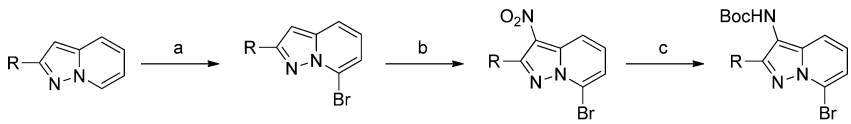
Scheme 1[a]



5 **6** **7**

[a]Reagents and conditions: (a) 1-Butyne, $PdCl_2(PPh_3)_2$, cat. CuI, Et_2NH, rt, 83%; (b) (i) *O*-mesitylenesulfonylhydroxylamine, CH_2Cl_2, 0 °C, (ii) MeONa, MeOH, 0 °C, 38%, 2 steps.

Scheme 2[a]

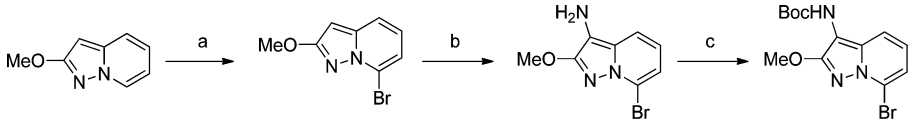


7 R = Et **8** R = Et **9** R = Et **10** R = Et
11 R = Me **12** R = Me **13** R = Me **14** R = Me

[a]Reagents and conditions: (a) BrF_2CCF_2Br, *n*-BuLi in hexane, THF, −78 °C–rt, 84–86%; (b) NO_2BF_4, CH_3CN, 0 °C, 57–60%; (c) (i) Zn, AcOH, EtOH, H_2O, 60 °C, 47–60%, (ii) Boc_2O, Et_3N, CH_2Cl_2, 0 °C–rt, 79–83%.

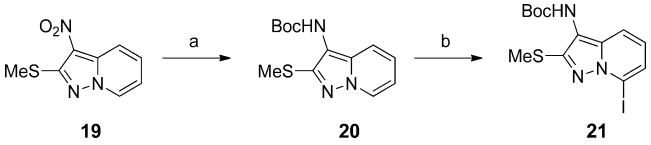
dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255–5269

Scheme 3[a]



[a]Reagents and conditions: (a) $BrCl_2CCCl_2Br$, *n*-BuLi in hexane, THF, −78 °C−rt, 65%; (b) $NaNO_2$, AcOH, H_2O, rt, then Zn, EtOH, H_2O, 60 °C, 70%; (c) Boc_2O, Et_3N, CH_2Cl_2, rt, 97%.

Scheme 4[a]



[a]Reagents and conditions: (a) (i) Zn, AcOH, EtOH, H_2O, 80 °C, (ii) Boc_2O, Et_3N, CH_2Cl_2, rt, 43%, 2 steps; (b) 1,2-diiodoethane, *n*-BuLi in hexane, THF, −78 °C, 69%.

Pharmacology. The affinity for human CRF_1 receptors was determined by competition with ^{125}I-CRF using cell membranes prepared from human CRF_1 receptor expressed in HEK293 cells; the functional antagonistic activities of CRF-stimulated cAMP production were determined in HEK293 cells expressing the human CRF_1 receptor.[32]

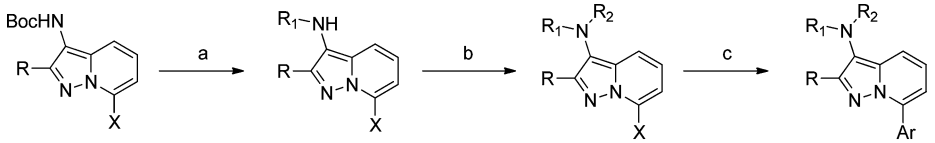
To evaluate the potential of the 5,6-fused heteroaromatic template, we first investigated pyrazolo[1,5-*a*]pyridine compounds **24a**, **24b**, **24f**, and **24g**, bearing 2,4-disubstituted phenyl rings at C-7 and dialkylamino groups at C-3 (Table 1). As can be seen, these pyrazolo[1,5-*a*]pyridine derivatives exhibited potent binding activity. In addition, 3-dicyclopropylmethylamino derivatives showed somewhat increased activity, especially functional antagonistic activity, when compared with di-*n*-propyl analogues. Other substitution patterns such as the methyl analogue **24c** and the trisubstituted analogues **24d** and **24e** also displayed significant binding affinity.

Regarding the functional antagonistic activities, trisubstituted analogue **24d**, with an *ortho* methoxy group, tended to be more potent than the disubstituted analogue **24c**. Unfortunately, **24d** exhibited high intrinsic clearance (hCLint; 1.12 mL/min/mg) in human liver microsomes, probably because of its high lipophilicity (clogP[33] = 6.8). Therefore, modification of **24d** became the focus for improvement of the drug-like properties, especially human metabolic stability.

Prediction of the metabolic pathways of **24d** highlighted the alkylamine moiety at C-3 as a possible cause for the metabolic instability. Thus, the side chain was modified at the 3-position, as summarized in Table 2. In order to improve hCLint, introduction of polar groups at one of the *N*-substituents was explored while retaining the other cyclopropylmethyl group. Although introduction of methoxy (**24h**) and fluorine (**24i**) slightly reduced lipophilicity, these compounds showed higher hCLint than **24d**. Subsequently, cyclic ether groups were introduced, based on the hypothesis that the cause of the metabolic instability might be *O*-demethylation in **24h**. Unfortunately, 3- and 2-THF analogues, **24j** and **24k**, respectively, showed high hCLint. However, 4-tetrahydropyranyl (4-THP) analogue **24l** displayed significant improvement in hCLint while maintaining potent binding affinity. Metabolism studies on these compounds showed mono- and didealkylation of the 3-amino side chain as prominent pathways; oxidation of the α-carbon to the oxygen atom on the cyclic ether could also contribute to metabolic instability. The improved stability of **24l** relative to **24j**/**24k** does not appear to stem from the overall lipophilicity of the molecule. We speculate that the position of the oxygen atom on the cyclic ether might contribute to reducing the metabolism at the α position of the THP ring.[34]

Since the 4-THP analogue **24l** had improved hCLint, modification of its 7-aryl ring was examined to enhance solubility[35] (Table 3). Replacement of the *ortho* methyl group with a methoxy group (**24m**) slightly improved solubility while maintaining comparable binding affinity and hCLint. However, regioisomer **24n** did not have improved solubility. Since **24m** showed higher *in vitro* affinity as well as better solubility than **24n**, modification of the *para* position of the C-7 *ortho* dimethoxyphenyl ring was explored. While the solubility of the methoxy derivative **24o** was almost identical to that of **24m**, introduction of hydroxymethyl (**24q**) greatly improved the solubility but only provided moderate affinity. However, the methoxymethyl analogue **24r** showed the appropriate balance

Scheme 5[a]

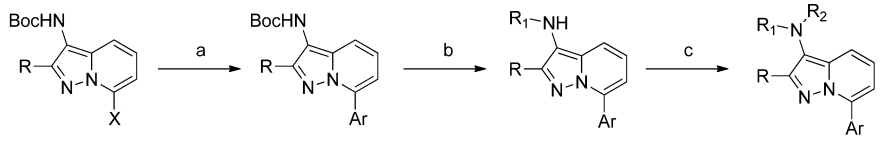


10 R = Et, X = Br	**22** R = Et, X = Br	**23** R = Et, X = Br	**24** R = Et
18 R = OMe, X = Br	**25** R = OMe, X = Br	**26** R = OMe, X = Br	**27** R = OMe
21 R = SMe, X = I	**28** R = SMe, X = I	**29** R = SMe, X = I	**30** R = SMe
14 R = Me, X = Br	**31** R = Me, X = Br	**32** R = Me, X = Br	**33** R = Me

[a]Reagents and conditions: (a) (i) R_1-Br or R_1-OTs, NaH, DMF, rt−40 °C, (ii) HCl in AcOEt, rt−40 °C, 77−100%, 2 steps; (b) Alkylaldehyde, $NaBH(OAc)_3$, THF, rt, 58−99%; (c) Ar−$B(OH)_2$, $Pd(PPh_3)_4$, $Ba(OH)_2{\cdot}8H_2O$, DME, H_2O, reflux, 37−98%, or Ar−$B(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, DME, H_2O, reflux, 61−93%.

dx.doi.org/10.1021/jm300259r | J. Med. Chem. 2012, 55, 5255−5269

Scheme 6[a]



10 R = Et, X = Br
18 R = OMe, X = Br
21 R = SMe, X = I

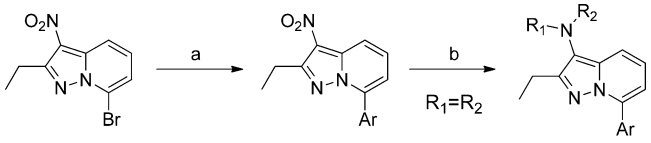
34 R = Et
36 R = OMe
38 R = SMe

35 R = Et
37 R = OMe
39 R = SMe

24 R = Et
27 R = OMe
30 R = SMe

[a]Reagents and conditions: (a) Ar−B(OH)$_2$, Pd(PPh$_3$)$_4$, DME, H$_2$O and Ba(OH)$_2$·8H$_2$O or K$_2$CO$_3$, 80−90 °C, 35−99%; (b) (i) R$_1$-Br or R$_1$-I or R$_1$-OMs, NaH, DMF, 40−50 °C, (ii) HCl in AcOEt, or TFA, CH$_2$Cl$_2$, rt−40 °C; (c) Alkylaldehyde, NaBH(OAc)$_3$, THF; or NaBH(OAc)$_3$, THF, AcOH; or NaBH$_4$, 3 M H$_2$SO$_4$, THF; or α-picoline-borane, MeOH, rt, 16−86%, 3 steps; (c) for **30c** (i) Alkylaldehyde, NaBH(OAc)$_3$, THF, rt, (ii) TBAF, THF, rt, 19%, 2 steps; (c) for **30g** (i) Alkylaldehyde, NaBH(OAc)$_3$, THF, AcOH, rt, 43%, (ii) TBAF, THF, rt, 78%, (iii) MeI, NaH, DMF, rt, 94%.

Scheme 7[a]



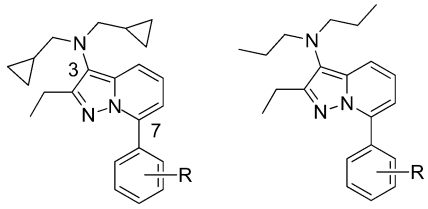
9 **40** **24**

[a]Reagents and conditions: (c) Ar−B(OH)$_2$, Pd(PPh$_3$)$_4$, Ba(OH)$_2$·8H$_2$O, DME, H$_2$O, 80 °C, 73−100%; (b) (i) Zn, AcOH, EtOH, H$_2$O, 60 °C, (ii) Alkylaldehyde, NaBH$_4$, 3 M H$_2$SO$_4$, THF, 0 °C−rt, 66−77%, 2 steps.

of solubility, potent activity, and improved hCLint. The fact that clogP is lower for **24r** (4.73) than for **24m** (5.43) might contribute to the improved hCLint. Regioisomer **24s** also showed comparable solubility but less activity than **24r**, indicating that bulky substituents might be less well tolerated at the *ortho* position than the *para* position. Compound **24t** showed identical affinity to **24r**, but somewhat lower solubility and increased hCLint. *n*-Propyl analogues **24v** and **24w** displayed 2-fold lower binding affinity and higher hCLint compared with **24m** and **24r**, respectively, suggesting that the cyclopropylmethyl group is preferable, in line with the results listed in Table 1.

Next, the substituent effects at the 2-position of the promising compound **24r** were examined (Table 4). The methoxy analogue **27a** showed comparable binding affinity and

Table 1. Initial Investigation of Pyrazolo[1,5-*a*]pyridine Derivatives



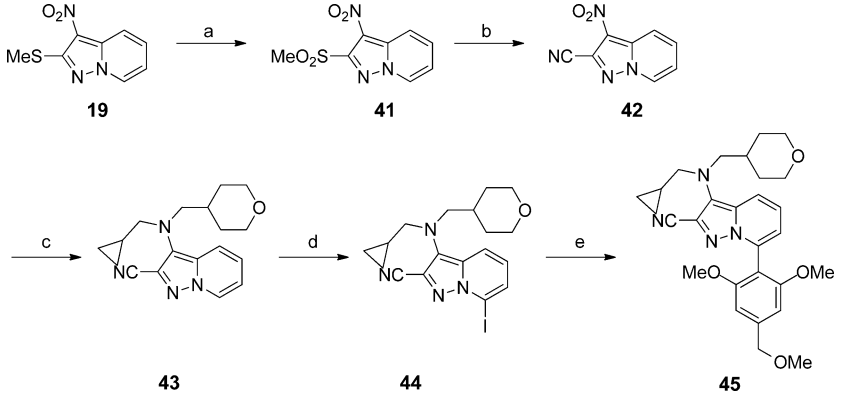
24a-e **24f,g**

compd	R	binding IC$_{50}$ (nM)[a]	cAMP IC$_{50}$ (nM)[a]
24a	2-Cl-4-MeO	10	100
24b	2-Me-4-MeO	16	150
24c	2-MeO-4-Me	12	100
24d	2-MeO-4,6-diMe	24	59
24e	4-MeO-2,6-diMe	13	90
24f	2-Cl-4-MeO	15	200
24g	2-Me-4-MeO	57	480

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

solubility, but displayed higher hCLint and weaker functional antagonism relative to **24r**. However, introduction of methylthio (**30a**) preserved the binding affinity and hCLint, but decreased the solubility. The solubilities of **24r**, **27a**, and **30a** were not correlated with the corresponding clogP values (4.7, 3.9, and 4.5, respectively). To confirm this, their relative

Scheme 8[a]



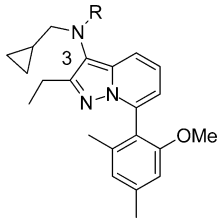
19 **41** **42**

43 **44** **45**

[a]Reagents and conditions: (a) alumina, oxone, H$_2$O, CHCl$_3$, 80 °C, 83%; (b) NaCN, THF, DMF, 80 °C, 69%; (c) (i) Pd/C, EtOAc, rt, 89%, (ii) *c*PrCHO, Ti(O*i*-Pr)$_4$, NaBH$_4$, THF, MeOH, rt; (iii) 4-THP-CHO, α-picoline-borane, MeOH, AcOH, rt, 37%, 2 steps; (d) 1,2-diiodoethane, *n*-BuLi in hexane, THF, −78−40 °C, 63%; (e) Ar−B(OH)$_2$, Pd(PPh$_3$)$_4$, 1 M Na$_2$CO$_3$, toluene, EtOH, 90 °C, 60%.

dx.doi.org/10.1021/jm300259r | J. Med. Chem. 2012, 55, 5255−5269

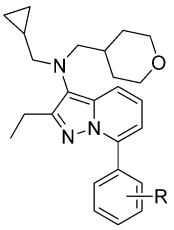
Table 2. Effects of Dialkylamino Side Chain at the 3-Position of Pyrazolo[1,5-*a*]pyridine Derivatives



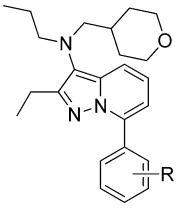
compd	R	binding IC_{50} (nM)[a]	hCLint (mL/min/mg)[b]	clogP
24d	$cPrCH_2$	24	1.12	6.8
24h	$MeOCH_2CH_2$	41	1.69	5.9
24i	$FCH_2CH_2CH_2$	20	1.41	6.3
24j	$3\text{-}THFCH_2$	38	1.35	5.7
24k	$2\text{-}THFCH_2$	34	1.23	6.3
24l	$4\text{-}THPCH_2$	22	0.45	6.1

[a]All values are the averages of two measurements. [b]Rate of human intrinsic clearance *in vitro*. Each value represents the mean of six measurements.

Table 3. Effects of 7-Phenyl Substituents of Pyrazolo[1,5-*a*]pyridine Derivatives



24l-u



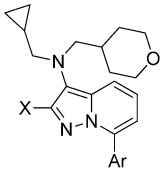
24v,w

compd	R	binding IC_{50} (nM)[a]	solubility (μM)[b]	hCLint (mL/min/mg)[c]
24l	2-MeO-4,6-diMe	22	0	0.45
24m	2,6-diMeO-4-Me	22	1.6	0.44
24n	2,4-diMeO-6-Me	40	0	0.45
24o	2,4,6-triMeO	42	2.5	0.45
24p	2,4,5-triMeO	130	3.6	0.60
24q	2,6-diMeO-4-$HOCH_2$	200	30	NT[d]
24r	2,6-diMeO-4-$MeOCH_2$	50	4.8	0.30
24s	2,4-diMeO-6-$MeOCH_2$	120	2.9	0.53
24t	2,6-diMeO-4-$EtOCH_2$	49	1.4	0.52
24u	2,6-diMeO	105	3.3	0.74
24v	2,6-diMeO-4-Me	40	1.3	0.89
24w	2,6-diMeO-4-$MeOCH_2$	90	3.1	0.70

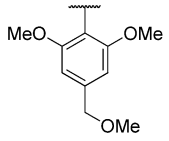
[a]All values are the averages of two measurements. [b]Kinetic solubility in phosphate buffer saline using 10 mM DMSO stock solution. [c]Rate of human intrinsic clearance *in vitro*. Each value represents the mean of six measurements. [d]NT = not tested.

lipophilicities were measured by reversed-phase high-pressure liquid chromatography (HPLC) using an octadecylsilyl column; the retention times of **27a**, **24r**, and **30a** were 85.9, 98.4, and 131.0, respectively, with reference to the t_0 marker (NO_3^-). Thus, **30a** is more lipophilic than **27a** or **24r**, in line with its reduced aqueous solubility. Replacement of the ethyl group

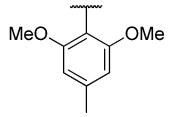
Table 4. Effects of 2-Substituents in Pyrazolo[1,5-*a*]pyridine Derivatives



Ar =



1



2

compd	Ar	X	binding IC_{50} (nM)[a]	solubility (μM)[b]	hCLint (mL/min/mg)[c]	cAMP IC_{50} (nM)[a]
24r	1	Et	50	4.8	0.30	23
27a	1	OMe	51	5.5	0.76	110
30a	1	SMe	35	0	0.34	30
33	1	Me	120	21	0.11	50
45	1	CN	52	1.7	1.10	52
24m	2	Et	22	1.6	0.44	20
27b	2	OMe	25	0.5	0.57	140
30b	2	SMe	47	0	NT[d]	39

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

with a methyl group (**33**) decreased the binding affinity by more than 2-fold. Although introduction of a cyano group (**45**) as a polar substituent resulted in a binding affinity comparable to that of **24r**, hCLint was poor. Modification of **24m** to 2-methoxy or 2-methylthio derivatives provided similar results to those for the methoxymethyl analogue **24r**.

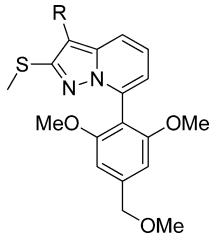
Because compound **30a** satisfied most of the targeted properties except solubility, modification of the 3-position was reinvestigated to explore the effects of additional polar groups on the solubility (Table 5). Introduction of OH (**30c**) or CN (**30d**), replacement of 4-THP with dioxane (**30e**, **30f**), and modification of cyclopropylmethyl to methoxyethyl (**30g**) all improved the solubility, as expected. Among them, **30e** and **30g** showed potent binding affinity but weaker functional antagonism (cAMP IC_{50}: **30e**, 180 nM; **30g**, 124 nM) compared with **30a** (30 nM). This suggests that a polar group might be not functionally well tolerated at this position and that comparable lipophilicity to **24r** might be necessary to acquire potent functional antagonistic activity.

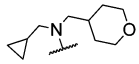
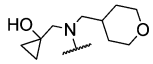
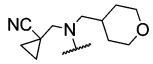
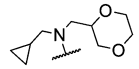
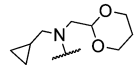
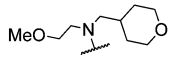
On balance, compound **24r** was determined to be the most promising compound from this work and was subjected to further detailed investigations. The functional antagonism of **24r** was also confirmed in a cAMP assay using the human neuroblastoma cell line IMR-32 expressing human CRF_1 receptors (data not shown). Screening for salt and crystal forms using various acids such as HCl, H_2SO_4, MsOH, *p*TsOH, and HBr identified the *p*TsOH salt **46** (E2508) as the most promising crystalline form. The measured K_i values of **46** were 11 nM and >10 μM for $hCRF_1$ and $hCRF_2$ receptors, respectively. Compound **1**, which was used as a positive control, displayed a K_i value of 8.3 nM in the assay.[36]

The anxiolytic efficacy of **46** was explored using the conventional light/dark test in mice (Figure 3).[37] The compound significantly increased both the time spent in the light box and the number of crosses at 10 mg/kg p.o., suggesting that **46** may have the potential to ameliorate anxiety in patients.

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

Table 5. Effects of 3-Dialkylamino Side Chain in 2-Methylthio Derivatives



compd	R	binding IC_{50} (nM)[a]	solubility (μM)[b]
30a		35	0
30c		98	26
30d		194	4.7
30e		27	5.6
30f		113	4.1
30g		34	13

[a]All values are the averages of two measurements. [b]Kinetic solubility in phosphate buffer saline using 10 mM DMSO stock solution.

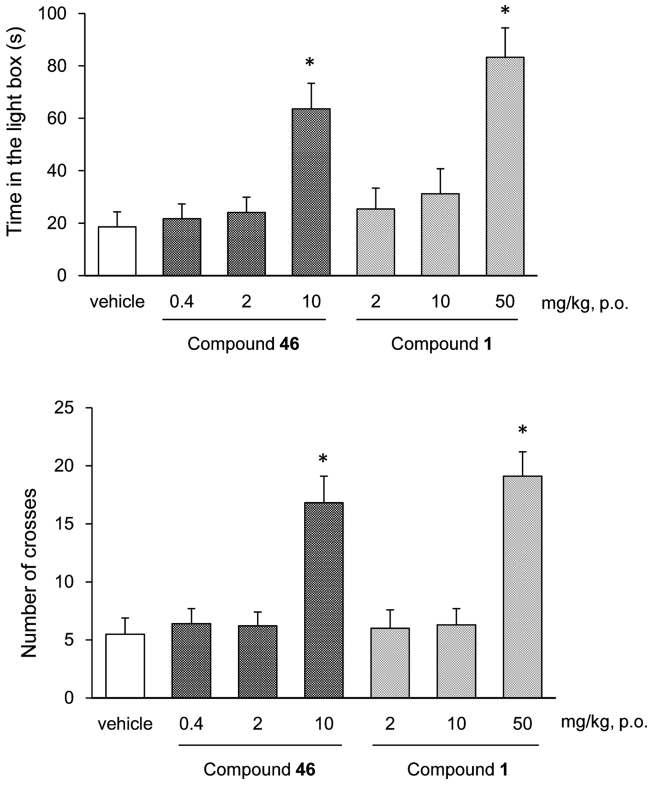


Figure 3. Effects of **46** on mice in a light/dark test with **1** as a positive control. Each value represents the mean ± SEM of 24 mice. *$p < 0.05$ vs vehicle (Dunnett multiple comparison test).

The pharmacokinetic properties of **46** were evaluated in male ICR mice after intravenous and oral administration at doses of 3 mg/kg i.v. and 10 mg/kg p.o. ($n = 4$), respectively (Table 6). The results indicated a T_{max} of 0.25 h, a half-life of 2.2 h, a plasma clearance of 2.6 L/h/kg, and an oral bioavailability of 31%. The hepatic clearances calculated from *in vitro* metabolic parameters were comparable with *in vivo* total clearances measured in preclinical species. These results indicate that the main elimination route of **46** in animals is probably hepatic metabolism. Therefore, oral bioavailability in humans was predicted based on *in vitro* metabolic parameters using human liver microsomes.

Table 6. Pharmacokinetic Parameters for 46 (i.v. and p.o.) in Male Mice[a]

i.v. (3 mg/kg)		p.o. (10 mg/kg)	
CL (L/h/kg)	2.6	C_{max} (μg/mL)	0.32
V_{dss} (L/kg)	3.74	T_{max} (h)	0.25
AUC(μg/mL·h)	0.85	AUC(μg/mL·h)	0.87
$T_{1/2}$ (h)	2.2	B.A. (%)	31

[a]Each value represents the mean of four animals.

Moreover, **46** is not a substrate of human Pgp, and its off-target liability panel screening did not show any significant affinity at various receptors and transporters up to 10 μM (data not shown). In addition, the inhibitory effect on the hERG channel was very weak (IC_{50} = 16.3 μM).

Based on these data, **46** was selected as a clinical candidate.

CONCLUSIONS

We discovered a novel series of pyrazolo[1,5-*a*]pyridines as potent, drug-like CRF_1 receptor antagonists. The combination of unique side-chains in the selected compound **46**, namely, 4-tetrahydropyranylmethyl and 2,6-dimethoxy-4-methoxymethyl-phenyl groups, afford improved human metabolic stability and solubility. **46** displays both high affinity and functional antagonism for the human CRF_1 receptor (K_i = 11 nM), exhibits significant anxiolytic activity in a light/dark test in mice at 10 mg/kg (p.o.), and has the appropriate drug-like properties. Hence, it has advanced into clinical trials, where we believe it will prove the clinical usefulness of CRF_1 receptor antagonists in the treatment of stress-related disorders such as depression and anxiety.

EXPERIMENTAL SECTION

Chemistry. ^{1}H NMR spectra were recorded on a Bruker Avance spectrometer (operating at 600 MHz) or Varian Mercury 400 spectrometer (operating at 400 MHz). ^{13}C NMR spectra were recorded on a Bruker Avance spectrometer (operating at 150 MHz) or JEOL JNM α400 spectrometer (operating at 100 MHz). Chemical shifts were calculated in ppm (δ) from the residual $CHCl_3$ signal at (δ_H) 7.26 ppm and (δ_C) 77.0 ppm in $CDCl_3$, or the residual C_5HD_4N signal at (δ_H) 8.71 ppm and (δ_C) 149.2 ppm or (δ_C) 123.5 ppm in C_5D_5N. High-resolution mass spectra (HRMS) were recorded on a ThermoFisherScientific LTQ-Orbitrap XL spectrometer (using electrospray ionization). For **46**, the melting point was determined by visual inspection according to the U.S. Pharmacopeia. The infrared (IR) spectrum was obtained on a FT/IR 620 spectrometer (JASCO, JAPAN). The carbon, hydrogen, and nitrogen elemental concentrations were measured on a vario EL III (Elementar, USA).

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

dx.doi.org/10.1021/jm300259r | J. Med. Chem. 2012, 55, 5255–5269

B; 8−11 min, 0% B; flow rate = 0.4 mL/min; detector: UV 254 nm; run time = 11 min.

Reagents were purchased from commercial sources. Chromatography was performed on silica gel using the solvent systems indicated below. For mixed solvent systems, the volume ratios are given.

2-(1-Butynyl)pyridine (6). To a solution of 2-bromopyridine (504.6 g, 3.2 mol) in Et_2NH (5 L) were added $PdCl_2(PPh_3)_2$ (22.5 g, 32 mmol) and copper iodide (3.0 g, 16 mmol), and the reaction mixture was stirred for 10 h at room temperature while introducing 1-butyne (400 g, 7.4 mol) as a gas. The reaction mixture was bubbled with nitrogen for 40 min, filtered through a pad of Celite to remove insoluble residue, and the filtrate washed with EtOAc. The organic extract was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:2) to afford **6** (380.5 g, 83%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (t, J = 7.6 Hz, 3H), 2.45 (q, J = 7.6 Hz, 2H), 7.16−7.20 (m, 1H), 7.35−7.38 (m, 1H), 7.59−7.63 (m, 1H), 8.53−8.54 (m, 1H).

2-Ethylpyrazolo[1,5-*a*]pyridine (7). To a solution of **6** (260 g, 1.98 mol) in CH_2Cl_2 (1.6 L) was added a solution of *O*-mesitylenesulfonylhydroxylamine (724 g, 2.6 mol, CAUTION!) in CH_2Cl_2 (900 mL) under ice cooling, and the reaction mixture was stirred for another one hour. Et_2O (24 L) was added to the reaction mixture to precipitate crystals. The filtrate was filtered off and then dried under reduced pressure to afford a crude product of *N*-amino-2-(1-butynyl)pyridinium mesitylenesulfonate as a pale yellow solid.

To the obtained crude product in MeOH (600 mL) was added sodium methoxide in MeOH (28%, 309 mL, 1.6 mol) dropwise at 0 °C, and the mixture was vigorously stirred for 30 min. Ice−water (1.4 L) was added, and the solvent was evaporated under reduced pressure. The aqueous layer was extracted with EtOAc. The obtained organic extract was dried over $MgSO_4$, filtered, and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 10:1) to afford **7** (88.2 g, 38%, 2 steps) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, J = 7.6 Hz, 3H), 2.86 (q, J = 7.6 Hz, 2H), 6.30 (s, 1H), 6.65 (ddd, J = 1.6, 6.8, 6.8 Hz, 1H), 7.04 (ddd, J = 1.2, 6.8, 8.8 Hz, 1H), 7.41 (ddd, J = 1.2, 1.2, 8.8 Hz, 1H), 8.37 (ddd, J = 1.2, 1.2, 6.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.8, 141.1, 128.2, 123.1, 117.3, 110.7, 94.5, 21.8, 13.9. HRMS calcd for ($C_9H_{10}N_2$) [M + H]+ 147.0917; found 147.0919.

7-Bromo-2-ethylpyrazolo[1,5-*a*]pyridine (8). To a solution of **7** (25.6 g, 175 mmol) in THF (750 mL) was added *n*-BuLi (1.56 M hexane solution: 146 mL, 228 mmol) dropwise at −78 °C under a nitrogen atmosphere, and the reaction mixture was further stirred for 1 h at the same temperature. A solution of 1,2-dibromo-1,1,2,2-tetrafluoroethane (59.2 g, 228 mmol) in THF (10 mL) was added dropwise to the reaction mixture, and stirred for 10 min at the same temperature. The mixture was warmed to 0 °C, and quenched carefully with a saturated NH_4Cl aqueous solution at an internal temperature of −10 °C. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 11:1) to afford **8** (340 g, 86%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (t, J = 7.6 Hz, 3H), 2.93 (q, J = 7.6 Hz, 2H), 6.49 (s, 1H), 6.94 (dd, J = 7.2, 8.4 Hz, 1H), 6.99 (dd, J = 1.6, 7.2 Hz, 1H), 7.44 (dd, J = 1.6, 8.4 Hz, 1H).

7-Bromo-2-ethyl-3-nitropyrazolo[1,5-*a*]pyridine (9). To a solution of **8** (102.9 g, 457.2 mmol) in CH_3CN (880 mL) was added nitronium tetrafluoroborate (76.7 g, 548.6 mmol) under cooling with ice water, and the reaction mixture was stirred for 10 min. The reaction mixture was then poured into crashed ice, and was extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 3:2) to give solid. The solid was washed with *n*-hexane to afford **9** (74.6 g, 60%) as a pale green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, J = 7.6 Hz, 3H), 3.27 (q, J = 7.6 Hz, 2H), 7.39 (dd, J = 1.2, 7.6 Hz, 1H), 7.50 (dd, J = 7.6, 8.8 Hz, 1H), 8.38 (dd, J = 1.2, 8.8 Hz, 1H).

***tert*-Butyl *N*-(7-bromo-2-ethylpyrazolo[1,5-*a*]pyridin-3-yl)-carbamate (10).** To a suspension of **9** (74.6 g, 276 mmol) in EtOH (1.3 L), water (650 mL), and AcOH (65 mL) was added zinc powder (75 g, 1.09 mol) at room temperature and the reaction mixture was stirred for 20 min at 60 °C. The reaction mixture was cooled and filtered through a pad of Celite to remove insoluble residue, and the filtrate was washed with EtOAc. The solvent was distilled off under reduced pressure, and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and filtered, and the solvent was distilled off under reduced pressure. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 4:1) to give 7-bromo-2-ethylpyrazolo[1,5-*a*]pyridine-3-amine (39.6 g, 60%) as a dark-brown solid.

To a solution of 7-bromo-2-ethylpyrazolo[1,5-*a*]pyridine-3-amine (39.6 g, 165 mmol) from the previous step in CH_2Cl_2 (330 mL) was added Et_3N (34.6 mL, 248 mmol), followed by Boc_2O (45.5 mL, 198 mmol) under ice cooling, and the reaction mixture was stirred for 16 h. To the reaction mixture were added a saturated $NaHCO_3$ aqueous solution and EtOAc. The mixture was filtered through a pad of Celite to remove insoluble residue, and the filtrate was washed with EtOAc. The solvent was evaporated, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 4:1) to afford **10** (46.8 g, 83%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (t, J = 7.6 Hz, 3H), 1.52 (br s, 9H), 2.87 (q, J = 7.6 Hz, 2H), 5.91 (br s, 1H), 6.92−7.04 (m, 2H), 7.40 (d, J = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.4, 153.3, 138.2, 123.4, 118.7, 115.3, 115.1, 108.0, 80.5, 28.3, 19.6, 13.4. HRMS calcd for ($C_{14}H_{18}BrN_3O_2$) [M + H]+ 340.0655; found 340.0660.

***tert*-Butyl [2-(methylthio)pyrazolo[1,5-*a*]pyridin-3-yl]-carbamate (20).** Compound **20** was prepared according to the procedure described for the synthesis of **10** using 2-methylthio-3-nitropyrazolo[1,5-*a*]pyridine **19** (400 mg, 1.9 mmol), zinc powder (800 mg, 12.2 mmol) in EtOH (20 mL), water (10 mL), AcOH (2 mL), Boc_2O (625 mg, 2.9 mmol), and Et_3N (0.40 mL, 2.9 mmol) in CH_2Cl_2 (5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) to afford **20** (230 mg, 43%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (br s, 9H), 2.60 (s, 3H), 6.00−6.15 (m, 1H), 6.69 (t, J = 6.8 Hz, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.40−7.50 (m, 1H), 8.83 (d, J = 6.8 Hz, 1H).

***tert*-Butyl [7-iodo-2-(methylthio)pyrazolo[1,5-*a*]pyridin-3-yl]carbamate (21).** Compound **21** was prepared according to the procedure described for the synthesis of **8** using **20** (21.6 g, 77.3 mmol) in THF (1 L), 1,2-diiodoethane (24.0 g, 85.0 mmol) in THF (50 mL) and *n*-BuLi (1.6 M hexane solution; 130 mL, 208.0 mmol). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) to afford **21** (21.5 g, 69%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.64 (s, 3H), 6.02−6.10 (m, 1H), 6.81 (dd, J = 7.2, 8.8 Hz, 1H), 7.22 (dd, J = 1.2, 7.2 Hz, 1H), 7.42−7.50 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.9, 145.3, 136.7, 123.7, 123.1, 116.5, 110.4, 91.9, 80.8, 28.2, 15.5. HRMS calcd for ($C_{13}H_{16}IN_3O_2S$) [M + H]+ 406.0081; found 406.0085.

7-Bromo-*N*-(cyclopropylmethyl)-2-ethylpyrazolo[1,5-*a*]-pyridin-3-amine (22a). To a solution of **10** (46.8 g, 138 mmol) in DMF (1 L) was added NaH (60%, 6.6 g, 179 mmol) at room temperature under a nitrogen stream, and the reaction mixture was stirred for 20 min. (Bromomethyl)cyclopropane (22.4 g, 166 mmol) was added at the same temperature, and the reaction mixture was stirred for 1 h at 40 °C. To the reaction mixture was gradually added ice. The mixture was extracted with EtOAc, and the organic layers was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a crude product, which was used in the next step without purification.

The crude product from the previous step was dissolved in 4 M HCl/EtOAc solution (500 mL) at room temperature, and the mixture was stirred for 30 min at 40 °C. 5 M NaOH aqueous solution was added to the reaction mixture at room temperature, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc =

dx.doi.org/10.1021/jm300259r | J. Med. Chem. 2012, 55, 5255−5269

3:1) to afford **22a** (40.2 g, 99%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.14−0.22 (m, 2H), 0.47−0.56 (m, 2H), 0.96−1.10 (m, 1H), 1.37 (t, J = 7.6 Hz, 3H), 2.88 (d, J = 6.8 Hz, 2H), 2.90 (q, J = 7.6 Hz, 2H), 6.83 (dd, J = 7.0, 8.8 Hz, 1H), 6.90 (dd, J = 1.3, 7.1 Hz, 1H), 7.43 (dd, J = 1.3, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.5, 135.8, 121.2, 120.6, 118.4, 115.0, 114.4, 56.0, 19.4, 14.0, 12.0, 3.4. HRMS calcd for ($C_{13}H_{16}BrN_3$) [M + H]+ 294.0600; found 294.0604.

7-Bromo-*N*-(cyclopropylmethyl)-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (23a). To a solution of **22a** (40.2 g, 136.6 mmol) in THF (680 mL) was added tetrahydro-2*H*-4-pyrancarbaldehyde (31.2 g, 273.2 mmol) in THF (30 mL), followed by $NaBH(OAc)_3$ (40.5 g, 191.2 mmol), and the mixture was stirred at room temperature for 30 min. To the reaction mixture was added ice, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) to afford **23a** (51.5 g, 96%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 2H), 0.33−0.43 (m, 2H), 0.75−0.88 (m, 1H), 1.20−1.34 (m, 2H), 1.38 (t, J = 7.6 Hz, 3H), 1.48−1.62 (m, 1H), 1.69−1.78 (m, 2H), 2.88 (d, J = 6.8 Hz, 2H), 2.91 (q, J = 7.6 Hz, 2H), 3.04 (d, J = 7.0 Hz, 2H), 3.30 (dt, J = 2.1, 12.0 Hz, 2H), 3.90−4.00 (m, 2H), 6.88 (dd, J = 7.1, 8.8 Hz, 1H), 6.96 (dd, J = 1.3, 7.1 Hz, 1H), 7.49 (dd, J = 1.3, 8.8 Hz, 1H). 155.0, 138.9, 122.6, 122.0, 119.1, 115.7, 114.6, 67.9, 62.0, 61.8, 34.4, 31.6, 20.0, 14.0, 10.4, 3.7. HRMS calcd for ($C_{19}H_{26}BrN_3O$) [M + H]+ 392.1332; found 392.1336.

7-Bromo-*N*,*N*-bis(cyclopropylmethyl)-2-ethylpyrazolo[1,5-*a*]pyridin-3-amine (23e). Compound **23e** was prepared according to the procedure described for the synthesis of **23a** using **22a** (150 mg, 0.51 mmol), cyclopropanecarboxaldehyde (76 μL, 1.0 mmol), and $NaBH(OAc)_3$ (216 mg, 1.0 mmol) in THF (1.7 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 25:2) to afford **23e** (166 mg, 93%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.04−0.07 (m, 4H), 0.28−0.40 (m, 4H), 0.72−0.86 (m, 2H), 1.37 (t, J = 7.6 Hz, 3H), 2.92 (q, J = 7.7 Hz, 2H), 2.92−3.02 (m, 4H), 6.83 (dd, J = 7.2, 8.7 Hz, 1H), 6.91 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 8.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.5, 139.0, 122.6, 121.7, 119.0, 115.9, 114.4, 60.6, 19.9, 14.0, 10.5, 3.6. HRMS calcd for ($C_{17}H_{22}BrN_3$) [M + H]+ 348.1070; found 348.1070.

7-(2-Chloro-4-methoxyphenyl)-*N*,*N*-bis(cyclopropylmethyl)-2-ethylpyrazolo[1,5-*a*]pyridin-3-amine (24a). To a suspension of **40a** (115 mg, 0.35 mmol) in EtOH (5 mL), water (5 mL), and AcOH (0.1 mL) was added zinc powder (115 mg, 1.67 mol) at room temperature, and the reaction mixture was stirred for 30 min at 60 °C. The reaction mixture was cooled and filtered through a pad of Celite to remove insoluble residue, and the filtrate was washed with EtOAc. The solvent was evaporated under reduced pressure, and transferred to a separatory funnel. The aqueous layer was extracted with EtOAc. The organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was concentrated under reduced pressure to give crude product, which was used in the next step without purification.

The crude product from the previous step was dissolved in THF (8 mL). To this mixture was added cyclopropylmethyl carboxaldehyde (0.13 mL, 1.74 mmol) and 3 M H_2SO_4 aqueous solution (0.58 mL, 1.74 mmol) followed by $NaBH_4$ (53 mg, 1.39 mmol) in five portions while vigorously stirring on ice, and stirring was continued for 20 min. Water was added to the reaction mixture, and the residue was transferred to a separatory funnel and was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 20:1) to afford **24a** (94 mg, 66%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 4H), 0.30−0.38 (m, 4H), 0.77−0.90 (m, 2H), 1.26 (t, J = 7.5 Hz, 3H), 2.80 (q, J = 7.5 Hz, 2H), 2.99 (d, J = 6.6 Hz, 4H), 3.86 (s, 3H), 6,55 (dd, J = 1.3, 6.8 Hz, 1H), 6.92 (dd, J = 2.6, 8.6 Hz, 1H), 6.99 (dd, J = 6.8, 9.0 Hz, 1H), 7.06 (d, J = 2.4 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.49 (dd, J = 1.3, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 154.5, 137.5, 135.1, 132.3, 125.7, 121.1, 120.9, 116.4, 115.3, 112.8, 112.2, 60.7, 55.6, 19.8, 14.2, 10.6, 3.6. HRMS calcd for ($C_{24}H_{28}ClN_3O$) [M + H]+ 410.1994; found 410.1997.

***N*,*N*-Bis(cyclopropylmethyl)-2-ethyl-7-(4-methoxy-2-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24b).** Compound **24b** was prepared according to the procedure described for the synthesis of **24r** using **23e** (27 mg, 0.078 mmol), (4-methoxy-2-methylphenyl)boronic acid (26 mg, 0.16 mmol), $Pd(PPh_3)_4$ (15 mg, 0.013 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (49 mg, 0.16 mmol) in DME (0.6 mL) and water (0.3 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 11:1) to afford **24b** (25 mg, 81%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 4H), 0.30−0.38 (m, 4H), 0.78−0.90 (m, 2H), 1.26 (t, J = 7.5 Hz, 3H), 2.08 (s, 3H), 2.81 (q, J = 7.5 Hz, 2H), 3.00 (d, J = 6.6 Hz, 4H), 3.86 (s, 3H), 6,49 (dd, J = 1.4, 6.7 Hz, 1H), 6.80−6.90 (m, 2H), 6.99 (dd, J = 6.8, 9.0 Hz, 1H), 7.34 (d, J = 8.2 Hz, 1H), 7.48 (dd, J = 1.4, 8.9 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.1, 154.7, 140.6, 139.7, 137.5, 131.1, 126.8, 121.1, 120.8, 115.7, 115.6, 111.6, 111.0, 60.7, 55.2, 19.9, 19.7, 14.3, 10.6, 3.6. HRMS calcd for ($C_{25}H_{31}N_3O$) [M + H]+ 390.2540; found 390.2541.

***N*,*N*-Bis(cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4-methylphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24c).** Compound **24c** was prepared according to the procedure described for the synthesis of **24a** using **40b** (70 mg, 0.22 mmol), zinc powder (70 mg, 1.1 mol) in EtOH (6 mL), water (3 mL), AcOH (1 mL), and cyclopropylmethyl carboxaldehyde (63 μL, 0.84 mmol), 3 M H_2SO_4 aqueous solution (0.28 mL, 0.84 mmol), $NaBH_4$ (21 mg, 0.56 mmol) in THF (3 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 10:1) to afford **24c** (58 mg, 68%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00−0.05 (m, 4H), 0.32−0.38 (m, 4H), 0.80−0.90 (m, 2H), 1.26 (t, J = 7.6 Hz, 3H), 2.43 (s, 3H), 2.80 (q, J = 7.6 Hz, 2H), 2.99 (d, J = 6.8 Hz, 4H), 3.75 (s, 3H), 6.59 (dd, J = 0.8, 6.8 Hz, 1H), 6.86 (s, 1H), 6.89 (br d, J = 7.6 Hz, 1H), 6.98 (dd, J = 6.8, 8.8 Hz, 1H), 7.41 (d, J = 7.6 Hz, 1H), 7.45 (dd, J = 0.8, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 154.0, 140.8, 137.9, 137.5, 130.9, 121.3, 121.0, 120.7, 120.7, 115.7, 112.7, 111.8, 60.7, 55.7, 21.8, 19.8, 14.2, 10.7, 3.6. HRMS calcd for ($C_{25}H_{31}N_3O$) [M + H]+ 390.2540; found 390.2544.

***N*,*N*-Bis(cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24d).** Compound **24d** was prepared according to the procedure described for the synthesis of **24h** using **23e** (40 mg, 0.12 mmol), (2-methoxy-4,6-dimethyllphenyl)boronic acid (42 mg, 0.23 mmol), K_2CO_3 (48 mg, 0.35 mmol), and $Pd(PPh_3)_4$ (20 mg, 0.017 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 10:1) to afford **24d** (43 mg, 93%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.04 (m, 4H), 0.30−0.36 (m, 4H), 0.80−0.90 (m, 2H), 1.22 (t, J = 7.6 Hz, 4H), 1.97 (s, 3H), 2.39 (s, 3H), 2.72−2.84 (m, 2H), 2.99 (d, J = 6.4 Hz, 2H), 3.68 (s, 3H), 6.48 (dd, J = 1.2, 6.8 Hz, 1H), 6.69 (s, 1H), 6.77 (s, 1H), 6.99 (dd, J = 6.8, 8.8 Hz, 1H), 7.46 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 154.3, 139.8, 139.0, 137.5, 136.7, 123.4, 120.9, 120.5, 120.5, 115.5, 112.3, 110.0, 60.7, 55.9, 21.8, 19.7, 19.2, 14.5, 10.7, 3.6, 3.5. HRMS calcd for ($C_{26}H_{33}N_3O$) [M + H]+ 404.2696; found 404.2701.

***N*,*N*-Bis(cyclopropylmethyl)-2-ethyl-7-(4-methoxy-2,6-dimethylphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24e).** Compound **24e** was prepared according to the procedure described for the synthesis of **24r** using **23e** (40 mg, 0.12 mmol), (2,6-dimethyl-4-methoxyphenyl)boronic acid (42 mg, 0.23 mmol), $Pd(PPh_3)_4$ (20 mg, 0.017 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (73 mg, 0.23 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 13:1) to afford **24e** (35 mg, 76%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 4H), 0.30−0.40 (m, 4H), 0.81−0.94 (m, 2H), 1.24 (t, J = 7.5 Hz, 3H), 2.04 (s, 6H), 2.81 (q, J = 7.5 Hz, 2H), 3.03 (d, J = 6.6 Hz, 4H), 3.87 (s, 3H), 6,45 (dd, J = 1.5, 6.8 Hz, 1H), 6.73 (s, 2H), 7.02 (dd, J = 6.8, 8.8 Hz, 1H), 7.49 (dd, J = 1.4, 8.9 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 159.6, 154.8, 139.1, 138.9, 137.5, 126.5, 121.0, 120.6, 115.5, 112.9, 111.9, 60.7, 55.1, 19.9, 19.6, 14.5, 10.6, 3.5. HRMS calcd for ($C_{26}H_{33}N_3O$) [M + H]+ 404.2696; found 404.2699.

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

7-(2-Chloro-4-methoxyphenyl)-2-ethyl-*N*,*N*-dipropylpyrazolo[1,5-*a*]pyridin-3-amine (24f). Compound **24f** was prepared according to the procedure described for the synthesis of **24a** using **40a** (90 mg, 0.27 mmol), zinc powder (180 mg, 2.8 mol) in EtOH (10 mL), water (5 mL), AcOH (2 mL), and propionaldehyde (58 μL, 0.81 mmol), 3 M H_2SO_4 aqueous solution (0.27 mL, 0.81 mmol), $NaBH_4$ (20 mg, 0.54 mmol) in THF (2 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 50:1) to afford **24f** (70 mg, 67%, 2 steps) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.6 Hz, 6H), 1.23 (t, *J* = 7.6 Hz, 3H), 1.34−1.44 (m, 4H), 2.76 (q, *J* = 7.6 Hz, 2H), 3.02 (t, *J* = 7.6 Hz, 4H), 3.87 (s, 3H), 6.56 (dd, *J* = 1.6, 6.4 Hz, 1H), 6.92 (dd, *J* = 2.8, 8.6 Hz, 1H), 6.99 (dd, *J* = 6.4, 8.6 Hz, 1H), 7.06 (d, *J* = 2.8 Hz, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 7.45 (dd, *J* = 1.6, 8.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.6, 154.3, 137.6, 137.4, 135.0, 132.4, 125.6, 120.9, 120.6, 116.2, 115.3, 112.8, 112.4, 58.2, 55.6, 22.1, 19.9, 14.3, 11.7. HRMS calcd for ($C_{23}H_{28}ClN_3O$) [M + H]+ 386.1994; found 386.1992.

2-Ethyl-7-(4-methoxy-2-methylphenyl)-*N*,*N*-dipropylpyrazolo[1,5-*a*]pyridin-3-amine (24g). Compound **24g** was prepared according to the procedure described for the synthesis of **24r** using **23g** (40 mg, 0.12 mmol), (4-methoxy-2-methylphenyl)-boronic acid (41 mg, 0.25 mmol), $Pd(PPh_3)_4$ (21 mg, 0.018 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (78 mg, 0.25 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 16:1) to afford **24g** (40 mg, 89%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (t, *J* = 7.6 Hz, 6H), 1.23 (t, *J* = 7.6 Hz, 3H), 1.34−1.44 (m, 4H), 2.10 (s, 3H), 2.75 (q, *J* = 7.6 Hz, 2H), 3.01 (t, *J* = 7.6 Hz, 4H), 3.86 (s, 3H), 6.49 (dd, *J* = 1.6, 6.8 Hz, 1H), 6.82 (dd, *J* = 2.8, 8.4 Hz, 1H), 6.86 (d, *J* = 2.8 Hz, 1H), 6.99 (dd, *J* = 6.8, 8.8 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 7.45 (dd, *J* = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.1, 154.5, 140.5, 139.7, 137.6, 131.2, 126.8, 121.2, 120.3, 115.6, 115.5, 111.8, 111.0, 58.3, 55.2, 22.1, 20.0, 19.8, 14.3, 11.7. HRMS calcd for ($C_{26}H_{33}N_3O_2$) [M + H]+ 366.2540; found 366.2538.

***N*-(Cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)-*N*-(2-methoxyethyl)pyrazolo[1,5-*a*]pyridin-3-amine (24h).** To a sulution of **23b** (40 mg, 0.11 mmol) in DME (1 mL) and water (0.5 mL) were added (2-methoxy-4,6-dimethylphenyl)boronic acid (41 mg, 0.23 mmol), K_2CO_3 (47 mg, 0.34 mmol), and $Pd(PPh_3)_4$ (20 mg, 0.017 mmol). The mixture was stirred at 90 °C for 14 h under a nitrogen stream and was then cooled and diluted with water. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 7:1) to afford **24h** (36 mg, 78%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.07−0.05 (m, 2H), 0.28−0.40 (m, 2H), 0.79−0.94 (m, 1H), 1.16−1.25 (m, 3H), 1.97 (s, 3H), 2.39 (s, 3H), 2.68−2.85 (m, 2H), 2.94−3.05 (m, 2H), 3.29 (s, 3H), 3.33−3.43 (m, 4H), 3.68 (s, 3H), 6.50 (d, *J* = 6.8 Hz, 1H), 6.69 (s, 1H), 6.76 (s, 1H), 7.01 (dd, *J* = 6.8, 8.7 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 154.3, 139.9, 139.0, 137.6, 136.7, 123.4, 121.3, 120.3, 119.6, 115.2, 112.6, 109.9, 71.9, 61.9, 58.7, 55.9, 54.8, 21.8, 19.5, 19.3, 14.4, 10.6, 3.5, 3.4. HRMS calcd for ($C_{25}H_{33}N_3O_2$) [M + H]+ 408.2646; found 408.2640.

***N*-(Cyclopropylmethyl)-2-ethyl-*N*-(3-fluoropropyl)-7-(2-methoxy-4,6-dimethylphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24i).** Compound **24i** was prepared according to the procedure described for the synthesis of **24h** using **23c** (40 mg, 0.11 mmol), (2-methoxy-4,6-dimethyllphenyl)boronic acid (40 mg, 0.22 mmol), K_2CO_3 (47 mg, 0.34 mmol), and $Pd(PPh_3)_4$ (20 mg, 0.017 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 11:1) to afford **24i** (42 mg, 91%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.02 (m, 2H), 0.32−0.38 (m, 2H), 0.80−0.90 (m, 2H), 1.20 (t, *J* = 7.6 Hz, 3H), 1.70−1.82 (m, 2H), 1.97 (s, 3H), 2.39 (s, 3H), 2.74 (q, *J* = 7.6 Hz, 2H), 2.92 (d, *J* = 6.8 Hz, 2H), 3.30 (t, *J* = 6.8 Hz, 2H), 3.68 (s, 3H), 4.55 (td, *J* = 6.0, 47.2 Hz, 2H), 6.51 (dd, *J* = 1.2, 6.8 Hz, 1H), 6.69 (s, 1H). 6.77 (s, 1H), 7.02 (dd, *J* = 6.8, 8.8 Hz, 1H), 7.44 (dd, *J* = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 154.1, 139.9, 139.0, 137.5, 136.8, 123.4, 121.4, 120.3, 119.3, 115.1, 112.6, 109.9, 82.5 (d, *J* = 163.7 Hz), 61.8, 55.9, 50.9 (d, *J* = 5.5 Hz), 30.0 (d, *J* = 18.8 Hz), 21.8, 19.7, 19.3, 14.4, 10.5, 3.6, 3.5. HRMS calcd for ($C_{25}H_{32}FN_3O$) [M + H]+ 410.2602; found 410.2600.

***N*-(Cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)-*N*-[(tetrahydrofuran-3-yl)methyl]pyrazolo[1,5-*a*]-pyridin-3-amine (24j).** Compound **24j** was prepared according to the procedure described for the synthesis of **24h** using **23f** (40 mg, 0.11 mmol), (2-methoxy-4,6-dimethyllphenyl)boronic acid (39 mg, 0.22 mmol), K_2CO_3 (44 mg, 0.32 mmol) and $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 5:1) to afford **24j** (42 mg, 92%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.03 (m, 2H), 0.33−0.40 (m, 2H), 0.80−0.90 (m, 1H), 1.21 (t, *J* = 7.2 Hz, 3H), 1.60−1.70 (m, 1H), 1.88−1.98 (m, 1H), 1.99 (s, 3H), 2.23−2.32 (m, 1H), 2.40 (s, 3H), 2.76 (q, *J* = 7.2 Hz, 2H), 2.92 (d, *J* = 6.4 Hz, 2H), 3.06−3.13 (m, 1H), 3.21−3.28 (m, 1H), 3.60−3.65 (m, 1H), 3.66−3.72 (m, 4H), 3.73−3.86 (m, 2H), 6.52 (br d, *J* = 6.8 Hz, 1H), 6.70 (s, 1H), 6.78 (s, 1H), 7.03 (dd, *J* = 6.8, 8.8 Hz, 1H), 7.45 (br d, *J* = 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 154.0, 139.9, 139.0, 137.6, 136.8, 123.4, 121.3, 120.2, 119.6, 115.1, 112.6, 109.9, 67.7, 58.7, 55.9, 38.7, 30.4, 21.8, 19.7, 19.3, 14.4, 10.6, 3.6, 3.6. HRMS calcd for ($C_{27}H_{35}N_3O_2$) [M + H]+ 434.2802; found 434.2807.

***N*-(Cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)-*N*-[(tetrahydrofuran-2-yl)methyl]pyrazolo[1,5-*a*]-pyridin-3-amine (24k).** Compound **24k** was prepared according to the procedure described for the synthesis of **24h** using **23d** (40 mg, 0.11 mmol), (2-methoxy-4,6-dimethyllphenyl)boronic acid (38 mg, 0.21 mmol), K_2CO_3 (44 mg, 0.32 mmol) and $Pd(PPh_3)_4$ (19 mg, 0.016 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 11:1) to afford **24k** (28 mg, 61%) as a pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.06−0.03 (m, 2H), 0.28−0.38 (m, 2H), 0.80−0.91 (m, 1H), 1.17−1.25 (m, 3H), 1.62−1.71 (m, 1H), 1.75−1.95 (m, 3H), 1.97 (br s, 3H), 2.39 (s, 3H), 2.70−2.83 (m, 2H), 2.93−3.00 (m, 2H), 3.03−3.11 (m, 1H), 3.40−3.46 (m, 1H), 3.65−3.74 (m, 1H), 3.67 (s, 3H), 3.80−3.93 (m, 2H), 6.50 (d, *J* = 6.8 Hz, 1H), 6.69 (s, 1H), 6.76 (s, 1H), 7.01 (dd, *J* = 6.8, 8.5 Hz, 1H), 7.48 (bd, *J* = 8.5 Hz, 1H). HRMS calcd for ($C_{27}H_{35}N_3O_2$) [M + H]+ 434.2802; found 434.2802.

***N*-(Cyclopropylmethyl)-2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]-pyridin-3-amine (24l).** To a solution of **34a** (300 mg, 0.76 mmol) in DMF (5 mL) was added NaH (60%, 46 mg, 1.14 mmol), followed by (bromomethyl)cyclopropane (87 mg, 0.91 mmol) at room temperature, and the mixture was stirred for 1 h at 40 °C. Water was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to afford a crude product, which was used in the next step without purification.

The crude product from the previous step was dissolved in EtOAc (10 mL). To this mixture was added a 4 M HCl/EtOAc solution (20 mL) at room temperature, and the mixture was stirred for 1 h at 40 °C. A 5 M NaOH aqueous solution was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give crude product, which was used in the next step without purification.

The crude product from the previous step was dissolved in THF (2 mL). To this mixture was added tetrahydro-2*H*-4-pyrancarbaldehyde (173 mg, 1.52 mmol), followed by $NaBH(OAc)_3$ (241 mg, 1.14 mmol), and the mixture was stirred at room temperature for 1 h. A saturated $NaHCO_3$ aqueous solution was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) to afford **24l** (134 mg, 39%, 3 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04−0.00 (m, 2H), 0.31−0.35 (m, 2H), 0.76−0.88 (m, 1H), 1.20 (t, *J* = 7.6 Hz, 3H), 1.24−1.34 (m, 2H), 1.54−1.65 (m, 1H), 1.72−1.80 (m, 2H), 1.98 (s, 3H), 2.39 (s, 3H), 2.74 (dq, *J* = 1.6, 7.6 Hz, 2H), 2.88 (d, *J* = 6.8 Hz,

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

2H), 3.04 (d, J = 6.8 Hz, 2H), 3.31 (dt, J = 2.0, 11.6 Hz, 2H), 3.68 (s, 3H), 3.92−3.98 (m, 2H), 6.51 (dd, J = 1.6, 6.8 Hz, 1H), 6.69 (s, 1H), 6.77 (s, 1H), 7.01 (dd, J = 6.8, 8.8 Hz, 1H), 7.44 (dd, J = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.6, 153.9, 139.9, 139.0, 137.5, 136.7, 123.4, 121.2, 120.6, 120.3, 115.2, 112.6, 109.9, 68.0, 62.0, 55.9, 34.5, 31.6, 21.8, 19.7, 19.3, 14.4, 10.6, 3.6. HRMS calcd for ($C_{28}H_{37}N_3O_2$) [M + H]+ 448.2959; found 448.2951.

***N*-(Cyclopropylmethyl)-7-(2,6-dimethoxy-4-methylphenyl)-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24m).** Compound **24m** was prepared according to the procedure described for the synthesis of **24l** using **34b** (420 mg, 1.0 mmol), NaH (60%, 61 mg, 1.5 mmol), (bromomethyl)-cyclopropane (117 μL, 1.2 mmol) in DMF (5 mL) and 4 M HCl/EtOAc (40 mL) in EtOAc (20 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (232 mg, 2.0 mmol), $NaBH(OAc)_3$ (432 mg, 2.0 mmol) in THF (5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 4:1) to afford **24m** (353 mg, 76%, 3 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.01−0.03 (m, 2H), 0.34−0.40 (m, 2H), 0.80−0.90 (m, 1H), 1.23 (t, J = 7.6 Hz, 3H), 1.20−1.34 (m, 2H), 1.56−1.62 (m, 1H), 1.74−1.80 (m, 2H), 2.45 (s, 3H), 2.77 (q, J = 7.6 Hz, 2H), 2.89 (d, J = 6.4 Hz, 2H), 3.06 (d, J = 6.8 Hz, 2H), 3.32 (dt, J = 2.0, 11.6 Hz, 2H), 3.71 (s, 6H), 3.92−3.98 (m, 2H), 6.53 (s, 2H), 6.60 (dd, J = 1.2, 6.8 Hz, 1H), 7.01 (dd, J = 6.8, 8.8 Hz, 1H), 7.45 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, C_5D_5N) δ 158.4, 152.8, 140.9, 137.2, 134.8, 121.1, 120.8, 115.2, 113.2, 109.5, 105.0, 67.2, 61.5, 61.3, 55.1, 34.1, 31.3, 21.4, 19.5, 13.8, 10.4, 3.4. HRMS calcd for ($C_{28}H_{37}N_3O_3$) [M + H]+ 464.2908; found 464.2908.

***N*-(Cyclopropylmethyl)-7-(2,4-dimethoxy-6-methylphenyl)-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24n).** Compound **24n** was prepared according to the procedure described for the synthesis of **24l** using **34c** (80 mg, 0.19 mmol), NaH (60%, 12 mg, 0.29 mmol), (bromomethyl)-cyclopropane (22 mg, 0.23 mmol) in DMF (2 mL) and 4 M HCl/EtOAc (2 mL) in EtOAc (1 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (66 mg, 0.58 mmol), $NaBH(OAc)_3$ (123 mg, 0.58 mmol) in THF (2 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 4:1) to afford **24n** (70 mg, 78%, 3 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.05−0.00 (m, 2H), 0.31−0.36 (m, 2H), 0.77−0.87 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H), 1.22−1.32 (m, 2H), 1.56−1.64 (m, 1H), 1.72−1.80 (m, 2H), 2.00 (s, 3H), 2.72−2.79 (m, 2H), 2.87 (d, J = 6.4 Hz, 2H), 3.04 (d, J = 6.8 Hz, 2H), 3.27−3.35 (m, 2H), 3.67 (s, 3H), 3.85 (s, 3H), 3.90−3.96 (m, 2H), 6.43 (d, J = 2.0 Hz, 1H), 6.47 (d, J = 2.0 Hz, 1H), 6.49 (dd, J = 1.2, 6.8 Hz, 1H), 6.99 (dd, J = 6.8, 8.8 Hz, 1H), 7.43 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.0, 158.9, 153.8, 140.2, 137.5, 136.6, 121.2, 120.6, 115.9, 115.2, 112.9, 106.8, 96.7, 68.0, 62.0, 55.9, 55.3, 34.5, 31.6, 31.6, 19.8, 19.7, 14.4, 10.6, 3.6. HRMS calcd for ($C_{28}H_{37}N_3O_3$) [M + H]+ 464.2908; found 464.2913.

***N*-(Cyclopropylmethyl)-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-7-(2,4,6-trimethoxyphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24o).** Compound **24o** was prepared according to the procedure described for the synthesis of **24l** using **34d** (100 mg, 0.23 mmol), NaH (60%, 14 mg, 0.35 mmol), (bromomethyl)cyclopropane (27 μL, 0.28 mmol) in DMF (2 mL) and 4 M HCl/EtOAc (2 mL) in EtOAc (1 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (107 mg, 0.94 mmol), $NaBH(OAc)_3$ (198 mg, 0.94 mmol) in THF (2 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 3:1) to afford **24o** (82 mg, 73%, 3 steps) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.03 (m, 2H), 0.34−0.40 (m, 2H), 0.80−0.90 (m, 1H), 1.23 (t, J = 7.6 Hz, 3H), 1.23−1.34 (m, 2H), 1.54−1.64 (m, 1H), 1.73−1.80 (m, 2H), 2.78 (q, J = 7.6 Hz, 2H), 2.89 (d, J = 6.8 Hz, 2H), 3.05 (d, J = 6.8 Hz, 2H), 3.27−3.36 (m, 2H), 3.71 (s, 6H), 3.89 (s, 3H), 3.92−3.97 (m, 2H), 6.26 (s, 2H), 6.59 (dd, J = 1.6, 6.8 Hz, 1H), 7.00 (dd, J = 6.8, 8.8 Hz, 1H), 7.43 (dd, J = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.2, 159.5, 153.2, 137.4, 134.1, 121.0, 120.4, 115.2, 113.4, 105.2, 91.4, 68.0, 62.2, 62.0, 56.0, 55.4, 34.5, 31.6, 19.7, 14.4, 10.6, 3.6. HRMS calcd for ($C_{28}H_{37}N_3O_4$) [M + H]+ 480.2857; found 480.2862.

***N*-(Cyclopropylmethyl)-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-7-(2,4,5-trimethoxyphenyl)pyrazolo[1,5-*a*]pyridin-3-amine (24p).** Compound **24p** was prepared according to the procedure described for the synthesis of **24r** using **23a** (60 mg, 0.15 mmol), (2,4,5-trimethoxyphenyl)boronic acid (39 mg, 0.18 mmol), $Pd(PPh_3)_4$ (35 mg, 0.031 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (58 mg, 0.18 mmol) in DME (10 mL) and water (5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 1:1) to afford **24p** (62 mg, 85%) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ −0.02−0.06 (m, 2H), 0.34−0.41 (m, 2H), 0.78−0.88 (m, 1H), 1.19−1.36 (m, 2H), 1.27 (t, J = 7.6 Hz, 3H), 1.52−1.63 (m, 1H), 1.71−1.79 (m, 2H), 2.79 (q, J = 7.6 Hz, 2H), 2.86−2.91 (m, 2H), 3.02−3.07 (m, 2H), 3.27−3.34 (m, 2H), 3.77 (s, 3H), 3.86 (s, 3H), 3.90−3.96 (m, 2H), 3.96 (s, 3H), 6.67 (s, 1H), 6.68 (d, J = 7.0 Hz, 1H), 7.01 (dd, J = 7.0, 8.6 Hz, 1H), 7.22 (s, 1 H), 7.44 (d, J = 8.6 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 153.5, 152.3, 150.6, 142.9, 137.7, 137.2, 121.3, 120.8, 115.3, 114.5, 114.3, 112.4, 98.3, 68.0, 62.1, 61.9, 56.8, 56.4, 56.1, 34.4, 31.6, 19.8, 14.1, 10.6, 3.7. HRMS calcd for ($C_{28}H_{37}N_3O_4$) [M + H]+ 480.2858; found 480.2857.

[4-(3-{(Cyclopropylmethyl)[(tetrahydro-2*H*-pyran-4-yl)-methyl]amino}-2-ethylpyrazolo[1,5-*a*]pyridin-7-yl)-3,5-dimethoxyphenyl]methanol (24q). Compound **24q** was prepared according to the procedure described for the synthesis of **24r** using **23a** (250 mg, 0.64 mmol), [4-(hydroxymethyl)-2,6-dimethoxyphenyl]-boronic acid (407 mg, 1.9 mmol), $Pd(PPh_3)_4$ (111 mg, 0.096 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (303 mg, 0.96 mmol) in DME (13 mL) and water (6.5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 1:3) to afford **24q** (300 mg, 98%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03−0.05 (m, 2H), 0.32−0.40 (m, 2H), 0.80−0.90 (m, 1H), 1.22 (t, J = 7.5 Hz, 3H), 1.22−1.35 (m, 2H), 1.53−1.66 (m, 1H), 1.72−1.81 (m, 2H), 1.96 (t, J = 5.6 Hz, 1H), 2.78 (q, J = 7.5 Hz, 2H), 2.86−2.92 (m, 2H), 3.02−3.09 (m, 2H), 3.28−3.38 (m, 2H), 3.74 (s, 6H), 3.90−4.00 (m, 2H), 4.76 (d, J = 5.6 Hz, 2H), 6.61 (dd, J = 1.3, 6.8 Hz, 1H), 6.72 (s, 2H), 7.02 (dd, J = 6.8, 8.8 Hz, 1H), 7.46 (dd, J = 1.3, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 153.4, 144.4, 137.4, 134.0, 121.2, 120.6, 115.4, 113.3, 110.9, 102.7, 68.0, 65.2, 62.1, 62.0, 56.1, 34.5, 31.6, 19.6, 14.4, 10.6, 3.6. HRMS calcd for ($C_{28}H_{37}N_3O_4$) [M + H]+ 480.2857; found 480.2848.

***N*-(Cyclopropylmethyl)-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24r).** To a solution of **23a** (60 mg, 0.15 mmol) in DME (2 mL) and water (1 mL) were added [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (45 mg, 0.20 mmol), $Pd(PPh_3)_4$ (35 mg, 0.030 mmol), and $Ba(OH)_2{\cdot}8H_2O$ (72 mg, 0.23 mmol). The mixture was stirred at 90 °C for 4 h under a nitrogen stream and was then cooled and diluted with water. It was subsequently filtered through a pad of Celite to remove insoluble residue, and the filtrate was washed with EtOAc. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 1:1) to afford **24r** (40 mg, 54%) as a light-yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.04 (m, 2H), 0.34−0.38 (m, 2H), 0.80−0.90 (m, 1H), 1.22 (t, J = 7.6 Hz, 3H), 1.24−1.34 (m, 2H), 1.54−1.64 (m, 1H), 1.74−1.80 (m, 2H), 2.77 (q, J = 7.6 Hz, 2H), 2.88 (d, J = 6.8 Hz, 2H), 3.05 (d, J = 7.2 Hz, 2H), 3.31 (t, J = 11.6 Hz, 2H), 3.49 (s, 3H), 3.73 (s, 6H), 3.90−4.00 (m, 2H), 4.53 (s, 2H), 6.59 (dd, J = 1.2, 6.8 Hz, 1H), 6.67 (s, 2H), 7.00 (dd, J = 6.8, 8.8 Hz, 1H), 7.44 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 153.3, 141.4, 137.3, 134.0, 121.0, 120.4, 115.4, 113.1, 111.3, 103.6, 74.9, 68.0, 62.2, 62.0, 58.4, 56.1, 34.5, 31.6, 19.7, 14.3, 10.6, 3.6. HRMS calcd for ($C_{29}H_{39}N_3O_4$) [M + H]+ 494.3013; found 494.3019.

***N*-(Cyclopropylmethyl)-7-[2,4-dimethoxy-6-(methoxymethyl)phenyl]-2-ethyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24s).** Compound **24s** was prepared according to the procedure described for the synthesis of **24r** using **23a** (60 mg, 0.15 mmol), [2,4-dimethoxy-6-(methoxymethyl)phenyl]boronic acid (104 mg, 0.46 mmol), $Pd(PPh_3)_4$ (35 mg, 0.030 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (72 mg, 0.23 mmol) in DME (10 mL) and water (5 mL). The product was purified

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

by column chromatography on silica gel (*n*-hexane:EtOAc = 3:1), follwed by column chromatography on NH silica gel (*n*-hexane:EtOAc = 3:1) to afford **24s** (38 mg, 50%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04−0.02 (m, 2H), 0.30−0.36 (m, 2H), 0.78−0.88 (m, 1H), 1.20 (t, J = 7.6 Hz, 3H), 1.20−1.32 (m, 2H), 1.52−1.64 (m, 1H), 1.72−1.80 (m, 2H), 2.70−2.80 (m, 2H), 2.87 (d, J = 6.8 Hz, 2H), 3.04 (d, J = 6.8 Hz, 2H), 3.19 (s, 3H), 3.28−3.34 (m, 2H), 3.68 (s, 3H), 3.88 (s, 3H), 3.90−3.98 (m, 2H), 3.98 (d, J = 12.8 Hz, 1H), 4.21 (d, J = 12.8 Hz, 1H), 6.51 (d, J = 2.4 Hz, 1H), 6.54 (dd, J = 1.6, 6.8 Hz, 1H), 6.76 (d, J = 2.4 Hz, 1H), 6.99 (dd, J = 6.8, 8.8 Hz, 1H), 7.44 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.5, 158.7, 153.8, 140.4, 137.5, 135.4, 121.1, 120.7, 115.5, 114.7, 113.1, 103.8, 98.5, 71.9, 68.0, 62.0, 62.0, 58.1, 56.0, 55.4, 34.5, 31.6, 31.6, 19.7, 14.3, 10.6, 3.6, 3.6. HRMS calcd for ($C_{29}H_{39}N_3O_4$) [M + H]+ 494.3013; found 494.3019.

***N*-(Cyclopropylmethyl)-7-[4-(ethoxymethyl)-2,6-dimethoxyphenyl]-2-ethyl-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24t).** Compound **24t** was prepared according to the procedure described for the synthesis of **24r** using **23a** (67 mg, 0.17 mmol), [4-(ethoxymethyl)-2,6-dimethoxyphenyl]boronic acid (104 mg, 0.46 mmol), $Pd(PPh_3)_4$ (40 mg, 0.034 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (65 mg, 0.21 mmol) in DME (10 mL) and water (5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 2:1), follwed by column chromatography on NH silica gel (*n*-hexane:EtOAc = 4:1) to afford **24t** (61 mg, 70%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.06 (m, 2H), 0.34−0.43 (m, 2H), 0.80−0.94 (m, 1H), 1.24 (t, J = 7.5 Hz, 3H), 1.33 (t, J = 7.0 Hz, 3H), 1.20−1.38 (m, 2H), 1.54−1.68 (m, 1H), 1.74−1.84 (m, 2H), 2.78 (q, J = 7.5 Hz, 2H), 2.90 (d, J = 6.6 Hz, 2H), 3.07 (d, J = 7.0 Hz, 2H), 3.33 (dt, J = 1.6, 12.0 Hz, 2H), 3.66 (q, J = 7.0 Hz, 2H), 3.75 (s, 6H), 3.92−4.02 (m, 2H), 4.59 (s, 2H), 6.61 (br d, J = 6.8 Hz, 1H), 6.71 (s, 2H), 7.03 (dd, J = 7.0, 8.8 Hz, 1H), 7.46 (br d, J = 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 153.3, 141.8, 137.3, 134.0, 121.0, 120.4, 115.4, 113.1, 111.2, 103.7, 73.0, 68.0, 66.0, 62.2, 62.0, 56.1, 34.5, 31.6, 19.7, 15.3, 14.3, 10.6, 3.6. HRMS calcd for ($C_{30}H_{41}N_3O_4$) [M + H]+ 508.3170; found 508.3167.

***N*-(Cyclopropylmethyl)-7-(2,6-dimethoxyphenyl)-2-ethyl-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24u).** Compound **24u** was prepared according to the procedure described for the synthesis of **24r** using **23a** (50 mg, 0.12 mmol), (2,6-dimethoxyphenyl)boronic acid (33 mg, 0.18 mmol), $Pd(PPh_3)_4$ (21 mg, 0.018 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (57 mg, 0.18 mmol) in DME (2.4 mL) and water (1.2 mL). The product was purified by column chromatography on NH silica gel (*n*-hexane:EtOAc = 5:1), follwed by column chromatography on silica gel (*n*-hexane:EtOAc = 2:1) to afford **24u** (46 mg, 85%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.05−0.05 (m, 2H), 0.31−0.40 (m, 2H), 0.79−0.90 (m, 1H), 1.20−1.36 (m, 2H), 1.21 (t, J = 7.6 Hz, 3H), 1.53−1.65 (m, 1H), 1.71−1.80 (m, 2H), 2.76 (q, J = 7.6 Hz, 2H), 2.85−2.91 (m, 2H), 3.01−3.08 (m, 2H), 3.26−3.35 (m, 2H), 3.71 (s, 6H), 3.87−4.00 (m, 2H), 6.60 (d, J = 6.4 Hz, 1H), 6.69 (d, J = 8.4 Hz, 2H), 7.01 (dd, J = 6.4, 8.7 Hz, 1H), 7.39 (t, J = 8.4 Hz, 1H), 7.44 (d, J = 8.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 153.3, 137.3, 134.1, 130.7, 121.0, 120.5, 115.4, 113.1, 112.2, 104.6, 68.0, 62.2, 62.0, 56.1, 34.5, 31.6, 19.7, 14.3, 10.6, 3.6. HRMS calcd for ($C_{27}H_{35}N_3O_3$) [M + H]+ 450.2751; found 450.2754.

7-(2,6-Dimethoxy-4-methylphenyl)-2-ethyl-*N*-propyl-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (24v). Compound **24v** was prepared according to the procedure described for the synthesis of **24l** using **34b** (120 mg, 0.29 mmol), NaH (60%, 16 mg, 0.40 mmol), iodopropane (34 μL, 0.35 mmol) in DMF (0.8 mL) and 4 M HCl/EtOAc (0.8 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (78 mg, 0.68 mmol), $NaBH(OAc)_3$ (87 mg, 0.41 mmol) in THF (1.4 mL). The residue was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:2) to afford **24v** (111 mg, 86%, 3 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 7.3 Hz, 3H), 1.20 (t, J = 7.6 Hz, 3H), 1.22−1.32 (m, 2H), 1.39 (ddq, J = 7.3, 7.3, 7.3 Hz, 2H), 1.51−1.63 (m, 1H), 1.70−1.78 (m, 2H), 2.43 (s, 3H), 2.74 (q, J = 7.6 Hz, 2H), 2.93−2.98 (m, 2H), 2.97 (dd, J = 7.3, 7.3 Hz, 2H), 3.26−3.35 (m, 2H), 3.70 (s, 6H), 3.90−3.97 (m, 2H), 6.51 (s, 2H), 6.59 (dd, J = 1.3, 6.8 Hz, 1H), 7.00 (dd, J = 6.8, 8.8 Hz, 1H), 7.41 (dd, J = 1.3, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.5, 153.3, 141.1, 137.3, 134.2, 121.0, 120.3, 115.2, 113.2, 109.3, 105.5, 68.0, 62.8, 59.1, 56.0, 34.5, 31.7, 22.4, 22.1, 19.7, 14.5, 11.7. HRMS calcd for ($C_{27}H_{37}N_3O_3$) [M + H]+ 452.2908; found 452.2900.

7-[2,6-Dimethoxy-4-(methoxymethyl)phenyl]-2-ethyl-*N*-propyl-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]-pyridin-3-amine (24w). Compound **24w** was prepared according to the procedure described for the synthesis of **24l** using **34e** (70 mg, 0.16 mmol), NaH (60%, 10 mg, 0.24 mmol), 1-bromopropane (17 μL, 0.19 mmol) in DMF (2 mL) and 4 M HCl/EtOAc (2 mL) in EtOAc (1 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (48 mg, 0.42 mmol), $NaBH(OAc)_3$ (90 mg, 0.42 mmol) in THF (2 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 2:1) to afford **24w** (49 mg, 63%, 3 steps) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, J = 7.6 Hz, 3H), 1.20 (t, J = 7.2 Hz, 3H), 1.23−1.32 (m, 2H), 1.36−1.45 (m, 2H), 1.52−.62 (m, 1H), 1.72−1.78 (m, 2H), 2.73 (q, J = 7.2 Hz, 2H), 2.96−3.00 (m, 4H), 3.27−3.35 (m, 2H), 3.49 (s, 3H), 3.73 (s, 6H), 3.90−3.97 (m, 2H), 4.53 (s, 2H), 6.60 (dd, J = 1.6, 6.8 Hz, 1H), 6.68 (s, 2H), 7.01 (dd, J = 6.8, 8.8 Hz, 1H), 7.42 (dd, J = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 153.3, 141.4, 137.3, 134.0, 121.0, 120.3, 115.3, 113.2, 111.2, 103.6, 74.9, 68.0, 62.8, 59.1, 58.5, 56.1, 34.5, 31.7, 22.1, 19.7, 14.4, 11.7. HRMS calcd for ($C_{28}H_{39}N_3O_4$) [M + H]+ 482.3013; found 482.3005.

***N*-(Cyclopropylmethyl)-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2-methoxy-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (27a).** Compound **27a** was prepared according to the procedure described for the synthesis of **24r** using **26** (48 mg, 0.12 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (36 mg, 0.16 mmol), $Pd(PPh_3)_4$ (28 mg, 0.024 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (58 mg, 0.18 mmol) in DME (2 mL) and water (1 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 2:1), follwed by column chromatography on NH silica gel (*n*-hexane:EtOAc = 5:1) to afford **27a** (53 mg, 89%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.04 (m, 2H), 0.30−0.36 (m, 2H), 0.80−0.92 (m, 1H), 1.24−1.36 (m, 2H), 1.52−1.64 (m, 1H), 1.74−1.82 (m, 2H), 2.84 (d, J = 6.8 Hz, 2H), 2.97 (d, J = 6.8 Hz, 2H), 3.32 (td, J = 2.0, 11.6 Hz, 2H), 3.51 (s, 3H), 3.76 (s, 6H), 3.87 (s, 3H), 3.90−3.98 (m, 2H), 4.55 (s, 2H), 6.51 (dd, J = 1.6, 6.8 Hz, 1H), 6.69 (s, 2H), 7.04 (dd, J = 7.2, 8.8 Hz, 1H), 7.33 (dd, J = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.2, 158.8, 141.3, 139.2, 133.7, 122.2, 114.0, 111.5, 111.3, 105.9, 103.4, 75.0, 68.1, 61.7, 61.2, 58.5, 56.1, 56.0, 34.2, 31.6, 10.4, 3.4. HRMS calcd for ($C_{28}H_{37}N_3O_5$) [M + H]+ 496.2806; found 496.2798.

***N*-(Cyclopropylmethyl)-7-(2,6-dimethoxy-4-methylphenyl)-2-methoxy-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (27b).** Compound **27b** was prepared according to the procedure described for the synthesis of **24l** using **36a** (250 mg, 0.61 mmol), NaH (60%, 36 mg, 0.91 mmol), (bromomethyl)-cyclopropane (90 μL, 0.91 mmol) in DMF (8 mL) and 4 M HCl/EtOAc (15 mL) in EtOAc (10 mL) and tetrahydro-2*H*-4-pyrancarbaldehyde (72 mg, 0.63 mmol), $NaBH(OAc)_3$ (24 mg, 0.63 mmol) in THF (5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 4:1) to afford **27b** (45 mg, 16%, 3 steps) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.04−0.08 (m, 2H), 0.28−0.40 (m, 2H), 0.80−0.94 (m, 1H), 1.20−1.38 (m, 2H), 1.52−1.70 (m, 1H), 1.74−1.84 (m, 2H), 2.47 (s, 3H), 2.84 (d, J = 6.6 Hz, 2H), 2.97 (d, J = 6.9 Hz, 2H), 3.33 (dt, J = 2.0, 12.0 Hz, 2H), 3.75 (s, 6H), 3.89 (s, 3H), 3.90−4.00 (m, 2H), 6.52 (dd, J = 1.4, 6.9 Hz, 1H), 6.54 (s, 2H), 7.04 (dd, J = 6.8, 8.8 Hz, 1H), 7.33 (dd, J = 1.4, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.2, 158.6, 141.0, 139.2, 133.9, 122.2, 113.8, 111.6, 109.4, 105.8, 105.3, 68.1, 61.7, 61.2, 56.0, 56.0, 34.2, 31.6, 22.4, 10.4, 3.4. HRMS calcd for ($C_{27}H_{35}N_3O_4$) [M + H]+ 466.2700; found 466.2694.

***N*-(Cyclopropylmethyl)-7-iodo-2-(methylthio)pyrazolo[1,5-*a*]pyridin-3-amine (28).** Compound **28** was prepared according to

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

the procedure described for the synthesis of **22a** using **21** (600 mg, 1.5 mmol), NaH (60%, 80 mg, 2.0 mmol), (bromomethyl)cyclopropane (0.22 mL, 2.2 mmol) in DMF (6 mL) and 4 M HCl/EtOAc (10 mL, 40 mmol) in EtOAc (1 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 3:1) to afford **28** (506 mg, 94%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16−0.24 (m, 2H), 0.48−0.56 (m, 2H), 1.00−1.10 (m, 1H), 2.60 (s, 3H), 2.96 (d, J = 6.0 Hz, 2H), 3.00−3.24 (m, 1H), 6.68 (ddd, J = 1.2, 6.8, 8.8 Hz, 1H), 7.17 (dd, J = 1.2, 6.8 Hz, 1H), 7.43 (dd, J = 1.2, 8.8 Hz, 1H).

***N*-(Cyclopropylmethyl)-7-iodo-2-(methylthio)-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (29).** Compound **29** (50 mg, 58%) was obtained as a yellow oil according to the procedure described for the synthesis of **23a** using **28** (300 mg, 1.0 mmol), tetrahydro-2*H*-pyran-4-carbaldehyde (56 mg, 0.49 mmol) and $NaBH(OAc)_3$ (103 mg, 0.49 mmol) in THF (2.5 mL), which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.06−0.04 (m, 2H), 0.30−0.38 (m, 2H), 0.74−0.86 (m, 1H), 1.20−1.32 (m, 2H), 1.40−1.60 (m, 1H), 1.66−1.80 (m, 2H), 2.69 (s, 3H), 2.85 (d, J = 6.8 Hz, 2H), 3.02 (d, J = 7.2 Hz, 2H), 3.22−3.32 (m, 2H), 3.86−3.94 (m, 2H), 6.72 (dd, J = 7.2, 8.8 Hz, 1H), 7.15 (dd, J = 1.2, 7.2 Hz, 1H), 7.40 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.3, 139.0, 123.0, 122.5, 121.8, 115.6, 92.4, 67.9, 61.1, 60.7, 34.2, 31.5, 14.3, 10.2, 3.6. HRMS calcd for ($C_{18}H_{24}IN_3OS$) [M + H]+ 458.0758; found 458.0764.

***N*-(Cyclopropylmethyl)-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2-(methylthio)-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (30a).** Compound **30a** was prepared according to the procedure described for the synthesis of **24r** using **29** (50 mg, 0.19 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (50 mg, 0.25 mmol), $Pd(PPh_3)_4$ (40 mg, 0.038 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (56 mg, 0.18 mmol) in DME (2 mL) and water (1 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 3:1), follwed by column chromatography on NH silica gel (*n*-hexane:EtOAc = 6:1) to afford **30a** (36 mg, 37%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.02−0.04 (m, 2H), 0.30−0.38 (m, 2H), 0.82−0.92 (m, 1H), 1.22−1.34 (m, 2H), 1.52−1.64 (m, 1H), 1.76−1.82 (m, 2H), 2.44 (s, 3H), 2.90 (d, J = 6.8 Hz, 2H), 3.06 (d, J = 7.2 Hz, 2H), 3.32 (td, J = 2.0, 12.0 Hz, 2H), 3.50 (s, 3H), 3.74 (s, 6H), 3.90−3.98 (m, 2H), 4.54 (s, 2H), 6.59 (dd, J = 1.6, 7.2 Hz, 1H), 6.67 (s, 2H), 7.05 (dd, J = 7.2, 8.8 Hz, 1H), 7.41 (dd, J = 1.6, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.8, 146.3, 141.5, 138.7, 133.8, 121.9, 120.8, 114.6, 113.0, 110.9, 103.2, 74.9, 68.0, 61.3, 61.2, 58.4, 56.0, 34.3, 31.6, 14.8, 10.4, 3.5. HRMS calcd for ($C_{28}H_{37}N_3O_4S$) [M + H]+ 512.2578; found 512.2582.

***N*-(cyclopropylmethyl)-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2-methyl-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine (33).** Compound **33** was prepared according to the procedure described for the synthesis of **24h** using **32** (40 mg, 0.11 mmol), [2,6-dimethoxy-4-(methoxymethyl)phenyl]boronic acid (48 mg, 0.21 mmol), K_2CO_3 (44 mg, 0.32 mmol), and $Pd(PPh_3)_4$ (18 mg, 0.016 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 45:55), follwed by column chromatography on NH silica gel (*n*-heptane:EtOAc = 7:3) to afford **33** (31 mg, 61%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.05−0.02 (m, 2H), 0.31−0.36 (m, 2H), 0.78−0.88 (m, 1H), 1.20−1.32 (m, 2H), 1.54−1.64 (m, 1H), 1.72−1.78 (m, 2H), 2.34 (s, 3H), 2.85 (d, J = 7.2 Hz, 2H), 3.03 (d, J = 7.2 Hz, 2H), 3.26−3.34 (m, 2H), 3.47 (s, 3H), 3.71 (s, 6H), 3.90−3.96 (m, 2H), 4.51 (s, 2H), 6.53 (dd, J = 1.2, 6.8 Hz, 1H), 6.66 (s, 2H), 7.00 (dd, J = 6.8, 8.8 Hz, 1H), 7.42 (dd, J = 1.2, 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 148.0, 141.5, 137.6, 133.9, 121.3, 121.0, 115.1, 113.0, 111.3, 103.6, 74.9, 68.0, 61.9, 61.7, 58.4, 56.1, 34.4, 31.6, 12.7, 10.5, 3.5. HRMS calcd for ($C_{28}H_{37}N_3O_4$) [M + H]+ 480.2857; found 480.2856.

***tert*-Butyl [2-ethyl-7-(2-methoxy-4,6-dimethylphenyl)-pyrazolo[1,5-*a*]pyridin-3-yl]carbamate (34a).** Compound **34a** was prepared according to the procedure described for the synthesis of **24h** using **10** (40 mg, 0.12 mmol), (2-methoxy-4,6-dimethyllphenyl)boronic acid (34 mg, 0.19 mmol), K_2CO_3 (26 mg, 0.19 mmol), and $Pd(PPh_3)_4$ (20 mg, 0.017 mmol) in DME (1 mL) and water (0.5 mL). The product was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 4:1) to afford **34a** (40 mg, 86%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, J = 7.6 Hz, 3 H), 1.54 (br s., 6H), 1.98 (s, 3H), 2.39 (s, 3H), 2.73 (q, J = 7.6 Hz, 2H), 3.65 (s, 3H), 5.88 (br s., 1H), 6.56 (d, J = 6.6 Hz, 1H), 6.68 (s, 1H), 6.76 (s, 1H), 7.13 (dd, J = 8.8, 6.6 Hz, 1H), 7.37 (d, J = 8.8 Hz, 1H). ^{13}C NMR (150 MHz, C_5D_5N) δ 157.6, 155.0, 151.7, 139.6, 138.5, 136.7, 136.6, 122.6, 122.0, 120.0, 114.8, 112.8, 109.4, 107.8, 78.3, 54.9, 27.8, 20.9, 19.6, 18.7, 13.1. HRMS calcd for ($C_{23}H_{30}N_3O_3$) [M + H]+ 396.2282; found 396.2278.

7-(2-Chloro-4-methoxyphenyl)-2-ethyl-3-nitropyrazolo[1,5-*a*]pyridine (40a). Compound **40a** was prepared according to the procedure described for the synthesis of **24r** using **9** (94 mg, 0.35 mmol), (2-chloro-4-methoxyphenyl)boronic acid (130 mg, 0.70 mmol), $Pd(PPh_3)_4$ (80 mg, 0.070 mmol) and $Ba(OH)_2{\cdot}8H_2O$ (220 mg, 0.70 mmol) in DME (15 mL) and water (7.5 mL). The product was purified by column chromatography on silica gel (*n*-hexane:EtOAc = 5:1) to afford **40a** (115 mg, quantitative yield) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (t, J = 7.6 Hz, 3H), 3.15 (q, J = 7.6 Hz, 2H), 3.90 (s, 3H), 6.96 (dd, J = 2.4, 8.4 Hz, 1H), 7.07 (dd, J = 1.6, 7.2 Hz, 1H), 7.10 (d, J = 2.4 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.68 (dd, J = 7.2, 8.8 Hz, 1H), 8.40 (dd, J = 1.6, 8.8 Hz, 1H).

2-(Methylsulfonyl)-3-nitropyrazolo[1,5-*a*]pyridine (41). A mixture of aluminum oxide (57.2 g, 561 mmol) and water (11.4 mL) was stirred to mix homogeneously. To the mixture were added **19** (14.3 g, 68.4 mmol) and $CHCl_3$ (343 mL), followed by potassium peroxomonosulfate compound (126 g, 205 mmol) at room temperature, and the reaction mixture was stirred for 8 h at 80 °C. The reaction mixture was cooled and filtered through a pad of Celite to remove insoluble residue The filtrate was concentrated *in vacuo* to give a crude product of **41** (13.7 g, 83%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (s, 3H), 7.08 (dd, J = 6.8, 8.4 Hz, 1H), 7.63 (dd, J = 8.4, 8.8 Hz, 1H), 8.27 (d, J = 8.8 Hz, 1H), 8.34 (d, J = 6.8 Hz, 1H).

3-Nitropyrazolo[1,5-*a*]pyridine-2-carbonitrile (42). To a solution of **41** (2.1 g, 8.6 mmol) in THF (16 mL) and DMF (3 mL) was added sodium cyanide (508 mg, 10.4 mmol) at room temperature, and the reaction mixture was stirred for 6 h at 80 °C. The mixture was cooled and filtered through a pad of Celite to remove insoluble residue. The filtrate was concentrated *in vacuo*, and then water was added. The resulting solid was collected by filtration and washed with water to give a crude product of **42** (1.1 g, 69%) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (dd, J = 6.8, 6.8 Hz, 1H), 7.81 (dd, J = 6.8, 9.2 Hz, 1H), 8.43 (d, J = 9.2 Hz, 1H), 8.61 (d, J = 6.8 Hz, 1H).

3-[(Cyclopropylmethyl)[(tetrahydro-2*H*-pyran-4-yl)methyl]-amino]pyrazolo[1,5-*a*]pyridine-2-carbonitrile (43). To a solution of **42** (1.1 g, 6.0 mmol) in EtOAc (30 mL) was added 10% Pd/C (0.98 g, 50% wet). The reaction mixture was treated under H_2 atmosphere (1 atm) for 2 h at room temperature. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo* to give a crude product (843 mg, 89%) as a yellow solid.

The crude product (527 mg, 3.3 mmol) from the previous step was dissolved in THF (11 mL). To this mixture was added cyclopropanecarboxaldehyde (249 μL, 3.3 mmol), followed by titanium(IV) isopropoxide (1.2 mL, 4.0 mmol). After stirring for 7 h at room temperature, to the mixture was added MeOH (11 mL), followed by $NaBH_4$ (126 mg. 3.3 mmol) at 0 °C, and the reaction mixture was stirred for 3 h at room temperature. Water and a small amount of aqueous ammonia solution was added. The mixture was filtered through a pad of Celite to remove insoluble residue, and was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 1:1) to give the secondary amine including impurity.

To a solution of impurity-containing product from the previous step in MeOH (7 mL) and AcOH (0.7 mL) were added tetrahydro-2*H*-pyran-4-carbaldehyde (317 mg, 2.8 mmol) and α-picoline-borane (223 mg, 2.1 mmol) at room temperature, and the reaction mixture was

dx.doi.org/10.1021/jm300259r | *J. Med. Chem.* 2012, 55, 5255−5269

stirred for 16 h. A saturated $NaHCO_3$ aqueous solution was added to the reaction mixture, and the residue was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (*n*-heptane:EtOAc = 1:1) to afford **43** (378 mg, 37%, 2 steps) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03−0.05 (m, 2H), 0.38−0.47 (m, 2H), 0.83−0.95 (m, 1H), 1.22−1.37 (m, 2H), 1.56−1.70 (m, 1H), 1.71−1.81 (m, 2H), 2.96−3.02 (m, 2H), 3.18−3.24 (m, 2H), 3.26−3.35 (m, 2H), 3.90−3.98 (m, 2H), 6.92 (dd, J = 6.8, 7.2 Hz, 1H), 7.15 (dd, J = 6.8, 9.2 Hz, 1H), 7.58 (d, J = 9.2 Hz, 1H), 8.35 (d, J = 7.2 Hz, 1H).

3-[(Cyclopropylmethyl)[(tetrahydro-*2H*-pyran-4-yl)methyl]-amino]-7-iodopyrazolo[1,5-*a*]pyridine-2-carbonitrile (44). To a solution of **43** (222 mg, 0.72 mmol) in THF (4 mL) was added *n*-BuLi (1.59 M hexane solution; 0.54 mL, 0.86 mmol) dropwise at −90 °C under a nitrogen stream, and the reaction mixture was stirred for 30 min at the same temperature. A solution of 1,2-diiodoethane (263 g, 0.93 mmol) in THF (4 mL) was added dropwise to the reaction mixture at −78 °C, and the mixture was allowed to warm to −40 °C over 2 h. The mixture was quenched carefully with a saturated NH_4Cl aqueous solution, and the internal temperature was allowed to room temperature. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (*n*-heptane:EtOAc = 2:1) to afford **44** (198 mg, 63%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.03−0.06 (m, 2H), 0.38−0.48 (m, 2H), 0.82−0.96 (m, 1H), 1.22−1.37 (m, 2H), 1.55−1.80 (m, 3H), 2.97−3.04 (m, 2H), 3.19−3.36 (m, 4H), 3.89−3.99 (m, 2H), 6.91 (dd, J = 7.0, 8.8 Hz, 1H), 7.52 (d, J = 7.0 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H).

3-[(Cyclopropylmethyl)[(tetrahydro-*2H*-pyran-4-yl)methyl]-amino]-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]pyrazolo-[1,5-*a*]pyridine-2-carbonitrile (45). To a sulution of **44** (103 mg, 0.24 mmol) in toluene (2 mL) and EtOH (1 mL) were added [2,6-dimethoxy-4-(methoxymethyl)phenyl)]boronic acid (103 mg, 0.45 mmol), $Pd(PPh_3)_4$ (27 mg, 0.024 mmol) and 1 M Na_2CO_3 aqueous solution (0.47 mL, 0.47 mmol), and the mixture was stirred at 90 °C for 3 h under a nitrogen stream. The reaction mixture was cooled and water was added. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on NH silica gel (*n*-heptane:EtOAc = 2:1), followed by normal silica gel (*n*-hexane:EtOAc = 1:1) to afford **45** (70 mg, 60%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ −0.07−0.03 (m, 2H), 0.35−0.44 (m, 2H), 0.85−0.97 (m, 1H), 1.22−1.36 (m, 2H), 1.59−1.73 (m, 1H), 1.73−1.83 (m, 2H), 2.95−3.02 (m, 2H), 3.17−3.24 (m, 2H), 3.26−3.36 (m, 2H), 3.48 (s, 3H), 3.73 (s, 6H), 3.89−3.98 (m, 2H), 4.53 (s, 2H), 6.87 (d, J = 6.8 Hz, 1H), 6.68 (s, 2H), 7.18 (dd, J = 6.8, 8.8 Hz, 1H), 7.55 (d, J = 8.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 142.6, 136.9, 134.7, 129.9, 123.1, 122.4, 117.6, 116.3, 115.1, 109.2, 103.2, 74.8, 67.9, 62.0, 60.6, 58.5, 56.0, 34.1, 31.3, 9.8, 3.5. HRMS calcd for ($C_{28}H_{34}N_4O_4$) [M + H]+ 491.2653; found 491.2660.

***N*-(Cyclopropylmethyl)-7-[2,6-dimethoxy-4-(methoxymethyl)phenyl]-2-ethyl-*N*-[(tetrahydro-*2H*-pyran-4-yl)methyl]pyrazolo[1,5-*a*]pyridin-3-amine 4-methylbenzenesulfonate (46).** To a solution of **24r** (1.6 g, 3.2 mmol) in EtOAc (25 mL) was added *p*-toluenesulfonic acid monohydrate (617 mg, 3.2 mmol), and the mixture was stirred at room temperature. The precipitate was filtered out to afford a crude product (2.18 g), which was recrystallized from a mixed solvent of *tert*-butyl methyl ether (640 mL) and EtOAc (770 mL) to afford **46** (1.9 g, 89%) as a white crystal. mp 201 °C. ^{1}H NMR (400 MHz, C_5D_5N) δ −0.03−0.07 (m, 2H), 0.30−0.40 (m, 2H), 0.70−0.82 (m, 1H), 1.22 (dddd, J = 5, 12, 13, 13 Hz, 2H), 1.32 (t, J = 8 Hz, 3H), 1.56 (ttt, J = 4, 7, 13 Hz, 1H), 1.68 (br d, J = 13 Hz, 2H), 2.16 (s, 3H), 2.79 (d, J = 7 Hz, 2H), 2.87 (q, J = 8 Hz, 2H), 2.95 (d, J = 7 Hz, 2H), 3.22 (ddd, J = 5, 12, 12 Hz, 2H), 3.38 (s, 3H), 3.52 (s, 6H), 3.95 (br dd, J = 2, 12 Hz, 2H), 4.50 (s, 2H), 6.85 (s, 2H), 6.88 (dd, J = 1, 7 Hz, 1H), 7.18 (d, J = 8 Hz, 2H), 7.21 (dd, J = 7, 9 Hz, 1H), 7.63 (dd, J = 1, 9 Hz, 1H), 8.37 (d, J = 8 Hz, 2H). ^{13}C NMR (100 MHz, C_5D_5N) δ 159.3, 153.5, 145.8, 142.7, 139.4, 137.9, 135.2, 129.0, 126.9, 121.8, 121.6, 116.1, 113.8, 111.8, 103.7, 74.7, 67.9, 67.9, 62.2, 62.0, 58.2, 55.8, 34.8, 32.0, 32.0, 21.1, 20.2, 14.5, 11.1, 4.1, 4.1. IR (KBr, cm^{-1}) 2629 (w), 1638 (w), 1612 (w), 1583 (w), 1542 (w), 1224 (m), 1159 (m), 1131 (m), 1119 (m), 1031 (w). Anal. Calcd for $C_{36}H_{47}N_3O_7S$: C, 64.94%, H, 7.11%, N, 6.31%; Found: C, 64.77%, H, 7.11%, N, 6.22%.

Biology. CRF_1 Receptor Antagonist Binding Assays. HEK293 cells expressing human CRF_1 receptor were cloned using essentially the same method as that described in literature.[32] CRF_1 receptor binding was performed by using the homogeneous technique of scintillation proximity (SPA, Amersham Pharmacia, UK) with 96-well plates. Cell membrane (5 μg/well), wheat germ agglutinin coated SPA beads (1 mg/well), [^{125}I] human/rat CRF (0.1 nM), and diluted test compound solution were suspended in 150 μL of assay buffer (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 1.5 mM KH_2PO_4, 10 mM $MgCl_2$, 2 mM EGTA, 1.5% bovine serum albumin (BSA), pH 7.0). Total binding and nonspecific binding were measured in the absence and presence of 0.2 μM unlabeled sauvagine, respectively. Plates were shaken gently and incubated for more than 2 h at room temperature. After centrifugation (260 *g*, 5 min, rt), radioactivity was detected using TopCount (Packard, USA, MA) with a 1 min counting time per well. Each count was corrected by subtracting nonspecific binding, and was represented as a percentage of total binding. The IC_{50} value of each compound was calculated using a concentration−response curve.

Compounds **46** and **1** were also examined by filtration methods, as described previously, but with some modification.[36] Diluted test compounds and 0.1 nM [^{125}I] human/rat CRF were incubated with cell membrane (5 μg/well) in 150 μL of assay buffer (0.2% BSA, 50 mM Tris-HCl, 10 mM $MgCl_2$, 2 mM EGTA, protease inhibitor cocktail, pH 7.1) for more than 2 h at room temperature. Bound and free radioligand were separated by filtration using a glass fiber filter (Whatman GF/C, presoaked with 1% BSA/PBS(-)). The filter was washed with 5 mL of PBS (-), and was measured with a gamma-counter (ARC-1000 M, Aloka, Japan). Nonspecific binding was defined using 0.4 μM sauvagine. The IC_{50} value of each compound was obtained as described above. The K_i value was calculated with the Cheng−Prusoff equation: $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration of [^{125}I]-CRF and K_d is the dissociation constant of the radioligand. The K_i value was obtained using MS *Excel 2000* (Microsoft Co., Redmond, WA, USA).

Functional Assay. To determine the antagonistic activities, their effects on CRF-stimulated intracellular cAMP accumulation was examined in HEK293 cells expressing human CRF_1 receptor, which were cloned as described in the literature.[32] cAMP was measured using an enzyme immunoassay kit (Amersham Pharmacia, UK) or a cAMP kit, which is a homogeneous time-resolved fluorescence system (CIS bio international, France). Similar results were obtained with these two assay kits. HEK293 cells expressing human CRF_1 receptor (50 000 cells/well) in Dulbecco's modified Eagle medium containing 0.1% fetal bovine serum and 1 mM 3-isobutyl-1-methylxanthine, which is a phosphodiesterase inhibitor, were seeded in 96-well plates. After preincubation for 30 min, diluted test compounds were added and incubated for 30 min at 37 °C. Cells were stimulated with 1 nM human/rat CRF for 30 min at 37 °C and collected by centrifugation (630 *g*, 5 min, 4 °C). After aspiration of the medium, cells were lysed with lysis buffer, and the amount of intracellular cAMP was measured according to the procedures detailed in the instruction manual of each kit. Each produced cAMP was corrected for basal cAMP production (i.e., in the absence of CRF) and was expressed as a percentage of total production. The IC_{50} value of each compound was calculated using a concentration−response curve.

Light/Dark Test in Mice. Male BALB/c mice (Charles River Japan Inc., Kanagawa, Japan) weighing 20−28 g were used. Test compounds (0.4, 2, 10 mg/kg for **46** and 2, 10, 50 mg/kg for **1**) were orally administered to the mice 1 h before the test. A control group received an equivalent volume of vehicle (0.5% methyl cellulose, 10 mL/kg). The test apparatus was a modified version of that described by Belzung et al.[37] It consisted of two acrylic boxes, one of which was a black darkened box (dark box; 10 × 15 × 20 cm^3 high), and the other

was a white open-top box (light box; 20 × 15 × 20 cm^3 high). A black acrylic tunnel (7 × 10 × 4.5 cm^3 high) separated the dark box from the light box. In order to record the behavior of animals, the front- and back-sides of the light box (20 × 20 cm^2) were made of clear acrylic glass. The light intensity on floor of the light box was fixed to 150 lx. At the beginning of the experiment, a mouse was placed in the dark box. Its behavior was recorded on video tape over a 5 min period and the following two parameters were noted by an observer who was unaware of the treatment each animal had received: (a) the time spent in the light box, and (b) the number of line crosses between the dark tunnel and the light box. A mouse whose four paws were in the light box was considered as being in the light box. Data are expressed as the mean ± standard error of the mean (S.E.M.). Differences between the vehicle control and **46** or **1** treated groups were evaluated by one-way analysis of variance, followed by Dunnett multiple comparison test. A value of $p < 0.05$ (two-sided) was considered statistically significant.

Determination of *In Vitro* Hepatic Clearance. The *in vitro* hepatic clearance data were determined by depletion in human liver microsomes. Pooled human liver microsomes (n = 150) were purchased from BD (MA, USA). The stability assay was conducted by using 0.3 μmol/L substrate and 0.1 mg/mL microsomal protein in which the final concentration of organic solvent was 0.03% DMSO. Incubation was conducted at 37 °C for 0 and 15 min by adding the NADPH generating system. After the incubation, the microsomal matrix was deproteinized by adding acetonitrile/methanol containing the internal standard. After centrifugation, the resulting supernatant was analyzed by LC/MS/MS.

Mouse Pharmacokinetic Study. Pharmacokinetic parameters were estimated in male fasted CD1 (ICR) mice (Charles River Japan Inc., Kanagawa, Japan) after intravenous (IV) (3 mg/kg, 10 mL/kg) and oral (PO) (10 mg/kg, 10 mL/kg) administration. Dosing solution was prepared in DMSO/5% glucose with 1/250 volume of 5 mol/L HCl (1:19 for IV and 1:9 for PO). Blood samples were collected from the vena cava at 0.083 (5 min; for IV), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after dosing (n = 4 at each time point). Plasma was separated by centrifugation and stored in a frozen state until analysis. Plasma concentrations were measured by an HPLC−fluorescence method. A plasma sample (50 μL), 50 μL of internal standard solution, and 500 μL of 0.1 mol/L phosphate buffer (pH 7.4) were mixed, followed by the addition of 2 mL of isopropyl alcohol/n-hexane (3:97, v/v). Samples were shaken for 10 min and centrifuged. The organic layer was transferred to a new tube to which 500 μL of 0.2 mol/L HCl was added. This tube was again shaken for 10 min and centrifuged. After centrifugation, the resulting water layer (50 μL) was injected into the HPLC column. Chromatography was performed in the reversed-phase mode with a Mightysil RP-8 GP (4.6 mm i.d. × 150 mm, Kanto Chemical Co., Ltd., Tokyo, Japan) using gradient elution with two mobile phases (A and B) at 1 mL/min. Mobile phase A consisted of distilled water/acetonitrile/60% $HClO_4$/$NaClO_4$ (800:200:1:5, v/v/v/w), and mobile phase B consisted of distilled water/acetonitrile/60% $HClO_4$/$NaClO_4$ (200:800:1:5, v/v/v/w). Compound **46** and the internal standard were detected using a fluorescence detector (Ex: 305 nm, Em: 410 nm).

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

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table of HPLC purity data, and experimental procedures for the preparation of compounds **30b−g** and the intermediates **12−14**, **16−18**, **22**, **23**, **25**, **26**, **31**, **32**, **34**, used for **24c**, **24 h−k**, **24g**, **24m−o**, **27a−b**, **30b−g**, **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel.: +81 (0)29 847 7395; Fax: +81 (0)29 847 4952; e-mail: y4-takahashi@hhc.eisai.co.jp.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank Dr. Kodo Shikata for performing the mouse light/dark tests; Mami Gomibuchi, for HRMS; Eri Ena and Yumi Yokoyama, for NMR analysis; and Dr. Taro Terauchi, Dr. Yuichi Suzuki, Dr. Noritaka Kitazawa, Dr. Luis Castro, and Dr. Teiji Kimura for their assistance in the preparation of the manuscript.

■ ABBREVIATIONS USED

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

■ REFERENCES

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

(2) Owens, M. J.; Nemeroff, C. B. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.* **1991**, *43*, 425−473.

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

(4) Kostich, W. A.; Chen, A.; Sperle, K.; Largent, B. L. Molecular identification and analysis of a novel human corticotropin releasing factor (CRF) receptor: the $CRF_{2\gamma}$ receptor. *Mol. Endocrinol.* **1998**, *12*, 1077−1085.

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

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

(7) Smith, G. W.; Aubry, J.-M.; Dellu, F.; Contrarino, A.; Bilezekijian, L. M.; Gold, L. H.; Chen, R.; archuk, Y.; Hauser, C.; Bentley, C. A.; Sawchenko, P. E.; Koob, G. F.; Vale, W.; Lee, K.-F. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* **1998**, *20*, 1093−1102.

(8) Timpl, P.; Spanagel, R.; 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.

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

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

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

(12) Zorrilla, E. P.; Koob, G. F. Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discovery Today* **2010**, *15*, 371−383.
(13) Ising, M.; Zimmermann, U. S.; Kuenzel, H. E.; Uhr, M.; Foster, A. C.; Learned-Coughlin, S. M.; Holsboer, F.; Grigoriadis, D. E. High-Affinity CRF_1 Receptor Antagonist NBI-34041: Preclinical and Clinical Data Suggest Safety and Efficacy in Attenuating Elevated Stress Response. *Neuropsychopharmacology* **2007**, *32*, 1941−1949.
(14) Heinrichs, S. C.; De Souza, E. B.; Schulteis, G.; Lapsansky, J. L.; Grigoriadis, D. E. Brain penetrance, receptor occupancy and antistress in vivo efficacy of a small molecule corticotropin releasing factor type I receptor selective antagonist. *Neuropsychopharmacology* **2002**, *27*, 194−202.
(15) Gutman, D. A.; Owens, M. J.; Skelton, K. H.; Thrivikraman, K. V.; Nemeroff, C. B. The Corticotropin-Releasing $Factor_1$ Receptor Antagonist R121919 Attenuates the Behavioral and Endocrine Responses to Stress. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 874−880.
(16) Mansbach, R. S.; Brooks, E. N.; Chen, Y. L. Antidepressant-like effects of CP-154,526, a selective CRF_1 receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21−26.
(17) Arborelius, L.; Skelton, K. H.; Thrivikraman, K. V.; Plotsky, P. M.; Schulz, D. W.; Owens, M. J. Chronic Administration of the Selective Corticotropin-Releasing Factor 1 Receptor Antagonist CP-154,526: Behavioral, Endocrine and Neurochemical Effects in the Rat. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 588−597.
(18) Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; chmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, F. D.; Winston, E. N., III; Chen, , Y.; Heym, J. CP-154526, a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477−10482.
(19) Li, Y.-W.; Hill, G.; Wong, H.; Kelly, N.; Ward, K.; Pierdomenico, M.; Ren, S.; Gilligan, P.; Grossman, S.; Trainor, G.; aub, R.; McElroy, J.; Zaczek, R. Receptor Occupancy of Nonpeptide Corticotropin-Releasing Factor 1 Antagonist DMP696: Correlation with Drug Exposure and Anxiolyic Efficacy. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 86−96.
(20) Chen, C.; Grigoriadis, D. E. NBI 30775 (R121919), an Orally Active Antagonist of the Corticotropin-releasing Factor (CRF) Type-1 Receptor for the Treatment of Anxiety and Depression. *Drug Dev. Res.* **2005**, *65*, 216−226.
(21) Zobel, A. W.; Nickel, T.; Künzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects of high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatric Res.* **2000**, *34*, 171−181.
(22) Binneman, B.; Feltner, D.; Kolluri, S.; Shi, Y.; Qiu, R.; Stiger, T. A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH_1 antagonist) in the treatment of major depression. *Amer. J. Psychiatry* **2008**, *165*, 617−620.
(23) Chen, C.; Wilcoxen, K. M.; Huang, C. Q.; Xie, Y.-F.; McCarthy, J. R.; Webb, T. R.; Zhu, Y.-F.; Saunders, J.; Liu, X.-J.; Chen, T.-K.; Bozigian, H.; Grigoriadis, D. E. Design of 2,5-dimethyl-3- (6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5-*a*] pyrimidine (NBI 30775/R121919) and structure-activity relationships of a series of potent and orally active corticotropin-releasing factor receptor antagonists. *J. Med. Chem.* **2004**, *47*, 4787−4798.
(24) Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; allaschun, R. J.; Schmidt, A.; Schultz, D. W. Synthesis and oral efficacy of a 4-(butylethylamino)pyrrolo[2,3-d]pyrimidine: a centrally active corticotropin-releasing factor1 receptor antagonist. *J. Med. Chem.* **1997**, *40*, 1749−1754.
(25) He, L.; Gilligan, P. J.; Zaczek, R.; Fitzgerald, L. W.; McElroy, J.; Shen, H.-S. L.; Saye, J. A.; Kalin, N. H.; Shelton, S.; Christ, D.; Trainor, G.; Hartig, P. 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-*a*]-1,3,5-triazine: a potent, orally bioavailable CRF_1 receptor antagonist. *J. Med. Chem.* **2000**, *43*, 449−456.
(26) Chen, Y. L.; Braselton, J.; Forman, J.; Gallaschun, R. J.; Mansbach, R.; Schmidt, A. W.; Seeger, T. F.; Sprouse, J. F.; Tingley, F. D.; Winston, E., III; Schulz, D. W. Synthesis and SAR of 2-aryloxy-4-alkoxy-pyridines as potent orally active corticotropinreleasing factor 1 receptor antagonists. *J. Med. Chem.* **2008**, *51*, 1377−1384.
(27) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. Corticotropin Releasing Factor (CRF) Receptor Modulators: Progress and Opportunities for New Therapeutic Agents. *J. Med. Chem.* **2000**, *43*, 1641−1660.
(28) Tamura, Y.; Minamikawa, J.; Sumoto, K.; Fujii, S.; Ikeda, M. Synthesis and some properties of O-acyl and O-nitrophenylhydroxylamines. *J. Org. Chem.* **1973**, *38*, 1239−1241.
(29) Tsuchiya, T.; Sashida, H.; Konoshita, A. Thermal Rearrangements of Cyclic Amine Ylides. III. Intramolecular Cyclization of 2-Ethynylpyridine N-Imides to 3-Azaindolizine Derivatives. *Chem. Pharm. Bull.* **1983**, *31*, 4568−5572.
(30) Ochi, H.; Miyasaka, T.; Kanada, K.; Arakawa, K. Studies of Heterocyclic Compounds. VIII. Synthesis and Tautomerism of 2-Hydroxypyrazolo[1,5-*a*]pyridine. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1980−1984.
(31) Fujito, H.; Tominaga, Y.; Matsuda, Y.; Kobayashi, G. Reaction of Pyridinium and Isoquinolinium *N*-imines with Ketenethioacetals. *Heterocycles* **1977**, *6*, 379−382.
(32) Chen, R.; Lewis, A. K.; Perrin, H. M; Vale, W. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8967−8971.
(33) ClogP was calculated by software of Daylight Chemical Information Systems, Inc.
(34) Stepan, A. F.; Karki, K.; McDonald, W. S.; Dorff, P. H.; Dutra, J. K.; DiRico, K. J.; Won, A.; Subramanyam, C.; Efremov, I. V.; O'Donnell, C. J.; Nolan, C. E; Becker, S. L.; Pustilnik, L. R.; Sneed, B.; Sun, H.; Lu, Y.; Robshaw, A. E.; Riddell, D.; O'Sullivan, T. J.; Sibley, E.; Capetta, S.; Atchison, K.; Hallgren, A. J.; Miller, E.; Wood, A.; Obach, R. S. Metabolism-Directed Design of Oxetane-Containing Arylsulfonamide Derivatives as γ-Secretase Inhibitors. *J. Med. Chem.* **2011**, *54*, 7772−7783.
(35) Sugaya, Y.; Yoshiba, T.; Kajima, T.; Ishihama, Y. Development of Solubility Screening Methods in Drug Discovery. *YAKUGAKU ZASSHI* **2002**, *122*, 237−246.
(36) Okuyama, S.; Chaki, S.; Kawashima, N.; Suzuki, Y.; Ogawa, A.; Nakazato, A.; Kumagai, T.; Okubo, T.; Tomisawa, K. Receptor binding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasing factor subtype 1 receptor antagonists CRA1000 and CRA1001. *J. Pharm. Exp. Ther.* **1999**, *289*, 926−935.
(37) Belzung, C.; Misslin, R.; Vogel, E. Behavioural effects of the benzodiazepine receptor partial agonist RO 16−6028 in mice. *Psychopharmacology* **1989**, *97*, 388−391.